

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Affinity-Based Methods for the Separation of Parasite Proteins

C.R. Alves, F.S. Silva, F.O. Oliveira Jr, B.A.S. Pereira,
F.A. Pires and M.C.S. Pereira
*Instituto Oswaldo Cruz – Fundação Oswaldo Cruz,
Rio de Janeiro, RJ,
Brasil*

1. Introduction

Affinity chromatography-based techniques have been developed to purify parasite proteins and improve our understanding of the parasite life cycle. These advances can be translated into concrete proposals for new drugs, diagnostic methods and vaccines for parasite diseases and help to reduce social inequality.

Affinity chromatography has been demonstrated to be a powerful tool for the isolation and purification of parasite proteins and has potential applications for diagnosis and therapy. Many studies have focused on parasite proteins that modulate host cell defense, as gp63, a glycoprotein from *Leishmania spp.*, that is involved in the cleavage of the complement factor C3b to iC3b, which promotes adhesion of promastigotes to macrophages via complement receptor 3 (Brittgham et al., 1995). This route of internalization does not lead to production of oxygen radicals or NO and favors parasite subsistence within the host cell. Another example is the cysteine protease B (CPB), an important virulence factor of the *Leishmania (L.) mexicana* complex, that inhibits lymphocytes Th1 and/or promotes the Th2 response either through proteolytic activity or through epitopes derived from its COOH-terminal extension (Pereira et al., 2011).

Due to the important role of these molecules, many researchers seek to develop specific and potent inhibitors for therapeutic strategies. Aspartic protease, a potential target for antiparasitic therapies, has been isolated from *Trypanosoma cruzi* by affinity chromatography using a specific inhibitor of this enzyme (Pinho et al., 2009); this enzyme is target for treatment of infections caused by HIV (Wlodawer & Vondrasek, 1998) and *Candida* (Hoegl et al., 1999). This enzyme has also been reported in *Plasmodium spp.* and *Schistosoma mansoni*, where it plays an important role in host hemoglobin degradation (Klemba & Goldberg, 2002). Additionally, specific inhibitors of plasmepsins and renin are viable drugs for the treatment of patients with malaria and high blood pressure.

These parasite proteins, along with others, have been tested as new targets for chemo- and immunotherapies for parasite diseases. They have been assessed by lectins or protease inhibitor affinity chromatography. The separation of sugars based on lectin affinity is one of main procedure that has been used. This technique is based on the ability of lectins to bind

specifically to certain oligosaccharide structures on glycoconjugates isolated from parasites. Parasite proteins are processed through a multi-lectin affinity column, and they bind to the immobilized lectins through their sugar chains. Certain glycoconjugates are important for the parasite life cycle, and lectin affinity chromatography can help to reveal their roles (Guha-Niyogi et al., 2001).

The use of protease inhibitors in affinity chromatography is another important approach for assessing parasite proteins. Proteases hydrolyze peptide bonds and can therefore degrade proteins and peptides that influence a broad range of biological functions, including the process of parasite infection (Mackeron et al., 2006). The specificity of the protease inhibitor used is an important aspect of this methodology; L- trans-epoxy-succinylleucylamido-(4-guanidino) butane (specific to cysteine-protease), pepstatin A (to aspartyl-protease) and aprotinin (to serine-protease) are frequently immobilized on a solid matrix for this technique.

Glycosaminoglycan (GAG) affinity is the only affinity chromatography method that is based on the sugar chains of lectin-like proteins. Some of these molecules (such as heparin sulfate, heparan sulfate, dermatan sulfate, keratan sulfate and chondroitin sulfate) contain complex oligosaccharide structures, which may be displayed on cell surfaces, incorporated into the extracellular matrix or attached to secreted glycoproteins, suggesting that they play structural roles (Dreyfuss et al., 2010). GAGs have been reported as potential candidates for therapeutic intervention against parasitic infections, such as leishmaniasis and Chagas diseases (Azevedo-Pereira et al., 2007; Oliveira-Jr et al., 2008).

According to the general principle of affinity chromatography (Fig. 1), a protein of interest is recovered based on its capacity to bind a specific functional group (ligand) that is immobilized on a bead material (matrix) that has been packed into a solid support (column). Although many ligands (enzymatic substrates, inhibitors of an enzyme, lectin, sugar residues, vitamins, enzyme cofactors, monoclonal antibodies) have been used to isolate proteins based on affinity, only lectin, an enzyme inhibitor and glycosaminoglycans have been used to obtain parasite proteins. The most commonly used matrix materials for the attachment of the ligand are polysaccharide derivatives (cellulose, dextran and agarose) and polyacrylamide.

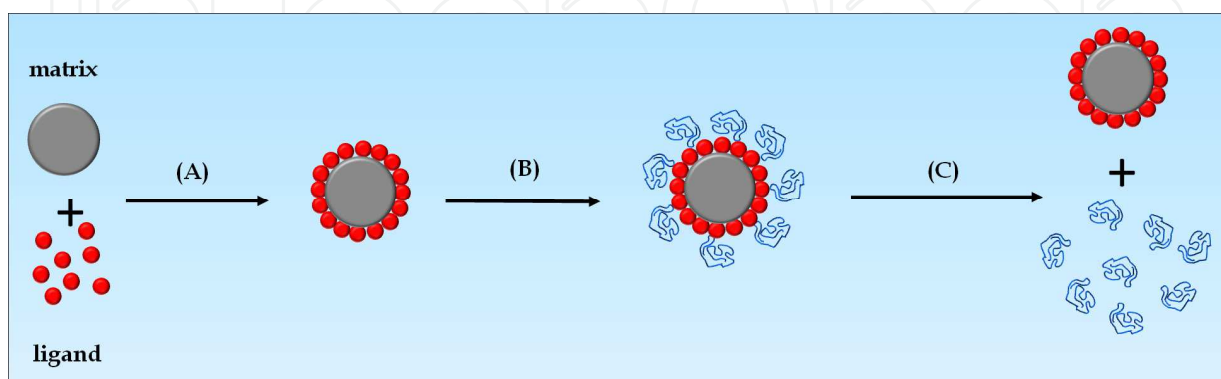


Fig. 1. The principle of affinity chromatography. The ligand is covalently bound to a matrix (A). The functionalized matrix is then able to bind to a target protein aided by a binding buffer (B). Afterwards, the bound proteins are eluted with a different buffer (C).

In these procedures, the soluble proteins are prepared from crude parasite lysates (or sub-cellular fractions) and loaded onto a column under chemical (buffer) and physical (temperature and pressure) conditions that promote the specific binding of the protein to the immobilized ligand (affinity) in what is known as the binding phase. Proteins that do not bind to the immobilized ligand under these conditions are removed from the solid phase by application of a constant liquid phase, which is referred to as the wash phase. Then, the bound protein can be recovered by changing the buffer conditions to favor desorption during the elution phase.

In this chapter, we describe the use of affinity chromatography to assess parasite proteins and the importance of these methods for public health. Several affinity chromatography protocols are considered. Additionally, we discuss our experience using affinity chromatography to obtain parasite proteins, and we include some unpublished results related to *Dermatobia hominis* third (L3) instar larvae proteases.

2. The use of affinity chromatography in parasite protein studies

2.1 Lectin affinity-based separation of parasite proteins

There are relatively few studies available in the current literature describing the use of lectins to affinity-purify glycosylated proteins from parasites. However, the reports on this subject demonstrate that this technique is useful for the retrieval of putative virulence factors or potential protective immunogens from a large array of parasites, including apicomplexan, trypanosomatids and nematodes (e.g., Fauquenoy et al., 2008, Gardiner et al., 1996, Smith et al., 2000). In addition to its utility in the isolation of parasite factors, lectin-based affinity chromatography is also a valuable resource for characterization of the structure of carbohydrates bound to proteins from these organisms due to the distinct specificities of the lectins that are available for this type of analysis.

Lectins are proteins that specifically bind to sugars, and they have been used for many types of studies, ranging from blood typing to immune regulation analysis (Rüdiger & Gabius, 2001). These proteins are generally isolated from plants (mostly legume seeds), where they can be found in abundance. Their usage is determined by the particular sugar structures that they are able to bind (Rüdiger & Gabius, 2001). The surveyed literature the use of six plant lectins [concanavalin A (Con A), ricin, jacalin, peanut agglutinin (PNA), wheat germ agglutinin (WGA) and Wisteria floribunda agglutinin (WFA)] in studies of parasites glycoproteins. Furthermore, one report described the use of *Biomphalaria alexandrina* lectin (BaII), which in contrast to the others is a lectin obtained from an animal.

Con A is a lectin that can be extracted from jack beans of the species *Canavalia ensiformis* (family Fabaceae). It binds to mannose or glucose residues and is thus characterized as a mannose-binding lectin. This lectin presents a high affinity for the oligosaccharide $\text{GlcNAc}\beta 2\text{Man}\alpha 6(\text{GlcNAc}\beta 2\text{Man}\alpha 3)\text{-Man}\beta 4\text{GlcNAc}$. It is also known to be a potent mitogen (Beckert & Sharkey, 1970; Rüdiger & Gabius, 2001).

Ricin, along with jacalin and PNA, is a lectin that binds to galactose. Specifically, it binds with high affinity to the motif $\text{Gal}\beta 4\text{GlcNAc}\beta 2\text{Man}\alpha 6(\text{Gal}\beta 4\text{-GlcNAc}\beta 2\text{Man}\alpha 3)\text{Man}\beta 4\text{GlcNAc}$. Ricin is highly toxic because it can impair ribosome activity through cleavage of the nucleobases of ribosomal RNA, and it has potential to be used as a biological

weapon. This lectin is extracted from *Ricinus communis* (Family Euphorbiaceae) (Rüdiger & Gabius, 2001; Lord et al., 2003).

Jacalin binds to galactose and N-acetylgalactosamine, and presents a high affinity for the motif Gal β 3GalNAc α . It is obtained from *Artocarpus integrifolia* (Family Moraceae). It is commonly used to isolate IgA from human plasma (Kabir, 1998, André et al., 2007).

Like Con A, PNA is a legume lectin and is isolated from plants that belong to the family Fabaceae. It is extracted from *Arachis hypogea* and binds specifically to the monosaccharide galactose and to the motif Gal β 3GalNAc α , similarly to the binding motif of jacalin. PNA is used as a marker of T-cell subpopulations and to differentiate between the stages of the Leishmania parasites life cycle (Dumont & Nardelli, 1979, Wilson & Pearson, 1984, Rüdiger & Gabius, 2001).

