Crystal Structure of Human Gastric Lipase and Model of Lysosomal Acid Lipase, Two Lipolytic Enzymes of Medical Interest*

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Fat digestion in humans requires not only the classical pancreatic lipase but also gastric lipase, which is stable and active despite the highly acidic stomach environment. We report here the structure of recombinant human gastric lipase at 3.0-Å resolution, the first structure to be described within the mammalian acid lipase family. This globular enzyme (379 residues) consists of a core domain belonging to the αβ hydrolase-fold family and a “cap” domain, which is analogous to that present in serine carboxypeptidases. It possesses a classical catalytic triad (Ser-153, His-353, Asp-324) and an oxyanion hole (NH groups of Gln-154 and Leu-67). Four N-glycosylation sites were identified on the electron density maps. The catalytic serine is deeply buried under a segment consisting of 30 residues, which can be defined as a lid and belonging to the cap domain. The displacement of the lid is necessary for the substrates to have access to Ser-153. A phosphonate inhibitor was positioned in the active site that clearly suggests the location of the hydrophobic substrate binding site. The lysosomal acid lipase was modeled by homology, and possible explanations for some previously reported mutations leading to the cholesterol ester storage disease are given based on the present model.

Since 1990, when the first three-dimensional structures of a fungal (Rhizomucor miehei lipase) and a mammalian lipase (human pancreatic lipase (HPL))1 were published, growing interest in lipolysis has led to the structural determination of several lipases of various origins, including those present in bacteria, fungi, and mammals. All the lipases investigated so far vary considerably in size and in their amino acid sequences. However, they are all serine esterases belonging to the αβ hydrolase superfamily (1) in which the nucleophilic serine, part of a Ser-His-(Asp/Glu) triad, is located in an extremely sharp turn (nucleophilic elbow). Another feature that is common to all the members of the αβ hydrolase superfamily as well as to proteases is the occurrence of an oxyanion hole, which stabilizes the transition state. Some organophosphorous compounds inhibit lipases in a similar way to what occurs in the case of serine proteases (2).

The three-dimensional structures of several complexes consisting of lipases bound to covalent inhibitors have been solved: R. miehei lipase (3, 4) and Candida antarctica B lipases bound to a C6-alkyl phosphonate (5), Candida rugosa lipase bound to long chain alkyl sulfonyl (6), Pseudomonas cepacia lipase (7) as well as cutinase (8) bound to a dialkylcarbamoylglycerophosphonate, and human pancreatic lipase-colipase complex bound to C11-alkyl phosphonate (9). It has been established that the covalently inhibited lipases are in the so called “open” conformation, i.e. that the lid has moved away to give free access to the active-site serine. It has been suggested that this mechanism may be instrumental in the binding of lipases to the water-lipid interface and that the presence of a lid in the structure of the enzyme may be involved in the interfacial activation process (4, 10).

Among the mammalian lipases, the acid lipases belong to a family of enzymes that have the ability to withstand acidic conditions. This family that includes the preduodenal lipases and human lysosomal lipase shows no sequence homology with any other known lipase families (11). The preduodenal lipases form a group of closely related enzymes originating either from the stomach, the tongue, or the pharynx (12). They all have a low pH optimum, and none of them require any specific protein cofactor. Human gastric lipase (HGL, EC 3.1.1.3) is secreted by the chief cells located in the fundic part of the stomach (13), where it initiates the digestion of triacylglycerols (14, 15). The maximum specific activities of HGL are 1160 units/mg on TC4 (pH 6.0), 1110 units/mg on TC8 (pH 6.0), and 600 units/mg on IntraplidTM (pH 5.0) (16). Native HGL has an apparent molecular mass of 50 kDa and is a highly glycosylated molecule with 4 potential N-glycosylation sites (17). The glycan moiety was estimated to account for around 15% of its total protein mass (18).

This enzyme plays a crucial role in newborns, because pancreatic lipase is not yet fully developed at this age (15). The physiological importance of gastric lipase has been suspected for some time, based on pathological situations involving pancreatic exocrine insufficiency, such as the late stage of chronic pancreatitis or cystic fibrosis. In these cases, even in the complete absence of pancreatic lipase, the patients still absorb a high percentage of their ingested dietary fat (19, 20). In substitutive enzymatic therapy, the use of acidic-resistant lipases should help to treat patients with various forms of pancreatic deficiency. Physiological studies have
shown that preduodenal lipases are capable of acting not only in the stomach but also in the duodenum in synergy with a pancreatic lipase (14). Various clinical studies have been conducted on both animals and humans to assess the efficacy of enzymatic replacement therapies using acid-resistant lipases to treat exocrine pancreatic insufficiency (21). This treatment significantly increased the weight and reduced the steatorrhea in dogs.

