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Structural relationship between lipases and peptidases of the prolyl oligopeptidase family

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In prolyl oligopeptidase and its homologues, which constitute a new serine protease family, the order of the catalytic Ser and His residues in the amino acid sequence is the reverse of what is found in the trypsin and subtilisin families. The exact position of the third member of the catalytic triad, an Asp residue, has not yet been identified in the new family. Recent determination of the three-dimensional structures of pancreatic and microbial lipases has shown that the order of their catalytic residues is Ser, Asp, His, and this fits the order Ser, His of prolyl oligopeptidase. However, there is no sequence homology between lipases and peptidases, except for a 10-residue segment, which encompasses the essential Ser, and for the immediate vicinity of the catalytic Asp and His residues. This comparison identifies the catalytic Asp residue in the prolyl oligopeptidase family. The relative positions of the three catalytic residues in peptidases and microbial lipases were the same and this indicated structural and possibly evolutionary relationship between the two families.

Serine protease family; Prolyl oligopeptidase; Lipase structure; Evolutionary relationship

1. INTRODUCTION

The amino acid sequence homology among pancreatic, hepatic and lipoprotein lipases clearly shows that all three lipases are members of a common protein family [1,2]. A short, about 10-residue, homologous segment of these enzymes, which contains the functional serine residue, is also encountered in lingual/gastric lipases [3,4], in prokaryotic lipases [2,5–7], and in lecithin-cholesterol acyltransferase [2,7,8]. Apart from the short region, the lingual/gastric lipases, the prokaryotic lipases and the lecithin-cholesterol acyltransferase do not show any significant sequence homology to the pancreatic, hepatic and lipoprotein lipase family.

Recent X-ray crystallographic studies have revealed the three-dimensional structures of human pancreatic lipase [9] and a triacylglycerol lipase from *Rhizomucor miehei* [10]. These studies have shown that both enzymes possess a triad of Ser...His...Asp, which is analogous to the catalytic residues of serine proteases [11,12]. No other structural relationship to the known serine proteases was found, however.

We have recently reported that some peptidases, including prolyl oligopeptidase, dipeptidyl peptidase IV and acylaminoacyl peptidase, constitute a new family of serine proteases that is not related to the well-known trypsin and subtilisin families [13]. Specifically, the order of the triad residues is different in the three protease families: $His^{57}...Asp^{102}...Ser^{195}$ in chymotrypsin, $Asp^{32}...His^{64}$ ...Ser^{221} in subtilisin, and Ser^{554}, His^{680} in prolyl oligopeptidase where the position of the Asp residue has not yet been established. The positions of the catalytically competent Ser and His residues in the members of the new family were elucidated not only by sequence alignment but also by chemical modifications [14–17]. It is important that the order of Ser and His in the oligopeptidase family is the reverse of that found in the trypsin and subtilisin families but identical with that reported for lipases: $Ser^{152}...Asp^{176}...His^{263}$ and $Ser^{144}...Asp^{203}...His^{257}$ for the pancreatic [9] and microbial [10] enzymes, respectively. This finding prompted us to examine whether or not a relationship between the lipase and prolyl oligopeptidase familes can be detected.

The basic features of the three-dimensional structures of the pancreatic and the *Rhizomucor miehei* lipase are similar and characterized by a central 8–9 stranded β-sheet associated with several α-helical segments [9,10]. However, computer search failed to show amino acid sequence homology between the mammalian and microbial lipases, apart from the short segment containing the essential Ser residue [2,7]. We could not detect sequence homology between lipases and peptidases either, except for the short Ser segments. However, a distant relationship between lipases and the members of the oligopeptidase family may be established by examination of the similarities between the short segments com-

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prising the three catalytic residues and by comparison of the topologies of the triads.

2. RESULTS AND DISCUSSION

2.1. *Comparison of lipases*

Fig. 1 illustrates the short segments containing the three catalytic residues. The enzymes are arranged in 5 groups: (i) the pancreatic/hepatic/lipoprotein lipase group; (ii) the lingual/gastric lipase group; (iii) the microbial lipases; (iv) the acyltransferase; and (v) the peptidases. The relationship among the different lipase groups is restricted to the Ser segments. It is apparent that the similarity extends over the most conservative hexapeptide containing the essential Ser. At position 4 there is a fairly conserved Val which is replaced by closely related Ile or Leu in a few cases. Position 6 is also occupied by residues with hydrophobic side chains, whereas a basic residue is often encountered at position 3, as well as at position 2. As for the Asp and His segments, homology may not be observed between the different groups, except for the microbial enzymes and the lecithin-cholesterol acyltransferase. The absence of significant homology between groups 1 and 3 is not due to misalignment because the members of their catalytic triad were identified by X-ray crystallography. In the Asp segment of group 2, however, the catalytic residue could not be identified unequivocally, and the second Asp (ADP) may be an alternative candidate.