WGA is obtained from the species *Triticum vulgare*. It presents a low affinity for N-acetylgalactosamine, but it binds to the sialic acid N-acetylneuraminic and to the motif GlcNAc β 4GlcNAc β 4GlcNAc β 4-GlcNAc β 4GlcNAc. This lectin has been shown to bind more avidly to activated human T lymphocytes (Hellström et al., 1976, Rüdiger & Gabius, 2001).

WFA is isolated from *Wisteria floribunda*, a woody liana of the family Fabaceae. Although some uncertainty regarding its binding specificity remains, it seems that this agglutinin binds preferentially to the monosaccharide N-acetylgalactosamine and to the motif GalNAc α 6GalNAc. WFA is used to fractionate lymphocyte populations, and although it is not mitogenic like Con A, it can induce lymphokine production in murine splenocytes (Jacobs & Poretz, 1980; Rüdiger & Gabius, 2001).

BaSII is a lectin that can be isolated from the snail *B. alexandrina*, an intermediate host of the trematoda parasite *Schistosoma mansoni*, the causative agent of schistosomiasis. It specifically binds to the motif Fuc α 1,2Gal β 1,4Glc (Mansour, 1996).

2.1.1 General procedures for the isolation of parasite proteins by lectin affinity

The rationale for lectin-based affinity chromatography is the same as for other types of affinity-based fractionation: a sample is exposed to a solid phase that has been coupled to an affinity separation molecule (a lectin, in this case) under conditions that are adequate for binding (Fig. 2A). The unbound material from the sample is washed away (generally using the same buffer applied to equilibrate the solid-phase), and in the final step, the affinity-bound fraction is recovered by altering the equilibrium conditions of the solid phase (by changing the system pH or salt concentration) or by adding a molecule that competes for the binding site of the ligand.

To provide several practical examples, a collection of lectin affinity-based methodologies used to isolate and/or characterize glycoproteins from distinct parasites is listed in the Table 1.

It is important to note that some techniques, such as metabolic radioactive labeling (by [3H]-myristic acid or [3H]-glucosamine, for example) and cell disruption (by Triton X-100, dioxane or hypotonic solution), must be applied prior to lectin chromatography to allow for the identification of molecules eluted from the column or the preparation of suitable samples for the chromatography column, respectively.

Ligand	Organism	Isolated protein	First phase		Bind	Wash	Elution	Second phase		References
			Matrix	Matrix				Methods	Methods	
WGA	<i>Trypanosoma cruzi</i>	85 kDa surface glycoprotein	Sepharose	10mM Tris-HCl (pH 7.2), 150 mM NaCl	10mM Tris-HCl (pH 7.2), 150 mM NaCl	0.1 M N-acetyl-D-glucosamine in 10mM Tris-HCl (pH 7.2), 150 mM NaCl	None	None	Couto et al., 1990	
			Agarose	10 mM Mops buffer (pH 6.9), 1mM MgSO ₄ , 1 mM EGTA, 0.2% (v/v) Triton X-100, 10 µg/ml DNase I, 0.05 mM leupeptin, 2.5 mM PMSF, 5 mM iodoacetamide, 0.05 mM TPCK	10 mM Mops buffer (pH 6.9), 1mM MgSO ₄ , 1 mM EGTA, 0.2% (v/v) Triton X-100, 10 µg/ml DNase I, 0.05 mM leupeptin, 2.5 mM PMSF, 5 mM iodoacetamide, 0.05 mM TPCK	0.2 M acetic acid in 10 mM Mops buffer (pH 6.9), 1mM MgSO ₄ , 1 mM EGTA, 0.2% (v/v) Triton X-100, 10 µg/ml DNase I, 0.05 mM leupeptin, 2.5 mM PMSF, 5 mM iodoacetamide, 0.05 mM TPCK	None	None	Brickman & Balber, 1993	
BaSII	<i>Schistosoma mansoni</i>	37 kDa glycoprotein	Sepharose	20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 2 mM PMSF, 1% (v/v) Triton X-100, 1 mM PCMB, 1 mM o-phen, 1 mM iodoacetamide	20 mM Tris-HCl (pH 7.3), 0.1% (v/v) Triton X-100, 150 mM NaCl, 1mM CaCl ₂ , 1mM MgCl ₂	300 mM L-fucose in 20 mM Tris-HCl (pH 7.3), 0.1% (v/v) Triton X-100, 150 mM NaCl, 1mM CaCl ₂ , 1mM MgCl ₂	HPLC	HPLC	Mansour, 1996	
			(Con A) or agarose (ricin)	10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM MgCl ₂ , 5 mM CaCl ₂ , 2% NP-40, 100 µg/ml of antipain, leupeptin and E-64	10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM MgCl ₂ , 5 mM CaCl ₂ , 2% NP-40, 100 µg/ml of antipain, leupeptin and E-64	0.5 M alpha-methyl mannoside (Con A) or 0.5 M galactose (ricin) in 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% NP-40, NaCl, 0.1% NP-40, and 50 µg/ml leupeptin and E-64	Dyalis	Dyalis	Gardiner et al., 1996	
Concanavalin A	<i>Trypanosoma congolense</i>	Variant Surface Glycoprotein	Sepharose	10mM Tris-HCl (pH 7.5), 150 mM NaCl, 1mM CaCl ₂ , 1mM MnCl ₂	10mM Tris-HCl (pH 7.5), 150 mM NaCl, 1mM CaCl ₂ , 1mM MnCl ₂	Isoelectric focusing	Gel filtration (Biogel P 30)	Gel filtration (Biogel P 30)	Gerold et al., 1996	
			Agarose	50 mM Tris-HCl (pH 7.4), 0.02% sodium azide	50 mM Tris-HCl (pH 7.4), 0.02% sodium azide	50 mM Tris-HCl (pH 7.4), 0.02% sodium azide, 100 mM GalNAc	None	None	Gerold et al., 1996	
Concanavalin A	<i>Trypanosoma brucei</i>	Invariant surface glycoprotein heavily N-glycosylated	Sepharose	50 mM Tris buffer (pH 7.5), 150 mM NaCl, 1% (w/v) Triton X-100	10mM Tris buffer (pH 6.5), 0.1% (w/v) Triton X-100	0.5 M α-methylmannoside in 10mM Tris buffer (pH 6.5) containing 0.1% (w/v) Triton X-100	Ion exchange chromatography (DEAE-52)	Ion exchange chromatography (DEAE-52)	Nolan et al., 1997	
			Agarose	50 mM Tris buffer (pH 7.5), 150 mM NaCl, 1% (w/v) Triton X-100	10mM Tris buffer (pH 6.5), 0.1% (w/v) Triton X-100	0.5 M α-methylmannoside in 10mM Tris buffer (pH 6.5) containing 0.1% (w/v) Triton X-100	None	None	Nolan et al., 1997	

Table 1. Lectin affinity-based

Ligand	Organism	Isolated protein	First phase		Second phase		References	
			Matrix	Bind	Wash	Elution		Methods
PNA or jacalin	<i>Haemonchus contortus</i>	Glycoprotein fractions	Agarose	10mM Tris-HCl (pH7.4, 0.5 M NaCl, 0.02% NaN ₃ , 100 mM Ca ²⁺ , 10 mM Mg ²⁺)	10mM Tris-HCl (pH7.4, 0.5 M NaCl, 0.02% NaN ₃ , 100 mM Ca ²⁺ , 10 mM Mg ²⁺)	0.5 M galactose (PNA) or 0.8 M galactose (jacalin)	Gel filtration (Sephadex G-25); Anion Exchange chromatography (Mono Q - jacalin-binding material only)	Smith <i>et al.</i> , 2000
			Agarose	10 mM Tris (pH 7.4) 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.5% Triton X-100	10 mM Tris (pH 7.4) 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.25% CHAPS	0.5 M galactose in 12.5 mM Tris (pH 7.4) 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.25% CHAPS	None	Redmond <i>et al.</i> , 2004
Ricin	<i>Trypanosoma brucei</i>	Glycoproteins presenting poly-N-acetyllactosamine carbohydrate chains	Agarose	50 mM Tris-HCl (pH 6.8), 400 mM NaCl, 0.8% Triton X-100, 0.1 M TLCK, 1 µg/ml leupeptin, 0.1% sodium azide	50 mM Tris-HCl (pH 6.8), 400 mM NaCl, 0.8% Triton X-100, 0.1 M TLCK, 1 µg/ml leupeptin, 0.1% sodium azide	30 mg/ml lactose and 30 mg/ml galactose in 12.5 mM Tris-HCl (pH 6.8), 100 mM NaCl, 0.2% Triton X-100, 25 mM TLCK, 0.25 µg/ml leupeptin, 0.025% sodium azide	None	Atrih <i>et al.</i> , 2005
			Agarose	10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM CaCl ₂ , 1 mM MnCl ₂ , 1% (v/v) Triton X-100, protease inhibitor mixture	10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM CaCl ₂ , 1 mM MnCl ₂ , 1% (v/v) Triton X-100	1% (w/v) SDS in 100 mM Tris-HCl (pH 7.4) or 0.5 M Tris-HCl (pH 7.4) or 0.5 M α-methyl-	None	Fauquenoy <i>et al.</i> , 2008
Concanavalin A	<i>Toxoplasma gondii</i>	N-linked glycoproteins	Sheparose	20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	50 mM α-Dmannose in 20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	Anion exchange chromatography (DEAE-Sepahcel)	Rebello <i>et al.</i> , 2009

Table 1. (continued)

During the affinity chromatography procedure, other methods, such as isoelectric focusing, may be used instead of the application of competing carbohydrates to elude the column-bound material. Furthermore, distinct affinity columns can be used in sequence to purify fractions with specific characteristics from a single sample.

As for the handling of the material that is eluted from an affinity column, many options for further purification are available, depending on the analysis method chosen for the study. Some of these options include: anion exchange chromatography, size exclusion chromatography and dialysis.

The combination of these accessible approaches allows for a vast array of study possibilities. Several examples of the results obtained by applying lectin-affinity chromatography in association with other techniques are described in the following paragraphs.

2.1.2 Parasite proteins isolated by lectin affinity chromatography

The structure of an N-linked oligosaccharide from a surface glycoprotein of *Trypanosoma cruzi*, an important human parasite that causes Chagas disease, was defined in a study using lectin chromatography (Couto et al., 1990). It was determined that the structure of this oligosaccharide is comprised of complex carbohydrate chains that possess a terminal sialic acid, α -L-fucose and a galactosyl(α 1,3)galactose unit.

The cellular localization of glycoproteins of *Trypanosoma brucei rhodesiense*, a subspecies of the parasite responsible for the African sleeping sickness, was analyzed using ricin-based chromatography (Brickman & Balber, 1993). It was observed that the ricin-binding proteins were primarily located in the vesicles of the lysosomal / endosomal system.

