

Subunit III of ruminant procarboxypeptidase A-S6 complexes and pancreatic proteases E

A new family of pancreatic serine endopeptidases?

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Subunit III (BSIII) of the bovine ternary complex of procarboxypeptidase A-S6 (PCPA-S6), a defective serine endopeptidase-like protein, actively synthesized by the pancreas of some ruminant species, is highly homologous to human protease E (HPE). Both proteins possess the same atypical disulfide bridge in position 98-99b. They are structurally related to porcine elastase 1 and human elastase 2 (about 56% identity). However, in contrast to those two enzymes which have an overall positive net charge, BSIII and HPE are negatively charged. Three-dimensional models of BSIII and HPE have been constructed from the crystallographic structure of porcine pancreatic elastase 1. The inhibitor-binding site for TFAI in these three proteins seems to be very similar; the atypical disulfide bridge does not seem to be involved in this binding site. The specific structural features of BSIII and HPE strongly support the assumption that BSIII is a truncated protease E and that both proteins belong to a separate serine endopeptidase family.

Serine protease; Inactive protease; Protease E; (Pancreas).

1. INTRODUCTION

Several families of zymogens of serine endopeptidases have been identified in pancreatic exocrine secretion. This family distinction is mainly based on the substrate specificity of the various proteases.

Protease E, the activation product of zymogen E, has been identified in human [1], porcine [2] and bovine [3] pancreas. Since this protease

catalyses esterolysis of the typical elastase model substrate, *N*-acetyl tri-L-alanine methyl ester, it has often been identified as an elastase [4]. Yet, in contrast to elastase 1 [5] and 2 [6], protease E has a low *pI* and fails to hydrolyze insoluble elastin [1,4,7].

Porcine protease E is the only member of the family for which the specificity has been delineated [7]. It is complementary to that of chymotrypsin: porcine protease E shows a pronounced specificity for cleaving after the carbonyl group of hydroxylamino acids (serine, threonine), valine and alanine, the latter being most favoured. The human enzyme has been reported to have a similar specificity [1].

Besides the protease E described by Kobayashi et al. [3], bovine pancreas produces large amounts of a non-covalent ternary complex (PCPA-S6) [8,9]. This association is formed of two zymogens, the

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Abbreviations: PCPA-S6, procarboxypeptidase A-S6 ternary complex; BSIII, bovine subunit III; HPE, human protease E; PE1, porcine elastase 1; TFAI, trifluoroacetyl-L-lysyl-L-alanyl *p*-trifluoromethylphenylanilide

