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Molecular evolution of the pancreatic lipase and two related enzymes towards different substrate selectivities

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Abstract

The pancreatic lipase gene family includes lipoprotein lipases, hepatic lipases, pancreatic lipases, phospholipases A1 from vespid venoms, and non-enzymatic proteins from *Drosophila*. Among the previous enzymes, the classical human pancreatic lipase (HPL), the guinea pig pancreatic lipase-related protein 2 (GPLRP2) and the phospholipase A1 from hornet venom (DolmI) illustrate three steps in the molecular evolution of the pancreatic lipase gene family towards different substrate selectivities for triglycerides and phospholipids. Based on the known 3D structure of HPL, sequence alignments and kinetic properties, we compared these three enzymes for a better understanding of their structure–function relationships. Three surface loops surrounding the active site ($\beta 5$ loop, $\beta 9$ loop, lid domain) are believed to play an important role in substrate selectivity.

Keywords: Lipase; Phospholipase; Pancreatic lipase gene family; Substrate selectivity

1. Introduction: The pancreatic lipase gene family

The pancreatic lipase was still recently considered to be well characterized with respect to structural and kinetic properties, and to be distinct among the lipase gene family which also includes lipoprotein lipase, hepatic lipase and *Drosophila* yolk proteins [1]. Several new members of the pancreatic lipase family have now been cloned, sequenced and partly characterized [2–6]. Primary structure analysis of these new lipases revealed that the pancreatic lipase family can be divided into three subgroups (Fig. 1): (i) classical pancreatic lipases, (ii) pancreatic lipase-related proteins 1 (PLRP1) and (iii) pancreatic lipase-related proteins 2 (PLRP2). This classification is supported by the biochemical characterization of these novel proteins. Among the RP1 subfamily, HPLRP1 and RPLRP1 have been expressed in vitro but no lipolytic activity was found up to now [3–7]. Within the PLRP2 subfamily, the main kinetic properties of three lipases found in the guinea pig (GPLRP2, [4]), the coypu (CoPLRP2, [6]) and the rat (RPLRP2, also named GP3, [7]) have been studied. These enzymes share an atypical enzymatic behavior when compared to the classical pancreatic lipases, and they challenge the classical distinction between lipases, esterases and phospholipases.

From sequence comparison, it appears that several structural elements, conserved in all

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classical pancreatic lipases, are poorly conserved within the RP2 pancreatic lipase subfamily [8]. The major mutations are found in GPLRP2 which can be considered as the best example of divergent evolution of pancreatic lipases towards phospholipase activity whereas the classical pancreatic lipases only hydrolyze triglycerides. Another step in the molecular evolution of the pancreatic lipase gene family has been reached with the phospholipases A1 from vespid venoms. These enzymes are about 40% homologous to the N-terminal catalytic domain of pancreatic lipases [9,10] but they are missing the entire C-terminal domain required for colipase binding.