Despite the close amino acid sequence similarities (59% of the amino acids are identical) between HGL (17) and human lysosomal acid lipase (HLAL, EC 3.1.1.3) (22, 23), HGL lacks the cholesteryl ester hydrolyase activity reported in HLAL. The latter enzyme hydrolyzes not only the triglycerides that are delivered to the lysosomes by low density lipoprotein receptor-mediated endocytosis but also cholesteryl esters (24). The cholesteryl released by this reaction plays an important regulatory role in cellular sterol metabolism. Defective HLAL activity has been found to be associated with two rare autosomal recessive traits, Wolman disease and cholesteryl ester storage disease. In Wolman disease (25), a lack of HLAL activity results in a pronounced accumulation of cholesteryl esters and triacylglycerols in the lysosomes in most of the body tissues. The patients usually succumb to hepatic and adrenal failure within the first year of life. Cholesteryl ester storage disease, the other clinically recognized phenotypic form of HLAL deficiency, follows a more benign clinical course (26), and a residual HLAL activity has been detected. Since the cloning of the cDNA and determination of the genomic organization of the gene (LIPA) located on chromosome 10, which encodes HLAL (22, 23, 27), some deleterious LIPA gene mutations have been identified (28–34). Most of these mutations affect either the mRNA splicing or the amino acid sequence of HLAL. The exact correlations between these mutations and the biochemical and clinical phenotypes still remains to be elucidated, however. In this study, the crystal structure of recombinant HGL at 3.0-Å resolution is determined, and a model of HLAL is discussed and used to possibly explain the previously reported cholesteryl ester storage disease mutations.

MATERIALS AND METHODS

Recombinant HGL (rHGL) Expression and Purification—rHGL was expressed in the baculovirus/insect cell system (48). The active enzyme was produced on a large scale (5–13 mg/liter) from recombinant baculovirus-infected insect cells using a bioreactor and its specific activity (μmol/min·mg−1) was around 700 units/mg (49). The amino acid sequence (KLHPG) of rHGL in the purified protein starts at residue 4.

rHGL Crystallization and Enzymatic Activity in the Crystal—Crystals were obtained by mixing 2 μl of a well solution (2 M ammonium sulfate, 1.4% tert-butanol, at pH 5.0) with 2 μl of a protein solution at 5–6 mg/ml. The crystals are cubic, space group I213 with cell dimensions a = 244.0 Å, b = c = 244.0 Å. The protein mass, 47,673 Da, was determined from collected crystals by matrix-assisted laser desorption ionization time-of-flight spectroscopy. There are two molecules in the asymmetric unit (see below), and the Vm was estimated to be 6.35 Å3 Da−1 (81% water content). For the mercury derivative preparation, the crystals were transferred in a synthetic liquor corresponding to the well solution containing 23 mM mercaptacetic acid.

To assess the catalytic activity of the crystallized enzyme, tests were performed on both dissolved and intact crystals. Ten crystals were washed three times in the crystallization buffer and were subsequently dissolved in 50 μl of water, and the protein concentration was estimated by absorbance at 230 nm. The lipase activity was measured titrimetrically at 37 °C using a pH stat (metrom) at pH 5.7 with a tributyrin emulsion as the substrate: 0.5 ml of tributyrin added to 14.5 ml of 150 mM NaCl, 2 mM taurodeoxycholate, and 2 mM bovine serum albumin (16).

The specific activity (μmol/min·mg−1) was calculated and found to be around 580 units/mg. To test the catalytic activity in situ, a single rHGL crystal was incubated in the crystallization solution containing 0.1 mM Nitroblue tetrazolium and 0.75 mM 5-bromo-chloro-3-indoxyl butyrate as the substrate. After 24 h of incubation, the crystal was intensely colored in blue-gray.

