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Lipoprotein lipases and vitellogenins in relation to the known three-dimensional structure of pancreatic lipase

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A 106-residue region of high similarity between lipoprotein/pancreatic/hepatic lipases and *Drosophila* vitellogenins encompasses four β-strands with all residues but one strictly conserved or conservatively replaced between the structures, and enclosing the putative active site Ser-152. The properties suggest a common folding pattern but the region probably does not function as an 'interface recognition site' in the lipases, although it might well bind fatty acid esters of ecdysteroids or single lipid molecules in the vitellogenins. C-terminally of this 106-residue region, a surface loop ('flap') covers the active site. No residue within this loop is conserved through all lipases, but adjacent segments exhibit 60–70% residue identity. Hepatic and lipoprotein lipases probably hydrolyze both soluble and emulsified substrates at the same site. They lack residues corresponding to a second active site postulated in pancreatic lipase to account for hydrolysis of soluble substrates. In addition, due to structural differences the flap could prevent entry of soluble substrate molecules into the active site of pancreatic lipase.

Lipoprotein lipase; Pancreatic lipase; Hepatic lipase; Vitellogenin; Amino acid sequence similarity; Active site; Three-dimensional structure

1. INTRODUCTION

Lipoprotein lipase (LPL), pancreatic lipase (PL) and hepatic lipase (HL) exhibit sequence homologies suggesting that they have evolved from a common ancestor [1] by two gene duplications, the first of which probably resulted in a digestive lipase, PL, and a lipoprotein-metabolizing enzyme [2]. A later gene duplication allowed further specialization into LPL and HL which fulfill partially overlapping steps in lipoprotein metabolism. This hypothesis is supported by exon/intron patterns of the lipase genes [3–5]. A segment of the lipase structures is recognized also in *Drosophila* vitellogenins and has been functionally interpreted [2,6–9], but at that stage no three-dimensional structure of these proteins was known.

Recently, the crystal structure was reported for PL [10], allowing further judgements of the relationships. The three-dimensional structure of PL has two folding units, an N-terminal domain with a Ser, Asp, His triad in a putative active site and a smaller, C-terminal domain. The postulated active site is covered by a surface loop, a 'flap', which presumably moves for substrates to enter.

The comparisons and the knowledge of the three-dimensional structure suggest that lipases and vitellogenins are similarly folded around an active site, which in the vitellogenins might act as an ecdysteroid ester binding site. In contrast, the 'flap' shows fewer similarities, in agreement with different catalytic properties of the lipases. A second hydrolytic site, postulated to account for hydrolysis of water-soluble substrates by pancreatic lipase [10], is not found in the other lipases.

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2. MATERIALS AND METHODS

Amino acid sequences of PL, HL, LPL, and *Drosophila* vitellogenins are from [2,10–15]. Pair-wise comparisons with 15–30-residue spans, performed as in [2], secondary structure predictions [16] and hydrophilicity profiles [17] were related to positions in the tertiary structure of PL [10]. Residue numbers refer to human PL [10], unless otherwise stated.

3. RESULTS AND DISCUSSION

3.1. *Central homology region*

A region with extensive similarity in primary structure between the three lipases and *Drosophila* vitellogenins corresponds to residues 125–230 in PL (Fig. 1), and is located in the centre of the major folding unit (Fig. 2). Hydrophilicity profiles also support this similarity (data not shown). Adjacent to this region there are no significant homologies to the vitellogenins, and less stringent conservation among the lipases. Thus, whereas there is a 33% residue identity in the known PL, HL and LPL sequences for the 106-residue 'central homology region', there is only 16% residue identity in the neighbouring segment 231–335, and 15% in the segment 36–124.

