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Inhibitors of Serine Proteinase – Application in Agriculture and Medicine

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1. Introduction

Inhibitors are important elements of regulation of enzyme activity. They can be named ubiquitous components in living organisms (Fan & Wu, 2005). There is a number of inhibitors, such as protein and nonprotein. Among all inhibitors, the most significant are inhibitors of hydrolytic enzymes. Hydrolases are enzymes that catalyze the hydrolysis of chemical bonds. For example, proteolytic enzymes (peptidases or proteinases) hydrolyse the peptide bond, glycosidic glycosidases, esterases hydrolytically cleave the ester bond (The Enzyme List, 2010). The inhibitors of hydrolases was found in bacteria, plants, fungi and animals (Rawlings et al., 2004).

In the homeostatic state the ratio of proteinases and their inhibitors in the tissues of animals and humans is balanced, but at various pathological conditions, this equilibrium is disturbed. For example, at carcinogenesis, seeking to destroy the tissue barrier, synthesized mainly protease (Duffy et al., 2002).

Changes in the ratio of activity of proteases and their inhibitors are observed in the fungal and bacterial skin lesions, traumatic lesions of the eye, burns, apoptosis and other pathological processes (Garcia-Carreno, 1996; Bohm et al., 1998; Thorburn et al., 2003; Wouters et al., 2008). Thus, some inhibitors are actively affected both the cellular environment and on the cell proteome, changing its composition (Vincent & Zivy, 2007).

Most of them are used for medical purposes. For example, inhibitors of thrombin, a key factor in blood clotting. Thrombin (clotting factor II) is the trypsin-like proteases, which circulate in the blood in an inactive zymogen form. The search for new thrombin inhibitors began long before receiving the full 3D structure of thrombin. Initially, were synthesized short peptides containing the amino acid arginine, for example, H-Gly-Pro-Arg-H or H-DPhe-Pro-Arg-H. Then were obtained effective non-specific serine protease inhibitors – ketone-derivatives of 4-amidinophenylpyruvic acid and 4-aminopyridine (Smith & Simons, 2004).

One of the key enzymes of hepatitis C virus replication is a trypsin-like serine protease NS3. Obtained multiple protease inhibitors NS3, including macrocyclic and peptidomimetics (Harper et al., 2009).

Inhibitors of hydrolytic enzymes also play an important role in plant protection. They are widely used use in protecting plants from fungal and bacterial diseases as well as in insect

control. Harvest losses associated with damage to plants by various pathogens, can reach 100%, leading to economic disaster (Mehrabadi et al., 2010). The Republic of Kazakhstan is also concerned about the problem of protecting crops from pests because it is one of durum wheat exporter. At this reason Kazakhstan is paying attention to the study of inhibitors contained in wheat, barley and rice. Besides studies in agriculture sector, studies in medicine sector are conducted, for example in the Scientific Centre for Anti-Infectious Drugs were developed iodine-polymer complexes as inhibitors of HIV integrase (Yuldasheva et al., 2011).

2. Serine proteinase inhibitors

Serine proteinase inhibitors long time were classified according to their characteristics, for example, inhibits the enzyme (trypsin inhibitors, subtilisin, etc.) or source selection - the chicken or ovomucoid pancreatic trypsin inhibitor or by the location of the amino acid residue of lysine or arginine in the reactive centre (Laskowski Jr. & Kato, 1980). This nomenclature is often confusing. One of the challenges ranges of inhibitors is the presence of several homologous domains in a single polypeptide chain (Fan & Wu, 2005; Hejgaard et al., 2005; Dunwiddie et al., 1989).

Typically, the inhibitors are divided by at least 10 families or types of proteinase inhibitors (Laskowski Jr. & Kato, 1980). Among the most studied can be noted soybeans Kunitz inhibitor. To the family of Kunitz inhibitors are proteins with a long polypeptide chain of about 170 - 200 amino acids that contain one or two disulfide bonds and a rather conservative N-site, and generally inhibit the activity of trypsin or chymotrypsin. One well-studied is the soybean Kunitz inhibitor, which is a protein type globulin with a molecular weight of about 21.5 kDa, contains carbohydrate residues, is stable over a wide pH range 1,0-12,0, at a temperature below 40^{o}C. Inhibitor molecule consists of 198 amino acids at the N-terminal aspartic acid is located on the C-terminal leucine. Reactive centre includes the residue arginine-64 (Valueva & Mosolov, 2002). By the way, due to the high content of inhibitor in soybeans, raw soybeans, and raw foods are warming, causing the digestive system in mammals (Duranti M., et al. 2003).

