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Effects of Soybean Trypsin Inhibitor on Hemostasis

Eugene A. Borodin, Igor E. Pamirsky,
Mikhail A. Shtarberg, Vladimir A. Dorovskikh,
Alexander V. Korotkikh, Chie Tarumizu,
Kiyoharu Takamatsu and Shigeru Yamamoto

Additional information is available at the end of the chapter

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1. Introduction

Soy bean trypsin inhibitor (SBTI) belongs to the family of serpins – serine protease inhibitors widely distributed in the nature [Silverman G.A. et al., 2004; 21]. Serpins participate in the regulation of proteolytic reactions underlying very important physiological and pathological processes such as digestion [16], blood clotting [3, 14, 17], immunity [44] apoptosis [36, 42], inflammation [10], dystrophy [31], carcinogenesis [22, 11] and so on. Despite the apparent importance of the correction of imbalances of the proteolysis in pathology, in fact only the pancreatic trypsin inhibitor (aprotinin) is used more or less widely as a protease inhibitor drug (Trasisol, Contrical, Gordox etc) in the treatment of some diseases [24, 13, 40]. Being the animal protein aprotinin possesses substantial disadvantages [23, 12] and attempts to develop new drug on the base of plant or recombinant proteins or peptidomimetics are undertaken [35, 27]. SBTI is one of the candidates for such a role [34].

Among proteolytic processes hemostasis is of special importance because of the high frequency of its disturbances, accompanied many pathological processes and diseases [20, 18]. At first sight it looks strange to expect that plant protease inhibitor SBTI should influence hemostasis in humans because blood clotting represents the cascade of proteolytic reactions catalyzed by highly specific proteases [43]. However, results of the very early studies support such possibility [26, 41]. The present study was aimed to investigate the influence of SBTI and aprotinin on hemostasis using modern bioinformatics approach and classical in vitro methods. The concrete purposes of the study included:

1. To establish the extent of structural homology, compare functional activities and evaluate potential targets of SBTI and aprotinin among the human proteases by bioinformatics (*in silico*) methods.
2. To investigate the influence of trypsin, SBTI and aprotinin on some indexes of blood clotting, fibrinolysis and platelet aggregation as well as on the hemolytic activity of the complement *in vitro*.
3. To elucidate the possibility of the regulation of the total proteolytic and tripsin-inhibiting activity of blood plasma *in vivo* by the consumption of soy foods enriched with soy protein isolate (SPI) possessing thermo-stable fraction of SBTI.

2. The study of aprotinin and SBTI *in silico*

In silico methods (bioinformatics) entails the creation and advancement of databases, algorithms, computational techniques to solve formal and practical problems arising from the management and analysis of biological data. One of the potential areas for exploiting these methods is computational drug design. We used *in silico* methods in our study for the comparison of structural homology, functional activities and evaluation of the potential targets of SBTI and aprotinin among the human proteases.

From fifty databases we had browsed in the INTRNET nine contained information on aprotinin and SBTI (Table 1). From these PDB (Protein Data Bank) and UniProt/Swiss-Prot were the most informative and we used these databases in our study.

Database	ID in the Database	
	Aprotinin	SBTI
UniProt-Swiss-Prot http://www.expasy.org	BPT1_BOVIN (P00974)	ITRA_SOYBN (P01070)
Blocks - most highly conserved regions of proteins http://www.ebi.ac.uk	P00974	IPR002160
COG - the database of Clusters of Orthologous Groups of proteins http://www.ncbi.nlm.nih.gov	Precursor P00974; NP_001001554;	-
GTOP - Genomes TO Protein structures and functions http://spock.genes.nig.ac.jp	btau0:ENSBTAG00000017328	?atha0:At1g17860.1
iProClass - an integrated, comprehensive and annotated Protein Classification database http://pir.georgetown.edu	P00974/BPT1_BOVIN; PIRSF001621	-
LIGAND - LIGAND chemical database for enzyme reactions	50059016; 3809839; bta:616039; 100156830; Bt.32343;BPT1_BOVIN	-

Database	ID in the Database	
	Aprotinin	SBTI
http://www.pasteur.fr		
MMDB - Molecular Modeling Data Base http://www.ncbi.nlm.nih.gov	P00974 (Precursor); 1QLQ0	1AVU
PDB - Protein Data Bank http://www.rcsb.org	1OA6	1AVU; 1BA7
MEROPS (peptidases) http://merops.sanger.ac.uk	102.001	103.001

Table 1. Databases containing the information on aprotinin and SBTI.

2.1. The homology of the primary structures of SBTI and aprotinin

To investigate the homology of the primary structures of SBTI and aprotinin we used BLAST (Basic Local Alignment Search Tool) algorithm, sequence alignment editor Bio Edit 5.0.9 and got information on these proteins from Protein Data Bank and Uni Prot using FASTA (Table 2), MOL and PDB format-files. The extent of homology of the total sequences of SBTI and aprotinin, calculated by the method of the multiple paired alignment, makes up 10% (Figures 1 and 2). The low homology of the total sequences of should be attributed to the different lengths of polypeptide chains these proteins – 58 amino acids in aprotinin [45] and 181 in SBTI [39].

Traditional abbreviation	Ala	Asn	Cys	Asp	Glu	Phe	Gly	His	Ile	Lis	Met
FASTA fromat	A	B	C	D	E	F	G	H	I	K	M
Traditional abbreviation	Asn	Pro	Gln	Arg	Ser	Tre	Val	Trp	Tyr	Glu	*
FASTA fromat	N	P	Q	R	S	T	V	W	Y	Z	-

Table 2. Designation of aminoacids in FASTA format. *- symbol in FASTA format corresponds to the gap of unlimited length

Comparison of the separate domains of SBTI and aprotinin reveals greater sequence similarity. Thus, the extent of homology of the N-terminal region of SBTI (5-60 amino acid residues) and the sequence of aprotonin without last two amino acids makes up 21%. The central region of SBTI (85-116 amino acid residues) and the region of aprotinin within 25-56 amino acids show 24% similarity. The highest extent of homology up to 35% is characteristic

for C-terminal region of SBTI (132-181 amino acid residues) and aprotinin sequence without six C-terminal amino acids.