The third group of Fig. 1 includes the microbial lipases. For the first enzyme (3a) the catalytic residues are known from X-ray diffraction measurements [10]. Homologous segments with the catalytic residues could also be observed in staphylococcal proteases (3b, 3c). However, we were not able to find the Asp and His region in the amino acid sequence of the lipase from *Pseudomonas fragi* (3d). This is consistent with the special structural feature of the enzyme, which is thought to originate from a preprotein consisting of only 135 amino acids, and has the essential serine at position 83 [18]. Since the shortest distance between Ser and His contains more than 100 residues in all lipases (see below), we conclude that, in contrast to the notion that this enzyme is the shortest lipase, the published amino acid sequence is incomplete, lacking the C-terminal region.

Segments of the catalytic residues

	Ser	Asp	His
Mammalian lipases I	*	*	*
1a Hum.pan.+	PSNVHVIGHSLGAHAAG	LDPAEPCFQ	ACNHLRS
1b Por.pan.	PSNVHVIGHSLGSHAAG	LDPAEPCFQ	ACNHLRS
1c Rat hep.	RSKVHLIGYSLGAHVSG	LDPAGPMFE	KCAHERS
1d Hum.lip.	LDNVHLLGYSLGAHAAG	LDPAGPNFE	KCSHERS
Mammalian lipases II			
2a Rat lin.	QEKIHYVGHSQGTTIGF	NDILADPQD	AKNHLDF
2b Hum.gas.	QKQLHYVGHSQGTTIGF	NDLLADPQD	FYNHLDF
Microbial lipases			
3a Rhiz.mi.+	SYKVAVTGHSLGGATAA	RDI-VPHLP	VLDHISY
3b Stap.au.	GKKVHLVGHSMGGQTIR	NDGVVPVIS	GWDHVDF
3c Stap.hy.	PGPVHFIGHSMGGQTIR	NDGLVSQIS	GWDHSDF
3d Pseu.fr.	AQRVNLIGHSQGALTAR		
Acyltransferase			
4a Hum.lec.	GKPVFLIGHSLGGLHLL	EDH-VFIST	IYDHGFP
Peptidases			
5a Hum.pro.	PKRLTINGGSNGGLLVA	DDRVVPLHS	KAGHGAG
5b F.m.pro.	KEYMALSGRSNGGLLVG	DDRVVPAHS	NAGHGAG
5c E.col.p.	PSLCYAMGGSAGGMLMG	-DSQVQYWE	DSGHGGK
5d Rat acy.	ARRVALMGGSHGGFLSC	EDRRVPFKQ	KSNHALS
5e Pig acy.	AGRVALMGGSHGGFLSC	EDRRVPFKQ	KSTHALS
5f Rat dip.	SKRVAIVGWSYGGYVTS	DDN-VHFQQ	DEDHGIA
5g Hum.dip.	NKRIAIVGWSYGGYVTS	DDN-VHFQQ	DEDHGIA
5h Yea.dip.	PQKISLFGWSYGGYLTL	DDN-VHFQN	DSDHSIR
	ss s i i is	i i	i

Fig. 1. Alignment of amino acids around the three catalytically important residues. * = member of the catalytic triad; + = the triad was identified by X-ray crystallography; i = identical residues in groups 3 and 5; s = similar residues (G,A,S,T; K,R; V,L,I; Y,F; D,E,N,Q) in groups 3 and 5. The most conservative region in Ser segments is underlined. Hum.pan., human pancreatic lipase; Por.pan, porcine pancreatic lipase; Rat hep., rat hepatic lipase; Hum.lip., human lipoprotein lipase; Rat lin., rat lingual lipase; Hum.gas., human gastric lipase; Rhiz.mi., *Rhizobium miehei* lipase; Stap.au., *Staphylococcus aureus* lipase; Stap.hy., *Staphylococcus hyicus* lipase; Pseu.fr., *Pseudomonas fragi* lipase; Hum.lec., human lecithin-cholesterol acyltransferase; Hum.pro., human prolyl oligopeptidase; Pig pro., pig prolyl oligopeptidase; F.m.pro., *F. meningosepticum* prolyl oligopeptidase; E.col.p., *E. coli* protease II; Pig acy., pig acylaminoacyl-peptidase; Rat acy., rat acylaminoacyl-peptidase; Rat dip., rat dipeptidyl-peptidase IV; Hum.dip., human dipeptidyl-peptidase IV; Yea.dip., yeast dipeptidyl-peptidase IV. Alignment of the sequences of members of prolyl oligopeptidase family (group 5) is from [19].