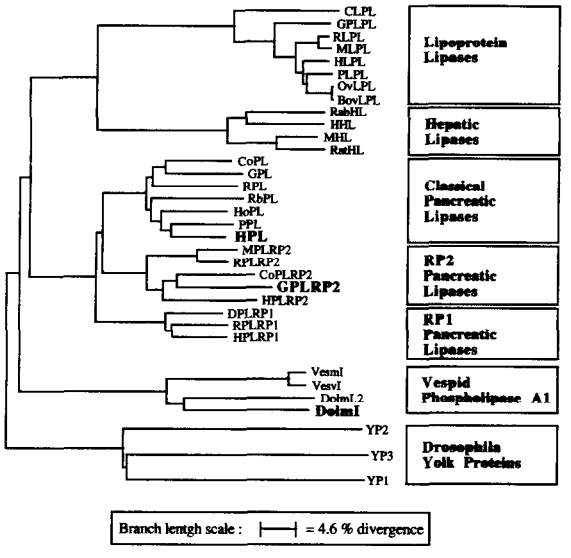

Fig. 1. Dendogram of sequence alignment. Up to now, the pancreatic lipase gene family is divided in seven subfamilies: (1) the lipoprotein lipases, from chicken (CLPL), guinea pig (GPLPL), rat (RLPL), mouse (MLPL), human (HLPL), pig (PLPL), ovine (OvLPL) and bovine (BovLPL), (2) the hepatic lipases, from rabbit (RabHL), human (HHL), mouse (MHL) and rat (RatHL), (3) the classical pancreatic lipases, from coypu (CoPL), guinea pig (GPL), rat (RPL), rabbit (RbPL), horse (HoPL), pig (PPL) and human (HPL), (4) the RP1 pancreatic lipases, from dog (DPLRP1), rat (RPLRP1) and human (HPLRP1), (5) the RP2 pancreatic lipases, from mouse (MPLRP2), rat (RPLRP2), coypu (CoPLRP2), guinea pig (GPLRP2) and human (HPLRP2), (6) the vespid phospholipases A1, from the yellow jackets (*Vespula maculifrons*, VesmI, and *Vespula vulgaris*, VesvI) and the white-faced hornet (*Dolichovespula maculata*, DolmI and DolmI.2), (7) the yolk proteins from *Drosophila melanogaster* (YP1, YP2, YP3).

Fig. 1 summarizes the present knowledge on the pancreatic lipase gene family which is now divided in seven subfamilies. The first six subfamilies regroup enzymes displaying various substrate selectivities for triglycerides and phospholipids, whereas the last one includes the non-enzymatic yolk proteins from *Drosophila*. These proteins represent the highest degree of divergence within the pancreatic lipase gene family (Fig. 1). They do not contain a catalytic triad and, therefore, do not display lipase activity, but the amino acid residues conserved between yolk proteins and pancreatic lipase surround the active site where there are interactions with lipids [11]. The likely reason for this sequence homology in the yolk proteins is to bind a steroid hormone and to store it under an inactive form until it is released during the embryogenesis of *Drosophila*.

In the present article, we review the catalytic and structural properties of the classical human pancreatic lipase (HPL), the guinea pig pancreatic lipase-related protein 2 (GPLRP2) and the phospholipase A1 from hornet venom (DolmI). Based on the know 3D structure of HPL, we compare the structure–function relationships of these three enzymes in order to understand their different substrate selectivities towards triglycerides and phospholipids.

2. The classical human pancreatic lipase

HPL is the major lipolytic enzyme involved in the digestion of dietary triglycerides [12] and it represents around 3% of the total proteins secreted by the exocrine pancreas [13]. HPL hydrolyzes primary ester bonds of tri and diglycerides, thus generating 2-monoglycerides and fatty acids which are absorbed through the intestinal barrier. Contrary to most of the pancreatic enzymes which are secreted as proenzymes and further activated by proteolytic cleavage in the small intestine, the pancreatic lipase is directly secreted as a 50 kDa active

enzyme consisting of a 449 amino acid polypeptide (Fig. 2) [14,15].

HPL is a soluble enzyme which differs from the classical esterases in that its natural substrate is insoluble into water. HPL carries out an heterogeneous catalysis and its activity is maximal only when the enzyme is adsorbed at an oil–water interface. This preference of HPL for an aggregated substrate can be demonstrated when using non-natural substrates possessing a low solubility into water, such as triacetin or triproprionin. The rate of breakdown of an isotropic solution of these short-chain triglycerides (monomers or micelles) is very slow.