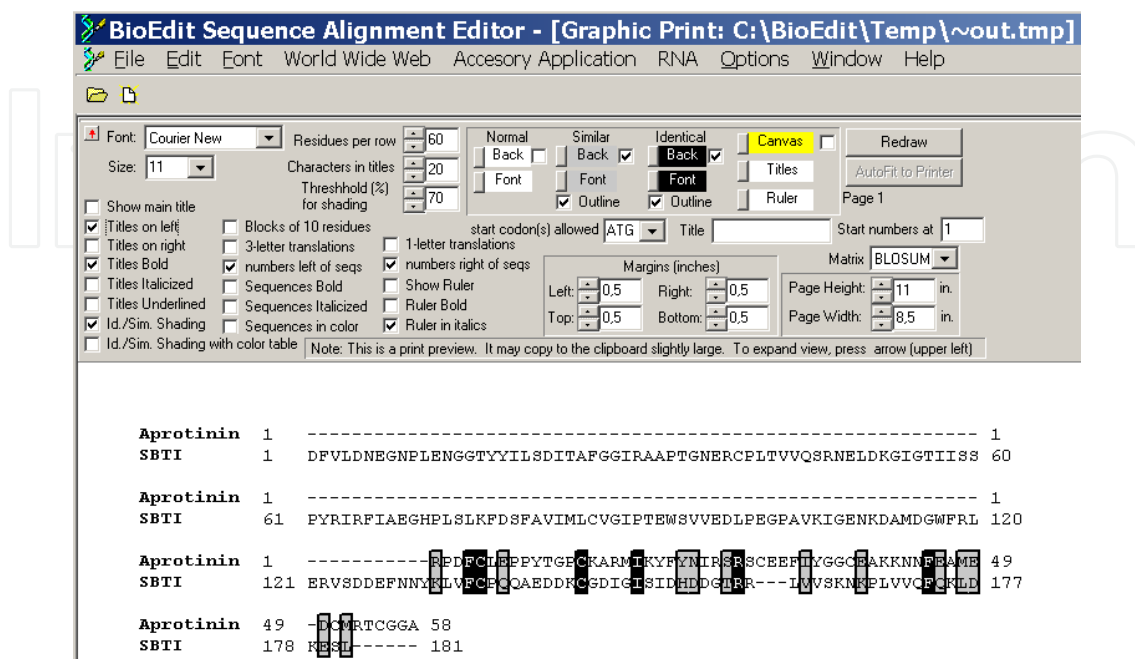


Figure 1. The screen of the Bio Edit 5.0.9 with the results of calculation of homology of Aprotinin and SBTI. The identical amino acids are marked with black and similar with gray. Amino acid sequence are represented in FASTA format.

```

Aprotinin 1  ~-RDFLCLEPPPYTGPCCKARMIKYFYNIKRSRSCSEEFYGGCEAKKNNFEAMDCMRTCGGA 58
SBTI      1  DFVLDNEGPLENGGTYIISDITAFGGIRAAAPTGNRCPLTVVQSRNELDRKIGITLISS 60

Aprotinin 1  RPDFCLEPPPYTGPCCKARMIKYFYNIKRSRSCSEEFYGGCEAKKNNFEAMDCMRTCGGA 58
SBTI      61  PYRIRFIAEGHPLSLKFDSPAVIMLCVGIPTETMSVVEDLEEGPAVKIGENKDKAMDGMFRL 120

Aprotinin 1  RPDFCLEPPPYTGPCCKARMIKYFYNIKRSRSCSEEFYGGCEAKKNNFEAMDCMRTCGGA 58
SBTI     132  KLVFCFCQAEDDRCSIDIGISIDHLDGIRR---LWVSKNKLPLVVCFCRLLD KESL----- 181
  
```

Figure 2. Homological regions of Aprotinin and SBTI (according to Bio Edit 5.0.9). The identical amino acids are marked with black and similar with gray.

2.2. Tertiary structures of SBTI and aprotinin

For the visualization of the 3D-structures of SBTI and aprotinin we used Chem Office 5.0 and Yasara 6.2.5 software. The files in MOL format, containing the information on the sequences of these protease inhibitors, were imputed into Chem Office 5.0 software and converted in PDB format. The obtained PDB files were imputed into Yasara 6.2.5 software. The results of the generation of the electronic 3D-structures of aprotinin and SBTI are presented in Figure 3. Apparently there there is similarity of 3D-structures of these proteins.

We failed to find molecular targets of SBTI and aprotinin because there was no necessary software in free excess.

