Hormone-sensitive Lipase Is Structurally Related to Acetylcholinesterase, Bile Salt-stimulated Lipase, and Several Fungal Lipases

BUILDING OF A THREE-DIMENSIONAL MODEL FOR THE CATALYTIC DOMAIN OF HORMONE-SENSITIVE LIPASE†

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Hormone-sensitive lipase is the key enzyme in the mobilization of fatty acids from adipose tissue, thereby playing a crucial role in the overall energy homeostasis in mammals. Its activity is stimulated by catecholamines through cAMP-dependent phosphorylation of a single serine, a process that is prevented by insulin. This regulatory property is unique to this enzyme among all known lipases and has been acquired during evolution through insertion of a regulatory module into an ancestral lipase. Sequence alignments have failed to detect significant homology between hormone-sensitive lipase and the rest of the mammalian lipases and esterases, to which this enzyme is only very distantly related. In the present work, we report the finding of a remarkable secondary structure homology between hormone-sensitive lipase and the enzymes from a superfamily of esterases and lipases that includes acetylcholinesterase, bile salt-stimulated lipase, and several fungal lipases. This finding, based on the identification of the secondary structure elements in the hormone-sensitive lipase sequence, has allowed us to construct a three-dimensional model for the catalytic domain of hormone-sensitive lipase. The model reveals the topological organization, predicts the components of the catalytic triad, suggests a three-dimensional localization of the regulatory module, and provides a valuable tool for the future study of structural and functional aspects of this metabolically important enzyme.

In mammals, free fatty acids derived from adipose tissue triglycerides are the most important fuel and provide more than half of the caloric requirements when dietary substrates are lacking. Hormone-sensitive lipase (HSL) plays a crucial role in the mobilization of free fatty acids from adipose tissue by catalyzing the first and rate-limiting step in the hydrolysis of stored triglycerides. Thus, HSL is a key enzyme not only in lipid metabolism but also in overall energy homeostasis. HSL is under acute hormonal and neural control. Its activity is stimulated by cAMP-dependent phosphorylation of a single serine residue (1), named regulatory site. This phosphorylation is catalyzed by cAMP-dependent protein kinase upon stimulation of β-adrenergic receptors by catecholamines. The antilipolytic action of insulin is mediated through a net dephosphorylation of the regulatory site of HSL (1). A second, cAMP-independent, phosphorylation site is present in the HSL protein, two residues C-terminal of the regulatory site (1, 2). Phosphorylation of this site (called the basal site) does not affect the catalytic activity but prevents phosphorylation of the regulatory site, thus having a potential antilipolytic role (3). The regulation of the enzyme activity by reversible phosphorylation is the most remarkable property of HSL, and unique among all known lipases. A second distinctive feature of HSL is its unusual substrate specificity, hydrolyzing cholesteryl esters and triglycerides at similar rates and showing 10 times higher hydrolytic activity toward diglycerides than toward triglycerides (4). The cholesteryl ester hydrolase activity, together with the reported presence of HSL protein in steroidogenic tissues (5), suggest a role of HSL also in the steroidogenic process (6), hydrolyzing stored cholesteryl esters to provide free cholesterol for the synthesis of steroids. A third distinctive feature of HSL is the retention of hydrolytic activity at low temperatures, at which other lipases are poorly active, a peculiarity that could be of importance in hibernating mammals (7).

Cloning of HSL from adipose tissue (7–9) showed that this enzyme shares virtually no homology with any other mammalian protein. A common origin with the other mammalian lipases and esterases is, however, ascertained by the presence of a Gly-Xaa-Ser-Xaa-Gly motif containing the catalytically active serine, and a few amino acids around a His-Gly dipeptide located approximately 80 residues N-terminal from this motif (10). The lack of further significant homology and the unique biological properties displayed by HSL indicate that this enzyme is only very distantly related to the other lipases and esterases found in mammals. However, HSL shares amino acid sequence homology with a few bacterial proteins (10, 11), of which the most significant is a lipase from the antarctic bacterium Moraxella TA144 (7). The homology spans the region of the HSL protein encoded by exons 5 to 9 (12) (Fig. 1), and includes the active site serine, whereas the region encoded by exons 1 to 4 shares no homology with any known protein. This organization suggests that HSL could be a mosaic protein,
assembled by the addition of an N-terminal domain to an ancestor catalytic domain closely related to the lipase from Moraxella TA144. Furthermore, the alignment of HSL and the Moraxella lipase shows an insertion of approximately 150 amino acids in the catalytic domain of HSL. The insertion contains the regulatory and basal phosphorylation sites, and therefore seems to be a module added during evolution, which conferred on HSL the regulatory properties mentioned above. The organization of the HSL molecule in (at least) two independent domains is supported by recent partial proteolysis and denaturation studies.