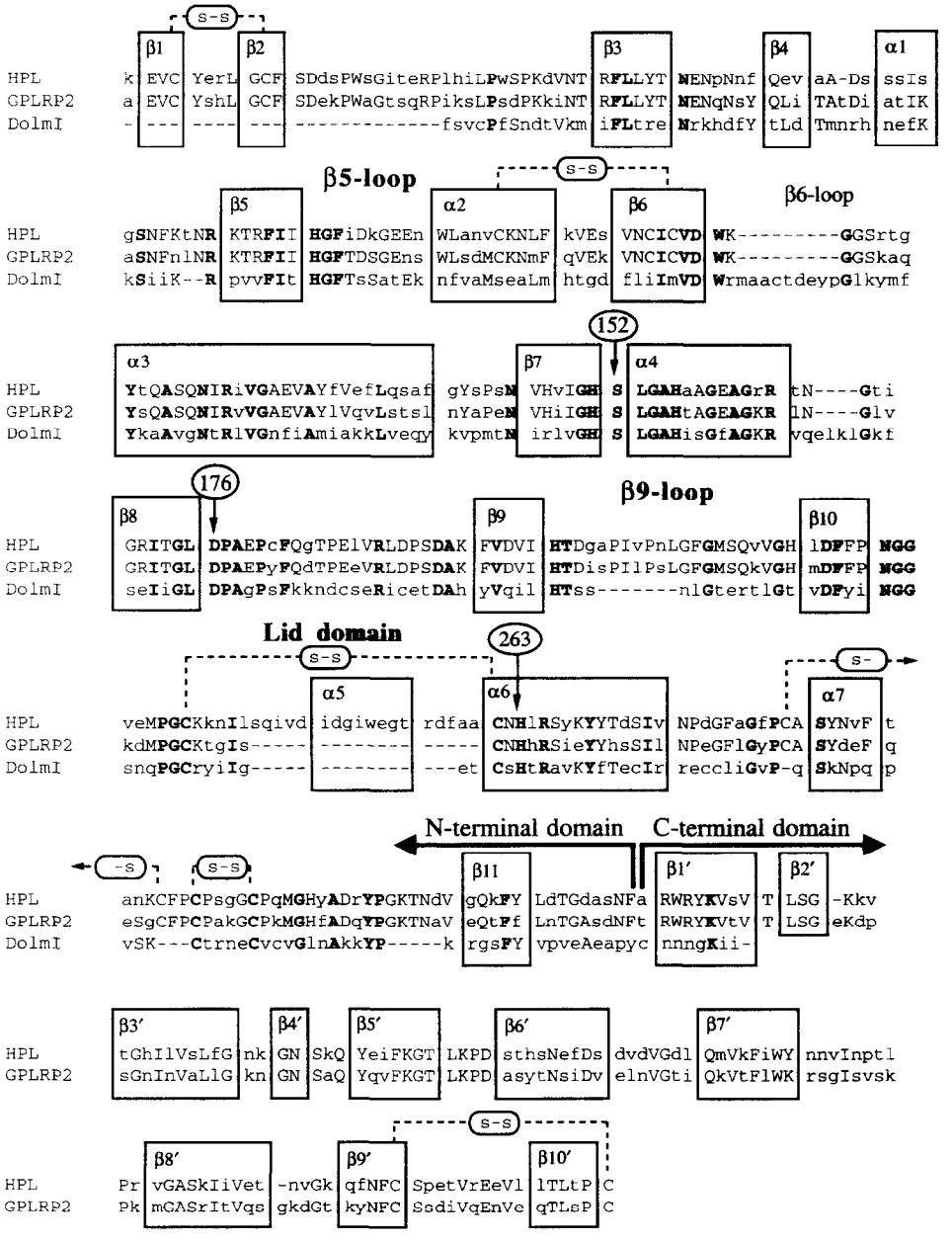

Fig. 2. Sequence alignment of HPL, GPLRP2 and DolmI PLA1. The secondary structures found in HPL are boxed and identified according to Winkler et al. [21].

There is however a sharp increase in the enzymatic activity once the substrate solubility is exceeded and a water–lipid interface appears. This kinetic property of classical pancreatic lipase, known as the phenomenon of interfacial activation [16], has been associated for many years with the definition of lipases. It is however clear today that some lipases — i.e. enzymes that can hydrolyze insoluble long chain triglycerides — do not display interfacial activation.

In addition to interfacial activation, the classical pancreatic lipase is also characterized by its behavior in the presence of bile salts [17]. It is inactive on an emulsified triglyceride substrate in the presence of micellar concentrations of bile salts such as those found in the small intestine during digestion. Bile salts are amphiphilic molecules mainly found adsorbed at the oil–water interface or dispersed as micelles in solution. The bile salt coating of triglyceride globules is inhibiting the pancreatic lipase adsorption and activation at the oil–water interface. However, a specific lipase-anchoring protein present in the exocrine secretion of pancreas, colipase, counteracts this effect through the formation of a specific 1:1 complex with lipase that facilitates adsorption at bile salt-covered lipid–water interfaces [18–20].