2.2. *Comparison of lipases and peptidases*

As for the 17-residue Ser segment, it is seen from Fig. 2 that the proteases are more related to the microbial lipases (residue identity: 5–7) than to the pancreatic lipase (identity: 3–4). It is important that the homology between peptidases and microbial lipases is only slightly different from that among the individual peptidases (identity: 6–7, 9 only for the two dipeptidyl-peptidases).

Fig. 2 clearly shows that homologous sequences between lipases and peptidases are also encountered with respect to the aspartic region. The closest similarity is found with the staphylococcal lipase and prolyl oligopeptidase, having 5 identical residues in the 9-residue segment (3b and 5a,b of Fig. 1). The consensus sequence for the three enzymes is XDXVVPXXS. The valine residue at position 5 is also conserved in groups 3, 4 and 5. It is interesting that the aspartic region of the microbial lipase is more related to that of prolyl oligopeptidase than of the pancreatic lipase (residue identity: 2). Moreover, this region is less conserved in the homologous peptidases, as well as in the microbial lipases.

As for the His region (Fig. 2), there is no homology between any two groups. Even within one group, only the same enzymes of different species, for example 5f, 5g and 5 h (Fig. 1), show an obvious relationship. Thus

Ser segment

	1a	3a	3b	4a	5a	5d	5f	5h
1a Hum.pan.	17							
3a Rhiz.mi.	8	17						
3b Stap.au.	7	8	17					
4a Hum.lec.	7	6	7	17				
5a Hum.pro.	4	5	5	5	17			
5d Rat acy.	4	6	6	5	7	17		
5f Rat dip.	4	7	6	5	7	7	17	
5h Yea.dip.	3	5	6	5	6	6	9	17

Asp segment

	1a	3a	3b	4a	5a	5d	5f	5h
1a Hum.pan.	9							
3a Rhiz.mi.	2	9						
3b Stap.au.	2	3	9					
4a Hum.lec.	1	2	2	9				
5a Hum.pro.	2	3	5	2	9			
5d Rat acy.	3	3	3	3	4	9		
5f Rat dip.	2	2	2	2	2	4	9	
5h Yea.dip.	1	2	2	2	2	3	7	9

His segment

	1a	3a	3b	4a	5a	5d	5f	5h
1a Hum.pan.	7							
3a Rhiz.mi.	2	7						
3b Stap.au.	2	1	7					
4a Hum.lec.	1	2	2	7				
5a Hum.pro.	1	1	1	2	7			
5d Rat acy.	3	1	1	1	2	7		
5f Rat dip.	1	2	2	3	2	1	7	
5h Yea.dip.	1	2	2	2	1	2	4	7

Fig. 2. Pair-wise comparisons of the segments containing the catalytic residues. The numbers of identical residues are shown. For abbreviations of the enzymes see Fig. 1. For a better overview, the identity numbers for comparisons between proteases and lipases including the acyltransferase are emphasized by rectangles.

a significant homology between the His segments of lipases and peptidases may not be expected, and this is consistent with the finding that the triad residues are located in loop regions [20] (see below) where mutations are rather frequent. However, a significant homology for certain enzymes of different groups can be observed in the immediate neighbourhood of the catalytic His. Thus, yeast dipeptidyl peptidase IV (5h) and the staphylococcal lipase (3c) have a common triad Asp-His-Ser, whereas Asp-His-Gly is common to the mammalian dipeptidyl peptidases (5f, 5g) and lecithin-cholesterol acyltransferase (4a). Accordingly, the loop regions of enzymes of different groups may be more conserved than the corresponding segments of the enzymes encountered in the same group.