Gardiner et al., (1996) characterized small glycoproteins isolated from the surface of *Trypanosoma vivax*, which causes bovine trypanosomiasis. That study was the first to detail the characteristics of a *T. vivax* Variable Surface Glycoprotein (VSG). The isolated protein, designated ILDat 2.1 VSG, presented a molecular mass of 40 kDa and contained mannose (or a derivative sugar) in small quantities, and it was poorly retained by the lectin affinity column. It is possible that carbohydrates comprise only the C-terminal anchoring structure of this protein.

The characteristics of a fucosyllactose determinant of a *S. mansoni* glycoprotein were identified using affinity chromatography based on a lectin that was isolated from a host of this parasite, *B. alexandrina*. This determinant is expressed in the outer chain of a single unit of complex type N-linked oligosaccharides (Mansour, 1996).

Additionally, the VSG glycosyl-phosphatidylinositol membrane anchors of *Trypanosoma congolense*, another trypanosomatid species that causes bovine trypanosomiasis, were studied by lectin affinity (Gerold et al., 1996) using a modification of the technique in which the bound proteins are electrophoretically desorbed (Reinwald et al., 1981). This analysis allowed for description of the VSG GPI-anchor in this parasite: it contains a β 1,6-linked galactose as the terminal hexose of the branch and an N-acetyl-glucosamine residue. Also, it was observed that *T. congolense* synthesizes two potential GPI-anchor precursors, one of which is insensitive to phospholipase C activity.

Nolan et al., (1997) identified a new invariant surface glycoprotein that is heavily N-glycosylated in the bloodstream forms of *Trypanosoma brucei* and designated it as ISG₁₀₀. This glycoprotein presents a large internal domain composed of a serine-rich repetitive motif, which was previously undescribed, and N-glycosylation sites on the N-terminal domain. Additionally, ISG₁₀₀ is encoded by a single gene, whereas the trypanosomal plasma membrane proteins are commonly encoded by tandemly repeated genes that are part of a multigene family.

Potentially protective glycoprotein fractions from *Haemonchus contortus*, a parasitic nematode in ruminants, were also obtained by lectin chromatography (Smith et al., 2000). The findings from that study confirmed the potential of the *H. contortus* PNA-binding glycoprotein fraction as an efficacious antigen against this parasite infection in sheep. Furthermore, this study identified another highly protective fraction that binds to jacalin. This second protective fraction presents sialylated versions of the same oligosaccharides that bound to the PNA column.

Another study on the protective properties of the glycoproteins of *H. contortus* was performed by the same group (Smith et al., 2003). The results showed that the four purified glycosylated zinc metalloproteinases from this parasite were such an efficacious antigen that, to an extent, they could account for most of the protection conferred by the urea-dissociated whole glycoproteins fraction. However, the role for the glycan moieties of these enzymes in the protection process was not clear.

The capacity of glycoproteins from *Caenorhabditis elegans*, a free living nematode, to induce protection from a challenge with *H. contortus* in sheep was assayed by Redmond et al. (2004). The lectin affinity methodology was able to identify glycoproteins with molecular masses between 25 and 200 kDa in extracts prepared from *C. elegans*, but the fractionated glycoproteins were not able to confer protection against an *H. contortus* challenge. These findings suggest that the conserved glycan moieties between these two species of worm are not solely responsible for the protection levels observed when native *H. contortus* antigens are used.

Trypanosoma brucei glycoproteins were shown to present distinctive structural features, such as the presence of giant poly-N-acetyllactosamine carbohydrate chains (Atrih et al., 2005). The recovered affinity-bound molecules were predominantly, but not exclusively, from the flagellar pocket. These glycoproteins carry massive glycans, representing the largest poly-LacNAc structures reported to that date, and they may produce a gel-like matrix in the lumen of the flagellar pocket and/or the endosomal/lysosomal system. Despite their remarkable size, these glycans present a very simple neutral structure, containing only mannose, galactose and N-acetylglucosamine.

Important glycoproteins from the apicomplexan parasite *Toxoplasma gondii* have also been analyzed by lectin affinity methods. It was shown that these components are pivotal factors for host invasion and intracellular development of parasites (Fauquenoy et al., 2008).

Cysteine proteinases from promastigotes of *Leishmania (Viannia) braziliensis* were shown to be anchored to the membrane by glycosylphosphatidylinositol structures in an analysis of the hydrophobic fraction of promastigote forms. These enzymes are suggested to play a role in the process of parasite survival inside its hosts (Rebello et al., 2009).

2.1.3 Remarks on the isolation of proteins by lectin affinity chromatography

These reports provide examples of the uses of lectin affinity chromatography to identify potentially antigenic fractions of parasites that could be used for vaccine development. Also, they point to the potential of this method to characterize glycoconjugates, such as the glycoproteins that are present on the parasite surface or secreted by these organisms. However, apart from these purely structural or clinically oriented applications, this method may also be relevant in other investigations, including studies of host-parasite interactions. This hypothesis is reinforced by reports indicating that lectin-glycan binding is important for the infection and virulence processes of some parasites, e.g. *Acanthamoeba castellanii* (Garate et al., 2006), *H. contortus* (Turner et al., 2008), *L. (V.) braziliensis* (Rebello et al., 2009) and *T. gondii* (Fauquenoy et al., 2008)

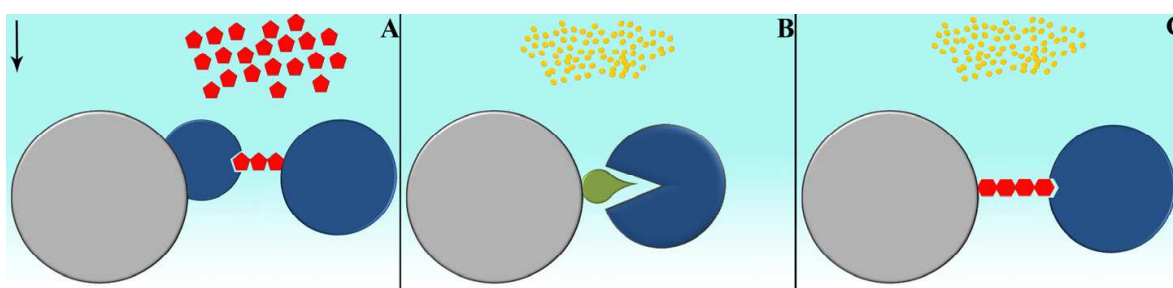


Fig. 2. Illustration of the affinity chromatography methodologies. The target molecules are bound to their ligands immobilized on a solid phase matrix. (A) Lectin affinity chromatography, (B) Protease inhibitor affinity chromatography and (C) Glycosaminoglycan affinity chromatography. Proteins = blue circle; carbohydrates = red pentagon and hexagon; protease inhibitors = green drop-like form; ions = yellow circles; and solid phase matrix beads = gray circle.

2.2 Protease inhibitors affinity-based separation of parasite proteins

Methodologies for the purification of parasite proteases have been applied in studies investigating the biological roles of these enzymes in parasite, including their participation in the infection process and in the survival of the parasites inside their hosts (McKerrow et al, 2006). Inhibitor affinity chromatography consists of the fractionation of parasite samples based on the reversible interactions between proteases and their specific inhibitors while the latter are covalently attached to a matrix (Fig. 2B). This technique can also be performed using irreversible inhibitors under particular conditions that will be described further in this section.

It is also interesting to note that, based on the specificity of the inhibitor used in the affinity chromatography, it is possible to suggest the enzyme class of the isolated protein. However, complementary analyses, such as characterization of the proteolytic activity, are often necessary to confirm these findings. Nevertheless, this purification strategy presents an initial advantage when compared to other methodologies.

In this section, fractionation approaches for serine-, aspartic acid- and cysteine proteases in specific parasites will be described. These approaches must take the class of the studied enzyme into consideration, as well as the inhibitor to be used and the characteristics of the mobile phase used for chromatography.

Ligand	Organism	Isolated protein	First phase		Wash	Elution	Second phase		References
			Matrix	Bind			Methods	Methods	
PNA or jacalin	<i>Haemonchus contortus</i>	Glycoprotein fractions	Agarose	10 mM Tris-HCl (pH 7.4), 0.5 M NaCl, 0.02% NaN ₃ , 100 mM Ca ²⁺ , 10 mM Mg ²⁺	10 mM Tris-HCl (pH 7.4), 0.5 M NaCl, 0.02% NaN ₃ , 100 mM Ca ²⁺ , 10 mM Mg ²⁺	0.5 M galactose (PNA) or 0.8 M galactose (jacalin)	Gel filtration (Sephadex G-25); Anion Exchange chromatography (Mono Q – jacalin-binding material only)	Smith <i>et al.</i> , 2000	
Concanavalin A	<i>Caenorhabditis elegans</i>	Glycoproteins	Agarose	10 mM Tris (pH 7.4), 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.5% Triton X-100	10 mM Tris (pH 7.4), 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.25% CHAPS	0.2 M methylmannopyranoside and 0.2 M methylglucopyranoside in 10 mM Tris (pH 7.4), 0.5 M NaCl, 10 mM CaCl ₂ , 100 mM MnCl ₂ , 0.25% CHAPS	None	Redmond <i>et al.</i> , 2004	
Ricin	<i>Trypanosoma brucei</i>	Glycoproteins presenting giant poly-N-acetyllactosamine carbohydrate chains	Agarose	50 mM Tris-HCl (pH 6.8), 400 mM NaCl, 0.8% Triton X-100, 0.1 M TLCK, 1 µg/ml leupeptin, 0.1% sodium azide	50 mM Tris-HCl (pH 6.8), 400 mM NaCl, 0.8% Triton X-100, 0.1 M TLCK, 1 µg/ml leupeptin, 0.1% sodium azide	30 mg/ml lactose and 30 mg/ml galactose in 12.5 mM Tris-HCl (pH 6.8), 100 mM NaCl, 0.2% Triton X-100, 25 mM TLCK, 0.25 µg/ml leupeptin, 0.025% sodium azide	None	Atrih <i>et al.</i> , 2005	
Concanavalin A	<i>Toxoplasma gondii</i>	N-linked glycoproteins	Agarose	10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM MnCl ₂ , 1% (v/v) Triton X-100, protease inhibitor mixture	10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM CaCl ₂ , 1 mM MnCl ₂ , 1% (v/v) Triton X-100	1% (w/v) SDS in 100 mM Tris-HCl (pH 7.4) or 0.5 M α-methyl-	None	Fauquenoy <i>et al.</i> , 2008	
Concanavalin A	<i>Leishmania (V) braziliensis</i>	Cysteine proteinases	Sheparose	20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	50 mM α-Dmannose in 20 mM Tris-HCl (pH 7.2), 5% (v/v) glycerol, 0.5% (v/v) CHAPS	Anion exchange chromatography (DEAE-Sepahcel)	Rebello <i>et al.</i> , 2009	

