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PLANT SERPINS AS POTENTIAL PHARMACEUTICALS FOR THE CORRECTION OF HEMOSTASIS AND FIBRINOLYSIS DISTURBANCES. BIOINFORMATIC STUDY

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Abstract. Protease inhibitors are protein molecules that inhibit the function of proteases. The vast majority of naturally occurring protease inhibitors are proteins widely distributed in living organisms and in particular in plants (1). The largest and most broadly distributed superfamily of protease inhibitors found in all domains of life (Archaea, Bacteria and Eukarya) are serpins (serine protease inhibitors) (2,3). The majority of serpins are irreversible inhibitors of serine proteinases of the chymotrypsin family. Examples of these enzymes from animals include trypsin, thrombin, elastase and blood coagulation factors such as factors Xa and VIIa. A minority of serpins have been evolved to inhibit the activity of serine proteinases other than those of the chymotrypsin family (such as subtilisins) and a few serpins inhibit cysteine proteinases (which have a similar catalytic mechanism to the serine proteinases) (4).

To determine whether serpin is capable of inhibiting the particular proteinase, the usual approach is to incubate the serpin with a range of different serine and cysteine proteinases, and to look for inhibition by a kinetic assay, or by formation of a covalent serpin–proteinase complex visualized by SDS gel electrophoresis (5). The other approach is to use bioinformatics. If the primary structure of the serpin is known, then an informed guess regarding the reactivity can be made based on identification of the reactive loop P1 and adjacent residues by alignment with other known serpin sequences.

Hemostasis and fibrinolysis, the biological processes that maintain proper blood flow, are the consequence of a complex series of cascading enzymatic reactions. Serine proteases involved in these processes are regulated by feedback loops, local cofactor molecules, and serpins (6). The principal serpins involved in the regulation of hemostasis and fibrinolysis are antithrombin, heparin cofactor II, protein Z-dependent protease inhibitor, a1-protease inhibitor, protein C inhibitor, a2-antiplasmin and plasminogen activator inhibitor-1. The delicate balance between proteolytic and inhibitory reactions in hemostasis and fibrinolysis, described by the coagulation, protein C and fibrinolytic pathways, can be disrupted, resulting in the pathological conditions of thrombosis or abnormal bleeding. Medicine capitalizes on the importance of serpins, using therapeutics to manipulate the serpin–protease reactions for the treatment and prevention of thrombosis and hemorrhage. However, at present only animal proteins such as antithrombin III and aprotinin (Trasisol, Contrical, Gordox etc) are used for the correction of the disturbances of hemostasis and fibrinolisis. Therefore, investigation of serpins, their cofactors, and their structure–function relationships is imperative for the development of state-of-the-art pharmaceuticals for the selective fine-tuning of hemostasis and fibrinolysis. Plant serpins are capable to inhibit proteases involved in blood clotting and fibrinolysis (7) and may be the candidates for such a role. We used bioinformatics approaches to compare plant serpins and serpins involved in the regulation of hemostasis, to reveal common and specific features in the in their primary and 3-D structures.

We used UniProt http://www.uniprot.org/ and NCBI Protein http://www.ncbi.nlm.nih.gov/protein databases to find primary structures, chartacteristic features, active sites or reactive bonds, functional activities, as well as for performing multiple and global pairwise alignment of the primary structures of serpins we are interested in. To perform local alignment and identify library sequences that resemble the query sequence by comparing a query sequence with a library or database of sequences we used UniProt BLAST algorithm. We found 3-D structures of serpins in RCSB PDB http://www.rcsb.org/pdb/home/home.do and aligned them with a help of PDBeFold http://www.ebi.ac.uk/msd-srv/ssm/

We chose the following serpins participating in hemostasis and fibrinolysis: antithrombin III, heparin cofactor II, protein Z-dependent protease inhibitor, a1-protease inhibitor, protein C inhibitor, a2-antiplasmin and plasminogen activator inhibitor-1 and compared them with plant protease inhibitors: Arabidopsis thaliana SERPIN1 Arabidopsis Thailana (Mouse-ear cress), Trypsin inhibitor A - Glycine max (Soybean), Trypsin/factor XIIA inhibitor - Zea mays (Maize), Trypsin inhibitor 1 - Momordica charantia (Bitter gourd), Cysteine proteinase inhibitor 1 - Oryza sativa subsp. japonica (Rice), Subtilisin-chymotrypsin inhibitor WSCI - Triticum aestivum (Wheat) and Bowman-Birk type proteinase inhibitor - Glycine max (Soybean) (Glycine hispida)