2.3. *The topology of the catalytic residues*

The three-dimensional structures of the mammalian and microbial lipases [9,10] show that the catalytic Ser and His residues occupy similar steric positions on the loops following strand 5 and strand 8, respectively. However, the Asp residue follows strand 6 in the mammalian enzyme but strand 7 in the microbial enzyme

	S-D		D-H		S-H	
1a Hum.pan.	S152	24	D176	87	H263	111
1c Rat hep.	S147	26	D173	84	H257	110
1d Hum.lip.	S132	24	D156	85	H241	109
2a Rat lin.	S153	131	D284	29	H313	160
3a Rhiz.mi.	S144	59	D203	54	H257	113
3b Stap.au.	S412	192	D604	41	H645	233
3c Stap.hy.	S369	190	D559	41	H600	231
4a Hum.lec.	S181	81	D262	67	H329	148
5a Hum.pro.	S554	88	D642	38	H680	126
5d Rat acy.	S587	88	D675	32	H707	120
5f Rat dip.	S631	79	D710	31	H741	110
5h Yea.dip.	S678	78	D756	32	H788	110

Fig. 3. Residues of the catalytic triad and the numbers of residues between them. For abbreviations of the enzymes see Fig. 1. S–D, D–H and S–H show the numbers of residues between the catalytic Ser and Asp, Asp and His, and Ser and His, respectively.

[20]. It is seen from Fig. 3 that the pancreatic lipase and its homologues in the first block have the shortest distance between the Ser and Asp residues, and the longest distance between the Asp and His residues, in agreement with the position of Asp being in the loop following strand 6. It is probable that in the other enzyme groups, in particular in microbial lipases and in peptidases where the distance between Asp and His is shorter (Fig. 3), Asp follows strand 7. The catalytic Asp in the prolyl oligopeptidase family has not been identified so far. In the alignment of the amino acid sequences [13] two Asp residues are conserved: Asp^{642} (prolyl oligopeptidase numbering) shown in Fig. 1 is located between Ser and His. The other conserved residue, Asp^{529} precedes the catalytic Ser^{554} in the amino acid sequence. Since the hydrophobicity of the environment of Asp^{642} varies greatly between family members relative to that of Asp^{529}, the latter was preferred as the third member of the catalytic triad [19]. The homology with the microbial lipases (Fig. 1) and the similar topology, however, indicate that Asp^{642} should serve as the catalytic residue.

It is an interesting structural feature of lipases that their catalytic triad is covered by a surface loop or flap [9,10], and the repositioning of this flap is necessary to render the catalytic site accessible to substrate. A similar flap may be conserved in the peptidases of the prolyl oligopeptidase family since we pointed out that the rate-limiting step in the catalysis of prolyl oligopeptidase should be a physical rather than chemical step, presumably a conformational change [21,22].

The existence of enzymes more distantly related to the five families discussed above is apparent from the recently reported three-dimensional structures of a lipase from the fungus *Geotrichum candidum* [23] and acetylcholinesterase [24]. Both enzymes have a central β-sheet characteristic of lipases and a catalytic triad with the acid after strand 7. However, the catalytic acid is Glu in these two enzymes rather than Asp.

A somewhat different β-sheet structure has also been found with wheat carboxypeptidase II [25], which is homologous to yeast carboxypeptidase Y and the barley carboxypeptidases I and II [26]. In these peptidases there is a crossing-over in the first part of the molecule so that strand 5 precedes strand 4 in the amino acid sequence. Nonetheless, the topology of the catalytic triad has not changed, the order of the residues is Ser^{146}, Asp^{338} and His^{397}. The conservative Gly in the Ser segments of all 5 groups of Fig. 1 (GXSXG) changed to Ala (GXSXA) in the carboxypeptidase group. However, the relationship is clearly seen in the Asp segment (DAVVPL), in particular when compared to that of the staphylococcal lipase (3b of Fig. 1, DGVVPV) and of prolyl oligopeptidase (5a of Fig. 1, DRVVPL). The homology of the His segment is as low as in the other cases. Hence the carboxypeptidases may constitute a subfamily of the prolyl oligopeptidase family.

It can be concluded that a central β-sheet structure serves as an ideal scaffold for holding the catalytic triad. However, the different lipases, proteases, cholinesterases, acyltransferases and carboxypeptidases have not necessarily diverged from an ancestral structure. It is possible that some of the different groups have arisen by convergent evolution as is known for the trypsin and subtilisin families [11,12]. Nonetheless, it cannot be excluded that the proteases of the prolyl oligopeptidase family and the microbial lipases are evolutionarily related. These two groups appear to display the least distant structural relationship, which is supported by: (i) the sequence homology of the catalytic segments, (ii) the similar topology of the triad, and (iii) the not entirely opened active site. It is interesting that the lecithin-cholesterol acyltransferase is more similar to the previous two groups than are the lingual/gastric lipases, having the same topology but sequentially unrelated Asp and His segments. The homologous of pancreatic lipase exhibit the most distant relationship as their catalytic Asp residue follows strand 6 rather than strand 7.

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