Table 1
Sequence alignment of PE1, BSIII and HPE

	16	20		30	36	a	b	c	37	40																													
PE1	V	V	G	G	T	E	A	Q	R	N	S	W	P	S	Q	I	S	L	Q	Y	R	S	G	S	S	W	A	H	T	C	G	G							
BSIII	.	.	N	-	E	D	-	V	P	Y	-	-	S	W	-	V	-	-	-	-	E	K	D	G	A	F	H	-	-	-	-	-							
HPE	V	V	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	S	-	S	-	Y	-	-	-	-								
																						*				*													
		50		60	65	66		70																															
PE1	T	L	I	R	Q	N	W	V	M	T	A	A	H	C	V	D	R	E	L	T	F	R	V	V	V	G	E	H	N	L	N	Q							
BSIII	S	-	-	A	P	D	-	-	V	-	-	G	-	-	I	S	T	S	R	-	Y	Q	-	-	L	-	-	Y	D	R	S	V							
HPE	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	S	-	W	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-						
		80		86	a	b	87	90		98	99b	100																											
PE1	N	N	G	T	E	Q	Y	V	G	V	Q	.	.	K	I	V	V	H	P	Y	W	N		T		D		D		V	A		A	G	Y	D	I		
BSIII	L	E	-	S	-	-	V	I	P	I	N	A	G	D	L	F	-	-	-	L	-	-		S		N	C	-	-		C	-	N	-	-				
HPE	K	-	-	P	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-		R		S	-	-	-	-	-	-	-	-	-			
	*																																						
		110		120				130																															
PE1	A	L	L	R	L	A	Q	S	V	T	L	N	S	Y	V	Q	L	G	V	L	P	R	A	G	T	I	L	A	N	N	S	P							
BSIII	-	-	V	K	-	S	R	-	A	Q	-	G	D	K	-	-	A	N	-	-	P	-	-	D	-	-	P	-	E	A	-								
HPE	-	-	I	-	-	-	-	-	-	-	-	-	-	A	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-						
		140		145	147	150		160																															
PE1	C	Y	I	T	G	W	G	L	T	R	T	N	G	O	L	A	Q	T	L	Q	Q	A	Y	L	P	T	V	D	Y	A	I	C							
BSIII	-	-	-	S	-	-	-	R	L	Y	-	G	-	P	-	P	D	K	-	-	E	-	L	-	-	V	-	-	-	E	H	-							
HPE	-	-	-	T	-	-	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
							*																																
		170	a	b	171			180		188	a	190																											
PE1	S	S	S	S	Y		W		G	S		T		V	K	N	S	M	V	C	A	G	G	N	G	V	R	S		G	C	Q	G	D	S		G	G	
BSIII	-	Q	W	D	W	-	-	I	-	-	-	I	-	-	K	T	-	-	-	-	-	-	-	D	.	T	-	-	-	N	-	-	-	-	-	-	-	-	
HPE	-	R	-	N	-	-	-	S		S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	I	-	-	-	-	-	-	-	-	-	-	-	-	-
	*	*																																					
		203	a		210			217	218	220	222																												
PE1	P	L	H	C	L	V	.	N	G	Q	Y	A	V	H	G	V		T	S	F	V	S	R		L	G	C	N	V	T	R	K	P	T					
BSIII	-	-	N	-	P	A	A	D	-	S	W	Q	-	-	-	-	-	-	-	-	-	-	-	A		F	-	-	T	I	K	-	-	-					
HPE	-	-	-	-	T	E	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R	R	-	-	-	-	-			
						*																																	
		230		240		245																																	
PE1	V	F	T	R	V	S	A	Y	I	S	W	I	N	N	V	I	A	S	N																				
BSIII	-	-	-	-	-	-	F	-	D	-	-	-	-	E	T	-	-	-	-																				
HPE	-	-	-	-	-	-	-	-	-	-	-	-	E	-	-	-	-	H																					
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(-) Identical residues between (i) PE1 and BSIII, (ii) BSIII and HPE; (.) missing residue (deletion); (| |) residues of the active site, within 5 Å from the inhibitor TFAI; (m) when residues are different, interaction involves main chain; (s) when residues differ, interaction involves side chain; (*) charge modification between BSIII and HPE

procarboxypeptidase A itself and a chymotrypsinogen of the C type, and a defective serine endopeptidase-like protein called subunit III [10–12]. This type of complex has only been found in some ruminant species [13]. Subunit III possesses a weakly functional active site unable to hydrolyze the specific substrates of the pancreatic serine endopeptidases [12,14]. All invariant residues essential to the activity of pancreatic serine endopeptidases are present in subunit III, except for the two strongly hydrophobic N-terminal residues characteristic of this class of enzymes [11]. As supported by three-dimensional modelling [12], the absence of these residues prevents the formation of the salt bridge (16–194) (chymotrypsinogen A numbering system) known to stabilize the active conformation of serine proteases. The very low catalytic efficiency of the protein would result from a defective binding site [12]. Another salient structural feature of BSIII is the presence of an atypical disulfide bridge in position 98–99b.

Sequence comparison clearly shows that BSIII is structurally close to an elastase (56% identity with PE1 and human elastase 2).

The recent elucidation of the human protease E sequence [15] allows one to observe a very high homology between BSIII and HPE. Here, we discuss the consequences of this finding and report more precise investigations of the active site of both proteins by computer modelling.

2. EXPERIMENTAL

2.1. Protein modelling

The models of BSIII and HPE have been derived from the crystallographic structure of porcine pancreatic elastase 1 [16] after the respective sequences were aligned (table 1). Construction of the three-dimensional molecular models was according to [12] through computer graphics techniques: sequence editing, modifications, insertions or deletions of amino acids and regularization of the structure were performed with the FRODO program [17] on a Silicon Graphics IRIS 3020 color display workstation. The resulting models have been refined through energy-minimization methods with the program EREF [18] on a DEC Microvax II.

2.2. Charge analysis

A set of programs which perform calculations of the centers of mass of negatively and positively charged residues and calculate their distance has been written. These programs have been interfaced with FRODO.