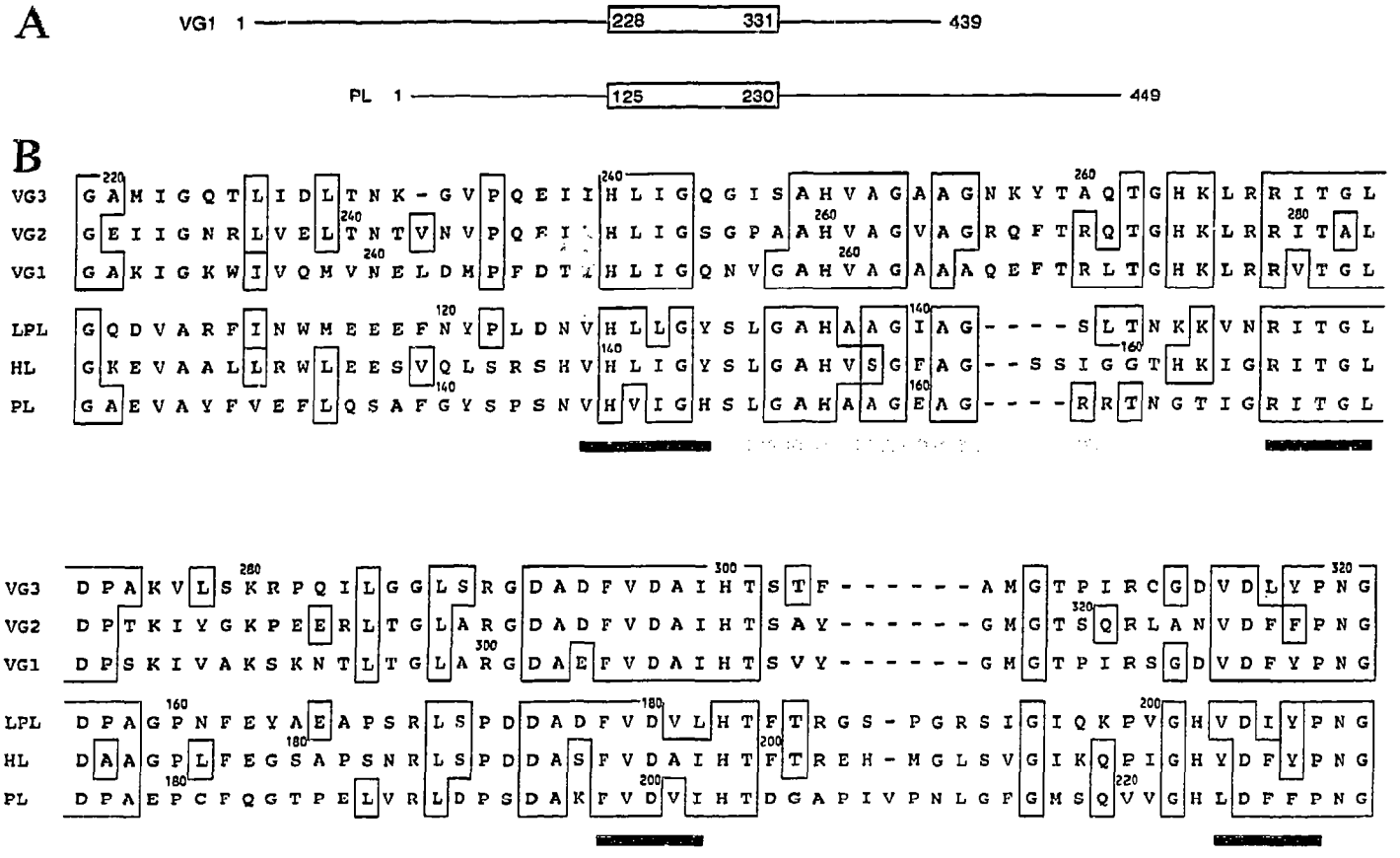

Fig. 1. Region of maximal similarity between lipases and *Drosophila* vitellogenins. A: shows location of the region; B: gives alignment with boxes showing identities between any of the lipases and the vitellogenins. Filled bars indicate β-sheets in pancreatic lipase, and the stippled bar indicates helix α4 [10]. LPL, lipoprotein lipase [11]; PL, pancreatic lipase [10]; HL, hepatic lipase [12], all human; VG1, 2 and 3, vitellogenins 1, 2 and 3 [6], all from *Drosophila*.