Inhibitors of Bowman-Birk types are widely distributed in plants and significantly different from the Kunitz inhibitor in their amino acid composition and are able to interact not only with trypsin, but with chymotrypsin (Valueva & Mosolov, 2002; Rawlings et al., 2004). Inhibitors of Bowman-Birk type characterized by the presence of two reactive centers on a single polypeptide chain rich in cysteine (7 or more residues in one polypeptide), and the lack of amino acid residues tryptophan and tyrosine. The molecular weight of such inhibitors can vary from 8 to 16 kDa. Sometimes there are inhibitors that contain two domains on one polypeptide chain and active only in relation to one type of enzyme (Valueva & Mosolov, 2002; Yan et al., 2009; Mosolov & Valueva, 2008).

Kazal family inhibitors - the type of trypsin inhibitor-like proteinase, commonly found in animals, including invertebrates. Molecules consist of one or more domains, and three disulfide bridges. The sequence of many often conservative, but the site of binding to the enzyme can be variable (Li et al., 2009; Rimphanitchayakit & Tassanakajon, 2010) Kazal inhibitors are involved in various pathologies of the pancreas. In particular inhibitor Kazal SPINK1 is expressed not only in normal cells of the pancreas, but also transformed. It is

assumed that SPINK1 is able to stimulate proliferation of pancreatic cancer through epidermal growth factor receptor / proteinkinase cascade. Inhibitor SPINK1 is also expressed in other organs and tissues. Apparently, this indicates that the value of SPINK1 is not only in preventing proteolysis non-secretory of proteinases in the gut, but he also has some regulatory function (Ozaki et al., 2009).

Seprpins (serin proteinase inhibitors) group of proteins, which have inhibitory activity, but it is not an inhibitor in the strict sense of the body and performs a different function. Among the most studied serpin antithrombin may be noted, ovalbumin, cortisol binding globulin, precursors of peptide hormones, etc. (Valueva & Mosolov, 2002; Rawlings et al., 2004). Serpins widespread in the proteome, and despite the name, serpins inhibit also other proteases, such as cysteine, aspartate etc.

The examples of classification and nomenclature of inhibitors, really confusing and complicated. Therefore, the team (Rawlings et al., 2004) developed a classification system of inhibitors based on sequence relationship and the relationship of protein fold on the inhibitory domain. An inhibitor domain is defined as the segment of the amino acid sequence containing a single reactive site after removal of any parts that are not directly involved in the inhibitor activity. Their searches of the amino acid sequence databases led to the retrieval of 2500 sequences homologous to those of known peptidase inhibitors. On the basis of sequence homologies of their inhibitor domain proteinase inhibitors been classified into 48 families. As expected, the greatest numbers of inhibitors with a similar sequence were in the family serpin, I4, with over 500 sequences. In turn, families are divided into clans. The study of inhibitors of proliferation of living organisms found, only three families are known so far from Archaea, two of which (I4 (serpins) and I42 (chagasin)) are present in all three superkingdoms. I4 is the most widespread of all, being found even in viruses (Rawlings et al., 2004).

3. The use of protease inhibitors in medicine

The discovery, development, and registration of a pharmaceutical are an immensely expensive operation and represent a rather unique challenge. For every 9000 to 10,000 compounds specifically synthesized or isolated as potential therapeutics, one (on average) will actually reach the market. Each successive stage in the process is more expensive, making it of great interest to identify as early as possible those agents that are likely not to go the entire distance, allowing a concentration of effort on the compounds that have the highest probability of reaching the market (Das & Suresh, 2008).