The structures of several lipases have been solved by x-ray crystallography, revealing that these enzymes share a similar three-dimensional fold, with different variants, called the α/β-hydrolase fold. Several molecular models have been constructed for lipases closely related to the ones with solved crystal structures. Examples of these are the models of lipoprotein lipase and salmon bile salt-stimulated lipase, based on the known structures of pancreatic lipase and acetylcholine esterase, respectively. However, the lack of significant sequence homology between HSL and other proteins of known three-dimensional structure has precluded modeling attempts for HSL.

In this work, we reveal a striking similarity in the organization of the secondary structure elements predicted for HSL and those actually found in the crystal structures of acetylcholine esterase (AChE) and two lipases from the fungi Geotrichum candidum and Geotrichum fujianense. This region was excluded from the modeling process.

Fig. 1. A, schematic representation of the HSL gene. Exons are represented as numbered boxes and introns as lines. Areas in black indicate the regions of the gene showing sequence homology with lipase 2 from Moraxella TA144 and encoding the catalytic domain of HSL, modeled in this work. Dotted boxes correspond to the regulatory module inserted during evolution. B, alignment of the catalytic domain of HSL with AChE and CRL based on secondary structure elements. The PHD program was used to obtain the secondary structure prediction for HSL. β-strands and α-helices are indicated with single and double underline, respectively. The nomenclature of the secondary structure elements follows that recommended for the carboxylesterase family (for a description, see Ref. 21).
The central β-sheet and the side chains of the residues in the catalytic triad (Ser-424, Asp-693, His-723) are shown by thick lines. The active site serine is indicated with an S. The numbered circles indicate the residues to which the regulatory module (not modeled) is connected. The sequence numbers of the first and last amino acids included in the model are shown.

thus close to each other in the tertiary structure. The only insertion in HSL compared to the other three proteins is the above mentioned regulatory module. All this region was eliminated from the alignment and not included in the modeling process (see “Results and Discussion”). The final alignment between HSL and CRL was used to generate the three-dimensional model of HSL. The modeling process was performed on a Silicon Graphics workstation with the molecular modeling program Modeller, which performs automated comparative modeling by satisfaction of spatial restraints (26). The model was further refined by energy minimization with X-PLOR (27), using parameter files by Engh and Huber (28). The final model was checked for stereochemical deviations with the Whatcheck (29) and Procheck (30) software packages.

RESULTS AND DISCUSSION

Identification of the Secondary Structure Elements of HSL and Sequence Alignment—Analysis of the human HSL protein sequence with the PHD secondary structure prediction program rendered a succession of strong and well defined predictions for α-helices and β-strands throughout the HSL sequence (not shown). When this prediction was carefully compared with the crystal structures of the lipases and esterases available at the Brookhaven protein data bank, a striking parallelism was found in the disposition of the secondary structure elements predicted for HSL and those present in AChE and CRL. On the basis of this observation, we performed a secondary structure-driven alignment of HSL with these two enzymes (Fig. 1b). Unexpectedly, the alignment revealed the presence of some conserved residues not previously detected with the usual sequence alignment algorithms (consensus line in Fig. 1b). Most of these conserved amino acids belong to a group of highly conserved residues previously identified by Cygler et al. (31) within the superfamily of lipases, esterases, and related proteins called the EST superfamily by Hemilä et al. (10). This implies that their placement is not merely coincidental, but important for the overall structure and/or function of the protein and indicates that the alignment shown in Fig. 1b is biologically sound. Furthermore, when compared at the DNA level, the regions around some of the predicted β-strands in HSL showed identity rates above 60% with the corresponding stretches in the DNA sequence from CRL and AChE (not shown), supporting a common evolutionary origin for these proteins.