Table 2. Protease Inhibitors affinity-based

Ligand	Organism	Isolated protein	First phase			Second phase		References
			Matrix	Bind	Wash	Elution	Methods	
Pepstatin A	<i>Neospora caninum</i>	52 kDa	Agarose	5 mM NaOAc, (pH 5.5)	5 mM NaOAc, (pH 5.5)	0.1 M Tris-HCl, 0.5 M NaCl, (pH 8.5)	None	Naguleswaran <i>et al.</i> , 2005
Aprotinin	<i>Leishmania amazonensis</i>	115 and 56 kDa	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 1,5 mM NaCl, (pH7,5)	None	Silva-Lopez <i>et al.</i> ; 2005
Bacitracin	<i>Trichomonas vaginalis</i>	60 and 30 kDa	Agarose	20 mM NaOAc, (pH 4.0)	20 mM NaOAc, (pH 4.0)	0.1 M Tris-HCl, 1.0 M NaCl 25% 2-propanol, (pH 7.0)	Bio-Gel P-60	Sommer <i>et al.</i> ; 2005
Aprotinin	<i>Leishmania braziliensis</i>	60 and 45 kDa	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 1,5 mM NaCl, (pH7,5)	None	Guedes HL <i>et al.</i> ; 2007
Aprotinin	<i>Trypanosoma cruzi</i>	75 kDa	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 1,5 mM NaCl, (pH7,5)	None	Silva-Lopez <i>et al.</i> ; 2008
Aprotinin	<i>Leishmania donovani</i>	115 kDa	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 1,5 mM NaCl, (pH7,5)	None	Choudhury <i>et al.</i> ; 2009
Pepstatin A	<i>Trypanosoma cruzi</i>	120, 48 and 56 kDa	Agarose	0.1 M NaOAc, 1.0 M NaCl, (pH 3.5)	0.1 M NaOAc, 1.0 M NaCl, (pH 3.5)	0.1 M Tris-HCl, 1.0 M NaCl, (pH 8.6)	HPLC gel filtration	Pinho <i>et al.</i> ; 2009
Aprotinin	<i>Leishmania donovani</i>	58 kDa	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,4)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,4)	10 mM Tris-HCl, 1 M NaCl, (pH7,5)	DEAE	Choudhury <i>et al.</i> ; 2010
Pepstatin A	<i>Rhipicephalus (B.) microplus</i>	42 kDa	Agarose	20 mM NaOAc, 1 M NaCl, (pH 5.3)	20 mM NaOAc, 1 M NaCl, (pH 5.3)	100 mM Tris-HCl, 1 M NaCl, (pH 8.6)	Mono-Q	Cruz <i>et al.</i> ; 2010
Benzamidine	<i>Plasmodium ookinete</i>	37 kDa	Sepharose	0.1% Triton X-100 in PBS	0.1% Triton X-100 in PBS	PBS	None	Li <i>et al.</i> ; 2010
Aprotinin	<i>Leishmania chagasi</i>	LCSII (105, 66, 60 kDa); LCSIII (60, 58 kDa) and LCSIII (76, 68 kDa)	Agarose	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 5 mM CaCl ₂ , (pH7,5)	10 mM Tris-HCl, 1,5 mM NaCl, (pH7,5)	HPLC	Silva-Lopez <i>et al.</i> ; 2010
E-64	<i>Trypanosoma cruzi</i>	60 kDa	Sepharose	20 mM Tris-HCl, 150 mM NaCl, 100 M PMSF, (pH 7.4)	20 mM Tris-HCl, 150 mM NaCl, 100 M PMSF, (pH 7.4)	20 mM Tris-HCl, 150 mM NaCl, 100 M PMSF, 2.0 M NaCl, (pH 7.4)	None	Bourguignon <i>et al.</i> 2011

Table 2. (continued)

Aprotinin and pepstatin A are examples of inhibitors that are frequently used in the isolation of serine- and aspartic acid proteases, respectively, from many parasite species (Bond & Beynon). Other inhibitors that have been previously described in the isolation of serine proteases include soybean trypsin inhibitor (SBTI) and chloromethylketone (CMK). As for the purification of cysteine proteases, the use of three other inhibitors has been reported: L-transepoxy succinyl-leucylamido-[4-guanidino]butane (E-64), bacitracin and glycyl-phenylalanyl-glycyl-semicarbazone (Table 2). It must be emphasized that these inhibitors cannot be used to isolate all of the proteases classes from parasites, as they present distinct affinities for members of different groups and families within these enzyme classes. Therefore, investigation of the possible variations present in the active site of these enzymes may prove useful.

The features of the buffer (temperature, pH and ionic strength) to be used may vary according to the ligand's physicochemical characteristics, the chemical environment of the parasite enzyme and the analyzed species of parasite. For example, distinct buffers were used for the purification of serine proteases from *S. mansoni* and *Trichinella spiralis* using benzamidine. It is also noteworthy that for each organism, a different matrix was used to immobilize the inhibitor, sepharose for *S. mansoni* and celite for *T. spiralis*. The use of distinct buffers in studies that are based on the same inhibitor is also noted in reports of SBTI, E-64, bacitracin and glycyl-phenylalanyl-glycyl-semicarbazone, all of which are cysteine protease inhibitors.

Affinity chromatography with an irreversible inhibitor has also been described previously; the cysteine-protease inhibitor is an example of this strong binding. In the interaction between E-64 and cysteine-protease, a covalent bond is established (Matsumoto, 1989). Therefore, a reaction between the epoxy groups of the inhibitor and the thiopropyl group of the sepharose matrix is necessary to bind E-64 to a solid support. This reaction prevents the reaction of E-64 with the cysteine residue at the protease catalytic center. However, this does not affect the bond between the inhibitor and cysteine-protease; instead, it only results in inhibition of the proteolytic activity (Govrin, 1999).

2.2.1 Parasite proteins isolated by cysteine-protease inhibitors affinity chromatography

There is only one published example of the use of E-64 affinity chromatography to assess cysteine-protease isolated from a parasite, and this study was conducted with the *T. cruzi* epimastigote. In this study, chromatography was useful for assessing the effects of β -Lapachone naphthoquinones on a 60 kDa cysteine-protease activity present in *T. cruzi*. The results demonstrated the potential of this protease inhibitor as a new antichagasic compound (Bourguignon et al., 2011). Another example of a cysteine-protease isolated by inhibitor affinity chromatography in parasites was described for *Plasmodium falciparum*. In this case, a glycyl-phenylalanyl-glycyl-semicarbazone-based column was used to isolate a protease with a molecular weight of 27 kDa, as determined by SDS-PAGE (Shenai et al, 2000).

2.2.2 Parasite proteins isolated by serine-protease inhibitors affinity chromatography

Aprotinin affinity-based chromatography was useful for the isolation of a serine-protease of 115 kDa (Silva-Lopez et al., 2005), a 68 kDa (Morgado- Diaz et al., 2004; Silva-Lopez et al., 2004) and a 56kDa (Silva-Lopez et al., 2004) from *L.(L.) amazonensis* compared to other

purification procedures that were used to isolate parasite serine peptidase enzymes (Kong et al., 2000; Ribeiro de Andrade et al., 1998). In *Leishmania (V) braziliensis* promastigotes, 60 kDa and 45 kDa enzymes were purified using the aprotinin affinity-based and activity esterase assessed against N-alpha-benzoyl-L-arginine ethyl ester hydrochloride and N-alpha-p-tosyl-L-arginine methyl ester hydrochloride (Guedes et al., 2007). Furthermore, three protein profiles were isolated from *Leishmania chagasi* promastigotes, including LCS I (58 and 60 kDa), LCS II (60, 66, 105 and kDa) and LCS III (68 and 76 kDa), which were characterized as serine-protease enzymes based on their activity toward α -N-p-tosyl-L-arginine methyl ester substrate (Silva-Lopez et al., 2010). Furthermore, serine proteases with molecular weights of 75 kDa (Silva-Lopez et al., 2008) and 115 kDa (Choudhury et al., 2009) were identified as excretory products of *T. cruzi* and components of the sub-cellular environment in *Leishmania donovani*, respectively, although the chromatography step was not able to produce a homogeneous fraction. Furthermore, an intracellular serine protease of 58 kDa was purified from *Leishmania donovani* (Choudhury et al., 2010).

In addition, the aprotinin affinity-based chromatography was useful for the isolation of serine-proteases of 35 kDa and 26 kDa from *Anisakis simpZex* (Morris et al, 1994), 43 kDa from *Candida albicans* (Morrison et al, 1993), 15 kDa from *Schistosoma mansoni* (Salter et al, 2000), 42 kDa from *Rhipicephalus (B.) microplus* (Cruz et al, 2010), 60 kDa and 30 kDa from *Trichomonas vaginalis* (Sommer et al; 2005) and 35 to 52 from *Caenorhabditis elegans* (Geier et al; 1999).

Benzamidine-celite was applied in the isolation of serine proteases among the excreted or secreted proteins of *T. spirali*. The recovered proteases were not purified to homogeneity, and they showed molecular masses of 18 kDa, 40 kDa and 50 kDa (Todorova & Stoyanov). A similar finding was reported for the serine-proteases of *Chrysomya bezziana* larvae by using an SBTI-based column to purify four proteins with molecular masses of 13 kDa, 16 kDa, 26 kDa and 28 kDa (Muharsini et al., 2000).

Because it is possible to isolate heterogeneous products using inhibitors for affinity-based chromatography, we assessed a serine-protease from the third (L3) instar larvae of *D. hominis*. This ectoparasite causes dermatobiose in vertebrates, including humans, and it is particularly relevant in cattle, where it can cause a drop in production of meat and milk, leather as well depreciation (Maia & Guimarães, 1985).

Due to the association of DEAE-Sephacel with aprotinin agarose, it was possible to assess a serine protease from L3 larvae (Fig. 3). The fractions obtained by ion change chromatography containing esterase activity were pooled and then fractionated on an aprotinin-agarose column. This fraction showed a profile with multiple bands by SDS-PAGE and silver staining, and only one band of enzyme activity (50 kDa) was detected by gelatin-SDS-PAGE at pH 7.5 (Fig. 3). Interestingly, this band of 50 kDa was not initially detected in the extracts from L3 by gelatin-SDS-PAGE. The expression of this enzyme is likely low in these larvae, and it can only be detected after concentration by chromatographic methods. The proposed strategy to isolate a serine protease allowed for the detection of a band of 50 kDa in extracts of L3 larvae, and this band had not been previously detected in the direct analysis of the total extract by gelatin-SDS-PAGE. Additionally, this fraction was found to have esterase activity (data not shown).