Data Collection and Heavy Metal Derivative Search—All data sets were collected at 100 K using a cryo-stream cooler from Oxford Cryosystems. A first native and a derivative data set were collected in-house using a 300-mm MAR Research imaging plate detector mounted on a RU200 rotating anode generator (Rigaku, Tokyo, Japan). The generator was operated at 3.2 kW with a focal spot size of 0.3 × 0.3 mm2. A second native data set was collected at LURE (Orsay, France) on DW32 beamline at 0.963 Å wavelength using a 345-mm MAR Research imaging plate. All data were collected in frames of 1.0 degree and processed with DENZO. The scaling was performed with SCALA (CCP4 (50)), and the derivative was merged with FIT2CALC. Data collection statistics are given in Table 1. The fact that most of the crystals diffracted only very poorly made the heavy metal derivative search laborious. In the end, it led to one mercury derivative diffraction to 3.6-Å resolution, isomorphous to the native crystal.

Phase Determination—The position of the heavy metal was determined using Patterson methods. The MLPHARE software program (51) was then used to refine the heavy atom parameters and calculate phases to 4.0 Å, taking into account both isomorphous and anomalous differences. Resulting single isomorphous replacement with anomalous scattering (SIRAS) phases were considerably improved after flattening 80% of the solvent using density modification. At this stage, the map indicated clearly that the handedness of the helices was wrong. Repeating the previous steps with the heavy atom position giving the negative coordinates yielded a suitable map for determining an envelope for each of the two molecules in the asymmetric unit. The noncrystalllographic symmetry operators were determined using both the GLRF program and the relation between the two molecular replacement solutions (automated molecular replacement density modification (52)) obtained from a search using a bones model (MAPMAN (53)) calculated on the basis of one molecule. The density modification program was then used again to improve the initial phases (from MLPHARE) by performing simultaneously solvent flattening, histogram matching, 2-fold noncrystallographic symmetry averaging, and phase extension. The best map was obtained when a suitable mask around one of the molecules was added to the program. The resolution of the derivative data set was actually better than that of the native one, and the map was therefore calculated using the derivative structure factor amplitudes with the phases extended to 3.6 Å.

Model Building, Refinement, and Analysis—The model was built with the program TURBO-FRODO (54) using the recently developed ab-initio building tools, which make it possible to build a model from planar pseudo-residues in a very short time. When the connectivity and the direction of the polypeptide chain have been determined, the pseudo-residues are automatically replaced by the actual residues in the sequence. Side-chain fitting is then performed manually. When most of the model had been built, a 3.0-Å native data set was collected on synchrotron radiation at the LURE. These better data were then used to calculate a new map, with which the model was completed (370 residues of 376 in the recombinant protein). The four sugar glycosylation sites were already clearly identified in this experimental electron density map. Refinement was carried out in X-PLOR (55) against the
3.0-Å data. Five percent of the data were set aside for calculating and monitoring of the free R-factor (R_free). Refinement involved cycles of simulated annealing using the slow-cool procedure interspersed with manual rebuilding. Noncrystallographic symmetry restraints were applied during the whole refinement procedure. Individual B-factor refinement was subsequently performed. The final model consisted of 6194 atoms; the final R-factor was 22.5%, and the R-free factor was 25.1%. The complete refinement statistics, given in Table I, the quality of the Ramachandran plot, and the electron density indicate that the model is better than expected for a structure at 3.0-Å resolution. Maps calculated from the derivative structure factor amplitudes showed a clear electron density for the mercury derivative bound to cysteine 244 but no significant movements of any part of the model. Fig. 1 was drawn up with Molscript (56) and Raster 3D (57), Fig. 2 with Clustal W (58) and Alscript (59), Figs. 3, 4, and 6 with Turbo-Frodo (54), and Fig. 5 with GRASP (60). The coordinates and structure factor amplitudes have been deposited in the Protein Data Bank with entry code 1HLG.

RESULTS AND DISCUSSION

After numerous trials using weakly diffracting crystal forms obtained from the native gastric lipases purified over the last 10 years from humans, rabbit, and dog (18), useful cubic crystals were finally obtained from rHGL expressed in the insect cell/baculovirus expression system. The structure of rHGL has been solved by a combination of SIRAS, solvent flattening, and 2-fold averaging at 4.5 Å resolution. Phase extension procedures have made it possible to extend the resolution to 3.6 Å. A preliminary model was constructed at this resolution. The final structure was refined to 3.0-Å resolution using restrained noncrystallographic symmetry between the two molecules. The crystallographic R-factor based on all the data between 15.0 and 3.0 Å is 22.6%, and the corresponding R-free is 25.1% with a model containing residues 9 to 53 and 57 to 379, 6 sugar residues located on the 4 potential N-glycosylation sites, and 46 water molecules/monomer.