Throughout most of history, humanity has used as medicines from natural sources, such as medicinal plants, by-products of animals and insects, etc. After isolation of these active substances and determine their molecular structure, drug manufacturing has become more ambitious. This is confirmed by the fact that all 250 of the main structures of existing drugs, as well as their prototypes, 60% were derived from natural sources or has a common molecular structure (Spainhour, 2005; Molinari, 2009). Only a small number have been developed and synthesized "from scratch" (Spainhour, 2005).

So, natural products have been the single most productive source of leads for the development of drugs. Over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infection (Harvey, 2008).

Mainly drugs are protein-based categories namely, recombinant proteins and enzymes, monoclonal antibodies and their derivatives, and synthetic peptides (Das & Suresh, 2008). Although there are studies that propose to use protein inhibitors as drugs. One of the problems of using proteins as drugs of instability in the gastrointestinal tract (GIT), and intravenous administration response from the immune system (Das & Suresh, 2008).

To protect the protein drugs from cleavage by enzymes in the GIT using of encapsulation or complexation with inhibitors. Some researchers propose to encapsulation with an inhibitor aprotinin (Larionova et al., 1999a). Aprotinin – basic pancreatic trypsin inhibitor, which inhibits trypsin and related proteolytic enzymes. By inhibiting kallikrein, aprotinin indirectly inhibits the formation of activated factor XII, a biochemical reaction normally amplified by inhibition of the contact system and of fibrinolysis by aprotinin. Aprotinin inhibits the initiation of both coagulation and fibrinolysis induced by the contact of blood with a foreign surface (Mannucci, 1998). Further studies have shown the effectivity aprotinin for suppressing viral replication rhinotracheitis and adenovirus in vitro (Larionova et al., 2000b).

Russian researchers (Valuev et al., 2001) have proposed as a protection from destruction of proteins in the digestive tract ovomucoid inhibitor included in the hydrogel. In the hydrogel also included insulin. As a result, the mechanism of action, this involves the inhibition of proteolysis of insulin due to neutralizing proteolytic enzymes and increasing the rate of diffusion of insulin from the particles through the small intestine. Rapid diffusion provided by interaction of the carbohydrate bonds of the hydrogel and of the ovomucoid with of lectins intestinal mucosa.

Inhibitors of serine proteinase can be used in the therapy of diseases associated with bowels surgery. Under intestinal resection, as well as ulcerative colitis, a high activity of proteolytic enzymes in the faeces are observed, which is highly undesirable (Bohe, 1987). Studies have shown that inhibitors of serine proteinases from potato significantly reduce the depression of the skin in the anogenital region in patients with intestinal resection, as well as healthy children, in which the activity of proteinases in the feces is physiologically increased (Ruseler-van Embden et al., 2004).

Proteinase inhibitors (aprotinin, ilomastat) can be used in the treatment of acute and chronic damage to lung tissue (Anderson et al., 2009; Weinberger et al., 2011). Chronic lung disease due to interstitial fibrosis can be a consequence of acute lung injury and inflammation. The inflammatory response is mediated through the migration of inflammatory cells (neutrophil granulocyte et al), actions of proinflammatory cytokines, and the secretion of matrix-degrading proteinases. The activation of circulating granulocytes was characterized by increased production of serine proteinases and reactive oxygen metabolites. After the initial inflammatory insult, successful healing of the lung may occur, or alternatively, dysregulated tissue repair can result in scarring and fibrosis. On the basis of recent insights into the mechanisms underlying acute lung injury and its long-term consequences, data suggest that proteinases may not only be involved in the breakdown and remodelling that occurs during the injury but may also cause the release of growth factors and cytokines known to influence growth and differentiation of target cells within the lung (Neumann et al., 1999; Winkler & Fowlkes, 2001).

The effectiveness of proteinase inhibitors as anti-oxidants has been investigated on a model venom-induced tissue damage in rats by Scorpion venom toxins (Fatani et al., 2006).As an antioxidant was used inhibitor aprotinin. Aprotinin alone significantly reduces the venom-elicited increase in glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities and the decrease in glutathione peroxidase levels. But the best results were obtained when the joint application of extract of *Gingko biloba* leaves and aprotinin (Fatani et al., 2006).

As we see the role of proteinase inhibitors in the pathogenesis of disease is significant. In our view, it is important to note the significance of inhibitors in tumours processes. Various types of proteinases are implicated in the malignant progression of tumours and increase oxidative stress (Koblinski et al., 2000; Aoshiba al., 2001). To be fair, it is not always proteases have been associated with cancer progression (Lypez-Otin & Matrisian, 2007).

Nevertheless, proteinase inhibitors may therefore be useful as therapeutic agents in anti-invasive and anti-metastatic treatment. Studies have shown that potato cysteine proteinase inhibitor PCPI 8.7 inhibited invasion B16 mouse melanoma cell by 21% and serine proteinase inhibitor reduced invasiveness by up to 24 % (Sever et al., 2005). Overexpression of cathepsin B excessively associated with adenocarcinoma of the esophagus and other cancers (Kos & Lah, 1998).

Trypsin inhibitor from soybean BBI 3 can suppress the growth of cancer cells (Armstrong et al., 2000). Because of its inhibitory properties BBI3 can be used inside in the form of capsules and tablets, dispersible in the gut, not worrying about what will be split by proteases of the gastrointestinal tract.

A very interesting example may be the use of Bowman-Birk Inhibitor (BBI) from soybeans in the treatment of multiple sclerosis. Proteases generated during inflammation are involved in the induction of tissue damage during inflammatory demyelination in the central nervous system. Both in vitro and ex vivo, BBI inhibited myelin basic protein-specific proliferation of lymph node cells. BBI reduced the activity of matrix metalloproteinase-2 and -9 in spleen cell supernatants and was detected in the central nervous system of treated rats. BBI suppresses experimental autoimmune encephalomyelitis, it can be administered orally, and it is safe and relatively inexpensive. It may have a therapeutic role in patients with MS (Gran et al., 2006).

Bifunctional inhibitor of xylanase and aspartic proteases from extremophilic microorganism Bacillus sp. aspartic proteinase inhibits HIV-1 (Dash et al., 2001).

The Bowman-Birk trypsin-chymotrypsin inhibitor (BBI) from soybean has been proposed as anticarcinogenic drugs. The BBI inhibited the growth of human colorectal adenocarcinoma HT29 cells in vitro (Clemente et al., 2005).

The trypsin inhibitor from *Peltophorum dubium* seeds (PDTI), as well as soybean trypsin inhibitor (SBTI), both belonging to the Kunitz family, have lectin-like properties and trigger rat lymphoma cell apoptosis. It was shown that PDTI and SBTI induce human leukemia Jurkat cell death. Induction of apoptosis was confirmed by flow cytometry (Troncoso et al., 2009).

In our research studies the cytotoxicity of trypsin inhibitor extract (Islamov & Fursov, 2007) from wheat seeds was evaluated by counting the cells at the time of harvest, with a

haemocytometr. Cytotoxicity in treated cultures was assessed by Relative increase in cell counts (Fellows & O'Donovan, 2007; Lorge et al., 2008). This study was made on L5178Y TK +/- clone 3.7.2c mouse lymphoma cells which were obtained from American Type Culture Collection. Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum, 200 U/ml penicillin, 50 µg/ml streptomycin, 2.5 µg/ml amphotericin B, 200 µg/ml L-glutamine, 200 µg/ml sodium pyruvate and 500 µg/ml pluronic acid. Cell cultures were grown in a humidified atmosphere with 5% CO2 in air at 37°C. Cells tested negative for mycoplasma contamination and were routinely passaged to maintain exponential growth. Three concentration of trypsin inhibitor substance was tested 1/1000, 1/100, 1/10 (v/v). The exposure was 24-h and 0-h recovery. Utilizing exponentially proliferating cells, 96 V-bottomed wells were seeded at a concentration of 6×10^4 cells per well. Each concentration was performed in triplicate. At the end of treatment time 20 µl of cells from each concentration was mix with trypan blue 1:1 (v/v). An aliquot of the cell suspension was then transferred to a hemacytometer for cell counts. The cell counts were used to determine Relative increase in cell counts. Representative results for trypsin inhibitor extract are shown in Table 1.

Concentration of trypsin inhibitors (µg/ml)	% Toxicity RICC*
Negative control	0
500	100%
50	91%
5	14%

*Relative increase in cell counts

Table 1. Cytotoxicity trypsin inhibitors from grain wheat

Our studies have shown the cytotoxic effect of trypsin inhibitors. Similar results were obtained in cell culture H28 of malignant mesothelioma. When adding Bowman-Birk protease inhibitor from soybeans at concentrations of 200-400 µg/ml Inhibition of cell growth (Kashiwagi et al., 2011). Currently, research is continuing to explore mechanisms of toxicity. Apparently, this may be related to the processes of apoptosis (Troncoso et al., 2009).

A completely different example of the serpin CrmA (cytokine response modifier A), which expresses Cowpox virus. The targets of CrmA are members of the caspase family of proteases that either initiate the extrinsic pathway of apoptosis (caspases 8 and 10) or trigger activation of the pro-inflammatory cytokines interleukin-1β and interleukin-18 (caspase 1). CrmA has the typical fold of a cleaved serpin, even though it lacks the N-terminal half of the A helix, the entire D helix, and a portion of the E helix that are present in all other known serpins. Thus, the inhibitory proteins can be not only drugs. They may also viral countermeasures to host defenses against infection may contribute significantly to the pathology associated with poxvirus infections (Renatus et al., 2000).

Such examples are very much (Polya, 2003). All this points to the crucial regulate the activity inhibitors of proteinase activity. In addition, the study of mechanisms of interaction enzyme with inhibitor can tell researchers how to develop new drugs - semi synthetic (including modified) and synthetic (non-protein). So, after some modification of the reactive site serpin,

they acquire the ability to block angiogenesis and induce tumor regression (Carrell, 1999). Successfully completed Phase 2 clinical trials engineered protein inhibitor of human neutrophil elastase (Dyax Corp. & Debiopharm). Human neutrophil elastase, an enzyme produced as part of the inflammatory response, is implicated in the loss of lung function in patients with cystic fibrosis and other clinical conditions. (Dyax Corp. & Debiopharm). The inhibitor of human neutrophil elastase is derived from the Kunitz-type domain of inter-α-inhibitor which is a natural inhibitor of human neutrophil elastase normally present in plasma (Grimbert et al., 2003).

There is enough work, in which information about the protease inhibitors and their application (Wenzel & Tschesche, 1995; Polya, 2003).

4. Inhibitors in agriculture

Considering the basic properties of inhibitors - inhibition of enzyme activity, they have successfully used to control pests. It is known that plants are often damaged by many different pathogens - fungi, bacteria and viruses (Selitrennikoff, 2001; Franco et al., 2002). Also, insects and parasitic worms of plants are cause illness and death of plants frequently, causing great agricultural economic losses. However, during infection of plants by infectious agents, not all plants of one population or species ill. This is due to phytoimmunity - the ability to resist pathogens (Valueva & Mosolov, 2002; Mosolov & Valueva, 2008). Protective mechanisms were elaborated in the long process of co-existence of host and parasite. The major components of the existing protection mechanisms are substances of protein nature. These include enzymes - glucanase, chitinase and protease inhibitors and α-amylase. For example, damage to tomato leaves (*Licopersicon esculentum* Mill.) by insects or microorganisms induce the synthesis of more than twenty different proteins, including inhibitors of serine, cysteine proteinases and aspartilnyh and α-amylase (Valueva & Mosolov, 2002).

Inhibitors of trypsin and chymotrypsin from soybeans, beans and potato to suppress proteinase from phytopathogenic fungus (*Fusarium solani*). Moreover, the inhibitors belonging to the family of Bowman-Birk inhibit the growth of hyphae and conidia germination of fungi *Fusarium solani*, *F. culmorum* and *Botrytis cinerea*, etc. (Loreti et al., 2002). Along with trypsin and chymotrypsin inhibitors in plants and detected proteins that inhibit proteases of microorganisms.

Quite interesting are bifunctional inhibitors. Bifunctional inhibitors - proteins that contain two active sites, interacting as one of the enzymes, eg trypsin/trypsin, and various enzymes - trypsin/chymotrypsin or proteinase/amylase (Sreerama et al., 1997; Valueva & Mosolov, 2002; Mosolov & Valueva, 2008).

Bifunctional (double-headed) inhibitors of proteases, traditionally, belong to the family Bowman-Birk inhibitors. Bifunctional inhibitors are assumed to have arisen by duplication of an ancestral single headed inhibitor gene and subsequently diverged into different classes and with a network of highly conserved disulfide bridges (Odani et al., 1983; Qi et al., 2005). However, interest bifunctional inhibitors active against both proteases and amylases (Table 2).

The first three bifunctional inhibitor (BASI, WASI and RASI) show significant similarities in the structure and properties (Ohtsubo & Richardson, 1992; Franco et al., 2002; Nielsen et al., 2004).

Inhibitors	Family or subfamily	Source	Molecular weight, kDa	Isoelectric points	Residue numbers	Reference
α-amylase/subtilisin (BASI)	I3A (Kunitz (plant)	*Hordeum vulgare* L	19,879	7,2	181	(Nielsen et al., 2004)
α-amylase/subtilisin (WASI)	I3A (Kunitz (plant)	*Triticum aestivum* L	20,5	7,2	187	(Mundy et al., 1984)
α-amylase/subtilisin (RASI)	I3A (Kunitz (plant)	*Orysa sativa* L.	21	9,05	189	(Yamasaki et al., 2006)
α-amylase/proteinase K (PKI3)	-	*Triticum aestivum* L	19,6	> 7	180	(Zemke et al., 1991)
α-amylase/trypsin (BIAT)	I6 (cereal)	*Triticum aestivum* L	14,0	10	-	(Islamov & Fursov, 2007)
α-amylase/trypsin (RBI)	I6 (cereal)	*Eleusine coracana* Gaertneri	13,3	10	122	(Maskos et al., 1996)

Table 2. Bifunctional inhibitors of amylase/protease of cereals

Thanks to the "dual" activity, the bifunctional inhibitor of amylase/proteinase appears to provide synergetic effect in protecting against pathogens. Bifunctional inhibitors of the subject of many articles and studies (Konarev et al., 1999; Franco et al., 2002; Birk, 2003; Salcedo et al., 2004; Pouvreau, 2004; Habib & Fazil, 2007).

The essential role of inhibitors in plant protection from insects and other pests inspired the creation of transgenic plants. Transgenic plants with enhanced resistance to insects, containing the genes of proteinase inhibitors. Trypsin inhibitor gene (SrTI) of cowpea (*Vigna unguiculata* L.) was transferred into tobacco plants (*Nicotiana tabacum* L.). Plants content SrTI reached 1% of total soluble protein, the defeat of the tobacco cutworm larvae plants decreased by 50% (Valueva & Mosolov, 2002). Along with transgenic plants containing genes of serine protease inhibitors were obtained plants expressing genes of inhibitors of cysteine proteinases. Extracts of potato containing the gene of LS-1, effectively inhibited the activity of proteinases from the digestive tract Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Mosolov & Valueva, 2008).

Differential expression of the CaMV proteinase inhibitor obtained the transfer of the corresponding gene using Agrobacterium transformation into the genome of tobacco culture (*Solanum nigrum*) and alfalfa (*Medicago sativa*). Expression was studied by measuring mRNA levels in leaves of CaMV. It was also shown the accumulation of inhibitors in the vacuoles of Solanum nigrum to 125 mg per 1 g of tissue, tobacco, 75 mcg / g, alfalfa 20mkg / g (Valueva & Mosolov, 2002). In a similar way shape transgenic peas (*Pisum sativum*), which is introduced into a plant α-amylase cDNA inhibitor I bean (*Phaseolus vulgaris*) and "substituted" under a strong promoter of phytohemagglutinin. As a result, the inhibitor

content increased to 3% of soluble protein, compared with the control (1%). Although I inhibitor inhibits α-amylase man, his concentration was not sufficient to disrupt the digestive proces (Schroeder et al., 1995).