Within the central homology region, the residue identities are particularly striking in five segments (Fig. 1B). Four of these coincide with β-sheets in the PL structure, β7 (146–151), β8 (171–175), β9 (198–202) and β10 (224–228) [10]. Thirteen of the 21 residues in these β-sheets are conserved in the other lipases and 12 are also conserved in *Drosophila* vitellogenins (Fig. 1B). All substitutions except one (at position 151) are conservative. Secondary-structure predictions suggest that these segments form β-structures also in LPL, HL and the vitellogenins. The fifth segment with strong sequence homology coincides with an α-helix in the PL structure, α4 (153–164). The homologous segments corresponding to β7 and α4 are joined by the sequence -G-X-S-X-G- found at the active site of many serine esterases, proteases and lipases [2,3]. The segment includes Ser-152, suggested [10] to be catalytic in PL.

These portions of the lipases are probably similarly folded and form the same type of active site in all three lipases. The presence of the same structural elements in *Drosophila* vitellogenins suggests that they also contain a similar, largely hydrophobic pocket, but without the catalytic Ser and therefore also without catalytic activity. However, vitellogenins may well be able to bind long-chain fatty acid esters of ecdysteroids [6]. Hence, one line of the protein family (the lipases) could have gained/kept the enzymatic property, whereas another line (the vitellogenins) could have kept a binding function. As pointed out before [2], this would be analogous to divergent properties between binding proteins and enzymes in other families, e.g. the haptoglobin/serine proteases [18].

3.2. *Flanking regions*

Immediately C-terminal of the central homology region in PL is a surface loop (between disulphide-bridged Cys-237 and Cys-261). This loop, or 'flap', covers the active site. Eight of the 14 residues immediately before the flap are identical in all lipases, and 10 of the 14 residues after the flap are identical in HL and LPL. The putative active site His-263 is located in the latter region. Within the flap itself, there is, however, not a single residue identity across all lipases. Of interest, trypsin nicks LPL between residues 230 and 231, indicating that this bond is particularly exposed [20]. It corresponds to the tip of the flap in PL (residues 251/252; Fig. 2).