Overall Structure—rHGL consists of one globular domain (Fig. 1) and belongs to the α/β hydrolase-fold family (1). The core domain, which is located between residues 9–183 and 309–379 (Figs. 1 and 2) contains a central β sheet composed of...
8 strands, 7 of which are parallel and 1 antiparallel (strand 2) with 1(-2)435678 connectivity, and 6 helices, 3 on each side of the β-sheet (Fig. 1C). Two segments are missing in the electron density maps. The first of these was from residues 4 to 9, because the electron density starts abruptly at residue 9. In the region of the second lacking segment (residues 54 to 56), some faint electron density was observed, suggesting that the loop may be intact but disordered (Fig. 1). The accessibility of this loop is consistent with the previously reported preferential trypsin cleavage site (Arg-55) of rabbit gastric lipase (35). In comparison with the canonical α/β hydrolase fold, an extra helix (α1) is present at the N terminus. Helix αA is shorter and has moved to the bottom of the structure, helix αB is replaced by two helices (αB1 and αB2), and helix αD is replaced by an extra domain (residues 184 to 308, Figs. 1 and 2) located between strands 6 and 7. Protrusions have been observed in other lipases, generally constituting the device covering the active site and called the lid. A “cap” domain occurs at the same location in wheat serine carboxypeptidase II (residues 181 to 311, WCSII) (36) and in human protective protein (residues 181 to 347, HPP) (37), two protease members of the α/β hydrolase-fold family. In HPL, the lid (237 to 261) lies between strands 8 and 9, and a β-sandwich domain extends the enzyme at the C terminus (336 to 449). In R. miehei lipase (4), a lid-containing extra domain formed by an α-helix and two short strands (82–109) can be observed between β4 and αB. The situation is more complex in C. rugosa lipase (6), where three protrusions have been observed that together cover the central core of the enzyme: between strands 1 and 2 (residues 30 to 98), between β6 and αE (residues 241 to 331), and between βD and αE (residues 339 to 415). In the latter enzyme, however, the lid covering the active site is composed of two helices belonging to the first protrusion and comprising residues 66 to 92.

The amino acid sequence of the enzyme includes four consensus N-glycosylation sites at Asn-15, -80, -252, and -308 (Fig. 2). Electron density patches attributable to the first sugar attached to Asn have been observed at all four sites (Fig. 1). GlcNAc residues were therefore introduced that nicely sustained the refinement. No electron density was observed in the case of the fucose residues attached at GlcNAc 1, in line with the fact that the expression of the enzyme was carried out in a weakly glycosylating cell type. The residual electron density made it possible to introduce a second GlcNAc residue at sites 80 and 252. Sites 15, 80, and 252 are located on one side of the molecule, whereas site 308 is on the other. Site 80 is located on the core of the protein, whereas site 252, which belongs to the cap domain, lies between helices αe5 and αe6. It is worth noting that the glycan chains on the above-mentioned sites are in close contact, which enhances the interactions between the core and the cap.

The amino acid sequence of HGL contains three cysteine residues, one of which is free, whereas the other two are in-
volved in a disulfide bridge (35, 38–40). Based on the present three-dimensional structure, the free cysteine can be unambiguously assigned to residue 244 (Fig. 3A), and the disulfide bridge, to residues 227–236 (Fig. 1), as previously suggested by Lohse et al. (40) using site-directed mutagenesis.