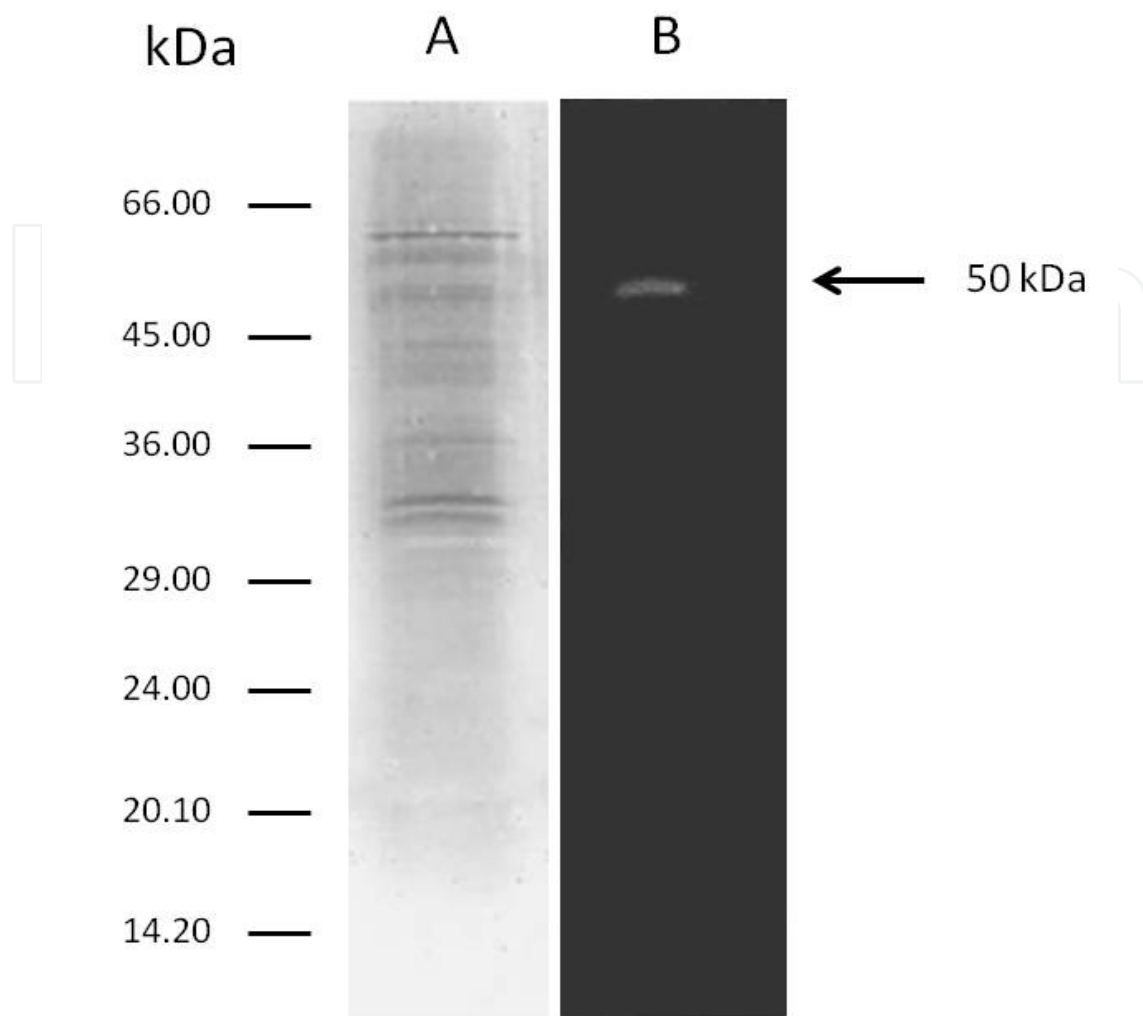


Fig. 3. Electrophoresis of proteins from L3 instar larvae of *Dermatobia hominis* eluted from a column of aproteinin-agarose. A total of 20 μg of protein from each fraction was resolved by SDS-PAGE (A) and gelatin-SDS-PAGE (B) and the bands were detected by silver staining and negative coloration, respectively. The arrow indicates a serine protease of 50 kDa. The molecular mass markers are indicated (kDa). These results are representative of two independent assays

2.2.3 Parasite proteins isolated by aspartyl-protease inhibitors affinity chromatography

Affinity-based chromatography based on pepstatine A was used to isolate a 52 kDa aspartyl protease from *Neospora caninum* tachyzoites (Naguleswaran et al., 2005) and a 45 kDa enzyme from *S.mansoni* (Valdivieso et al., 2003). In *Trypanosoma cruzi* epimastigotes, two aspartyl proteases were isolated (cruzipsin-I and cruzipsin-II). The molecular mass was estimated to be 120kDa by high performance liquid chromatography gel filtration, and the activities of these enzymes were detected in a doublet of bands (56 kDa and 48 kDa). These findings demonstrate that both proteases are novel *T. cruzi* acidic proteases. The physiological function of these enzymes in *T. cruzi* is not completely defined (Pinho et al., 2009).

An aspartyl protease with molecular mass of 37 kDa (plasmepsin) was isolated from the surface of *Plasmodium ookinete*, and its sequence was determined by mass spectrometry (Li et al., 2010). This protease was purified by using a benzamidine affinity-based column, which is typically used for the isolation of serine proteases. Structural similarity between the active site residues of the serine- and aspartyl proteases is possible, as some hydrogen-bonded residues can be arranged without any strain, such as in the formation of an oxyanion hole, in a manner that resembles the active site of a serine protease (Andreeva et al., 2004)

2.2.4 Remarks on the isolation of proteins by protease inhibitors affinity chromatography

Although the studies that have been conducted to isolate parasite proteases are of great medical interest, no parasiticide drug has been proposed thus far. In general, the chromatography methods involving inhibitor-based affinity-capture have been useful only to describe these enzymes in parasites and to establish their biochemical properties, their functions and their application in drugs tests.

Furthermore, the heterogeneous material obtained from affinity-based chromatography may require additional procedures for purification of the enzyme. Thus, other techniques must be applied to obtain proteases with greater purity, including molecular exclusion and ion exchange chromatography.

2.3 Glycosaminoglycans affinity-based separation of parasite proteins

Microbes have developed different strategies to gain access into mammalian cells (Bermúdez et al., 2010; Caradonna & Burleigh 2011; Soong et al., 2011). The first step involves the recognition of molecules at the surface of the target cell, which triggers the activation of signaling pathways that are implicated in the parasite internalization (Abban & Meneses 2010; Epting et al., 2011). Host cell surface sulfated proteoglycans have been implicated as key molecules at the host cell-parasite interface, mediating the adhesion and invasion of numerous parasitic microorganisms (Jacquet et al., 2001; Kobayashi et al., 2010; O'Donnell & Shukla 2010).

2.3.1 Structure of glycosaminoglycans

Proteoglycans (PGs) are composed of core proteins that are covalently linked to glycosaminoglycan (GAG) chains. As components of the extracellular matrix, the structural diversity of PGs depends on the identity of the core protein and the GAG composition. GAGs are linear polysaccharides comprised of disaccharide repeats containing uronic acid and hexosamine. GAGs vary in the type of hexosamine, hexose or hexuronic acid unit. The sulfated GAGs are classified as heparin [2-O-sulfo- β -D-glucuronic acid (GlcUA-2S) or 2-O-sulfo- α -L-iduronic acid (IdoUA-2S) and N-acetylglucosamine (GlcNAc) or N-sulfoglucosamine (GlcNS)], heparan sulfate [GlcUA, IdoUA or IdoUA-2S and GlcNAc or GlcNS], chondroitin sulfate [GlcUA and N-acetylgalactosamine (GalNAc)], dermatan sulfate [GlcUA or IdoUA and GalNAc] and keratan sulfate [galactose (Gal) and GlcNAc]. In fact, the structural diversity of PGs may provide sites of affinity for different ligands and, therefore, function as co-receptors or receptors for GAG-binding proteins (Dreyfuss et al., 2009; Ly et al., 2010). Although heparin is not found on the cell surface, this GAG has being

commonly used as tool for pathogen-host cell interaction assays. Heparins are negatively charged structures and native heparin presents molecular weights in the range of 5 to 30 kDa, whereas commercial heparin preparations are in the range of 12 kDa to 15 kDa.

2.3.2 Role of heparin-binding proteins in pathogen-host cell

Many pathogens express surface proteins that interact with GAGs in different stages of their life cycle. Although some parasites can bind to multiple GAGs (Coppi et al., 2007; Fallgren et al., 2001), heparan sulfate proteoglycan (HSPG) has been implicated in the recognition and/or invasion process of a wide range of pathogens, including viruses, bacteria and protozoan parasites (Bambino-Medeiros et al., 2011; Dalrymple & Mackow 2011; Yan et al., 2006). Despite the role of heparin-binding proteins in many physiological and pathological processes, the basis of the heparin-protein interaction at the molecular level is still unclear.

Thus, efforts have been concentrated to enhance methods for the isolation and characterization of heparin-binding proteins, and, in parallel, to determine the role of this GAG in pathogen-host cell interaction. Currently, heparin affinity chromatography has been applied to the purification of GAG-binding proteins from different pathogens (Table 3). In these chromatography assays, the heparin is covalently coupled to agarose or sepharose beads and its sulfates and carboxylates chains are able to bind many proteins by basic amino acids (Fig. 2C).

This technique has been used to isolate heparin-binding proteins without loss of their biological activity, leading to a better understanding of the mechanism involved in the parasite invasion process. For example, chlamydial outer membrane complex (OmcB), a 60 kDa cysteine-rich protein, displays a protein motif (50-70OmcB peptide) that acts as an acceptor molecule to bind heparan sulfate (HS) and promote *Chlamydia* invasion in eukaryotic cells (Stephens et al., 2001). Attachment of *Helicobacter pylori* to gastric epithelial cells also involves HS recognition. Two major proteins, one with a molecular mass of 71.5 kDa and pI 5.0 (HSBP50) and the other with a molecular mass of 66.2 kDa and pI 5.4 (HSBP54), have been identified on the surface of bacterial cells that are able to bind HS. The amino acid sequences of these proteins (HSBP50 - VPERAVRAHT; HSBP54 - VHLPADKTNV) are not homologous with bacterial adhesins or other HS-binding proteins (Ruzi-Bustos et al., 2001). Other proteins with the ability to bind heparin (66 and 60 kDa) have been identified in *Staphylococcus aureus*. The partial characterization of the amino acid sequences, which consist of DWTGWLAAA for the 66 kDa protein and MLVT for the 60 kDa protein, revealed no identity with HBPs from *Chlamydia* or *Helicobacter pylori*. HBPs from *S. aureus* have been demonstrated to be sensitive to heat and proteases, such as pronase E, proteinase K, pepsin and chymotrypsin (Liang et al., 1992). Interestingly, a 17-kDa heparin-binding protein with pI 4.6 has also been isolated from *S. epidermis* and *S. haemolyticus*, but the amino acid sequence similarity is low between these two organisms (MXTAHSYTXKYNGYTAN and MATQTKGYYSYNGYV, respectively) and other bacterial HBPs (Fallgren et al., 2001).