The previous properties clearly show the adaptation of HPL to the physiological conditions found in the small intestine where pancreatic and biliary secretions are mixed with dietary lipids. Through the phenomenon of interfacial activation and the anchoring effect of colipase, HPL displays a high selectivity to-

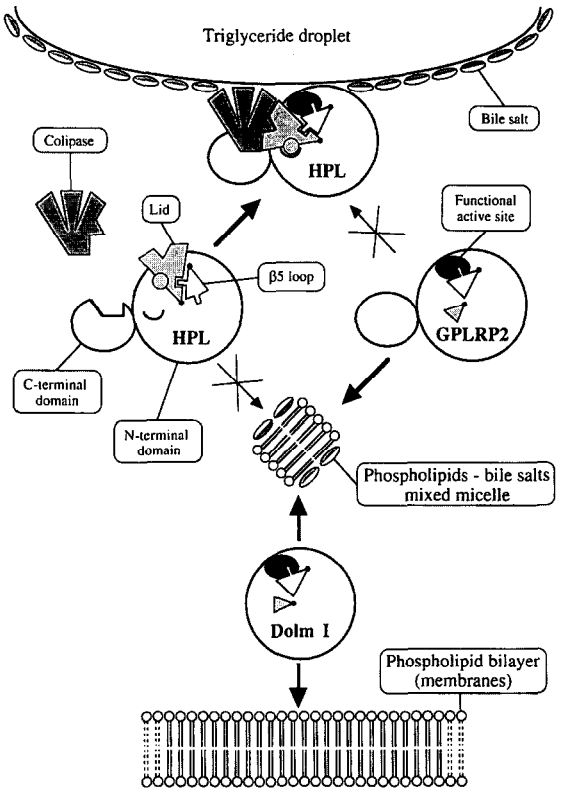

Fig. 3. A schematic representation of the structure–function relationships of HPL, GPLRP2, and DolmI PLA1.

Table 1
Lipase and phospholipase A1 activities of HPL, GPLRP2, and DolmI PLA1

Enzyme	Lipase activity on tributyrin (U/mg)	Phospholipase activity on egg yolk lecithins (U/mg)	Phospholipase-to-lipase activity ratio
HPL	12800 [a]	0 [b]	0
GPLRP2	1690 [a]	570 [b]	0.33
DolmI	33 [c]	280 [c]–1100 [d]	8.5–33.3

Lipase activity on tributyrin and phospholipase A1 activity on egg yolk lecithins were assayed in bulk using the pHstat method. The lipolytic activities are expressed in international units (U) per mg of enzyme. One unit = 1 μmol of fatty acid released per minute

[a] The tributyrin assay was performed under the optimal conditions for measuring HPL activity: 0.5 ml of tributyrin was emulsified into a reaction vessel containing 14.5 ml of 0.28 mM Tris buffer, 1.4 mM $CaCl_2$, 150 mM NaCl, 0.5 mM sodium taurodeoxycholate, pH 7.5. In the case of HPL, the reaction buffer was supplemented with colipase at a molar excess of 2.

[b] Egg yolk assay was performed according to [29].

[c] From [9].

[d] From [35].

wards insoluble triglycerides and does not hydrolyze phospholipids such as phosphatidylcholine (Table 1). The structural study of the HPL has revealed the complex catalytic machinery by which the classical pancreatic lipase displays its high substrate selectivity (Fig. 3).

3. Structure–function relationships of the classical human pancreatic lipase

The resolution of the first 3D structure by Winkler et al. [21] demonstrated the functional organization of HPL in several structural domains (Figs. 2 and 3): a large N-terminal domain (residues 1–336) and a smaller C-terminal domain (residues 337–449). The amino acid homology observed within the lipase gene family supports the view that this particular architecture is also common to LPL and HL. The large N-terminal domain belongs to the α/β hydrolase fold [22] (Fig. 4). It contains the active site with a catalytic triad formed by Ser 152, Asp 176, His 263 (Figs. 2, 4 and 5), all of which are conserved in LPL, HL and vespid phospholipases A1. This catalytic triad is chemically analogous to that originally described in serine proteases such as chymotrypsin [23] but is structurally distinct. The β-strand/$_{\varepsilon}$Ser/α-helix structural motif including the Gly–X–Ser–X–Gly consensus sequence has only been found in lipases and esterases so far [24,25].