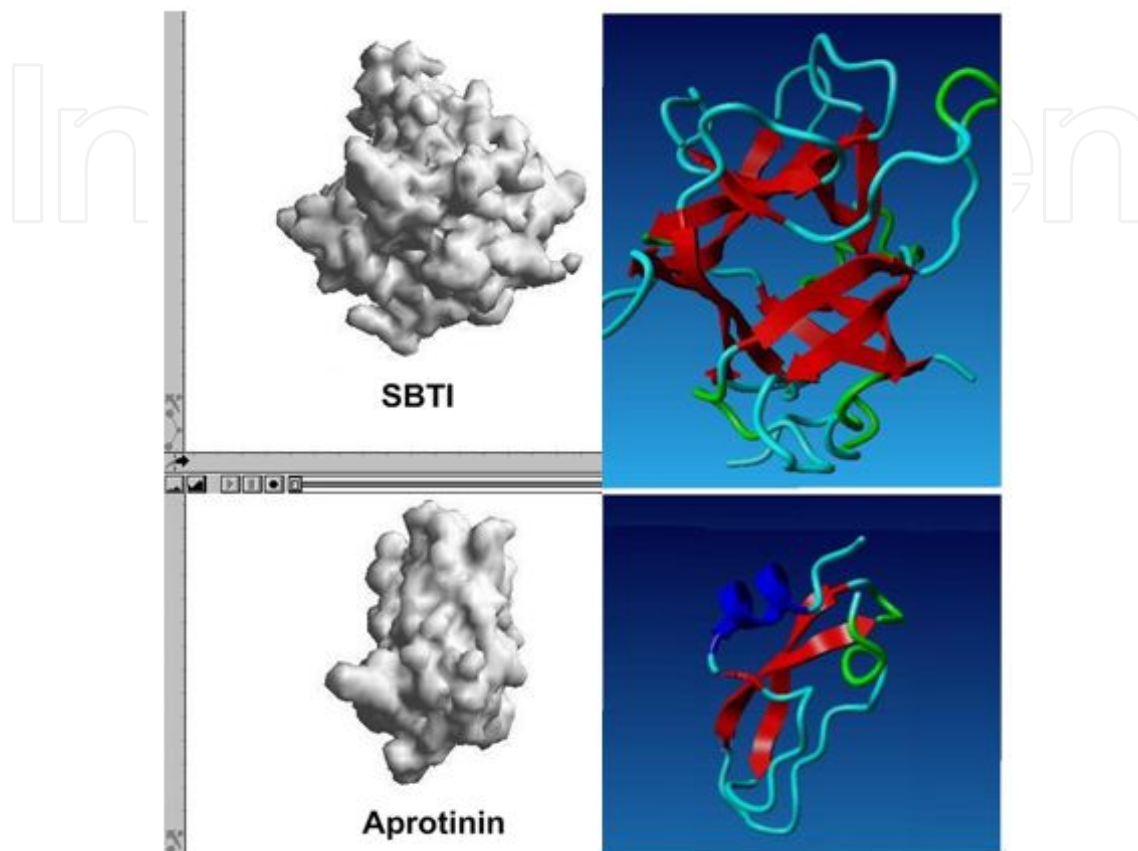


Figure 3. Electronic 3D-structures of SBTI and aprotinin. Left – model with the single surface (Chem Office 5.0). Right-ribbon diagram (Yasara 6.2.5).

2.3. Spectra of biological activity of SBTI and aprotinin

The exploiting the PASS software shows four potential activities of aprotinin and three for SBTI. Both SBTI and aprotinin may inhibit rennin as well as angiotensin- and endothelin-converting enzymes according to the revealing of the possible biological activities of these protease inhibitors by ISIS/Draw 2.4 and PASS Professional chemical structure drawing programs (Figure 4). The possibility of exerting of such effects (drug-likeness) is nearly the same in both protease inhibitors. Aprotinin may inhibit neutral endopeptidase, also. For all the abovementioned activities probability of the activity (P_a) is higher than the probability of the lack of activity (P_i). SBTI and aprotinin should not reveal serious toxicity, mutagenic, carcinogenic and teratogenic effects according to in silico, in vitro and in vivo studies.

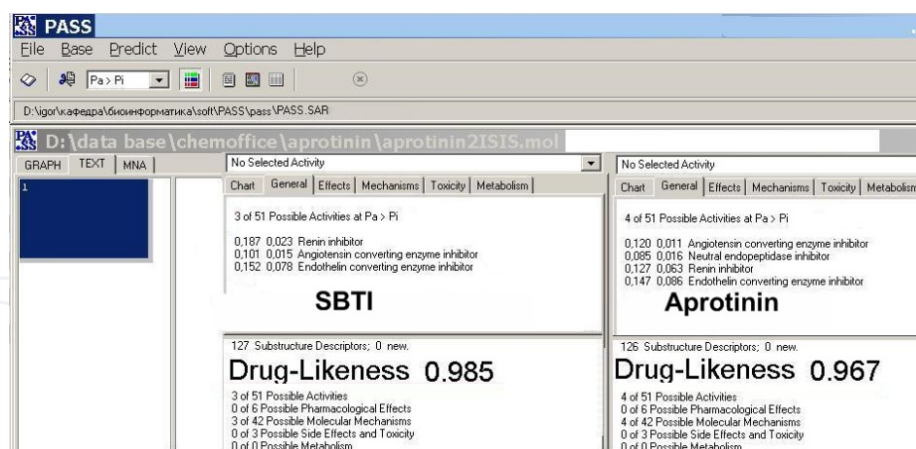


Figure 4. Spectra of the biological activities of aprotinin and SBTI according to PASS software. Drug-Likeness – probability of exerting the effect. Probability of the activity (P_a) and the lack of activity (P_i). The average accuracy of the prognosis makes up more than 85%.

3. The study of aprotinin and SBTI *in vitro*

In silico methods testify the common features in the primary structure, 3D-structures, functional activities of t SBTI and aprotinin and allow to propose that SBTI should influence processes of hemostasis similar to aprotinin. To prove this assumption we studied the influence of trypsin, SBTI and aprotinin on blood clotting, fibrinolysis and platelets aggregation *in vitro*.

3.1. Trypsin-inhibitory activity of SBTI and aprotinin

We measured typsin-inhibitory activity of aprotinin, SBTI and low molecular weight protease inhibitors (ϵ -aminocaproic acid and D-lysine) by their ability to inhibit BAEE-esterase activity of the trypsin [33]. The results are presented in the table 3.