Trypanosomatidaes also exploit HS for successful parasite attachment to and/or invasion of the mammalian and vector hosts. The adhesion of *Leishmania* amastigotes to macrophages is mediated by HS, but not other sulfated polysaccharides (Love et al., 1993). Two heparin-

binding proteins, (65 and 54.5 kDa) from *L. (V.) braziliensis* promastigotes (HBP-Lb) recognize several molecules in the gut of *Lutzomyia intermedia* and *Lutzomyia whitmani* (Azevedo-Pereira et al., 2007). The biochemical characterization of these proteins revealed that only the 65-kDa HBP-Lb has metallo-proteinase activity, and this protein is primarily localized at the flagellar domain of the promastigotes. Surface plasmon resonance (spr) also demonstrated high-affinity binding at the flagellar domain, which forms a stable binding complex (Côrtés et al., 2011). In *T. cruzi*, HBPs also mediate parasite adhesion by recognition of PGHS on the surface of the target cells (Bambino-Medeiros et al., 2011; Calvet et al., 2003; Oliveira-Jr et al., 2008; Ortega-Barria & Pereira, 1991). Currently, three HBPs have been described in this parasite: a 60-kDa protein named penetrin (Ortega-Barria & Pereira, 1991) and two other proteins of 65.8 and 59 kDa that bind heparin, HS and chondroitin sulfate (CS). These proteins have been identified in both trypomastigotes and amastigotes (Oliveira-Jr et al., 2008). Interestingly, the HBP-HS binding is related to a specific region of the HS chain, the N-acetylated/N-sulfated HS domain, which promotes parasite attachment and invasion (Oliveira-Jr et al., 2008). Although only HS binding triggers *T. cruzi* invasion of mammalian cells (Ortega-Barria & Pereira, 1991; Calvet et al., 2003; Oliveira-Jr et al., 2008; Bambino-Medeiros et al., 2003), the multiple GAG recognition may provide an efficient association with other GAGs within the parasite life cycle. Recently, it has been demonstrated that sulfated proteoglycans are involved in the adhesion of epimastigotes to the luminal midgut epithelial cells of *Rhodnius prolixus* (Gonzalez et al., 2011).

2.3.3 Remarks on the isolation of proteins by glycosaminoglycans affinity chromatography

While the application of affinity chromatography has provided advances in our understanding of heparin-binding proteins, a large number of studies have focused on the parasite-host cell interface to improve our comprehension of the mechanisms that are activated by the receptor-ligand interaction (reviewed by Chen et al., 2008). The binding of Dengue virus to HS, for example, seems to result in the accumulation of virions at the surface of the human hepatoma cell line HuH-7 and elicit clathrin-dependent endocytosis (Hilgard & Stockert 2000). In addition to promote attachment and parasite invasion, HSPG also seems to be involved in the tropism of pathogen to specific tissues. The degree of HSPG sulfation guides the migration of *Plasmodium* sporozoites and the invasion of hepatocytes. Highly sulfated heparan sulfate at the surface of hepatocytes seems to regulate the proteolytic activity of the calcium-dependent protein kinase-6 on the CSP, which triggers the invasion of the parasite (Coppi et al., 2007).

Another interesting phenomenon is the release of syndecan-1, a transmembrane PGHS, as a mechanism of host defense inhibition. *Pseudomonas aeruginosa* induces syndecan-1 shedding through the enzymatic activity of LasA, leading to an enhancement of bacterial virulence (Park et al., 2001). A similar mechanism has been described for *Staphylococcus aureus* in which β -toxin, a secreted virulence factor, also induces syndecan-1 shedding by activating a metallo-proteinase involved in the host cell shedding mechanism, leading to enhancement of bacterial virulence due to the recruitment of inflammatory cells (Hayashida et al., 2009). Because heparan sulfate has been shown to be a receptor for a variety of pathogens, HS-binding polypeptides have been the subject of intense research and provide possibilities for drug intervention.

Organism	Isolated proteins	First phase			Second phase		References
		Matrix	Bind	Wash	Elution	Methods	
<i>Trypanosoma cruzi</i>	Penetrin – 60 kDa	Sepharose	PBS, pH 7.2	PBS containing 0.05% Triton X-100, pH7.2	1.5M guanidine-HCl 2.0M potassium thiocyanate and NaCl gradient (0.25-3.0M)	None	Ortega-Barria and Pereira 1991
<i>Staphylococcus aureus</i>	<i>S. aureus</i> -HBP – 66 kDa and 60 kDa	Sepharose	PBS containing 100mM PMSF, pH6.0	PBS containing 100mM PMSF, pH6.0	NaCl gradient (0-1M), followed by 2M NaCl in 200mM Tris-HCl pH 8.0	None	Liang et al., 1992
<i>Trypanosoma cruzi</i>	Penetrin – 60 kDa	Sepharose	PBS, pH 7.2	PBS containing 0.05% Triton X-100, pH7.2	1.5M guanidine-HCl 2.0M potassium thiocyanate and NaCl gradient (0.25-3.0M)	None	Ortega-Barria and Pereira 1991
<i>Chlamydial</i>	Outer membrane complex (COMC) - 60 kDa	Agarose	50 mM DTT and 2% Triton X-100	PBS	2% SDS in PBS	None	Stephens et al., 2001
<i>Helicobacter pylori</i>	Outer membrane protein (OMP) - 71.5 kDa and 66.2 kDa	Sepharose	0.05M sodium acetate pH5.0	0.1M Sodium acetate, pH5.0	NaCl gradient (0-2M)	None	Ruzi-Bustos et al., 2001
<i>Staphylococcal</i>	HBP – 17 kDa	Sepharose	PBS	None	NaCl gradient (0-2M)	None	Fallgren et al., 2001
<i>Leishmania (V.) braziliensis</i>	HBP-Lb – 65 kDa and 54.5 kDa	Sepharose	PBS containing 0.5% glycerol and 0.5% Chaps, pH7.2	PBS containing 0.5% glycerol and 0.5% Chaps, pH7.2	PBS containing 0.5% glycerol, 0.5% Chaps and 2M NaCl, pH7.2	None	Azevedo-Pereira et al., 2007
<i>Trypanosoma cruzi</i>	HBP-Tc – 65.8 kDa and 59 kDa	Sepharose	PBS and 0.5% glycerol, pH 7.2	PBS, pH 7.2	NaCl gradient (0-1M)	None	Oliveira-Jr et al., 2008

Table 3. Heparin affinity-based

3. Conclusion

The chromatographic procedures described here maintain the minimal amount of native folding necessary for proteins to retain their biological and biochemical activities. Thus, the materials used as supports for packed affinity columns, including agarose, sepharose and celite (from diatomaceous earth), to immobilize ligands, such as lectins, protease inhibitors and glycosaminoglycans, do not interfere with the functional properties of these proteins.

Furthermore, proteins obtained by affinity-based procedure have been useful in understanding the biological processes related to the life cycles of parasites and in the interaction with hosts. These studies are essential to developing strategies, such as the use of vaccines and drugs, to control the parasite diseases.

4. Acknowledgements

We acknowledge the financial support Brazilian funding agencies, including CAPES, CNPq, FAPERJ and PAPES (CNPq/Fiocruz). Dr. Carlos Roberto Alves and Dr. Mirian Claudia de Souza Pereira are research fellows of CNPq

5. References

- Abban CY, & Meneses PI. 2010. Usage of heparan sulfate, integrins, and FAK in HPV16 infection. *Virology*. 403(1):1-16.
- André S, Sanchez-Ruderisch H, Nakagawa H, Buchholz M, Kopitz J, Forberich P, Kemmner W, Böck C, Deguchi K, Detjen KM, Wiedenmann B, von Knebel Doeberitz M, Gress TM, Nishimura S, Rosewicz S, & Gabius HJ. 2007. Tumor suppressor p16INK4a--modulator of glycomic profile and galectin-1 expression to increase susceptibility to carbohydrate-dependent induction of anoikis in pancreatic carcinoma cells. *FEBS J*. 274(13):3233-56.
- Andreeva N, Bogdanovich P, Kashparov I, Popov M, & Stengach M. 2004. Is Histoaspartic Protease A Serine Protease With A Pepsin-Like Fold? *Proteins*. 55(3):705-10.
- Atrih A, Richardson JM, Prescott AR, & Ferguson MA. 2005. *Trypanosoma brucei* glycoproteins contain novel giant poly-N-acetylglucosamine carbohydrate chains. *J Biol Chem*. 280(2):865-71.
- Azevedo-Pereira RL, Pereira MC, Oliveria-Junior FO, Brazil RP, Côrtes LM, Madeira MF, Santos AL, Toma L, & Alves CR. *Vet Parasitol*. 2007. Heparin binding proteins from *Leishmania (Viannia) braziliensis* promastigotes. 145(3-4):234-9.
- Bambino-Medeiros R, Oliveira FO, Calvet CM, Vicente D, Toma L, Krieger MA, Meirelles MN, & Pereira MC. 2011. Involvement of host cell heparan sulfate proteoglycan in *Trypanosoma cruzi* amastigote attachment and invasion. *Parasitology*. 138(5):593-601.
- Beckert WH, & Sharkey MM. 1970. Mitogenic activity of the jack bean (*Canavalia ensiformis*) with rabbit peripheral blood lymphocytes. *Int Arch Allergy Appl Immunol*. (4):337-41.
- Bermúdez A, Alba MP, Vanegas M, & Patarroyo ME. 2010. 3D structure determination of STARP peptides implicated in *P. falciparum* invasion of hepatic cells. *Vaccine*. 28(31):4989-96.
- Bond JS, & Beynon RJ., eds. 1989. *Proteolytic enzymes: A practical approach*. IRL Press/Oxford University Press, Oxford, United Kingdom