In the structure resolved by Winkler et al [21], the active site is covered by a surface loop, the so-called 'lid domain', found in between the disulphide-bridged Cys 237 and 261 (Figs. 2–4). This surface loop includes a short one-turn α-helix with a tryptophan residue (Trp 252) completely buried and sitting directly on top of the active Ser 152. Under this closed conformation, the 'lid' prevents access of substrate to the active site. It was then proposed that interfacial activation involves a substantial conformational change in the structure observed before the substrate can bind in the active site. Later, spectroscopic studies of tryptophan fluorescence clearly showed large spectral changes accompanying the conformational change of HPL induced by acylation with the inhibitor tetrahydrolipstatin in the presence of bile salt micelles

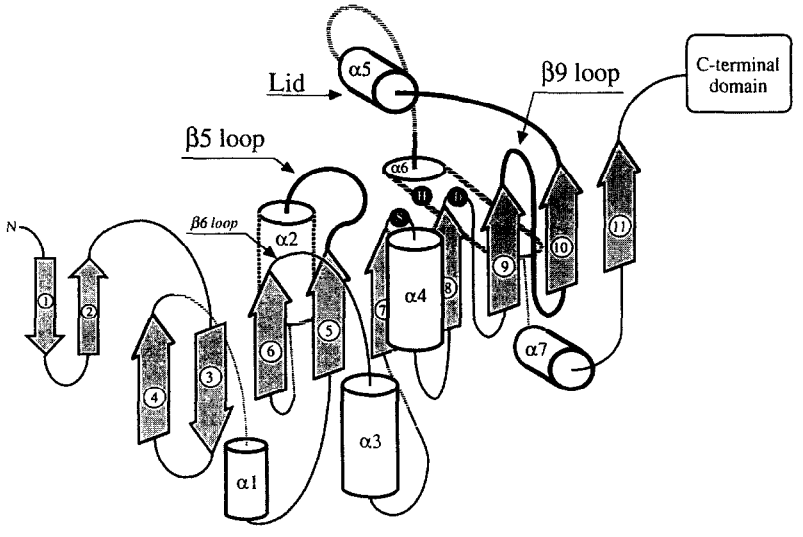

Fig. 4. A schematic diagram of the catalytic N-terminal domain of HPL. This structural domain shows the fingerprint of the α/β fold, including the β-strands 3 to 10. The elements of secondary structure are numbered according to Winkler et al. [21]. The arrows and cylinders indicate β-strands and α-helixes, respectively.

[26]. Finally, by crystallizing the pancreatic lipase–procolipase complex in the presence of mixed lipid micelles of bile salts and phosphatidylcholine, it was definitively shown that the 'lid' is displaced to one side, thus exposing both the active site and a larger hydrophobic surface [27]. The open and closed structures of HPL have suggested a structural basis for 'interfacial activation'.