3. RESULTS

3.1. Sequence comparison

Venot et al. [11] reported a detailed comparison of the sequences of BSIII and PE1 and Shen et al. [15] carefully studied the sequence homologies between HPE and rat and porcine elastases 1 and 2. Both proteins were shown to contain all the residues which contribute to catalysis as well as the four disulfide bridges characteristic of rat [19] and porcine [20] elastase 1. However, some discrepancies were noted and the sequence identity between BSIII or HPE and the above-mentioned elastases was no more than 56–57% (table 1). As reported by Venot et al. [11], BSIII possesses an additional atypical disulfide bridge (98–99b) which has been found in no other pancreatic serine endopeptidases. The presence of two cysteine residues at the same position in the mRNA-based sequence of HPE [15] is in strong support of the same disulfide bridge existing in this protease. Moreover, comparison of the sequences of HPE and BSIII reveals a striking homology between those two proteins, since out of 242 residues only 34 are different (including the two missing N-terminal residues in BSIII), leading to a score of 86% identity.

3.2. Three-dimensional modelling and active-site mapping

The modelling of BSIII [12] and HPE to PE1 indicates a close conformational fit between the tertiary structures of the three proteins, as expected from their high degree of homology. A more precise investigation of the topology of the binding site of BSIII and HPE has also been performed by inserting an elastase inhibitor (TFAI) into the models.

The coordinates for the crystallographic structure of the PE1-TFAI inhibitor complex [21] were obtained from the Protein Data Bank. Subsequently, the coordinates of TFAI were inserted into the models of BSIII and HPE. For the three proteins, 17 residues were found at less than 5 Å from any atom of the TFAI, defining the inhibitor-binding site. This could be done without further remodelling, since TFAI–protein distances were kept within a normal Van der Waals contact range. Inspection of table 1 shows that 3 residues of this site differ between PE1 and BSIII, 2 between BSIII and HPE and 4 between PE1 and HPE. Never-

Table 2

Charge analysis for porcine elastase 1, subunit III and human protease E

	PE1	BSIII	HPE
K + R (+1)	15	14	15
H (+0.5)	6	7	6
Sum +	18	17.5	18
D + E (-1)	11	25	24
Net charge	+7	-7.5	-6
$d(\text{CM}^+ - \text{CM}^-)$ (Å)	3.7	5.0	7.5

$d(\text{CM}^+ - \text{CM}^-)$, distance between centers of mass of positive and negative charges

theless, these changes either imply the main chain of the residue or are quite conservative (S-T or Q-N). Therefore, the inhibitor-binding site of these 3 proteins should be equivalent.

3.3. Charge analysis

HPE as well as BSIII exhibits a negative net charge (table 2). In contrast, PE1 and human elastase 2 have an overall positive charge. Out of the 34 residues substituted from BSIII to HPE, 13 correspond to a charge alteration leading to a slightly different localization of charges. This is also evident when considering the distances between the centers of mass of respectively positive and negative charges (table 2).

4. DISCUSSION

The striking structural similarities between BSIII and HPE clearly indicate that the two proteins are very closely related and strongly support the assumption that subunit III is a truncated protease E.

The lack of activity of BSIII has already been attributed to the absence of residues 16 and 17 which, by preventing the formation of the ion pair (N-terminal)-(Asp-194), should lead to an altered active site. The existence of an active protein very close to BSIII but bearing residues 16 and 17 confirms our hypothesis. Moreover, the presence of the atypical disulfide bridge cannot be responsible for the inactivity of BSIII, as previously suggested [12], since it is expected to exist in active protease E.

The identification of BSIII as a protease E is fur-

ther supported by reassociation experiments showing that BSIII and porcine zymogen E bind to the same (or part of the same) site on bovine and porcine procarboxypeptidases A [13]. The binding between BSIII and bovine procarboxypeptidase A mainly involves electrostatic interactions [22]. Nevertheless, due to the large number of charged residues substitutions between BSIII and HPE, it does not seem that the charge recognition pattern is very specific.

The classification of pancreatic serine endopeptidases is not easy, as illustrated by the case of elastases. A first distinction should be made between true elastases able to digest insoluble elastin (including 2 families [23]: a pancreatic elastase family represented by porcine and rat elastase 1 and a chymotrypsin-like elastolytic protease family represented by porcine and human elastase 2) and non-elastolytic proteases resembling elastase which hydrolyse only elastase synthetic substrates.

Although hydrolysing the synthetic substrate of elastase and bearing some of its structural characteristics, protease E is a non-elastolytic enzyme. The specific structural features of BSIII and HPE (including the S-S bridge pattern) strongly support the assumption that both proteins belong to a separate pancreatic serine endopeptidase family, the protease E family.

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