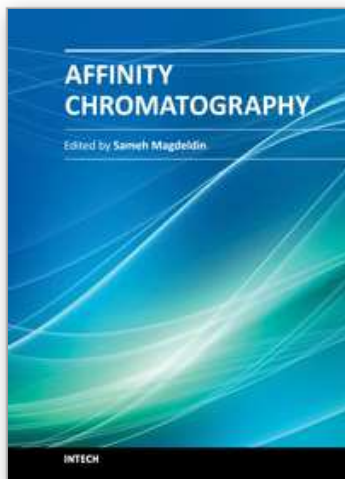
- Bourguignon SC, Cavalcanti DF, de Souza AM, Castro HC, Rodrigues CR, Albuquerque MG, Santos DO, da Silva GG, da Silva FC, Ferreira VF, de Pinho RT, & Alves CR. 2011. *Trypanosoma cruzi*: insights into naphthoquinone effects on growth and protease activity. *Exp Parasitol*. 127(1):160-6.
- Brickman MJ, & Balber AE. 1993. *Trypanosoma brucei rhodesiense*: membrane glycoproteins localized primarily in endosomes and lysosomes of bloodstream forms. *Exp Parasitol*. 76(4):329-44.
- Brittingham, A, Morrison, CJ, McMaster, WR, McGwire, BS, Chang, KP, & Mosser, DM. (1995). Role of the *Leishmania* surface protease gp63 in complement fixation, cell adhesion, and resistance to complement-mediated lysis. *J of Immunol* 155 (6) :3102-11.
- Calvet CM, Toma L, Souza FR, Meirelles MNL, & Pereira, MCP. 2003. Heparan Sulfate Proteoglycans mediate the invasion of cardiomyocytes by *Trypanosoma cruzi*. *J Eukariot Microbiol*. 50: 97-103.
- Caradonna KL, & Burleigh BA. 2011. Mechanisms of Host Cell Invasion by *Trypanosoma cruzi*. *Adv Parasitol*. 76:33-61.
- Chen Y, Götte M, Liu J, & Park PW. 2008. Microbial subversion of heparan sulfate proteoglycans. *Mol Cells*. 26(5):415-26.
- Choudhury R, Bhaumik Sk, De T, Chakraborti, T. 2009. Identification, Purification & Characterization of a secretory serine protease in an indian strain of *Leishmania donovani*. *Mol Cell Biochem*. 320(1-2):1-14
- Choudhury R, Das P, De T, Chakraborti T. 2010. Immunolocalization and characterization of two novel proteases in *Leishmania donovani*: putative roles in host invasion and parasite development. *Biochimie*. 92(10):1274-86.
- Coppi A, Tewari R, Bishop JR, Bennett BL, Lawrence R, Esko JD, Billker O, & Sinnis P. 2007. Heparan sulfate proteoglycans provide a signal to *Plasmodium* sporozoites to stop migrating and productively invade host cells. *Cell Host Microbe*. 2(5):316-27.
- Côrtes LMC, Pereira MCS, Olivera-Jr FOR, Suzana Corte-Real, Silva FS, Pereira BAS, Madeira MF, Moraes MTB, Brazil RP, & Alves RA. 2011. *Leishmania (Viannia) braziliensis*: insights on subcellular distribution and biochemical properties of heparin-binding proteins. *Parasitology*. 7: 1-8.
- Couto AS, Gonçalves MF, Colli W, & de Lederkremer RM. 1990. The N-linked carbohydrate chain of the 85-kilodalton glycoprotein from *Trypanosoma cruzi* trypomastigotes contains sialyl, fucosyl and galactosyl (alpha 1-3)galactose units. *Mol Biochem Parasitol*. 39(1):101-7.
- Dalrymple N, & Mackow ER. 2011. Productive dengue virus infection of human endothelial cells is directed by heparan sulfate-containing proteoglycan receptors. *J Virol*. 85(18):9478-85.
- Ribeiro de Andrade AS, Santoro MM, De Melo MN, & Mares-Guia M. 1998. *Leishmania (Leishmania) amazonensis*: purification and enzymatic characterization of a soluble serine oligopeptidase from promastigotes. *Exp Parasitol*. 89(2):153-60.
- Dreyfuss JL, Regatieri CV, Jarrouge TR, Cavalheiro RP, Sampaio LO, & Nader HB. 2009. Heparan sulfate proteoglycans: structure, protein interactions and cell signaling. *An Acad Bras Cienc*. 81(3):409-29.
- Dreyfuss JL, Veiga SS, Coulson-Thomas VJ, Santos IA, Toma L, Coletta RD, & Nader HB. 2010. Differences in the expression of glycosaminoglycans in human fibroblasts

- derived from gingival overgrowths is related to TGF-beta up-regulation. *Growth Factors*. 28(1):24-33.
- Dumont F, & Nardelli J. 1979. Peanut agglutinin (PNA)-binding properties of murine thymocyte subpopulation. *Immunology*. 37(1):217-24.
- Epting CL, Coates BM, & Engman DM. 2011. Molecular mechanisms of host cell invasion by *Trypanosoma cruzi*. *Exp Parasitol*. 127(2):607.
- Fallgren C, Utt M, & Ljungh A. 2001. Isolation and characterisation of a 17-kDa staphylococcal heparin-binding protein with broad specificity. *J Med Microbiol*. 50(6):547-57.
- Fauquenoy S, Morelle W, Hovasse A, Bednarczyk A, Slomianny C, Schaeffer C, Van Dorsselaer A, & Tomavo S. 2008. Proteomics and glycomics analyses of N-glycosylated structures involved in *Toxoplasma gondii*-host cell interactions. *Mol Cell Proteomics*. 7(5):891-910.
- Garate M, Marchant J, Cubillos I, Cao Z, Khan NA, & Panjwani N. 2006. In vitro pathogenicity of *Acanthamoeba* is associated with the expression of the mannose-binding protein. *Invest Ophthalmol Vis Sci*. 47(3):1056-62.
- Gardiner PR, Nene V, Barry MM, Thatthi R, Burleigh B, & Clarke MW. 1996. Characterization of a small variable surface glycoprotein from *Trypanosoma vivax*. *Mol Biochem Parasitol*. 82(1):1-11.
- Geier G, Banaj HJ, Heid H, Bini L, Pallini V, & Zwillig R. 1999. Aspartyl proteases in *Caenorhabditis elegans*. Isolation, identification and characterization by a combined use of affinity chromatography, two-dimensional gel electrophoresis, microsequencing and databank analysis. *Eur J Biochem*. 264(3):872-9.
- Gerold P, Striepen B, Reitter B, Geyer H, Geyer R, Reinwald E, Risse HJ, & Schwarz RT. 1996. Glycosyl-phosphatidylinositols of *Trypanosoma congolense*: two common precursors but a new protein-anchor. *J Mol Biol*. 261(2):181-94.
- Gonzalez MS, Silva LC, Albuquerque-Cunha JM, Nogueira NF, Mattos DP, Castro DP, Azambuja P, & Garcia ES. 2011. Involvement of sulfated glycosaminoglycans on the development and attachment of *Trypanosoma cruzi* to the luminal midgut surface in the vector, *Rhodnius prolixus*. *Parasitology*. 9:1-8.
- Govrin E, & Levine A. 1999. Purification of active cysteine proteases by affinity chromatography with attached E-64 inhibitor. *Protein Expr Purif*. 15(3):247-50.
- Guedes HL, Rezende JM, Fonseca MA, Salles CM, Rossi-Bergmann B, & De-Simone SG. 2007. Identification of serine proteases from *Leishmania braziliensis*. *Z Naturforsch C*. 62(5-6):373-81.
- Guha-Niyogi A, Sullivan DR, & Turco SJ. 2001. Glycoconjugate structures of parasitic protozoa. *Glycobiology*. 11: 45R-59R.
- Hayashida A, Bartlett AH, Foster TJ, & Park PW. 2009. *Staphylococcus aureus* beta-toxin induces lung injury through syndecan-1. *Am J Pathol*. 174(2):509-18.
- Hellström U, Dillner ML, Hammarström S, & Perlmann P. 1976. Fractionation of human T lymphocytes on wheat germ agglutinin-sepharose. *J Exp Med*. 144(5):1381-5.
- Hilgard P, & Stockert R. 2000. Heparan sulfate proteoglycans initiate dengue virus infection of hepatocytes. *Hepatology*. 32(5):1069-77.
- Hoegl L, Korting HC, & Klebe G. 1999. Inhibitors of aspartic proteases in human diseases: molecular modeling comes of age. *Pharmazie*. 54(5):319-29.

- Jacobs DB, & Poretz RD. 1980. Lectin induction of lymphokines in cultured murine leukocytes. *Cell Immunol.* 51(2):424-9.
- Jacquet A, Coulon L, De Nève J, Daminet V, Haumont M, Garcia L, Bollen A, Jurado M, & Biemans R. 2001. The surface antigen SAG3 mediates the attachment of *Toxoplasma gondii* to cell-surface proteoglycans. *Mol Biochem Parasitol.* 116(1):35-4
- Kabir S. 1998. Jacalin: a jackfruit (*Artocarpus heterophyllus*) seed-derived lectin of versatile applications in immunobiological research. *J Immunol Methods.* 212(2):193-211.
- Klemba M, & Goldberg DE. 2002. Biological roles of proteases in parasitic protozoa. *Annu Rev Biochem.* 71:275-305
- Kobayashi K, Kato K, Sugi T, Takemae H, Pandey K, Gong H, Tohya Y, & Akashi H. 2010. *Plasmodium falciparum* BAEBL Binds to Heparan Sulfate Proteoglycans on the Human Erythrocyte Surface. *J Biol Chem.* 285(3): 1716-1725.
- Kong, H H, Kim, T H & Chung, D-Ii. 2000. Purification And Characterization of A Secretory serine protease of *Acanthamoeba Healyi* isolated from gae. *Journal of Parasitology.* 86, 12-17.
- Li F, Patra KP, Yowell CA, Dame JB, Chin K, & Vinetz JM. 2010. Apical surface expression of aspartic protease plasmepsin 4, A Potential. *J Biol Chem.* 285(11):8076-83.
- Liang OD, Ascencio F, Fransson LA, & Wadström T. 1992. Binding of heparan sulfate to *Staphylococcus aureus*. *Infect Immun.* 60(3):899-906.
- Lord MJ, Jolliffe NA, Marsden CJ, Pateman CS, Smith DC, Spooner RA, Watson PD, & Roberts LM. 2003. Ricin. Mechanisms of cytotoxicity. *Toxicol Rev.* 22(1):53-64.
- Love DC, Esko JD, & Mosser DM. 1993. A heparin-binding activity on *Leishmania amastigotes* which mediates adhesion to cellular proteoglycans. *J Cell Biol.* 123(3):759-66.
- Ly M, Laremore TN, & Linhardt RJ. 2010. Proteoglycomics: recent progress and future challenges. *OMICS.* 14(4):389-99.
- Maia, A.A.M. & Guimarães, M.P. Distribuição Sazonal De Larvas De *Dermatobia Hominis* (Linnaeus Jr., 1781) (Diptera: Cuterebridae) Em Bovinos De Corte Da Região De Governador Valadares - Minas Gerais. *Arquivo Brasileiro De Medicina Veterinária E Zootecnia, Brasil.* , 1985. V. 37, N. 5, P. 469-475.
- Mansour MH. 1996. Purification and characterization of SM 37: a fucosyllactose determinant-bearing glycoprotein probed by a *Biomphalaria alexandrina* lectin on adult male shistosomes. *J Parasitol.* 82(4):586-93.
- Matsumoto K, Yamamoto D, Ohishi H, Tomoo K, Ishida T, Inoue M, Sadatome T, Kitamura K, & Mizuno H. 1989. Mode of binding of E-64-c, a potent thiol protease inhibitor, to papain as determined by X-ray crystal analysis of the complex. *FEBS. Lett* 245, 177-180.
- McKerrow JH, Caffrey C, Kelly B, Loke P, & Sajid M. 2006. Proteases in parasitic diseases. *Annu Rev Pathol.* 1:497-536.
- Morgado-Díaz JA, Silva-Lopez RE, Alves CR, Soares MJ, Corte-Real S, & De Simone SG. 2005. An intracellular serine protease of 68 kDa in *Leishmania (Leishmania) amazonensis* promastigotes. *Mem Inst Oswaldo Cruz.* 100(4):377-83
- Morris SR, & Sakanari JA. 1994. Characterization of The serine protease and serine protease inhibitor from the tissue-penetrating nematode *Anisakis Simplex*. *J Biol Chem.* 269(44):27650-6.