In solution (absence of interface), the classi-

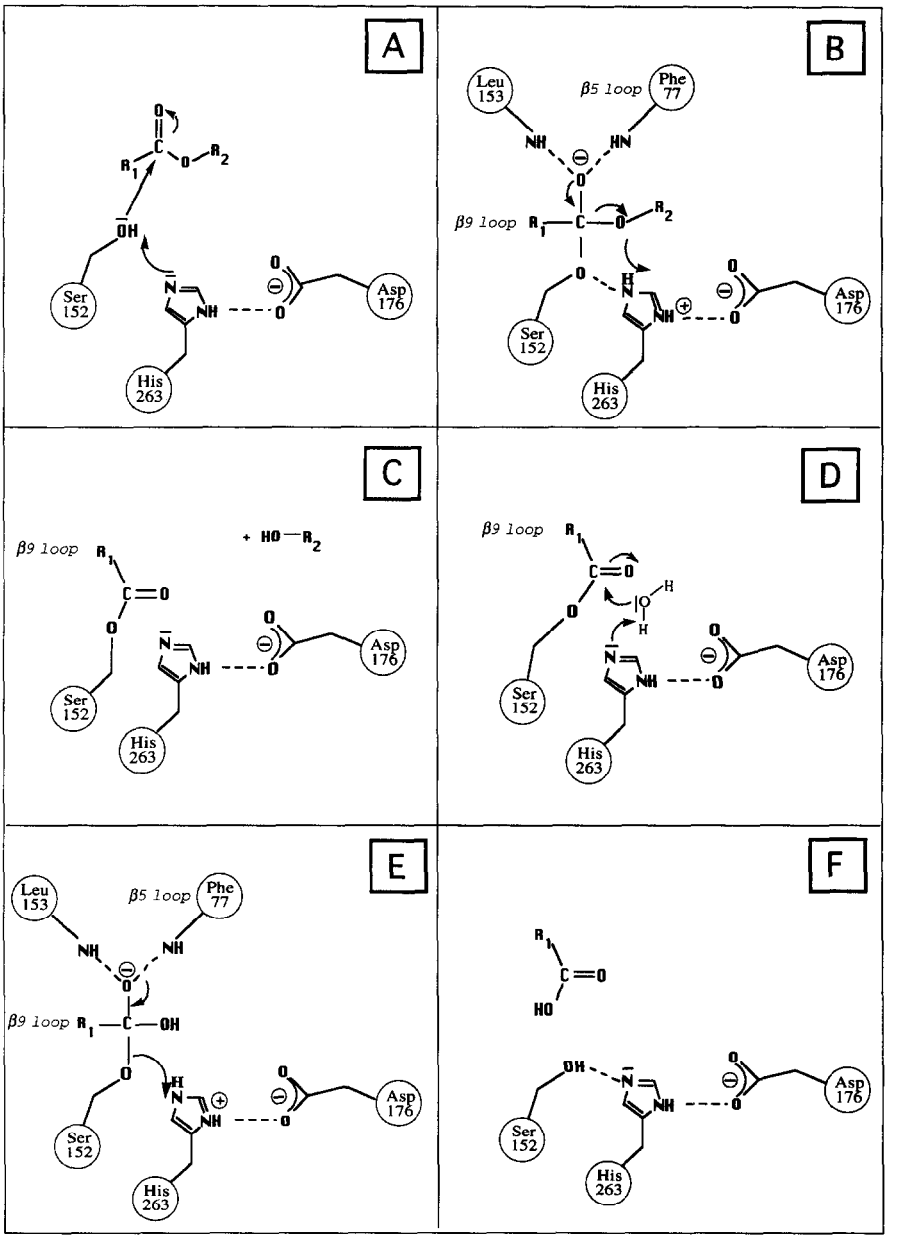

Fig. 5. Hydrolysis mechanism of a carboxylic ester by the catalytic triad of HPL. (a) Native conformation of the enzyme and nucleophilic attack of the substrate. (b) Formation of the first tetrahedral intermediate and stabilization by the oxyanion hole. (c) Alcohol release and formation of the acyl-enzyme. (d) Nucleophilic attack of the acyl-enzyme by a water molecule. (e) Formation of the second tetrahedral intermediate and stabilization by the oxyanion hole. (f) Carboxylic acid release and regeneration of the enzyme in the native form.

cal HPL is probably found in the closed conformation [21] (Fig. 3). The active center is completely inaccessible to solvent due to the conformation of the lid domain which also interacts with two other surface loops, the $\beta 5$ and the $\beta 9$ loops (Figs. 2 and 4). The indole ring of Trp 252 from the lid is packed against Phe 77 from the $\beta 5$-loop. In the presence of an interface, the lid domain as well as the $\beta 5$-loop undergo large conformational changes, creating a hydrophobic active-site groove and adjusting the hydrolytic machinery (Fig. 3), while the $\beta 9$ loop remains unchanged [27].

The opening of the active site is caused by a structurally complicated reorganization of the complete lid domain between Cys 237 and Cys 261. As mentioned before, this surface loop contains a short α-helix (residues 248 to 255, Fig. 2) covering the active site under the close conformation of HPL (Fig. 4). In presence of lipids, this helix partially unwinds and two new helices are formed (residues 241 to 246, and residues 251 to 259). The conformational change of the lid results in a maximal main chain movement of 29 Å for Ile 248. The substrate has now access to the active site. Trp 252, particularly, is conveyed to the surface of the molecule and is involved in a new interaction with the core of the protein.

Another consequence of the lid reorganization is the conformational change of the $\beta 5$-loop. In the closed conformation of the HPL-procolipase complex, this loop makes van der Waals contact exclusively with the lid. In the open conformation, these interactions are lost and the $\beta 5$-loop lofts back onto the core of the protein. This movement creates an electrophilic region close to the active site Ser 152 and creates the oxyanion hole which stabilizes the transition-state intermediate formed during catalysis. The main-chain nitrogen of Phe 77 from the $\beta 5$-loop moves to an ideal position to stabilize the negative charge developed during ester hydrolysis. The main-chain nitrogen of Leu 153 is also involved in the formation of the oxyanion hole (Fig. 5).