Trypsin inhibitor	Aprotinin	SBTI	ϵ -aminocaproic acid	D-lysine
Trypsin-inhibitory activity (IU/mg)	141 \pm 13	61 \pm 0,9	1,36 \pm 0,04	1,31 \pm 0,02
Trypsin-inhibitory activity (IU/nmole)	0,922 \pm 0,085	1,23 \pm 0,02	-	-

Table 3. Trypsin-inhibitory activity of aprotinin, SBTI, ϵ -aminocaproic acid and D-lysine.

Trypsin-inhibitory activity aprotinin, SBTI, ϵ -aminocaproic acid and D-lysine was measured as their ability to inhibit BAEE-esterase activity of trypsin.

Typsin-inhibitory activity, calculated per mg of inhibitor, decrease down in the order: aprotinin > SBTI > aminocaproic acid > D-lysine. Thus, typsin-inhibitory activity of aprotinin is

2-times higher than similar activity of SBTI. However, taking into account that molecular weight of SBTI (20100) is 3-times higher than molecular weight of aprotinin (6514), it should be concluded that the trypsin-inhibitory activity of SBTI, calculated per mole of the inhibitor, should be nearly 1,5-times higher than trypsin-inhibitory activity of aprotinin. Trypsin-inhibitory activity of ϵ -aminocaproic acid and D-lysine when calculated per mole of the inhibitor is negligibly low because of the low molecular weight.

3.2. The influence of SBTI and aprotinin on coagulation hemostasis

The following indexes, characterizing mainly the first and the second phases of blood clotting, were measured: prothrombin time (reflects activity of coagulation factors VII, V, X and II), activated partial thromboplastin time (reflects activity of coagulation factors VII and VIII), activated clotting time (a measure of the anticoagulation affects of heparin) and thrombin time (time of the formation of fibrin clot). Results are summarized in the table 4.

Indexes	Time,sec			
	Plasma (control) (1)	Plasma + trypsin (2)	Plasma + aprotinin (3)	Plasma + SBTI (4)
Prothrombin time	20±0,9	13±0,9 P ₁₋₂ <0,0001	21±0,9 P ₁₋₃ ^{ns} /0,05	31±0,9 P ₁₋₄ <0,0001
Activated partial thromboplastin time	36±1,7	3±0,9 P ₁₋₂ <0,0001	113±1,8 P ₁₋₃ <0,0001	105±2,7 P ₁₋₄ <0,0001
Thrombin time	16±0,9	2,5±0,5 P ₁₋₂ <0,0001	24±0,9 P ₁₋₃ <0,0001	∞
Activated clotting time	88±9,7	58±4,7 P ₁₋₂ <0,002	157±12 P ₁₋₄ <0,0001	258±17 P ₁₋₄ <0,001
Euglobulin lysis time	400±34,7	182±7 P ₁₋₂ <0,0001	No lysis of blood clot	No lysis of blood clot

Table 4. The influence of trypsin, SBTI and aprotinin on the indexes of hemostasis *in vitro*. The final concentration of trypsin in the incubation medium consisted of 0,01%, SBTI and aprotinin - 0,1%..

Prothrombin time decreases 33-35% in the presence of trypsin from 20±0,9 to 13±0,9 sec, increases insignificantly in the presence of aprotinin and increases 1,5-times up to 31±0,9 sec in the presence of SBTI. Activated partial thromboplastin time sharply decreases by trypsin from 36±1,7 sec in the control samples to 3,0±0,9 sec and increases 3-times by aprotinin (113±1,8 sec) and SBTI (105±2,7 sec). The changes of the activated clotting time under the influence of trypsin, aprotinin and SBTI are similar. Thrombin time decreases nearly 7-fold in the presence of trypsin from 16±0,9 to 2,5±0,5 sec and increases on 50% by aprotinin to 24±0,9 sec. SBTI completely blocked the formation of fibrin clot at least within 10 min. Similar effects trypsin and it's inhibitors exerts on fibrinolysis. Euglobulin lysis time (factor XII-

callicrein-dependent fibrinolysis) decreases 60% by trypsin from 400 ± 35 to 182 ± 7 sec. Aptotinin and SBTI in the abovementioned concentrations entirely inhibit the lysis of fibrin clot (Table 4).

3.3 The influence of SBTI and aprotinin on platelet aggregation

Results of the study of the influence of SBTI and aprotinin on platelets aggregation are presented in the table 5 and Figures 5-8.

	Plasma (control) (1)	Plasma + aprotinin (2)	Plasma + SBTI (3)	Plasma + trypsin (4)
Reversible (ADP)	$22\pm 1,8$	$14\pm 0,9$ $P_{1-2}<0,01$	$15\pm 0,9$ $P_{1-3}<0,01$	$34\pm 0,9$ $P_{1-4}<0,01$
T_{MA}	$55\pm 4,7$	$55\pm 4,7$	$55\pm 4,7$	65 ± 15
Two-phase (ADP):				
First phase				
MA	$48\pm 1,8$	$35\pm 0,9$ $P_{1-2}<0,01$	34 ± 1 $P_{1-3}<0,01$	57 ± 3 $P_{1-4}<0,01$
TMA	$85\pm 4,7$	$68\pm 10,5$	70 ± 9	80 ± 10
Second phase				
MA	70 ± 2	56 ± 2 $P_{1-2}<0,02$	58 ± 1 $P_{1-3}<0,05$	99 ± 1 $P_{1-4}<0,02$
TMA	$350\pm 9,2$	$325\pm 9,2$	$320\pm 9,3$	$310\pm 9,2$
Irreversible (ADP)				
MA	$49\pm 0,9$	$33\pm 0,9$ $P_{1-2}<0,02$	$29\pm 0,9$ $P_{1-3}<0,02$	100 $P_{1-4}<0,001$
T_{MA}	400 ± 18	$410\pm 9,3$	$410\pm 9,3$	$210\pm 26,4$ of
Two-phase (adrenalin):				
First phase				
MA	$23\pm 0,9$	$16\pm 1,8$ $P_{1-2}<0,02$	$16\pm 1,8$ $P_{1-3}<0,02$	$32\pm 0,9$ $P_{1-4}<0,01$
TMA	$110\pm 9,3$	$125\pm 13,1$	$125\pm 13,1$	$130\pm 9,3$
Second phase				
MA	$47\pm 2,4$	44 ± 2 $P_{1-2}"/0,05$	$44\pm 1,8$ $P_{1-3}"/0,05$	$55\pm 0,9$ $P_{1-4}<0,02$
TMA	$390\pm 9,3$	430 ± 18	430 ± 18	$435\pm 16,2$