- Morrison CJ, Hurst SF, Bragg SL, Kuykendall RJ, Diaz H, McLaughlin DW, & Reiss E. 1993. Purification and characterization of the extracellular aspartyl proteinase of *Candida albicans*: removal of extraneous proteins and cell wall mannoprotein and evidence for lack of glycosylation. *J Gen Microbiol.* 139 Pt 6:1177-86.
- Muharsini S, Sukarsih, Riding G, Partoutomo S, Hamilton S, Willadsen P, & Wijffels G. 2000. Identification and characterisation of the excreted/secreted serine proteases of larvae of the old world screwworm fly, *Chrysomya Bezziana*. *Int J Parasitol.* 30(6):705-14.
- Naguleswaran A, Alaeddine F, Guionaud C, Vonlaufen N, Sonda S, Jenoe P, Mevissen M, & Hemphill A. 2005. *Neospora caninum* protein disulfide isomerase is involved in tachyzoite-host cell interaction. *Int J Parasitol.* 35(13):1459-72.
- Nolan DP, Jackson DG, Windle HJ, Pays A, Geuskens M, Michel A, Voorheis HP, & Pays E. 1997. Characterization of a novel, stage-specific, invariant surface protein in *Trypanosoma brucei* containing an internal, serine-rich, repetitive motif. *J Biol Chem.* 272(46):29212-21.
- O'Donnell CD, & Shukla D. 2008. The importance of separan sulfate in herpesvirus infection. *Virology.* 23(6):383-393.
- Oliveira FO Jr, Alves CR, Calvet CM, Toma L, Bouças RI, Nader HB, Côrtes LMC, Krieger MA, Meirelles MN, & Souza Pereira MC. 2008. *Trypanosoma cruzi* heparin-binding proteins and the nature of the host cell heparan sulfate-binding domain. *Microb Pathog.* 44(4):329-38
- Ortega-Barria E, & Pereira EA. 1991. A novel *T. cruzi* heparin-binding protein promotes fibroblast adhesion and penetration of engineered bacteria and trypanosomes into mammalian cells. *Cell.* 67: 411-421.
- Park PW, Pier GB, Hinkes MT, & Bernfield M. 2001. Exploitation of syndecan-1 shedding by *Pseudomonas aeruginosa* enhances virulence. *Nature.* 411(6833):98-102.
- Pereira BA, Silva FS, Rebello KM, Marín-Villa M, Traub-Cseko YM, Andrade TC, Bertho AL, Caffarena ER, & Alves CR. 2011. In silico predicted epitopes from the COOH-terminal extension of cysteine proteinase B inducing distinct immune responses during *Leishmania (Leishmania) amazonensis* experimental murine infection. *BMC Immunol.* 2011,12: 44.
- Pinho RT, Beltramini LM, Alves CR, & De-Simone SG. 2009. *Trypanosoma cruzi*: isolation and characterization of aspartyl proteases. *Exp Parasitol.* 122(2):128-33.
- Rebello KM, Côrtes LM, Pereira BA, Pascarelli BM, Côte-Real S, Finkelstein LC, Pinho RT, d'Avila-Levy CM, & Alves CR. 2009. Cysteine proteases from promastigotes of *Leishmania (Viannia) braziliensis*. *Parasitol Res.* 106(1):95-104.
- Redmond DL, Geldhof P, & Knox DP. 2004. Evaluation of *Caenorhabditis elegans* glycoproteins as protective immunogens against *Haemonchus contortus* challenge in sheep. *Int J Parasitol.* 34(12):1347-53.
- Reinwald E, Rautenberg P, & Risse HJ. 1981. Purification of the variant antigens of *Trypanosoma congolense*: a new approach to the isolation of glycoproteins. *Biochim Biophys Acta.* 668(1):119-31.
- Rüdiger H, & Gabius HJ. 2001. Plant lectins: occurrence, biochemistry, functions and applications. *Glycoconj J.* 18(8):589-613.

- Ruiz-Bustos E, Ochoa JL, Wadström T, & Ascencio F. 2001. Isolation and characterisation of putative adhesins from *Helicobacter pylori* with affinity for heparan sulphate proteoglycan. *J Med Microbiol.* 50(3):215-22.
- Shenai BR, Sijwali PS, Singh A, & Rosenthal PJ. 2000 Characterization of native and recombinant falcipain-2, a principal trophozoite cysteine protease and essential hemoglobinase of *Plasmodium falciparum*. *J Biol Chem.* 275(37):29000-10.
- Silva-Lopez RE, & Giovanni-De-Simone S. 2004. *Leishmania (Leishmania) amazonensis*: Purification and characterization of a promastigote serine protease. *Exp Parasitol.* 107(3-4):173-82.
- Silva-Lopez RE, Coelho MG, & De Simone SG. 2005. Characterization of an extracellular serine protease of *Leishmania (Leishmania) amazonensis*. *Parasitology.* 131(Pt 1):85-96.
- Silva-López RE, Dos Santos TR, Morgado-Díaz JA, Tanaka MN, & De Simone SG. 2010. Serine protease activities in *Leishmania (Leishmania) chagasi* promastigotes. *Parasitol Res.* 107(5):1151-62
- Silva-Lopez RE, Morgado-Díaz JA, Alves CR, Côrte-Real S, & Giovanni-De-Simone S. 2004. Subcellular localization of an extracellular serine protease in *Leishmania (Leishmania) amazonensis*. *Parasitol Res.* 93(4):328-31
- Silva-Lopez RE, Morgado-Díaz JA, Dos Santos PT, & Giovanni-De-Simone S. 2008. Purification and subcellular localization of a secreted 75 KDa *Trypanosoma cruzi* serine oligopeptidase. *Acta Trop.* 07(2):159-67.
- Smith WD, Newlands GF, Smith SK, Pettit D, & Skuce PJ. 2003. Metalloendopeptidases from the intestinal brush border of *Haemonchus contortus* as protective antigens for sheep. *Parasite Immunol.* 25(6):313-23.
- Smith WD, Smith SK, Pettit D, Newlands GF, & Skuce PJ. 2000. Relative protective properties of three membrane glycoprotein fractions from *Haemonchus contortus*. *Parasite Immunol.* 22(2):63-71.
- Soong G, Martin FJ, Chun J, Cohen TS, Ahn DS, & Prince A. 2011. *Staphylococcus aureus* Protein A mediates invasion across airway epithelial Cells through activation of RhoA GTPase signaling and proteolytic activity. *J Biol Chem.* 286(41):35891-8.
- Stephens RS, Koshiyama K, Lewis E, & Kubo A. 2001. Heparin-binding outer membrane protein of chlamydiae. *Mol Microbiol.* 40(3):691-9.
- Todorova VK, & Stoyanov DI. 2000. Partial Characterization of Serine proteinases secreted By Adult *Trichinella Spiralis*. *Parasitol Res.* 86(8):684-7.
- Turner DG, Wildblood LA, Inglis NF, & Jones DG. 2008. Characterization of a galectin-like activity from the parasitic nematode, *Haemonchus contortus*, which modulates ovine eosinophil migration in vitro. *Vet Immunol Immunopathol.* 122(1-2):138-45.
- Valdivieso E, Bermudez H, Hoebeke J, Noya O, & Cesari IM. 2003. Immunological similarity between *Schistosoma* and bovine cathepsin D. *Immunol Lett.* 89(1):81-8.
- Wlodawer A, & Vondrasek J. 1998. Inhibitors of HIV-1 protease: a major success of structure-assisted drug design. *Annu Rev Biophys Biomol Struct.* 27:249-84.
- Wilson ME, & Pearson RD. 1984. *Infect Immun.* Stage-specific variations in lectin binding to *Leishmania donovani*. 46(1):128-34.
- Yan Y, Silvennoinen-Kassinen S, Leinonen M, & Saikku P. 2006. Inhibitory effect of heparin 41 sulfate-like glycosaminoglycans on the infectivity of *Chlamydia pneumoniae* in HL 42 cells varies between strains. *Microbes Infect.* 8:866-872



Affinity Chromatography

Edited by Dr. Sameh Magdeldin

ISBN 978-953-51-0325-7

Hard cover, 368 pages

Publisher InTech

Published online 21, March, 2012

Published in print edition March, 2012

Most will agree that one major achievement in the bio-separation techniques is affinity chromatography. This coined terminology covers a myriad of separation approaches that relies mainly on reversible adsorption of biomolecules through biospecific interactions on the ligand. Within this book, the authors tried to deliver for you simplified fundamentals of affinity chromatography together with exemplarily applications of this versatile technique. We have always been endeavor to keep the contents of the book crisp and easily comprehensive, hoping that this book will receive an overwhelming interest, deliver benefits and valuable information to the readers.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

C.R. Alves, F.S. Silva, F.O. Oliveira Jr, B.A.S. Pereira, F.A. Pires and M.C.S. Pereira (2012). Affinity-Based Methods for the Separation of Parasite Proteins, Affinity Chromatography, Dr. Sameh Magdeldin (Ed.), ISBN: 978-953-51-0325-7, InTech, Available from: <http://www.intechopen.com/books/affinity-chromatography/affinity-based-methods-in-separation-of-parasites-proteins>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen