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Potential Interaction Between Presenilin and Metacaspase on the Mechanism of Programed Cell Death in *Leishmania infantum*

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ABSTRACT

Proteases have been considered as promising targets for anti-parasitic agents, these enzymes occur in all organisms from prokaryotes to eukaryotes to viruses. The aim of the present study was to provide, through bioinformatics techniques, potential promising targets related to the apoptosis mechanism, in order to develop a vaccine and new anti-parasitic drugs. For the identifying of the hydrophobic regions, the Kyte and Doolittle methodology was utilized. The nine hydrophobic regions identified in the presenilin, based on the physicochemical properties, suggested the occurrence of transmembrane regions that were confirmed as helices scattered in the membrane by THMM. In the metacaspase structure of *L. chagasi*, besides the occurrence of four hydrophobic regions, the THMM analyses predicted just one helix, placed in the N-terminal portion. The analyzes of hydrophilicity through B-EpiPred Server, indicated the occurrence of several residues localized in external regions, showing that both molecules have significant numbers of fragments with high antigenic propensity. The prediction of epitopes on the tertiary structure was obtained by the I-TASSER server. In the present paper we are suggesting potential availability of a hybrid peptide originated from the presenilin and metacaspase of the *Leishmania* for the developing of new drugs or vaccine.

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1. INTRODUCTION

Leishmaniasis, a devastating disease caused by several species of a protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), it is endemic in 62 countries with epidemiological peculiarities according to factors such as human activity, parasite subpopulation and wild and domestic fauna composition, among others. In spite of being described as endemic in 29 countries, 90% of visceral leishmaniasis cases occur in India, Bangladesh, Nepal, Sudan and Brazil [1]. In the American continent, the disease, named American Visceral Leishmaniasis (AVL) occurs in the tropical and subtropical regions of Latin America. In Brazil, besides being a rural zoonosis, Visceral Leishmaniasis is becoming a peri-urban and even urban zooanthroponosis [2].

In spite of leishmaniasis have been discovered at beginning of the last century, the drugs utilized as the basis of treatment are still in use since 1940s. Currently, the two formulations adopted have comparable efficacy but considerable toxicity. The treatment course should last at least 20 days preferably 28 days and common side effects include nausea and vomiting, arthralgia, hepatitis, pancreatitis and cardiac dysrhythmias [3].

In function of the above stated difficulties, there is a great demand on the identification of targets that could have a key role on the parasite virulence and consequently on the pathogenesis of the disease.

Proteases have been considered as promising targets for anti-parasitic agents, these enzymes occur in all organisms from prokaryotes to eukaryotes to viruses. They are involved in a multitude of physiological processes from digestion to highly regulated cascades, such as: protein degradation, cell signaling and apoptosis [4].

Apoptosis is a ubiquitous kind of cell death characterized by specific biochemical and morphological changes it is strictly regulated and essential for the cellular homeostasis and development of organisms.

Among the various situations where the apoptosis plays some role on the progress of a pathogenesis, the Alzheimer's disease may be considered as an example. Here, the cysteine-aspartic proteases (caspase) that are centrally involved in apoptosis phenomenon as a rule, besides of promoting apoptosis can also cleave presenilin 1 (PSEN) and nicastrin, on the ϒ-secretase complex resulting in increased Aβ production and causing disease by the accumulation of amyloid plaque in the human brain [5, 6].

Additionally, it was observed in experiments with neuroblastoma cells, that presenilin, Nicastrin, Aph-1 and Pen-2 may interact forming active ϒ-secretase complexes in apoptotic cells. In the same experiments, the authors stated that the C terminal fragment (CTF) of some caspases may interact with the presenilin, becoming part of the ϒ-secretase complex and probably influencing different signaling pathways by the cleavage of ϒ-secretase substrates [7].

Apoptosis-like programmed cell death (PCD) has been also described in a great variety of taxonomic groups of unicellular organisms, including parasitic protozoan such as: *Plasmodium*, *Trypanosoma* and *Leishmania* [8-10]. Coincidentally, those organisms exhibit several morphological features similar from metazoans, for instance: chromosomal condensation, nuclear DNA fragmentation, cell shrinkage, loss of mitochondrial membrane potential, formation of apoptotic bodies, and the externalization of phosphatidylserine [11, 12].

In *Leishmania*, an apoptosis-like process can influence the progress of the infection through several factors including: an altruist trait to control parasite numbers to prolong survival of both the host and parasite, differentiation in response to stress (heat shock) and modulation of the host immunity [13, 14]. *Leishmania* can also induce distinct neutrophil phenotypes in resistant or susceptible mice [15] and increase the human neutrophils life span until two days, inhibiting the processing of procaspases in the infected cells [16].

The combination of the basic features of apoptosis, that have been maintained across diverse taxonomic groups throughout the evolution, associated with the possibility to draw a parallel from studies on cell death pathways and the proteases implicated on those processes in parasitic protozoan, may be an useful approach for the identification of effective targets.

The employment of bioinformatics has been proving as a very important methodology on the production of new insights on the clarification of diverse biological mechanisms in parasites; consequently, it can be a very useful tool for the identification of the above-mentioned targets.

Taking into account the key role of apoptosis in numerous biological processes among trypanosomatids, including in *Leishmania* and considering that the presenilin and metacaspases could present a correlated function on those processes like in other organisms. The aim of the present paper we are suggesting potential availability of a hybrid peptide originated from the presenilin and metacaspase of the *Leishmania* for the developing of new drugs or vaccine.

2. RESEARCH METHOD

2.1. Protein sequence analysis

The sequences of presenilin of *Leishmania infantum* and metacaspase of *Leishmania chagasi (infantum)* were analyzed by the UniProt [17].

2.2. Antigenicity prediction

Epitopes were identified through antigenic prediction program using the B-EpiPred Server methods [18], Hopp and Woods [19].

2.3. Detection of accessible regions

The prediction of membrane-spanning domains, potential antigenic sites and regions that could likely be exposed on the protein surface, were performed by the location of hydrophobic and hydrophilic scales in solvent accessible regions, according the methods of Kyte and Doolittle [20] and Eisenberg et al [21].

2.4. The transmembrane sequence prediction

The estimation of transmembrane domains was achieved by program Prediction of transmembrane helices in proteins (TMHMM) according to Möller et al [22].

2.5. Prediction of MHC binding peptides

NetMHCpan Server is a method that generates quantitative predictions related to the binding specificity of any peptide–MHC class. The prediction of the epitopes from presenilin and metacaspase presenting binding specificity with molecules of the MHC I - HLA- A02:01, was carried out with accuracy 0.853.

Concerning to the MHC II, for the presenilin the average was -0.853, minimum: -3.484, maximum: 1.627, threshold: 0.350. For the metacaspase the average was: 0.335, minimum:-2.051, maximum: 3.3, threshold: 0,350 [23].

2.6. Cytotoxity T Lymphocytes (CTL) epitope prediction

The CTL Epitopes of both, presenilin and metacaspase from *Leishmania infantum* were obtained from MHCBN comprehensive database of MHC binding and non-binding peptides using two different methods: firstly with Support Vector Machine (Cut off is 0.36) and then by Artificial Neural Network (Cut off is 0.51).

The predicted MHC-Peptide binding considered was a log-transformed value related to the IC50 values in nM units.

The average accuracy of Support Vector Machine (SVM) based epitope prediction method was ~86% at cut off 0.36. SVM has been trained on the binary input of single amino acid sequence. In Case of Artificial Neural Network ANN based epitope prediction method the average accuracy was ~78% at the cut off score 0.51 [24].

2.7. Transporter associated with Antigen Peptide (TAP) binding prediction

The Prediction was based on cascade SVM, using properties of amino acid sequence at correlation coefficient of 0.88 as per Jack-Knife validation test [25].

2.8. Tertiary structure prediction of the predicted epitopes

The primary sequences of presenilin and metacaspase (access codes: A4HWP2 and B6DU87) were submitted to I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) [26, 27]. The visualization was completed in Visual Molecular Dynamic (VMD) 1.9 [28].

2.9. Hybrid protein construction (NTF Psen + CTF metacaspase)

The hybrid protein was formed by a part of the presenilin N – terminal fragment, (from 1aa to 117aa) linked with the C-terminal fragment of the metacaspase (from 299aa to 448aa) as represented below in blue and red:

Psen

MSSRPLLDDAHLSRGVGIRFVSLVVPVTATMLAVVWSLSCLSPIYVNSQVPPLPVVVNENDAATAGEKF
VYSLVAALIVVGCVVAATFATVLLYHFHLQFVLYGWLAFSAVSMFFMLLWIWLDLFCTYFQIPYNVIS
MGIFVWNFGVVGLIALFCYSHPTVTQVYLVIASILTAWSLTALPEWSTWSLLICIATYDILAVLWQQGPL
HRLIKIAQERDEPIPGFVYSSAHSIVPITQPATASSARVPATAAESFMWTVQHATPFKLGLGDFIFYSLLV
GRASFSGFVSWSFCMVSILAGMLGTLLSLLLFRNSLRALPALPCSIFLSTVVFVLCRLIVESLSSFTSHHLL
VL

Metacaspase

MADLFDILGIGAVASLIPMLANGLLLVDRPKRVDINAGRRLIHTVRPMIPYRAPVPYTGGRVRALFIGIN
YTGMRNALRGCVNDVSSMLGTLQQISFPISECCILVDDPSFPGFCGMPTRDNIIKHMLWLTGDVRPGDV
LFFHFSGHGGQTKATRDSEEKYDQCLIPLDHVKNGSILDDDLFLMLVAPLPSGVRMTCVFDCCHSASM
LDLPFSYVAPRVGGGGAREYMQQVRRGNFSNGDVVMFSGCTDSGTSADVQNGGHANGAATLAFTWS
LLNTHGFSYLNILLKTREELRKKGRVQVPQLTSSKPIDLYKPFSLFGMITVNASMMHCVPQQYQQRPQS
LPPQAMPPPAGYPVHVPPPPQGYYPPPPQGYYPPPQRPGWGLGYPAPGYPAQGIPVQQATLGVSRCPPS
QYLPAPPPA LYAPPPPGQHGPPQPPPAQYTFSPLPPR

NTF Psen + CTF metacaspase

MSSRPLLDDAHLSRGVGIRFVSLVVPVTATMLAVVWSLSCLSPIYVNSQVPPLPVVVNENDAATAGEKF
VYSLVAALIVVGCVVAATFATVLLYHFHLQFVLYGWLAFSAVSMFFMLVPQLTSSKPIDLYKPFSLFG
MITVNASMMHCVPQQYQQRPQSPPQAMPPPAGYPVHVPPPPQGYYPPPPQGYYPPPQRPGWGLGYPAP
GYPAQGIPVQQATLGVSRCPPSQYLPAPPPALYAPPPPGQHGPPQPPPAQYTFSPLPPR

2.10. Predition of Potential Cleavage Sites by proteases

The preditions were performed through the Expasy (peptide cutter tool) and the folowing enzimes were utilized: Arg-C proteinase, Caspase from 1 to 10, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, Trypsin, Lys C, Lys N.

3. RESULTS AND ANALYSIS

4.

3.1. Proteins sequences and structural analysis

The sequences of the *Leishmania infantum* presenilin (A4HWP2) and *Leishmania chagasi (infantum)* metacaspase (B6DU87) consist respectively of 352 and 448 amino acids as follows:

Presenilin:

MSSRPLLDDAHLSRGVGIRFVSLVVPVTATMLAVVWSLSCLSPIYVNSQVPPLPVVVNENDAATAGEKF
VYSLVAALIVVGCVVAATFATVLLYHFHLQFVLYGWLAFSAVSMFFMLLWIWLDLFCTYFQIPYNVIS
MGIFVWNFGVVGLIALFCYSHPTVTQVYLVIASILTAWSLTALPEWSTWSLLICIATYDILAVLWQQGPL
HRLIKIAQERDEPIPGFVYSSAHSIVPITQPATASSARVPATAAESFMWTVQHATPFKLGLGDFIFYSLLV
GRASFSGFVSWSFCMVSILAGMLGTLLSLLLFRNSLRALPALPCSIFLSTVVFVLCRLIVESLSSFTSHHLL
VL.

Metacaspase:

MADLFDILGIGAVASLIPMLANGLLLVDRPKRVDINAGRRLIHTVRPMIPYRAPVPYTGGRVRALFIGIN
YTGMRNALRGCVNDVSSMLGTLQQISFPISECCILVDDPSFPGFCGMPTRDNIIKHMLWLTGDVRPGDV
LFFHFSGHGGQTKATRDSEEKYDQCLIPLDHVKNGSILDDDLFLMLVAPLPSGVRMTCVFDCCHSASM
LDLPFSYVAPRVGGGGAREYMQQVRRGNFSNGDVVMFSGCTDSGTSADVQNGGHANGAATLAFTWS
LLNTHGFSYLNILLKTREELRKKGRVQVPQLTSSKPIDLYKPFSLFGMITVNASMMHCVPQQYQQRPQS
LPPQAMPPPAGYPVHVPPPPQGYYPPPPQGYYPPPQRPGWGLGYPAPGYPAQGIPVQQATLGVSRCPPS
QYLPAPPPA LYAPPPPGQHGPPQPPPAQYTFSPLPPR.

3.2. Prediction of antigenic regions

The analyzes of hydrophilicity through B-EpiPred Server, revealed four residues in the presenilin and nine in the metacaspases localized in the external regions of both molecules, comprising the highest values of local hydrophilicity observed (Figure 1a/b), as confirmed through the method of Hoop and Woods (data not shown).

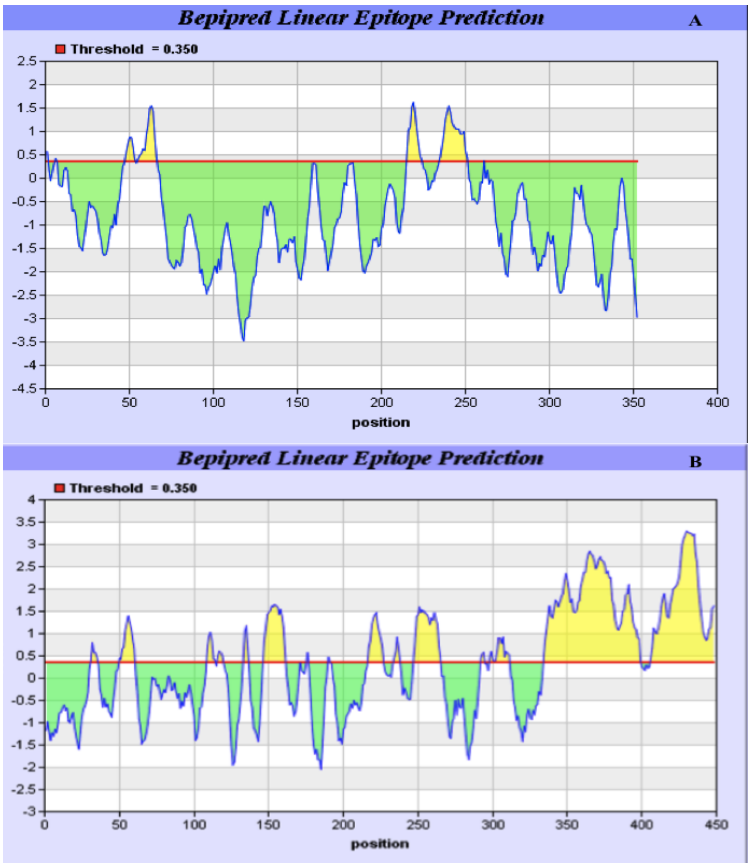

Figure 1 a. Prediction of continuous B-cell epitopes in antigenic sequences of *Leishmania infantum* presenilin. b. Prediction of continuous B-cell epitopes in antigenic sequences of *Leishmania chagasi* metacaspase.

3.3. Hydropathicity Analyses

The analyses of hydrophobic characteristics based on the physicochemical properties of the amino acids, showed nine hydrophobic and four highly hydrophilic regions in the presenilin while in the metacaspase were respectively four hydrophobic and several highly hydrophilic regions, figure 2 a/b.

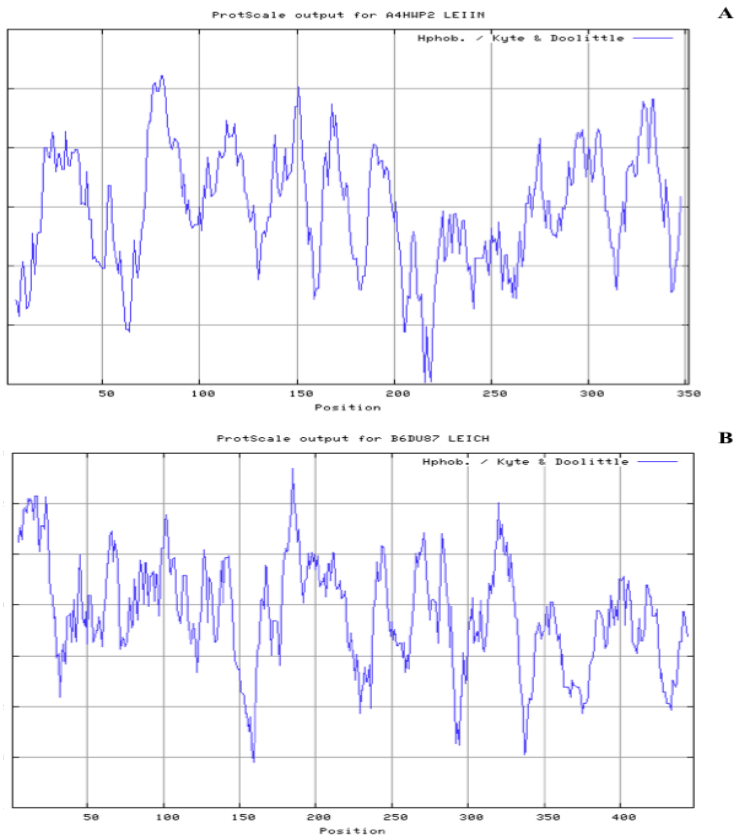

Figure 2 a. Hydrophobicity plot of Kyte and Doolittle (1982) of *Leishmania infantum* presenilin. b. Hydrophobicity plot of Kyte and Doolittle (1982) of *Leishmania chagasi* metacaspase.

3.4. THMM

The THMM analyses of the PSEN, showed a possible occurrence of nine transmembrane helices with seven areas with a high probability of being scattered in the membrane. Differently, in the metacaspase it was perceived just one helice, likely assigned in the N-terminal portion of the molecule (Figure 3 a/b).

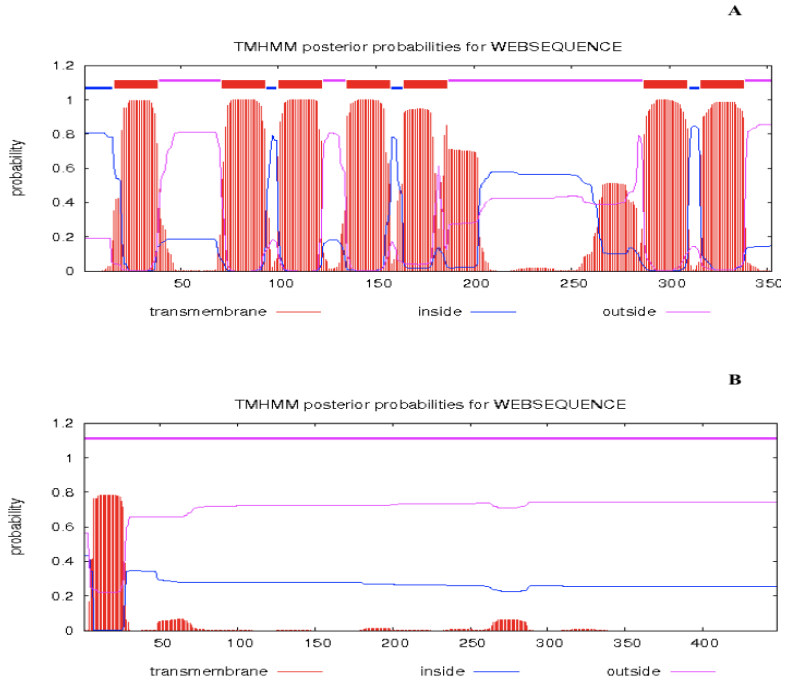

Figure 3 a. The prediction of transmembrane domains of the presenilin by program TMHMM, demonstrated the occurrence of nine transmembrane insertions. b. The prediction of transmembrane domains of the metacaspase by program TMHMM, demonstrated the occurrence of one transmembrane insertions.

3.5. Prediction of MHC binding peptides

Concerning MHC I, the presenilin presented 14 sequences with strong affinity, being four (28,6%) in the N-terminal portion and six (43%) in the C-terminal, with the remnants in the central part (Table 1). The metacaspase showed five sequences, all of then localized in the central portion of the molecule (Table 2).

Table 1. Epitopes prediction of presenilin from MHC I. Estimated prediction accuracy 0.853 (using nearest neighbor HLA – A02:01).

Residue number	peptide	1-log50k(aff)	Affinity(nM)	%Rank	Strong binding peptides
30	MLAVVWSLSCLSPI	0.750	14.93	0.80	X
36	SLSCLSPIYVNSQV	0.679	32.28	1.50	X
39	CLSPIYVNSQVPPL	0.676	33.37	1.50	X
104	WLAFSAVSMFFMLL	0.671	35.33	1.50	X
128	FQIPYNVISMGIFV	0.819	7.10	0.25	X
178	ALPEWSTWSLLICI	0.689	28.97	1.50	X
186	SLLICIATYDILAV	0.743	16.22	0.80	X
187	LLICIATYDILAVL	0.706	24.00	1.0	X
264	KLGLGDFIFYSLLV	0.649	44.46	1.50	X
295	ILAGMLGTLLSLLL	0.675	33.56	1.5	X
306	LLLFRNSLRALPAL	0.652	42.98	1.5	X
315	ALPALPCSIFLSTV	0.694	27.38	1.00	X
318	ALPCSIFLSTVVFV	0.803	8.47	0.40	X
324	FLSTVVFVLCRLIV	0.732	18.10	0.80	X

Table 2. Epitopes prediction of metacaspase from MHC I. Estimated prediction accuracy 0.853 (using nearest neighbor HLA – A02:01).

Residue number	peptide	1-log50k(aff)	Affinity(nM)	%Rank	Strong binding peptides	Weak binding peptides
2	DLFDILGIGAVASL	0.458	352.39	4.00		X
6	ILGIGAVASLIPML	0.556	122.08	3.00		X
39	RLIHTVRPMIPYRA	0.502	218.15	3.00		X
63	ALFIGINYTGMRNA	0.579	95.14	3.00		X
85	SSMLGTLQQISFPI	0.445	407.00	4.00		X
92	QQISFPISECCILV	0.612	66.43	2.00		X
103	ILVDDPSFPGFCGM	0.564	111.32	3.00		X
126	MLWLTGDVRPGDVL	0.508	206.16	3.00		X
175	ILDDDLFLMLVAPL	0.831	6.20	0.2	X	
181	FLMLVAPLPSGVRM	0.673	34.46	1.50	X	
184	LVAPLPSGVRMTCV	0.455	364.84	4.00		X
193	RMTCVFDCCHSASM	0.455	362.74	4.00		X
205	SMLDLPFSYVAPRV	0.865	4.32	0.12	X	
269	FTWSLLNTHGFSYL	0.782	10.52	0.50	X	
272	SLLNTHGFSYLNIL	0.541	143.87	3.00		X
273	LLNTHGFSYLNILL	0.659	40.00	1.50	X	

On the MHC II, the presenilin showed four sequences, being two (50%) in the N-terminal, one (25%) in the C-terminal and one in the central part of the molecule. In the presenilin the epitopes were: 48 – SQVPPL, 55 – VVVNENDAATAG, 215 – QERDEPIPGF, 235 - ITQPATASSARVPATAA. In the metacaspase, nine sequences were identified: four (44,4%) occurring in the N-terminal portion, two (22,2%) in the C-terminal and the tree leftovers, in the molecule's center. In the metacaspase the epitopes were: 31 – KRVDI, 50 – PYRAPVPYTGG, 109 – PSFPGF, 147 – HGGQTKATRDSEEKY, 217 – PRVGGGGARE, 234 – GNFSN, 247 – CTDSGTSADVQNGGHANGA, 298 – RVQVPQLTSSKPID, 334 – QQYQQRPQSLPPQAMPPPAGYPVHVPPPPQGYYPPPPQGYYPPPQRPGWGLGYPAPGYPAQGIPVQ.

3.6. Cytotoxity T Lymphocytes (CTL) epitope prediction

Considering the SVM based Predicted CTL epitopes:

In the presenilin the epitopes were: 13 – SRGVGIRFV (score-1.143), 110- AVSMFFMLL (score-1.084), 145 – FGVVGLIAL (score-1.083), 193 – ATYDILAVL (score-1.043), 328 – TVVFVLCRL (score-1.022), 15 – GVGIRFVSL (score-1.008), 137 – SMGIFVWNF (score-0.960), 158 – HPTVTQVYL (score-0.953).

In the metacaspase the epitopes were: 187 – APLPSGVRM (score-1.337), 77 – ALRGCVNDV (score-1.140), 29 – RPKRVDINA (score-1.093), 207 – MLDLPFSYV (score-1.083), 61 – RVRALFIGI (score-1.044), 19 – MLANGLLLV (score-0.955), 32 – RVDINAGRR (score-0.934), 160 – KYDQCLIPL (score- 0.917).

Based in the ANN Predicted CTL- epitopes:

In the presenilin, the epitopes were: 50 – VPPLPVVVN (score-1.000), 25 – VPVTATMLA (score-0.990), 74 – VAALIVVGC (score-0.990), 256 – WTVQHATPF (score-0.990), 285 – GFVSWSFCM (score-0.990), 290 – SFCMVSILA (score-0.990), 295 – SILAGMLGT (score-0.990), 58 – NENDAATAG (score-0.980).

In the metacaspase, the epitopes were: 94 – QISFPISEC (score-1.000), 412 – QYLPAPPPA (score-1.000), 50 – PYRAPVPYT (score-0.990), 76 – NALRGCVND (score-0.990), 156 – DSEEKYDQC (score-0.990), 390 – GYPAQGIPV (score-0.990), 33 – VDINAGRRL (score-0.980), 148 - GGQTKATRD (score-0.980).

The reliability of prediction was further improved by cascade SVM that uses the sequence and features of amino acids along with sequence based High affinity TAP epitopes of *L. infantum* presenilin and metacaspase (table 3 and 4).

Under that methodology, in the presenilin, 34 sequences were identified with high affinity to TAP binders while in the metacaspase were 23 sequences.

Table 3. Identification of epitopes from presenilin of *L. infantum* recognized as high-efficiency binders by Transporter associated with Antigen Peptide (TAP) through Cascade Support Vector Machine (SVM).

Peptide rank	Start position	Sequence	Score
1	86	ATFATVLLY	9.352
2	112	SMFFMLLWI	9.097
3	184	STWSLLICI	8.797
4	117	LLWIWLDLF	8.452
5	113	MFFMLLWIW	8.163
6	63	ATAGEKFVY	7.868
7	33	AVVWSLSCL	7.802
8	193	ATYDILAVL	7.801
9	98	LQFVLYGWL	7.433
10	114	FFMLLWIWL	7.422
11	272	IFYSLLVGR	7.330
12	285	GFVSWSFCM	7.126
13	169	ASILTAWSL	7.112
14	93	LYHFHLQFV	7.041
15	308	LLFRNSLRA	6.776
16	327	STVVFVLCR	6.683
17	107	AFSAVSMFF	6.530
18	120	IWLDLFCTY	6.521
19	251	AESFMWTVQ	6.517
20	95	HFHLQFVLY	6.513
21	309	LFRNSLRAL	6.480
22	18	IRFVSLVVP	6.401
23	314	LRALPALPC	6.390
24	250	AAESFMWTV	6.387
25	343	SFTSHHLLV	6.378
26	92	LLYHFHLQF	6.316
27	134	NVISMGIFV	6.304
28	181	PEWSTWSLL	6.278
29	110	AVSMFFMLL	6.125
30	256	WTVQHATPF	6.106
31	281	ASFSGFVSW	6.082
32	323	SIFLSTVVF	6.072
33	344	FTSHHLLVL	6.017
34	32	LAVVWSLSC	6.002

Table 4. Identification of epitopes from metacaspase of *L. chagasi* recognized as high-efficiency binders by Transporter associated with Antigen Peptide (TAP) through Cascade Support Vector Machine (SVM).

Peptide rank	Start position	Sequence	Score
1	122	NIIKHMLWL	11.345
2	39	RRLIHTVRP	8.802
3	225	REYMQQVRR	8.361
4	43	HTVRPMIPY	7.986
5	224	AREYMQQVR	7.623
6	44	TVRPMIPYR	7.384
7	406	SRCPPSQYL	7.086
8	153	ATRDSEEKY	7.075
9	326	ASMMHCVPQ	6.941
10	378	QRPGWGLGY	6.764
11	204	SASMLDLPF	6.682
12	325	NASMMHCVP	6.614
13	206	SMLDLPFSY	6.472
14	70	NYTGMRNAL	6.463
15	346	QAMPPPAGY	6.399
16	157	SEEKYDQCL	6.330
17	36	NAGRRLIHT	6.315
18	38	GRRLIHTVR	6.309
19	274	LLNTHGFSY	6.271
20	290	REELRKKGR	6.229
21	119	TRDNIIKHM	6.167
22	268	LAFTWSLLN	6.147
23	305	TSSKPIDLY	6.099

3.7. Tertiary structure prediction of the predicted epitopes

The model showed correct topology with TM-score values greater than 0.50 and reasonable quality with c-score in limit of the acceptance that showed more than 90% of prediction that was correct for the model (c-score = -0.37) (Figures 4 and 5).

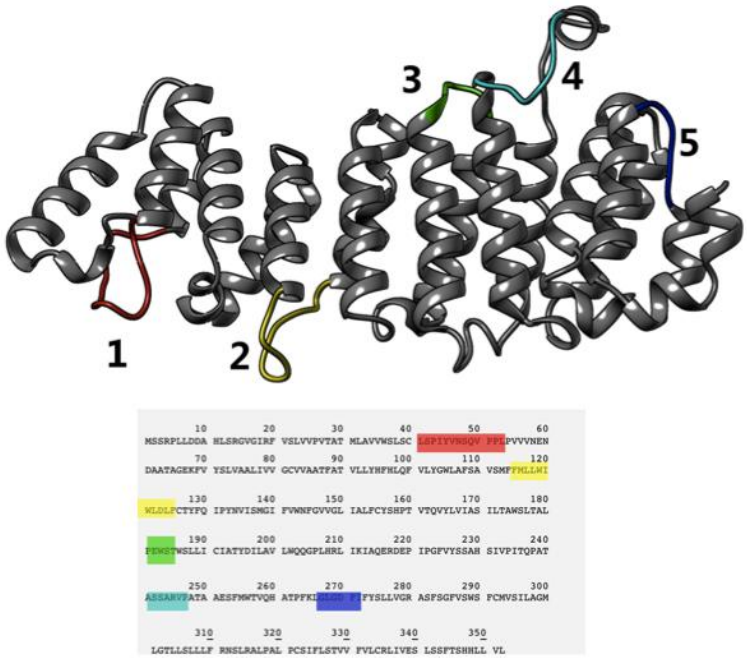

Figure 4. Predicted epitopes of the presenilin of *Leishmania infantum* (colors) represented in the molecule sequence (grey box) and in the tertiary structure (above).

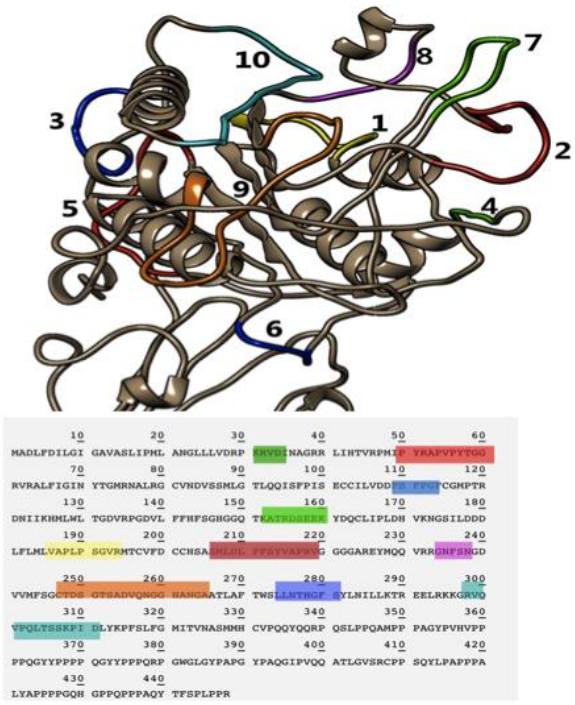

Figure 5. Predicted epitopes of the metacaspase of *Leishmania chagasi* (colors) represented in the molecule sequence (grey box) and in the tertiary structure (above).

3.8. Blast of the Hybrid protein (NTF Psen + CTF metacaspase)

The Blast of the hybrid molecule besides of the identity related to their original fragments respectively of the presenilin and metacaspase, also showed a high identity percentage with the anexin from several species of parasitic fungus such as: *Trichophyton equinum* (41%), *T. rubum* (43%), *Togninia minima* (43%) and *Penicillium marneffei* (44%).

3.9. Predition of Potential Cleavage Sites by proteases

The presenilin of *Leishmania* like its human counterparts could be cut for the following enzymes: Arg-C proteinase, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, Trypsin, Lys C, Lys N. Nevertheless, the presenilin of *Leishmania* differently from the human molecule was not cleaved by any caspase.

During the last decade, the countless importance on the identification of certain molecular features, like: hydrophilicity, flexibility, accessibility, turns, exposed surface and polarity, followed by the association with antigenic propensity of polypeptides chains, have been proven through the prediction of quite a lot of suitable continuous epitopes with potential use for diagnosis, treatment and vaccine.

In the present paper, the topological characteristics of both proteins were determined by the combination of prediction data from Hydropathic Character of the structures as well as of transmembrane helices.

The nine hydrophobic regions identified in the presenilin, based on the physicochemical properties of the amino acids, suggested the occurrence of a correspondent number of transmembrane regions that were confirmed as helices scattered in the membrane by THMM. Those structural characteristics of *L. infantum* presenilin presented here, they confirm it is a highly conserved molecule in the evolutionary scale, taking into consideration it maintains those features from protozoan to mammals [29].

In the metacaspase structure of *L. chagasi*, besides the occurrence of four hydrophobic regions, the THMM analyses predicted just one helix, placed in the N-terminal portion of the molecule.

Taking into account that the *Leishmania* species we have utilized, present a high sequence homology (94.0%) with *L. major*. Through comparison with the results of Meslin et al [30] it was possible identify the N-terminal domain containing a putative mitochondrial-localization signal, a central domain comprising the conserved catalytic dyad histidine and cysteine and the proline-rich C-terminal domain, which probably plays a role in protein-protein interactions.

It was already suggested, that the metacaspase of *L. major* has different domains that could be relevant to both its trafficking inside the cell and function [31], so the same properties could probably also occur in *L. chagasi*.

Our results related to the hydrophilicity indicated the occurrence of several residues localized in external regions, showing that both molecules have significant numbers of fragments with high antigenic propensity.

Considering the identification of binding peptides to major histocompatibility complex molecules (MHC-I), the presenilin presented most of the sequences with strong affinity in the terminal portions of the molecule. In contrast, the metacaspase showed all of then localized in the central part (Table 2).

Those results show a potential employment for such epitopes concerning the study of molecular interaction. They could also represent important tools for the development of vaccines and targets for immunotherapy, considering the role of MHC class I molecules on the immunological response against infectious pathogens, taking part on crucial steps in the immunological response during transportation of peptides from the cytosol to be recognized by CD8+ T cells [32].

The occurrence of binding peptides to the MHC II molecules of the two proteins, distributed in all portions of both molecules, showed their great immunogenic potential, considering that the recognition of epitopes presented by MHC class II molecules followed by the activation of CD4+ helper T cells, are crucial steps for the adaptive immunity against pathogens [33].

Since MHC class II molecules are highly polymorphic, many promiscuous peptides that can bind to several molecules from that class, have been deemed as a leading targets for vaccine and immunotherapy [33].

The Blast of the hybrid fragment formed by N-terminal of the presenilin plus C-terminal of the metacaspase, showed some interesting aspects, suggesting that distinctive parts of both molecules could have different evolutionary traits.

So, although the fragment have presented no identity with any amoeba's molecule, the results related to the whole molecule of *L. chagasi* presenilin showed a considerable identity (36%) with presenilins from several strains of *E. histolytica* and *E. nuttalli*.

Correspondingly, it was observed a significant identity of the C-terminal moiety of the fragment with the anexin from several species of parasitic fungus such as, *Trichophyton equinum* (41%), *T. rubrum* (43%), *Togninia minima* (43%) and *Penicillium marneffei* (44%). Nonetheless, the whole metacaspase molecule showed no identity with any anexin molecule.

It was already observed in *Plasmodium falciparum*, the presence of a splice variant that leads to the expression of an isoform modified of a Golgi protein that displays a different N-terminus similar to those found in fungi and phylogenetic analyses between the resembling proteins of numerous taxa point to an independent evolution of the unusual N-terminus [34, 35].

The relative identity of the C-terminal of the fragment with annexin of fungi could indicate a probable role for certain cleavage products, formed by the molecular interaction. Until now there is no record about the presence of annexins in Kinetoplastida, but it is important to mention that besides of being used as apoptosis marker because its capability of interact with phosphatidylserine, they are a large family of proteins, which are broadly expressed throughout all eukaryotes and play key roles in a range of essential biological activities.

In parasites, annexins play critical roles in mechanisms linked to their survival, including the maintenance of cell structure integrity and modulation of the immune responses of the vertebrate hosts. Due to their location at the host-parasite interface and their immunogenic properties, these parasite annexins, have been proposed as potential targets for the development of novel drug and vaccine candidates [36].

It is worth to emphasize, that during the first steps to establishment of the infection, the interaction between the *Leishmania* and neutrophils is quite important and the parasite may persuade the host cell behavior modulating the programmed cell death process [37, 38]. Moreover human neutrophils contains in their cytosol, large amounts of annexin 1 (between 2% and 4%) and the human recombinant annexin 1 and its peptido mimetics were able to inhibit neutrophil recruitment in several models of acute inflammation as well as act as an endogenous ligand that mediates apoptotic cell engulfment.

That relative identity of certain parts of molecules among different taxa must be expected, since a protein domain may be considered an independent evolutionary unit that can form a single-domain protein or be part of one or more different multi-domain proteins. Furthermore, domains can either have an independent function or contribute to the function of a multidomain protein in cooperation [39].

The simulation on the potential incidence of caspases cleavage sites in the presenilins showed that, while the human molecule could be cleaved only by the caspase 1, the presenilin of *L. infantum* presented no cleavage sites for all the human caspases. On the other hand, the parasite's metacaspase could probably cut both presenilins, since they presented several cleavage sites when tested against enzymes related to the metacaspase activity, such as: Lys C, Lys N, Arg-C proteinase as well as the trypsin.

Furthermore, it was already showed, a metacaspase of *Leishmania* presenting a trypsin-like activity with a putative role in programmed cell death [40], suggesting that our results could indicate a probable interaction between the metacaspase and the presenilin during the process of apoptosis in *Leishmania*.

5. CONCLUSION

Besides the common functional features, the several distinctive structural characteristics of the proteases involved on the mechanism apoptosis make their epitopes very interesting targets for several purposes this approach can be applied for designing subunit and synthetic peptide vaccines. In the present paper we are suggesting potential availability of a hybrid peptide originated from the presenilin and metacaspase of the *Leishmania* for the developing of new drugs or vaccine.

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