All of the insertions and deletions observed in HSL compared to AChE or CRL occur in surface areas of these two enzymes and, in most of the cases, they coincide with small loops that are also variable between AChE and CRL. Only two large regions were found to be different between HSL and the other two enzymes. The first of these regions is the connection between the catalytic core of HSL and the regulatory module. All of this region was eliminated from the alignment and not included in the modeling process (see “Results and Discussion”).
between β6 and β7 (Fig. 1b, delimited by brackets), corresponding to a regulatory module inserted in HSL during the course of evolution. Its three-dimensional disposition is discussed below.

The second divergent region is the connection between β7 and β8. In CRL, this region consists of a group of α-helices and connecting loops that are absent from HSL. These structures form an external hairpin module with the N and C termini very close to each other in the tertiary structure (Fig. 4). Therefore, a shorter connection is possible without disturbance of the αβ fold. In fact, this module is also absent from some other lipases, the overall tertiary structure of which is otherwise similar to that of CRL, as is the case of lipase B from C. antarctica (32).

The good alignment of secondary structure elements and the presence of conserved residues shown in Fig. 1b support the distant evolutionary relationship between HSL and the EST superfamily previously suggested by Hemila et al. (10) and must reflect a similar three-dimensional fold in the region aligned. Thus, the building of a three-dimensional model showing the overall topology of the catalytic domain of HSL becomes feasible, despite the minimal amino acid sequence homology shared by these proteins.

Overall Structure of the Model—The region of the HSL protein modeled comprises the central core of the catalytic domain, from β2 to β6 and from β7 to α′,9 (Fig. 1b). The region located between β6 and β7 (Fig. 1b, delimited by brackets) contains the regulatory module and is highly divergent between HSL and CRL; any attempt to model it would be only tentative. This region was, therefore, not included in the modeling process. A stereoscopic view of the Ca trace of the HSL sequence is shown in Fig. 2. Bond lengths, bond angles, torsion angles, and ϕ/ψ plots from the model showed distributions in accordance with those expected for a naturally folded protein (not shown). An expected exception was the serine in the active site (Ser-424, numbering from the human HSL sequence), which showed divergent ϕ/ψ angles (14). Fig. 3 shows a schematic representation of the topology of the model. Positioning of the exon boundaries shows that the core of the αβ-hydrolase fold is encoded by exons 5, 6, and 9 and the last amino acids of exon 8 (Figs. 1 and 3). Exon 7 and most of exon 8 encode an external module that contains the regulatory and basal phosphorylation sites.

We have not included the 62 amino acids encoded by exon 4 in the building of the model, but it is likely that these residues are part of the catalytic domain of HSL. The PHD program predicts three β-strands and one α-helix in this part of the HSL protein (not shown). The corresponding regions in AChE and CRL contain the most external β-strand (β1) of the central β-sheet and a two-stranded β-sheet located almost perpendicular to the central one (19, 21). Because a definite correlation could not be established between the predicted β-strands in HSL and the ones in AChE or CRL, we decided not to include this region in the model. It is of relevance to note that the loop-helix-loop structure that constitutes the lid in CRL (21, 22) is also located in this part of the sequence. An amphipathic α-helix is predicted in HSL immediately before β2, in a position analogous to part of the lid in CRL (not shown). However, the loop connecting this helix and β2 is too short in HSL to allow the helix to cover the active site in a putative “closed” conformation of HSL, arguing against this region being a functional lid in HSL.

Catalytic Triad—As mentioned above, the active site of lipases and some esterases consists of a catalytic triad of serine, histidine, and a carboxylic acid (14). The side chains of these residues adopt a three-dimensional conformation that allows hydrogen bonding between them, increasing the nucleophilic character of the serine residue that performs the cleavage of the ester bond in the substrate. The first important implication of our model is the suggestion for the catalytic triad of HSL. Whereas Ser-424 (corresponding to Ser-423 in the rat sequence) was known to be the active site serine (33) and previous alignments suggested His-723 as the most likely histidine in the active site (10, 12), no good suggestion could be given for the acidic residue of the triad before this work. Our model confirms His-723 and predicts Asp-693 (located in the loop immediately after β7) as the residues completing the triad with Ser-424 in human HSL. The geometry of the side chains of these three residues is adequate for the stabilization of the triad through hydrogen bonding.