The Catalytic Machinery—In lipases, as well as in serine proteases, the catalytic machinery consists of a triad and an oxyanion hole (2, 4). The present three-dimensional structure of rHGL is also equipped with this machinery (Fig. 3B). The nucleophilic serine belonging to the usual consensus sequence G\textsubscript{X1}S\textsubscript{X2}G (X\textsubscript{1} and X\textsubscript{2} being His and Gln, respectively, see Fig. 2) is located at position 153, between β5 and αC. This Ser-153 has an ε conformation (41), which is a characteristic feature of all the enzymes within the α/β hydrolase-fold family (1). His-353 (between β8 and αF) and Asp-324 (between β7 and αE) are the other two residues forming the catalytic triad, as previously identified by site-directed mutagenesis (42). The rHGL triad is almost superimposable on all the other lipase catalytic triads. The nucleophilic serine is covered by the cap (184–308) and is therefore not freely accessible to lipidic substrate molecules (Fig. 1). The environment of the catalytic triad seems not to display any special features. However, one might expect His-353 to show local pK\textsubscript{a} decrease to be able to explain the acidic pH optimum of rHGL. There are no charged residues within a 10-Å sphere centered on the Ser-153 O\textsubscript{γ} atom. This absence makes it difficult to suggest the mechanism possibly responsible for the local pK\textsubscript{a} modulation.

Cys-244 was found to be very close to Ser-153 and His-353, and O\textsubscript{γ} and S\textsubscript{γ} are at a distance of only 5.2 Å. This cysteine, which is completely buried, has nevertheless permitted the binding of the mercury acetate derivative. In the presence of specific cysteine reagents (C12-docylthiobis-5,5'-dithiobis(2-nitrobenzoic acid) or 4,4'-dithiopyridine), it has been established that a single cysteine can react

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**Fig. 3. The active site.** A, stereo view of the electron density map around the active site residues Ser-153, His-353, and the single cysteine 244 (The density has been contoured at 1 σ level). B, representation of the HGL catalytic triad and of the oxyanion hole (Gln-154, Leu-67) superposed to the same part of HPL (green, in Cα tracing).
stoichiometrically with a concomitant loss of enzymatic activity (38). Another member of the acid lipase family (calf pregastric esterase) has a threonine at this position (Fig. 2). Furthermore, rHGL Cys-244 Thr mutant has been constructed, and no loss of activity was observed in the purified enzyme. In view of all the above findings, the possibility that cysteine 244 may participate directly in the catalytic mechanism can be ruled out. Based on the three-dimensional structure, however, the specific inhibition observed with sulfhydryl reagents was attributed to a steric hindrance occurring at the level of the active site (see Fig. 3A).

The oxyanion hole is a device that stabilizes the oxyanion transition state via hydrogen bonds with two main-chain nitrogen (2). It is often evidenced by using organophosphate or organophosphonate complexes in which the two main chain NH groups establish short hydrogen bonds with an oxygen belonging to the phosphonate group. In rHGL, the oxyanion hole has been identified on the basis of comparisons with other esterases or lipases. Within the α/β hydrolase-fold family, one mandatory component of the oxyanion hole is the NH group of the residue following the nucleophile Ser (Gln-154 in rHGL). The second NH group occupies a different position along the sequence but always originates from the same spatial region. In some lipases, the oxyanion hole is not preformed in the closed conformation but results from the concerted movements during the opening process of the lid or of a small loop bearing the nitrogen atom of the main chain. Comparisons between rHGL and the open form of the C11-phosphonate HPL-collipase ternary complex indicates that the second oxyanion NH group belongs to Leu-67 (Fig. 3B). The Ca tracing of the loop bearing Leu-67 is fully superimposable on that of the open HPL (Fig. 3B). It can therefore be concluded that the oxyanion hole of rHGL is preformed as in esterases, cutinase (43), and C. rugosa lipase (6). The second oxyanion hole residue can be of very diverse nature in lipolytic enzymes. R. miehei lipase (4) or cutinase (43) possess a polar noncharged residue, Ser-82 and Ser-42, respectively. Furthermore, the Ser-42 side chain in cutinase has been found to be a third essential component of the oxyanion hole (44). It seems, however, that the Leu-67 side chain in rHGL may play a similar role to that of Phe-77 in the open form of HPL, leading to an exquisite interaction with the alkyl group of the phosphonate inhibitor (see below).