Inhibitors of proteolytic enzymes may play an important role in plant protection not only from harmful insects, but also other pests: nematodes, the nematodes. Many nematodes are parasitic on plants and cause significant damage to agricultural production. In the gut of nematodes contains active cysteine and serine proteases, including chymotrypsin and kallikrein is similar. It is interesting that when infected with worms, among transcripts of tomato were found proteins related to the family of Kunitz inhibitor (SBTI) (Valueva & Mosolov, 2002; Schroeder et al., 1995).

Expression of proteinase inhibitor chimera-II-CAT in tissues of potato tobacco prevailed 50 times under the influence of 1% sugar (maltose, glucose and fructose) in the farmed environment. Adding mannitol to the nutrient medium in place of these sugars does not affect the synthesis inhibitor-II-CAT (Valueva &Mosolov, 2002). The resulting transgenic plants from cell cultures of tobacco leaves revealed sensitivity to a 3% sucrose, which results in increased synthesis inhibitor-II-CAT 3-fold (Mundy et al., 1986). In addition, a mechanical failure of one of the leaves of the plant stimulates the transcription of genes encoding proteinase inhibitors are not only damaged but also in intact tissues. Systemic induction of gene expression was also observed with wounds or infected tomatoes (Valueva & Mosolov, 2002). Similar results, mostly on potatoes are presented in studies and reviews (Rolland et al., 2002).

In the process of co-existence of plants and their vermins, every organism has developed a protection system, and ways to overcome this protection. Plants synthesize a large number of different inhibitors, which makes them not edible. In response, many vermin have developed a system of protection against these inhibitors. Especially, began to secrete enzymes are not susceptible to inhibition by inhibitors of the existing (Maarten & Jules, 2011). For example, instead of trypsin, which is actively suppressed by inhibitors of trypsin and which are enriched with plants, insects began to synthesized chymotrypsin-like enzymes (Valueva & Mosolo, 2002). So, in future research the question of the co-evolution of insect proteases and plant inhibitors should be better approached from a systems level.

Apart from the use of inhibitors in the creation of transgenic plants resistant to certain pathogens, they are also of interest as genetic markers to study diversity, evolution and plant breeding. In many Compositae plants shows monogenic control, while interline crosses, loci coding for many protein inhibitors, have demonstrated linked inheritance. For example, the cyclic inhibitor, with a mass of 1.5 kDa and trypsin inhibitor, three groups of sunflowers, which, according to the results of mating, gave the values of the recombination frequencies for different pairs of loci from 0.23 to 0.40 in F2. Small molecule inhibitors of the information on the TI better match the taxonomy of Asteridae, based on data for molecular markers than the classical (Lawrence & Koundal, 2002).

A number of cereal caryopsis contains two clearly divided groups of inhibitors with a mass of 12 and 24 kDa. A comparative study of intraspecific polymorphism of these proteins and their components of different species showed that the evolution of the spectrum there is a complication of inhibitors and most complete range of typical soft hexaploid wheat (Schroeder et al., 1995).

In the works of A. Konarev (Konarev et al. 1999; Konarev et al., 2004) shows in detail the variability of inhibitors of trypsin-like proteinases in cereals due to resistance to various grain pests. So in wheat trypsin inhibitors are represented by several genetically independent systems of proteins controlled by the genome and B chromosomes 1D (endosperm), 3Dβ (aleurone layer), 1DS and 3Aβ (leaf). Trypsin inhibitors of rye are controlled chromosome 3R and barley 3H. The most complex structure of inhibitors was wheat leaves, with the genomic formula AABBDD. In general, it is the sum of the spectra of trypsin inhibitors from several tetraploid (T. turgidum) (AABB) and (Aegilops tauschii Coss.) (DD) (Konarev, 1986; Konarev et al., 2004).

In addition to the trypsin inhibitors are very interesting inhibitors of chymotrypsin and subtilisin, which are the most heterogeneous structure and variability of all species of wheat and corn and barley, which are controlled by fifth chromosome, while the wheat and rye - I homoeologous group of chromosomes of different genomes. In diploid wheat, a number of species (*T. timopheevii*) identified the protein components capable of inhibiting both subtilisin and chymotrypsin, which are characterized by low variability (Konarev et al., 2004).