A second important aspect of the catalytic process is the stabilization of the reaction intermediates by means of hydrogen bonding to residues of the so-called oxyanion hole (14). By
analogy with CRL, the oxyanion hole in HSL is most likely formed by the amides of Ala-425 and Gly-353, the latter found in a conserved Gly-Gly-Gly motif. The three-dimensional position of these residues in the model is compatible with this suggestion.

Regulatory Module—As mentioned above, HSL activity is regulated by reversible phosphorylation, a feature that is unique to this enzyme amongst all known lipases. The molecular mechanism underlying the activation upon phosphorylation is unknown, but alignment of HSL with the lipase from Moraxella TA144 clearly indicates that HSL acquired this feature by the insertion of a regulatory module into the catalytic core of the ancestral lipase (7, 10, 12). Because the inserted amino acids are located in the primary structure between the serine and the histidine that appeared to be the most likely candidates to form the catalytic site (10), the insertion must have taken place without disturbing the original architecture of the lipase. The topological organization of the model (Fig. 3) shows that the regulatory module is located between β6 and β7 in the HSL sequence, one of the two divergent regions as compared to the template enzymes. It is interesting to note that the corresponding region in AChE and CRL (i.e. the approximately 100 amino acids connecting β6 and β7) consists of an external group of loops and α-helices that constitute a module relatively independent of the central α/β-hydrolase fold (Fig. 4, yellow structures). Therefore, it is a suitable area for the insertion of new modules without affecting the folding of the catalytic core. Even if reliable modeling of the regulatory module is not possible, it is tempting to speculate about its putative spatial localization and function. The presence of conserved residues allowed us to identify two of the predicted α-helices in HSL as α1.6,7 and α2.6,7 (Fig. 1b). These helices are encoded by exon 7 and precede the predicted α-helix that contains the phosphorylation sites, which is encoded at the beginning of exon 8. If the three-dimensional localization of α1.6,7 and α2.6,7 is equivalent in HSL and CRL (see Fig. 4), then the position of the helix with the regulatory serine (Ser-552) would be constrained to the side of the molecule that faces the lipid surface during lipolysis. Conformational changes of this helix upon phosphorylation could then have dramatic effects on the interaction of the enzyme with the lipid droplet.

Mutagenesis Data—We have some preliminary site-directed mutagenesis data that are consistent with the overall structure of the model. According to the model, the side chain of Cys-680 (located in β7; see Fig. 1b) points toward the Asp and His residues of the catalytic triad. Changing this residue into alanine had no effect on HSL activity, but replacing it with the bulky amino acids tyrosine or tryptophan rendered the enzyme completely inactive with regard to both lipase and esterase activity. A similar result was obtained with Ala-451. This residue is predicted to be in the C-terminal end of β6 (Fig. 1b), very close in the tertiary structure to the catalytic triad. In fact, the corresponding position in CRL is a serine that participates in the stabilization of the triad by hydrogen bonding with the active site glutamic acid (34). Mutating Ala-451 into tyrosine, tryptophan, or aspartic acid completely inactivated HSL, presumably by steric hindrance with the residues of the triad, whereas replacing it with serine only caused a minor reduction in activity. A triple point mutation in α1.6,7 (Phe-Leu-Thr-Leu mutated to Ala-Ala-Thr-Ala or Gly-Gly-Thr-Gly), originally designed to check the putative lipid binding ability of this region (7, 25), led to a completely inactive enzyme (toward both water soluble and lipidic substrates). The immediate proximity of this helix to the predicted active site His-723 is consistent with this result because substitutions in the helix might displace it from its normal position, thus moving the histidine from the active site.

In summary, the present work describes, for the first time, the structural characteristics of the catalytic domain of HSL. The components of the α/β-hydrolase fold are identified in the HSL sequence, and a three-dimensional model showing the topology of the catalytic domain of this enzyme is presented, which represents an important breakthrough in our knowledge of this lipase. The similarity found in the organization of secondary structure elements and the finding of structurally important conserved residues between HSL and the EST superfamily support the distant evolutionary link between these proteins (10). Efforts to obtain the crystal structure of the HSL protein are currently in progress. Meanwhile, the model represents a valuable tool to design site-directed mutagenesis experiments aimed at clarifying some of the biochemical and physiological properties of this metabolically important lipase.