The Cap and the Putative Lid—The cap domain fold (residues 184–308) is an intricate mixture of 8 helices, turns, and random coils (Figs. 1 and 2). When this domain is removed from rHGL on a display, the active site becomes accessible to solvent. An apparently sufficient degree of accessibility can be achieved, however, by removing a shorter stretch of residues between residues 215 and 244 (Fig. 4). The flanking residues of this stretch are Pro-214 and Gly-245. It has been suggested that the trans-cis-proline isomerization may be one of the main factors involved in the C. rugosa lipase lid conformational changes (45). This, together with the flexibility of the glycine residue, suggests that these residues might act as hinges in the opening of the lid. It seems unlikely, however, that this lid displacement might occur as a rigid body movement, as described previously in R. miehei lipase (4). Given the complex folding of this lid, the opening of rHGL is probably a more complex process than those described previously. Cysteine 244 would therefore be the last residue in the lid, before the hinge. The resulting displacement of the chemically modified Cys-244, which occurs upon the lid opening, might therefore be too limited to abolish the steric hindrance during the formation of the lipase-substrate complex.

Cap domains of this kind have been found to have the same topology in WSCI (36), and in the related HPP, a serine carboxypeptidase zymogen occurring in lysosomes (37). In HPP, the protruding domain is located, as in rHGL, between strands 6 and 7, comprising residues 181–347. The putative excision peptide is thought to be situated between residues 285 and 298. When this excision peptide is not removed, the active-site serine remains covered (Fig. 4A), in the same way as the putative lid covers the rHGL serine (Fig. 4B). In both cases, the rest of the cap domain forms a ring around the active site, ensuring the substrate specificity in the case of HPP and the lipid affinity in the case of rHGL. In the framework of the above hypothesis, the lid remains covalently attached to the body of rHGL.
after opening, in contrast to HPP.

Active-site Accessibility and Model of a Phosphonate Complex—On the water-accessible surface of rHGL, a large number of cavities that are accessible to water can be observed in the structure (not shown). These are predominantly located at the interface between the core and the cap domains, which suggests that their packing may be imperfect. Of particular interest is the small cavity (23 Å³) located above the active-site serine. Another cavity of 115 Å³, lined by residues 274 to 278 and 284 to 287, makes it possible for the solvent to gain access to Ser-153, provided that side-chain displacements can occur.

The question therefore arises as to whether or not the above channels might make it possible for substrates or inhibitors to reach to the active site. There exist several arguments against this hypothesis. It is in fact impossible for a bulky long chain triglyceride to travel through these channels to be accommodated in the closed active site. When the putative lid domain was removed on the display, from residues 215 to 244, a large hydrophobic surface appears around the active site and can act as a lipid binding site (Fig. 5). As observed in the three-dimensional structures of open lipases, this hydrophobic surface may face the lipid interface after the lid opening. Accordingly, the complementary surface part of the lid domain is also composed of hydrophobic residues facing the putative lipid binding site in the closed form of rHGL.

When positioning the two enantiomers of the C11-phosphonate inhibitor, keeping their spatial position as observed in the three-dimensional structure of the HPL-C11-phosphonate complex, it was noted that both molecules fit well against the bottom of the active-site cleft (Figs. 5B and 6). The C11 enantiomer corresponding to the hydrolyzable triglyceride acyl chain fits nicely into the putative acyl binding site and interacted only with hydrophobic residues (Fig. 6). The other phosphonate enantiomer is surrounded by a more polar environment, as in the HPL-C11 phosphonate complex.

The Lysosomal Acid Lipase—HLAL displays 59% identity and 75% homology with HGL, without any insertion or deletion (Fig. 2). A straightforward homology model of HLAL was built, and this model was examined in the light of the mutations, resulting in a clinical phenotype in humans. Besides mutants bearing large deletions, few single mutations associated with cholesteryl ester storage disease have been described. The deletion of fragment 205 to 253 and fragment 254 to 277 affect the cap domain (29, 31, 32, 46). It is possible that the residual activity reported in the latter study (5–10%) might be because of the fact that the α/β hydrolase fold of the catalytic domain has remained intact. The mutation Leu-273 → Ser creates a new potential glycosylation site (NMS), and this mutant expressed in HeLa cells has been found to have a higher molecular mass than that of the wild type HLAL expressed in the same system (29, 30). This residue is water-exposed, and the bulky glycan moiety may prevent either the substrate binding or the movement of the lid. The mutation Leu-336 → Pro is located just inside helix αE and probably destabilizes its structure, leading to an inactive enzyme as initially proposed by Seedorf et al. (32). The mutation Gly-66 → Val is easily interpretable in structural terms, because the valine side chain clashes with the active-site serine 153 and also partly blocks the putative triglyceride binding site, on similar lines to what has been found to occur with pancreatic lipase RPI (47).

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