Table 5. The effects of aprotinin, SBTI and trypsin on the aggregation of platelets initiated by ADP (reversible, two-phase and irreversible aggregation) and adrenalin (two-phase aggregation). MA – maximum aggregation (transmittance, %), T_{MA} – time of accomplishment of maximum aggregation (sec).

The effects of trypsin, SBTI and aprotinin on the aggregation of platelets initiated by ADP (reversible, two-phase and irreversible aggregation) and adrenalin (two-phase aggregation) are similar to their effects on blood coagulation and fibrinolysis. Trypsin increases platelet aggregation (the increase of the maximum of all types of aggregation and decrease of maxi-

imum aggregation time of irreversible ADP-initiated aggregation). SBTI and aprotinin reveals anti-aggregation effect manifested by the decrease of maximum aggregation.

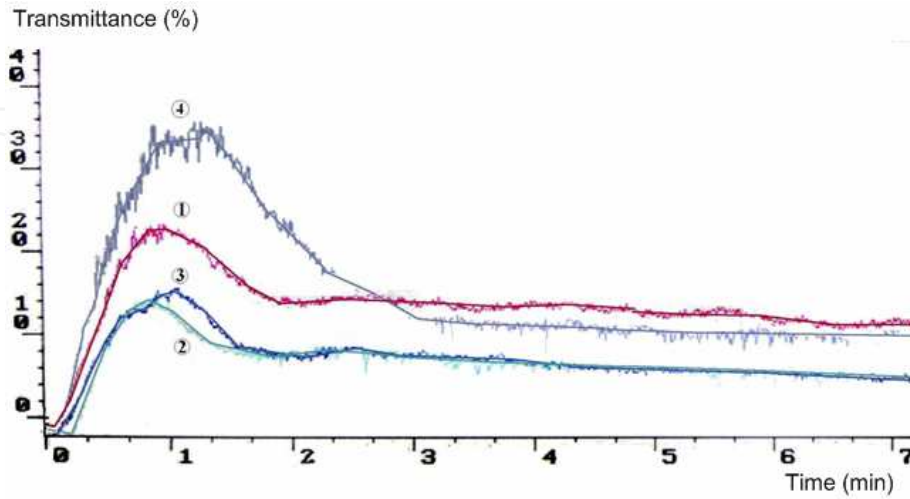


Figure 5. ADP-initiated reversible aggregation of platelets in the presence of aptotinin, SBTI and trypsin in vitro. The platelet aggregation was initiated by the addition of the 5 mkM ADP. 1 – controle; 2 – aprotinin - 1%; 3 – SBTI - 1%; 4 – trypsin - 0,1%.

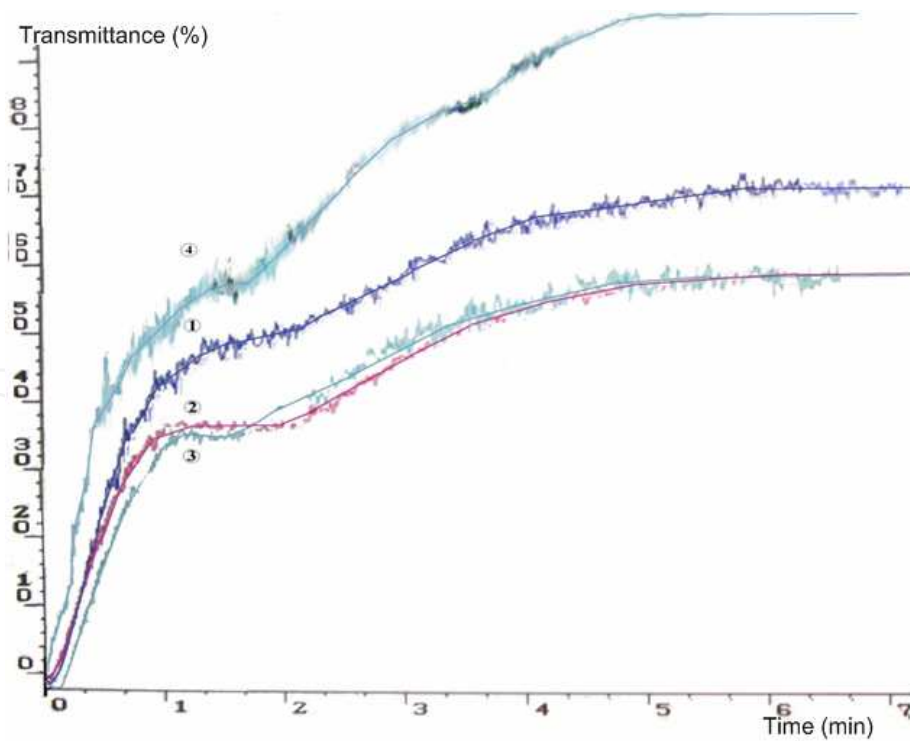


Figure 6. ADP-initiated two-phase aggregation of platelets in the presence of aptotinin, SBTI and trypsin in vitro. The platelet aggregation was initiated by the addition of the 5 mkM ADP. 1 – controle; 2 – aprotinin - 1%; 3 – SBTI - 1%; 4 – trypsin - 0,1%.