Investigation of the stability of different wheat varieties to pest insect (*Rhyzopertha dominica* F) showed some dependence on composition of trypsin inhibitors and chymotrypsin. Grades Kirghiz 16, Saratov 41 had low levels of trypsin inhibitors and demonstrated the instability to the pest insect. Whereas grade Kalayan Sona, Saba, Diamond, Aurora 44, Zernogardskaya 39 were resistant to grain pest insect. This is also confirmed by the fact that part of the digestive enzymes *R. dominica* F. are trypsin-like proteases. In addition, a variety Zernogardskaya 39 contained a number of specific inhibitors of α-amylase insects (Konarev, 1986; Konarev et al., 1999; Konarev, 2006).

As we see the potential use of inhibitors in agriculture and in particular, to protect plants from pests wide. Such an approach to crop protection against loss of environmentally safer and economically more advantageous. Since there is no need to conduct expensive toxicological studies of new insecticides. The products of these plants are safe for humans and animals. And the use of transgenic technology will accelerate the development of new, resistant to insect pests, plant varieties.

5. Conclusion

Presented data from studies of protein inhibitors of serine proteases confirm their crucial role in the regulation of proteolytic enzymes. Examples of their use as drugs, crop protection agents, antioxidants, etc. are given in this chapter. No less interesting is the diversity of the whole family of inhibitory proteins - Serpin. Nevertheless, there are several obstacles to widespread use of inhibitors:

1. Several inhibitors of serine proteases can cause allergies.
2. Intravenous application of inhibitors is extremely difficult because of their antigenic properties.
3. Increased production of one of the inhibitor in the plant is inefficient, since most vermins are able to quickly adapt to it.

At the same time, there are positive aspects for the development of research inhibitors. It should also be noted.

1. Studying the mechanisms of interaction of natural protein inhibitors of enzymes provides an opportunity to develop approaches to the preparation of synthetic, small molecule inhibitors.
2. The prevalence of proteinase inhibitors in all kingdom, from viruses to eukaryotes, and indicates their important role in the regulation of proteolysis.
3. The use of two or more inhibitors at the same time to protect plants from pests, reducing the ability of pathogens to adapt.

So, enzyme – inhibitor interactions are very important process into living organisms. To date, a variety of genetic and biochemical methods exist for studying enzyme - inhibitor interactions and identifying of such interactions.

As mentioned earlier, of methods of the quantum chemistry, not only managed to learn in detail about HIV integrase, but also to develop an algorithm for obtaining drugs based on organic complexes of iodine. The next step is to study the inhibition of organic complexes of iodine with HIV proteinase.

Quite promising direction is also in search of new plants, small inhibitors. Examples of the serpin showed that any protein can potentially be an inhibitor that expands the range of functional significance of the proteome.

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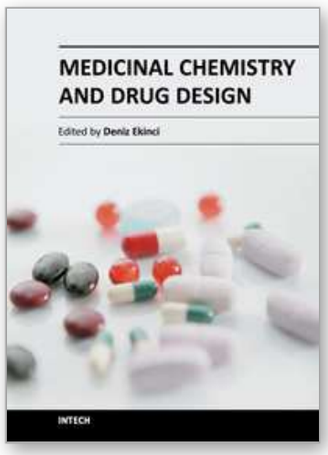

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Over the recent years, medicinal chemistry has become responsible for explaining interactions of chemical molecules processes such that many scientists in the life sciences from agronomy to medicine are engaged in medicinal research. This book contains an overview focusing on the research area of enzyme inhibitors, molecular aspects of drug metabolism, organic synthesis, prodrug synthesis, in silico studies and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in medicinal chemistry and drug design. Particular emphasis is devoted to both theoretical and experimental aspect of modern drug design. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in associated areas. The textbook is written by international scientists with expertise in chemistry, protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the textbook will enhance the knowledge of scientists in the complexities of some medicinal approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of medicinal chemistry and drug design.

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