In PL, disulphides link residues 237–261, 285–296

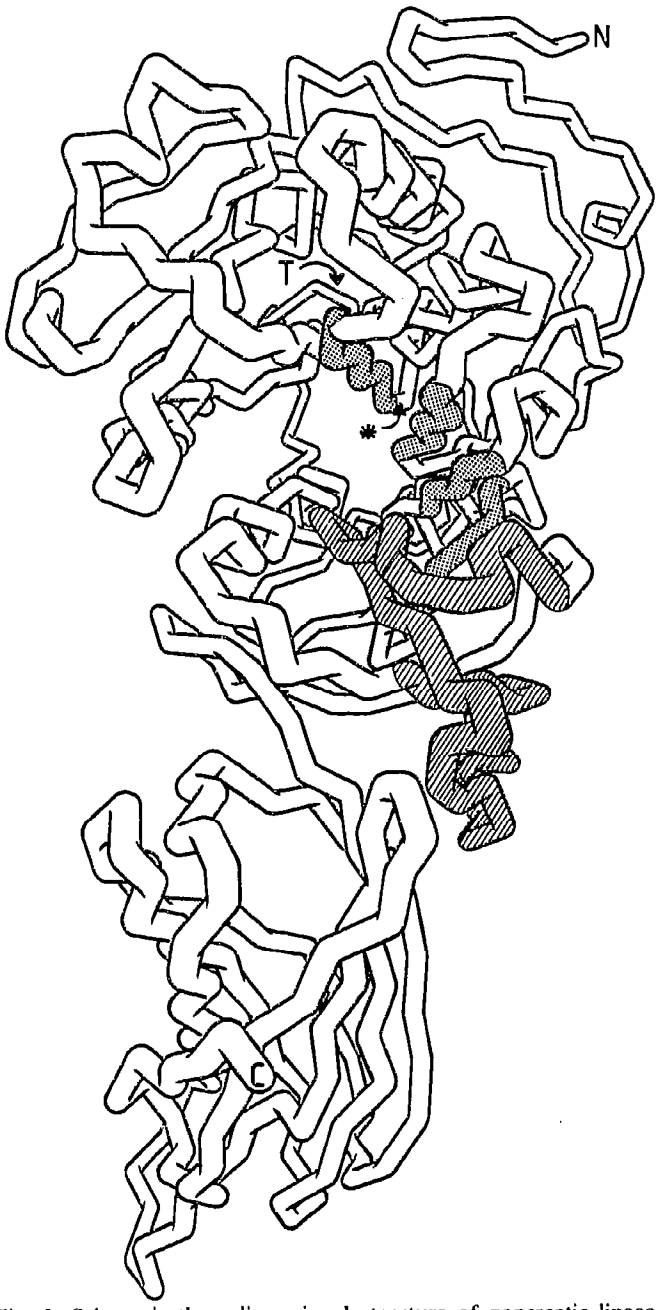

Fig. 2. Schematic three-dimensional structure of pancreatic lipase with markings of the segments discussed. Drawing by Hans Nilsson and representing an artist's interpretation of the three-dimensional figure in [10]. Stippled segments show the four β-sheets concluded (alignment in Fig. 1B) to be conserved also in the vitellogenins. The hatched region corresponds to the segment postulated to be heparin-binding in lipoprotein lipase. The arrow with asterisk marks active site Ser-152. Arrow marked T indicates the cleavage site for trypsin in lipoprotein lipase [20] in the 'flap region' pointing downwards in this projection. N and C indicate the corresponding termini.

and 299-304 [10]. For bovine LPL, another arrangement has been reported, corresponding to 237-261, 285-304 and 296-299 [19], suggesting that the two last disulphides have exchanged partners. The three-dimensional structure shows that this shift could be accomplished without major refolding.

The heparin-binding region of bovine LPL, first ascribed this function from results of limited proteolysis [20] and localized early [2], corresponds to positions 281-320 in PL (Fig. 2), as deduced from the distribution of positively charged residues. Residues 283-342 in PL form a surface loop between the two major folding units and include the two disulphides that appear to have changed partners in LPL. Charged residues within this region could be available for binding to a heparin chain [21].

In PL, residues 336-449 form a second folding unit (domain) which is of the β-sandwich type. The homologies to HL and LPL are relatively weak here. Of the 114 residues only 10 are conserved in all sequences. Further indications of divergence are that several of the substitutions are non-conservative and that gaps must be introduced to align the sequences, some of them fairly long.

3.3. *Postulated second active site*

A second active site has been postulated in PL to account for hydrolysis of soluble substrates, comprising Lys-373, and His-354 or His-382 [10]. Lys-373 is stoichiometrically acetylated after prolonged incubation of PL or its C-terminal fragment with *p*-nitrophenylacetate [22]. This Lys residue and the two His residues are not conserved in LPL or HL. In LPL, the activities against soluble and emulsified substrates are lost in parallel upon treatment with guanidine-HCl [23]. Hence, HL and LPL lack the postulated second active site, and probably hydrolyze both soluble and emulsified substrates at the same site. This difference could relate to the structural differences in the flap, which prevents entry of substrate molecules into the active site of PL, and presumably moves away only after the lipase has adsorbed to a lipid-water interface.

It has been suggested that the conserved residues around Ser-152 form part of an 'interface recognition site' which serves to anchor the lipase to the lipid/water interface of substrate emulsions [1]. The three-dimensional structure [10] shows that Ser-152 and adjoining residues are at the bottom of a pocket and seem to rule out that they interact directly with the interface (Fig. 2). This probability invalidates the suggestion [1] that there is a second 'interface recognition site' in HL centered around Ser-268.

While the catalytic mechanisms of the lipases may be the same, the functional properties of PL, LPL and HL are not. Pancreatic lipase is adapted to hydrolyze lipids in the intestine environment, while LPL and HL are not capable of hydrolysis under those conditions. On the other hand PL cannot hydrolyze lipids carried in lipoproteins of the plasma environment. It is tempting to speculate that this difference relates to the divergent structures of the flap, opening up for catalysis under different physico-chemical conditions.

A recent study suggests that the preferred route for

substrate entry into LPL and HL at lipid–water interfaces is from below, i.e. from the core of the lipid droplet, rather than from the side, i.e. along the interface [24]. This is in accord with the interpretation of catalysis from the three-dimensional structure of PL. On adsorption to the interface the flap presumably moves aside to allow access to the largely hydrophobic active site pocket. A similar sequence of events has been reported to apply to the three-dimensional structure of *M. miehei* lipase [25], which also shows a flap that shields the active site pocket in solution.

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