Animal serpins we chose belong to the different clades: A, B, C, D, E, F, G, according to the A-P classification which divides Eukaryota serpin superfamily into 16 clades. Antithrombin III (SERPINC1), heparin cofactor II (SERPIND1), protein Z-dependent protease inhibitor and a1-protease inhibitor (SERPINA10 and SERPINA1), plasma protease C1 inhibitor (SERPING1), a2-antiplasmin (SERPINF2) and plasminogen activator inhibitor 1 (SERPINE1), plasminogen activator inhibitor 2 (SERPINB2), where letters A – G are the clades and the numbers – position in the clades.

Plant protease inhibitors are classified according to MEROPS database (8). Selected plant protease inhibitors belong to following families and subfamilies: Arabidopsis thaliana SERPIN1 Arabidopsis Thailana (Mouse-

ear cress) , Soybean trypsin inhibitor A - I3A, Maize Trypsin/factor XIIA inhibitor – I6, Bitter gourd trypsin inhibitor 1 – I7, Rice cysteine proteinase inhibitor 1 – I25B, Wheat subtilisin-chymotrypsin inhibitor WSCI – I13 and Soybean Bowman-Birk type proteinase inhibitor – I12.

Bioinformatics study reveals common and particular features in the primary and 3D-structures of selected animal and plant serpins. Plant serpins aminoacid chain usually consists of 100-200 amino acids. Animal serpins – components of hemostasis and fibrinolysis systems typically are nearly two times larger and contain 400-500 amino acids in comparison with protease inhibitors such as basic pancreatic trypsin inhibitor (BPTI), which is about 60 amino acids. In spite of the poor sequence homology between family members, serpins share a highly conserved core structure near C-terminal that is critical for their functioning as serine protease inhibitors. This conserved domain consists of nearly 20 amino acids and include reactive center loop (RCL) or reactive site loop (RSL). For Conserved Protein Domain Family C1 the following sequence of amino acids is very typical GVEAAAASAISVARTILLVFEVQQP (in FASTA format). It is common that the active site of serpins contains two amino acids (P2-P1). The positively charged residue Arg is by far the most common P1 residue among plant serpins and that a large number of serpins have Leu at P2. The "LR serpins", which are those with P2– P1 Leu-Arg-X, where X is a small residue (Ser, Cys, Ala, Gly), are very widespread in the plant kingdom. Nearly 40% of the LR serpins have a positively charged residue at P3, and most of the remainder have hydrophobic or small/polar residues at this position. 3D-structures of serpins are made up of three β sheets (A, B and C) and 8-9 α helices (termed hA-hI) and a RLC containing a specific bait sequence for the serpin's target proteinase(s).

The common features in the structures of plant serpins and serpins of hemostasis and fibrinolysis revealed with a help of bioinformatic approaches proves the possibility of creating of new drugs designed for the correction of hemostasis and fibrinolysis disturbances on the base of plant serpins.

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ENZYME-LINKED IMMUNOSORBENT ASSAY IN THE DIAGNOSIS OF HELMINTHIASIS IN CHILDREN

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The topic is relevant due to the fact that against the background of economic change, environmental degradation, inadequate and unbalanced nutrition, psychological stress associated with school pressures and social tensions, widespread use of various medications are more common disturdanges of the adaptation processes and many well-known diseases change their clinical picture, including diseases caused by various parasites. Over the past 15 years this index has increased by 85.6% and in 2006 amounted to – $ 84.1 per 100 thousand population and has no tendency to decrease. Among patients more than 60% are children, the incidence of cases since 1991 has increased 2.5 times and in 2006 amounted to 355,8 per 100 thousand children under 14 years. Young children are affected 3 times more often than adults.

Distinguish risk factors that contribute to invasion: poor maintenance of the population with safe drinking water, pollution of open waters untreated sewage, unbalanced nutrition in the form of increased consumption of easily digestible carbohydrates on the background of a significant deficit of protein, hypochlorhydria, dyscholia, high intensity parietal digestion, inherent in children, intestinal microflora after treatment with antibiotics (especially with multiple courses).