A further characterization of a C_{11} phosphonate inhibitor in the HPL active site [28] allowed the assignment of the residues involved in the stabilization of the inhibitor alkyl chain on either the open lid or the $\beta 9$ loop, and confirmed the location of the two acyl binding sites first identified by van Tilbeurgh et al. [27]. The $\beta 9$ loop is in interaction with the inhibitor alkyl chains via hydrophobic contacts with Leu 213 and Phe 215. These residues are probably involved in the stabilization of the leaving acyl chain during the hydrolysis of glycerides.

The β-sandwich C-terminal domain of pancreatic lipase is required for colipase binding as shown by the 3D structure of HPL-porcine procolipase complex [20]. Procolipase is a 'three finger' protein topologically comparable to snake toxins even though these proteins do not share any sequence homology. Procolipase is an amphiphilic protein with the tips of the fingers containing most of the hydrophobic amino acids and forming the interfacial binding site. Lipase binding occurs at the opposite side to this site and involves polar interactions. In absence of interface, no conformational change in the lipase molecule is induced by the binding of procolipase. Under the open conformation of the HPL-procolipase complex, procolipase is also binding to the re-organized lid domain (Fig. 3).

The structure of the lipase–procolipase complex illustrates how the procolipase might anchor the lipase at the interface in presence of bile salts (Fig. 3): procolipase binds to the non-catalytic β-sheet of the C-terminal domain of HPL and exposes the hydrophobic tips of its fingers at the opposite site of its lipase-binding domain. This hydrophobic surface helps to bring the catalytic N-terminal domain of HPL into close contact with the interface where the opening of the lid domain probably occurs. As a result of all these conformational changes, the open lid and the extremities of the procolipase fingers form an impressive continuous hydrophobic plateau, extending over more than 50 Å. This surface might be able to interact strongly

with a lipid–water interface and could explain the procolipase effect in the presence of bile salts.

4. GPLRP2: A pancreatic lipase with atypical kinetic properties and a new physiological role

In contrast to the classical pancreatic lipases, GPLRP2 displays no interfacial activation, i.e. this enzyme is already fully active on an isotropic solution of a partly soluble triglyceride and there is no jump in the hydrolysis rate once the solubility is exceeded. In GPLRP2, the lid domain is shortened to 5 residues instead of 23 in HPL (Fig. 2) and the active center is envisioned to be freely accessible from the modelling based on HPL 3D structure [4] (Fig. 3).

It was also demonstrated that colipase does not reactivate GPLRP2 in the presence of high bile salt concentrations [6]. The lack of colipase effect in GPLRP2 probably results from several key mutations: among the 12 residues of the HPL C-terminal domain involved in colipase binding [20], 9 are different in GPLRP2 [8]. Moreover, colipase also interacts with the lid-domain of HPL in its open conformation [27]. These interactions cannot exist in a lipase with a 'mini-lid' such as GPLRP2.

GPLRP2 displays a high phospholipase activities of 570 U/mg (1 unit = 1 μmol of fatty acid released per minute), using egg yolk lecithin as substrate (Table 1) [4]. For comparison, the classical porcine pancreatic phospholipase A2 displays a specific activity of 700 U/mg under similar conditions [29], whereas HPL showed no measurable activity on this substrate (Table 1).

Even though GPLRP2 is able to display both lipase and phospholipase activity in vitro (Table 1), it can only act on phospholipids in vivo (Fig. 3). Due to the lack of colipase effect on GPLRP2, this enzyme will not bind to the dietary fat droplets in the presence of bile salts and, therefore, it will not hydrolyze triglycerides in the small intestine. In the guinea pig, the hydrolysis of triglycerides can be carried out by a classical pancreatic lipase (GPL) which is produced at the same level as GPLRP2 [30]. The physiological role of GPLRP2 as a phospholipase is supported by the fact that the guinea pig pancreas does not produce the classical pancreatic phospholipase A2 [31]. This feature seems to be a characteristic property of the hystricomorph rodents [6,32]. It is worth noticing however that GPLRP2 can also hydrolyze galactolipids [33]. Since the guinea pig is mainly an herbivore, GPLRP2 might be involved in the digestion of these major lipids from plant membranes.