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REFERENCES
1. Strålfors, P., Björgell, P., and Belfrage, P. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 3317–3321
2. Gerton, A. J., Campbell, D. G., Carling, D., Hardie, D. G., Colbran, R. J., and Yeaman, S. J. (1989) Eur. J. Biochem. 179, 249–254
3. Gerton, A. J., and Yeaman, S. J. (1990) Eur. J. Biochem. 191, 245–250
4. Fredrikson, G., Strålfors, P., Nilsson, N. O., and Belfrage, P. (1981) J. Biol. Chem. 256, 6111–6120
5. Holm, C., Belfrage, P., and Fredrikson, G. (1987) Biochem. Biophys. Res. Commun. 148, 99–105
6. Yeaman, S. J. (1990) Biochim. Biophys. Acta 1052, 128–132
7. Langin, D., Laurell, H., Stenson Holst, L., Belfrage, L., and Holm, C. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 4897–4901
8. Holm, C., Kirchgessner, T. G., Svensson, K. L., Fredrikson, G., Nilsson, S., Miller, C. G., Shively, J. E., Heinzmann, C., Sparkes, R. S., Mohandas, T., Lasius, A. J., Belfrage, P., and Schotz, M. C. (1988) Science 241, 1503–1506
9. Li, Z. G., Sumida, M., Birchbauer, A., Schotz, M. C., and Reue, K. (1994) Geonetics 24, 259–265
10. Hemila, H., Koivivaara, T., and Palva, I. (1994) Biochim. Biophys. Acta 1210, 249–253
11. Langin, D., and Holm, C. (1993) Trends Biochem. Sci. 18, 466–467
12. Østerlund, T., Danielsson, B., Degerman, E., Contreras, J. A., Edgren, G., Davis, R. C., Schotz, M. C., and Holm, C. (1996) Biochim. Biophys. Acta 991, 411–420
13. Ollis, D., Cheah, E., Cygler, M., Dijkstra, B., Frolow, F., Franke, S. M., Harel, M., Remington, S. J., Silman, I., Schrag, J. D., Sussman, J. L., Verscheure, K. H. G., and Goldman, A. (1992) Protein Eng. 5, 197–211
14. Derewenda, Z. S. (1993) in Advances in Protein Chemistry (Schumaker, V. N., ed), Vol. 45, pp. 1–52, Academic Press, Inc., New York
15. van Tilbeurgh, H., Roussel, A., Lalouel, J.-M., and Cambilau, C. (1994) J. Biol. Chem. 269, 4626–4633
16. Gjelstad, D. R., Lorenz, J. B., and Male, R. (1994) Eur. J. Biochem. 226, 603–612
17. Winkler, F. K., D’Arcy, A., and Hunziker, W. (1990) Nature 343, 771–774
18. Tilbeurgh, H., Sardis, L., Verger, R., and Cambilau, C. (1992) Nature 359, 159–162
19. Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., and Silman, I. (1991) Science 253, 872–879
20. Schrag, J. D., and Cygler, M. (1995) J. Mol. Biol. 230, 575–591
21. Grochulski, P., Li, Y., Schrag, J. D., Bouthillier, F., Smith, P., Harrison, D., Rubin, B., and Cygler, M. (1993) J. Biol. Chem. 268, 12843–12847
22. Bruenger, A. T. (1992) X-PLOR (Version 3.1), A System for X-ray Crystallography and NMR, Yale University Press, New Haven, CT
23. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. Sect. A 47, 392–400
24. Fragic, G. (1990) J. Mol. Graph. 8, 52–56
25. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
26. Cygler, M., Schrag, J. D., Sussman, J. L., Harel, M., Silman, I., Gentry, M. K., and Doctor, B. P. (1993) Protein Sci. 2, 366–382
27. Uppenberg, J., Hansen, M. T., Patkar, S. and Jones, T. A. (1994) Structure 2, 293–308
28. Holm, C., Davis, R. C., Østerlund, T., Schotz, M. C., and Fredrikson, G. (1994) FEBS Lett. 344, 234–238
29. Vernet, T., Zwieck, E., Recktenwald, A., Schrag, J. D., de Montigny, C., Tessier, D. C., Thomas, D. Y., and Cygler, M. (1993) J. Biol. Chem. 268, 26212–26219
30. Au-Young, J., and Fielding, C. J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 4094–4098