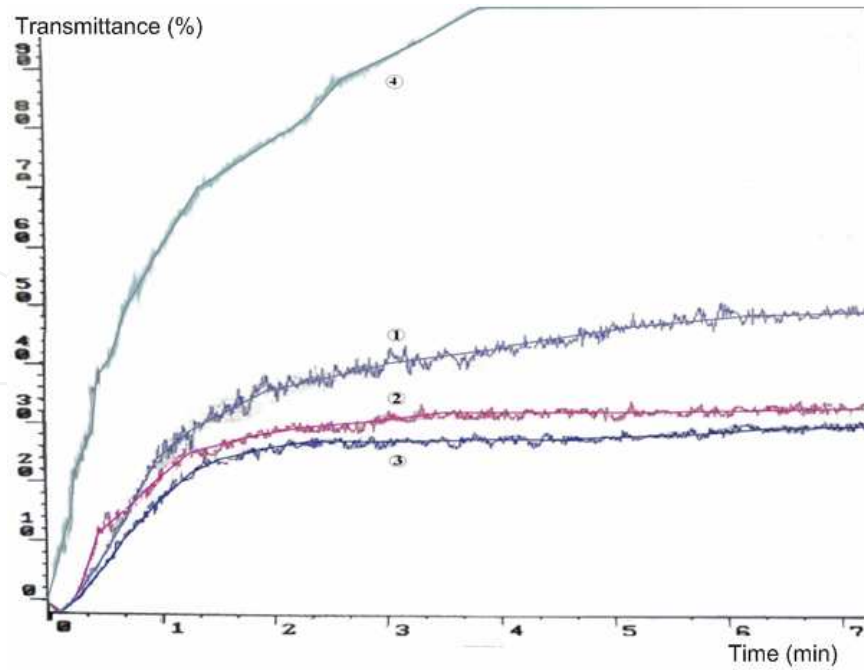


Figure 7. ADP-initiated irreversible aggregation of platelets in the presence of aptotinin, SBTI and trypsin in vitro. The platelet aggregation was initiated by the addition of the 25 mkM ADP. 1 – controle; 2 – aprotinin - 1%; 3 – SBTI - 1%; 4 – trypsin – 0,1%.

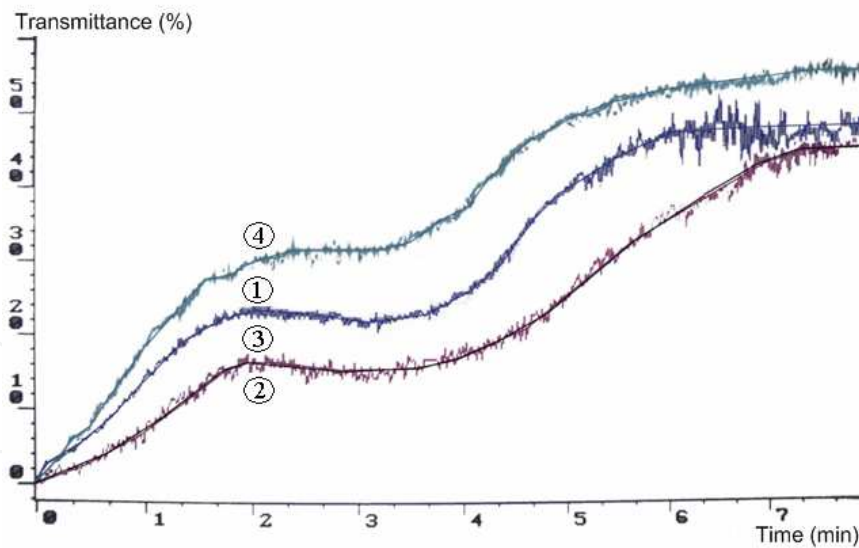


Figure 8. Adrenalin-initiated two-phase aggregation of platelets in the presence of trypsin, aptotinin and SBTI in vitro. The platelet aggregation was initiated by the addition of the 25 mkM adrenalin. 1 – controle; 2 – trypsin - 0,1%; 3 – aprotinin - 1%; 4 – SBTI - 1%.

So, the results of *in vitro* experiments proves the ability of SBTI to influence the hemostatsis predicted by the *in silico* studies.

3.4. The influence of SBTI and aprotinin on the hemolytic activity of the complement

Limited cascade proteolysis underlies the activation of the complement system, which includes nearly 20 individual proteins [30, 37]. Majority of these proteins belongs to the serine protease family (subunits C1r and C1s, C3/C5-convertase of the classical way of activation, factors I and D) [15]. While complement system does not have direct relation to hemostasis and targeted on alien cells and microorganisms, in some conditions complement system may play a role in the disruption of intrinsic cells of the organism and among them blood cells [15, 37]. Because of this we investigated the influence of trypsin, SBTI and aprotinin on the hemolytic activity of the complement assuming that SBTI and aprotinin may retard hemolysis of red blood cells by inhibiting activation of some components of the complement system. Results are presented in the table 6 and Figures 9-10.