On a structural point of view, the reason for the high phospholipase activity of GPLRP2 remains unclear. This enzyme displays a high phospholipase activity towards lecithins but also towards other phospholipid classes [4], whereas classical pancreatic lipases can only hydrolyze negatively charged phospholipids with a very low specific activity [34]. From the GPLRP2 modelling based on HPL 3D structure, the core of the N-terminal domain appears to be conserved overall, with the exception of the lid-domain. Furthermore, within a 10 Å radius sphere of the active site serine, there are no insertions/deletions and only four minor residue changes pointing away from the active site. Thus, we speculated that the lid domain was also involved in substrate selectivity towards phospholipids [4]. The lack of high phospholipase activity in classical pancreatic lipases might be viewed as a depressed action on phospholipids due to the steric hindrance of the lid-domain, rather than a total absence of catalysis [4]. One can notice that the other characterized PLRP2 possess a full-length lid domain of 23 residues. The PLRP2 from coypu and rat still hydrolyze phospholipids but their phospholipase-to-lipase activity ratio is reduced as compared to that of GPLRP2 [6,7]. Accordingly, a large lid domain is one of the structural elements controlling substrate selectivity, but its

presence is not sufficient to completely suppress the phospholipase activity.

5. The phospholipase A1 from the White-faced hornet

The phospholipase A1 from vespid venoms catalyzes the hydrolysis of the sn-1 acyl group of phospholipids (PLA1) and their direct action on membrane phospholipids leads to an extremely high hemolytic activity (Fig. 3). These PLA1 are major allergens from vespid venoms and they display the highest catalytic activity among all phospholipases [35,36]. On the contrary, their lipase activity is extremely low as illustrated in Table 1 in the case of the DolmI PLA1 from the white-faced hornet (*Dolichovespula maculata*).

The sequence comparison of DolmI PLA1 [9] with GPLRP2 and HPL is shown in Fig. 2. Four stricking deletions can be observed in the DolmI PLA1: (i) the C-terminal domain is totally missing, (ii) the N-terminal part of the catalytic N-terminal domain is also absent, (iii) the lid domain is shortened to 7 residues instead of 5 residues in GPLRP2, (iv) part of the $\beta 9$ loop is deleted. Interestingly, all the vespid phospholipases A1 show similar deletions [9,10,37]. Among the observed insertions, one can be particularly noticed within the $\beta 6$ loop (Fig. 2). In the structure of HPL, the $\beta 6$ loop is bordering the active site and is facing the $\beta 9$ loop (Fig. 4). Such an insertion in DolmI PLA1 may have some implication in the catalytic mechanism.

6. Conclusions

The $\beta 5$ and $\beta 9$ loops, and the lid domain are believed to play an important role in catalysis and substrate selectivity. These loops are bordering the catalytic pocket (Fig. 4) and it has been shown in the case of HPL that acyl chains can interact with the $\beta 9$ loop and the lid domain, whereas residues of the $\beta 5$ loop are involved in the oxyanion hole [27,28].

The $\beta 9$ loop and the full-length HPL lid are highly hydrophobic and are proposed to determine the recognition of a triglyceride–water interface. The absolute selectivity of HPL for triglycerides (Table 1) is probably resulting from both a higher affinity for triglyceride droplets (adsorption step of catalysis) and the fact that the lid is supposed to act as an important selectivity filter depressing the phospholipase activity. In the case of GPLRP2, the lid is partly deleted (Fig. 2) and the $\beta 9$ loop alone remains sufficient for adsorption to the triglyceride matrix, as demonstrated by the lipase activity which is still high (Table 1). In DolmI PLA1, both the lid and the $\beta 9$ loop are deleted (Fig. 2) and the lipase activity is drastically reduced (Table 1).

As we wrote previously, the lid is only one of the structural elements controlling the phospholipase activity. A better understanding of the phospholipase activity of both GPLRP2 and DolmI PLA1 deserves further investigations.

7. Methods

All the sequences used to build the dendogram have been selected through a literature search and through searches of the GENEBANK and SWISSPROT data bases. The sequence comparison has been performed using the CLUSTAL package of multiple alignment programs [38]. The sequence alignment of HPL, GPLRP2 and DolmI has been further adjusted based on the known 3D structure of HPL [21].

Acknowledgements

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