	Lag-phase (min)	Total time of hemolysis, including lag-phase (min)
Control (1)	3,5±0,5	8,5±1,5
Aprotinin (1%) (2)	3,2±0,15 P ₁₋₂ "/0,05	7,25±0,25 P ₁₋₂ "/0,05
SBNI (1%) (3)	3,2±0,15 P ₁₋₃ "/0,05	7,25±0,25 P ₁₋₃ "/0,05
Trypsin(0,01%) (4)	4,25±0,25 P ₁₋₄ <0,02	15,5±0,5 P ₁₋₄ <0,02

Table 6. The influence of aprotinin, SBTI and trypsin on the hemolytic activity of complement *in vitro*

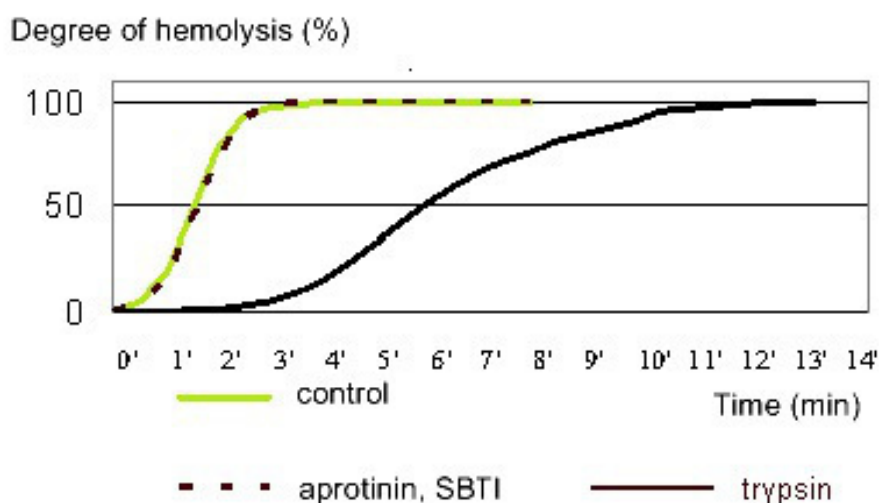


Figure 9. The hemolytic activity of the complement in the presence of aprotinin, SBTI and trypsin *in vitro*. The lag-phase is not shown. 1 – controle; 2 – aprotinin - 1%; 3 – SBTI - 1%; 4 – trypsin – 0,1%.

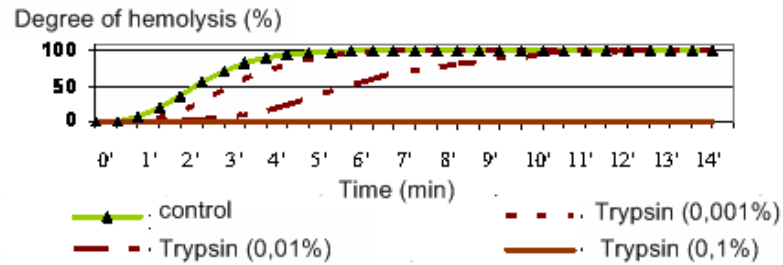


Figure 10. The hemolytic activity of the complement in the presence of different concentrations of trypsin in vitro. The lag-phase is not shown.

SBTI and aprotinin in the final concentrations 0,001-0,1% do not influence hemolysis time and the only statistically insignificant effect consists of small decrease of lag-phase from $3,5\pm 0,3$ to $3,2\pm 0,15$ min ($p>0,05$) and total time of hemolysis from $8,5\pm 1,5$ to $7,25\pm 0,15$ min ($p>0,05$). Trypsin suppresses the hemolytic activity of the complement in the concentration-dependent manner. In the presence of 0,0001% and 0,001% trypsin the lag-phase of the hemolysis increases to $4,25\pm 0,25$ min ($p<0,02$) and total time of hemolysis to $11,5\pm 0,5$ min ($p<0,02$) and $15,5\pm 1,6$ min ($p<0,01$), respectively. 0,01% trypsin entirely blocks the hemolysis. The inhibiting effect of trypsin should be attributed to the disruption of C3 component of the complement system.

Thus, the results obtained show that aprotinin and SBTI in the abovementioned concentrations do not influence the hemolytic activity of the complement.

4. The influence of the consumption of soy protein isolate by healthy persons on the indexes of proteolysis in the blood serum

Soya is cultivated as a foodstuff, having favorable effect on human health, more than 3000 years [28]. Within the last decades soy foods have started to be used in Europe, USA and in Russia as dietotherapy means at a number of diseases [6, 29]. In particular, we have shown the cholesterol-lowering effect of soy protein, resulting from the consumption of SPI enriched cookies by persons with modern hypelipidemia [7, 9]. The consumption of soy foods is followed by the antioxidant effect [Santana et al., 2008, 8, 9] due to the high antioxidant content in these foods [2, 9]. Antioxidant effect is important for the treatment of diseases followed by the development of the oxidative stress [1, 38].

The other possible use of soy foods in dietotherapy may be the correction of disturbances of proteolytic processes. Activation of the tissue proteolysis accompanies different diseases and pathological processes [34]. Soy protein foods contain Bouman-Birk type trypsin inhibitor – SBTI [4]. This protease inhibitor has been officially recognized as a component of foodstuff [25]. There are data showing the possibility of absorption of SBTI in the intestine after the consumption of soy protein foods [22]. So, it seems reasonable to assume that the con-

sumption of soy foods will follow anti-proteolytic effect. Because of this it was interesting to elucidate whether prolonged consumption of the cookies enriched with soy protein isolate (SPI) will influence total proteolytic and trypsin-inhibiting activity of the blood plasma in humans or not?

4.1. Tripsin-inhibiting activity of SPI

According to the characteristics, specified by manufacturer, protein content of SPI consists of 90%. From this 10% belongs to protease inhibitors [32]. Within the processing of soy beans into SPI the former are exposed to high temperatures, which inactivates the major part of soy proteins. However, about 5-20 % of SBTI are represented by the thermostable fraction [19, 22]. The measurement of the trypsin-inhibiting activity of SPI shows that trypsin-inhibiting activity of SPI consists of $1,4 \pm 0,1$ IU/mg of SPI. For example 1 mg of pure SBTI possesses activity equal nearby 60 IU. Thus, the active SBTI makes up 2,7% from SPI mass.

4.2. The influence of the consumption of SPI by healthy persons on the total proteolytic and trypsin inhibiting activity of blood serum

30 adult people aged 35-67 years without the expressed signs of chronic diseases consumed cookies, enriched with SPI (30% protein content), for two months [7]. Fasting blood samples were drawn before and after the dietary treatment. Serum samples were frozen and analyzed for total proteolytic (BAEE-esterase activity) and tripsin-inhibiting activity [33]. Daily intake of 100g of cookies corresponds to consumption of 30g of SPI and about 0,85g of the active SBTI (nearly 52 00 IU). The total consumption of SPI for two months consists of 1,8 kg or 50 g of an active SBTI. Twenty-eight participants (19 females and 9 males) could complete the trial. Results are presented in the table 7.

	Total proteolytic activity (relative units)	Trypsin-inhibitory activity (IU/ml)
Before the consumption	$0,343 \pm 0,010$	$113 \pm 3,6$
After the consumption	$0,282 \pm 0,008$ $p < 0,05$	$137 \pm 5,3$ $p < 0,01$

Table 7. Total proteolytic and trypsin-inhibitory activity of blood serum of healthy persons before and after two months consumption of soy protein isolate.

Total proteolytic activity of blood serum was measures as serum BAEE-esterase activity and trypsin-inhibitory activity by inhibiting by serum of BAEE-esterase activity of trypsin.

Two months long SPI consumption was followed by 18% decrease of total proteolytic activity of blood serum from $0,343 \pm 0,010$ to $0,282 \pm 0,008$ relative units ($p < 0,05$) and by 21% increase of trypsin-inhibiting activity from $113 \pm 3,6$ to $137 \pm 5,3$ IU/ml ($p < 0,01$). The results obtained show possibility of the regulation of proteolysis *in vivo* by the consumption of soy foods.

5. Discussion

The disturbances of the hemostasis accompany many pathological processes and diseases [20, 18]. Both blood clotting and fibrinolysis represents the cascade of proteolytic reactions catalyzed by highly specific proteases [43]. To correct disturbances of the fibrinolysis as well as imbalances of the other proteolytic processes the animal trypsin inhibitor aprotinin is used as a drug (Trasisol, Contrical, Gordox etc) [24, 13, 40]. The effectiveness of the aprotinin in the treatment of some diseases has been questioned recently because of its substantial disadvantages [23, 12]. Following consultation with the German Federal Institute for Drugs and Medical Devices, the U.S. Food and Drug Administration, Health Canada, and other health authorities, the producer of Trasylol - Bayer announced in 2007 that it has elected to temporarily suspend worldwide marketing of Trasylol® (aprotinin injection) until final results from the Canadian BARTtrial can be compiled, received and evaluated. Information regarding the decision has been posted to Bayer's websites [5]. Because of this attempts to develop new protease inhibitor drug are undertaken [35, 27]. One of the candidates for such a role is plant protease inhibitor - SBTI.

In the present study we exploited the advantages of the modern bioinformatics for establishing the extent of structural homology, comparing functional activities and evaluating potential targets of SBTI and aprotinin among the human proteases. The results of *in silico* study testifies apparent homology of SBTI and aprotinin manifested by the common features in the primary structure, 3D-structures, functional activities of these proteins and allow us to propose that SBTI should influence processes of hemostasis similar to aprotinin. The investigation of the influence of SBTI and aprotinin on coagulation and thrombocyte hemostasis by *in vitro* methods prove this assumption and show that both proteins inhibit blood clotting, fibrinolysis and platelet aggregation which is evident from the increase of prothrombin time, activated partial thromboplastin time, activated clotting time, thrombin time and inhibition of fibrinolysis. We investigated the influence of SBTI and aprotinin on the hemolytic activity of the complement assuming that SBTI and aprotinin may retard hemolysis of red blood cells by inhibiting activation of some components of the complement system. However, both inhibitors do not influence hemolysis time.

While the major part of SBTI is disrupted by heating, nearly 20% of inhibitor are thermostable and remains active in soy foods [19]. Part of the consumed SBTI are absorbed in the intestine after the consumption of soy foods [22]. Because of this we elucidated whether prolonged consumption of the cookies, enriched with SPI, will influence total proteolytic and trypsin-inhibiting activity of the blood plasma in humans or not? Daily intake of 100g of cookies corresponds to consumption of about 0,85g of the active SBTI. The total consumption of SBTI for the study period consists of 50 g of an active inhibitor. The consumption of 100 g of cookies daily for two months was followed by 18% decrease of total proteolytic activity of blood serum and by 21% increase of trypsin-inhibiting activity. The results obtained testify possibility of the soft regulation of proteolysis *in vivo* by the consumption of soy foods.

At first, the ability of SBTI to inhibit blood clotting was shown more than half a century ago [26, 41]. However, these results had no any practical consequences and in fact were forgot-

ten for a long time. Our study represent new attempt to revive interest to SBTI as possible protease inhibiting drug.

Author details

Eugene A. Borodin^{1*}, Igor E. Pamirsky², Mikhail A. Shtarberg¹, Vladimir A. Dorovskikh¹, Alexander V. Korotkikh¹, Chie Tarumizu³, Kiyoharu Takamatsu⁴ and Shigeru Yamamoto³

*Address all correspondence to: borodin@amur.ru

1 Amur State Medical Academy, Blagoveshchensk, Russian Federation

2 Institute of Geology and Natural Management of the Far Eastern Branch of Russia Academy of Sciences, Russian Federation

3 Asian Nutrition and Food Culture Research Center, Jumonji University, Saitama, Japan

4 Fuji Oil Co., Ltd Osaka, Japan

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