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Mutation of Tryptophan Residues in Lipoprotein Lipase

EFFECTS ON STABILITY, IMMUNOREACTIVITY, AND CATALYTIC PROPERTIES*

(Received for publication, August 6, 1996, and in revised form, September 22, 1996)

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Previous studies had pointed to an important function of a putative exposed loop in the C-terminal domain of lipoprotein lipase for activity against emulsified lipid substrates. This loop contains 3 tryptophan residues (Trp³⁹⁰, Trp³⁹³, and Trp³⁹⁴). We have expressed and characterized lipase mutants with tryptophan to alanine substitutions at positions 55, 114, 382, 390, 393, and 394 and a double mutant at residues 393 and 394. The substitutions in the N-terminal domain (W55A and W114A) led to poor expression of completely inactive lipase variants. Heparin-Sepharose chromatography showed that mutant W114A eluted at the same salt concentration as inactive wild-type monomers, indicating that this substitution prevented subunit interaction or led to an unstable dimer. In contrast, all mutants in the C-terminal domain were expressed as mixtures of monomers and dimers similarly to the wild-type. The dimers displayed at least some catalytic activity and had the same apparent heparin affinity as the active wild-type dimers. The mutants W390A, W393A, W394A, and W393A/W394A had decreased reactivity with the monoclonal antibody 5D2, indicating that the 5D2 epitope is longer than was reported earlier, or that conformational changes affecting the epitope had occurred.

The mutants W390A, W393A, W394A, and W393A/W394A had decreased catalytic activity against a synthetic lipid emulsion of long-chain triacylglycerols (Intralipid[®]) and in particular against rat lymph chylomicrons. The most pronounced decrease of activity was found for the double mutant W393A/W394A which retained only 6% of the activity of the wild-type lipase, while 70% of the activity against water-soluble tributyrilglycerol was retained. In the case of chylomicrons also the affinity for the substrate particles was lowered, as indicated by severalfold higher apparent K_m values. This effect was less prominent with the synthetic lipid emulsion.

We conclude that the tryptophan cluster Trp³⁹⁰-Trp³⁹³-Trp³⁹⁴ contributes to binding of lipoprotein lipase to lipid/water interfaces. Utilizing different lipid substrates in different physical states, we have demonstrated that the tryptophan residues in the C-terminal

domain may have a role also in the productive orientation of the enzyme at the lipid/water interface.

Lipoprotein lipase (LPL)¹ belongs to the mammalian triacylglycerol lipase gene family, which also contains pancreatic lipase and hepatic lipase (1). Based on the crystal structure of pancreatic lipase and sequence homologies, it can be assumed that LPL has a similar three-dimensional structure with an N-terminal folding domain containing the active site and a C-terminal folding domain consisting of β -structures (2–4). Active LPL is a noncovalent dimer, which readily dissociates into inactive monomers (3–6). A site for interaction with the low density lipoprotein receptor-related protein (LRP) has been localized to the C-terminal domain of LPL (7, 8). This region is also involved in the interaction with lipoproteins (9). The LRP-binding site and the lipoprotein-binding site were found to partly interfere with each other (10). Important structures for both sites appear to be present in, or close to, a predicted exposed loop in the C-terminal domain round amino acid residue 388. This region is sensitive to chymotrypsin (9). Cleavages of the peptide bonds between Phe³⁸⁸-Ser³⁸⁹ and Trp³⁹⁰-Ser³⁹¹ in bovine LPL (two residues longer than human LPL) resulted in reduced activity with synthetic emulsions of long-chain triacylglycerols, and activity against chylomicrons was totally abolished (9). It was shown that the truncated LPL did not bind to chylomicrons. In contrast, the cleavage had little effect on the activity toward short-chain substrates like tributyrilglycerol (9).

In the present study we have investigated the role of tryptophan residues in the C-terminal domain of LPL for binding to lipid substrates. We chose to replace the tryptophans by alanine, since it is known that these residues are well suited to mediate interactions to a lipid/water interface (11) and since there is a cluster of presumably exposed tryptophan residues in the C-terminal domain of LPL.

EXPERIMENTAL PROCEDURES

Materials—Lipoprotein lipase was purified from bovine milk (12). Human apolipoprotein CII was isolated from plasma by adsorption to Intralipid[®], followed by gel filtration and ion exchange chromatography as described (12). Intralipid[®] (10%) was from KABI Pharmacia Parenterals, Stockholm, Sweden. Heparin-Sepharose was made as described previously (13). [9,10-³H]Trioleoylglycerol was synthesized by Dr. Lenart Krabish, Lund University, Lund, Sweden. The labeled trioleoylglycerol was incorporated into Intralipid[®] 10% by custom emulsion preparation at Pharmacia Hospital Care, Stockholm, Sweden. Tributyril glycerol and bovine serum albumin (fraction V) were from Sigma. Labeled tributyril glycerol (glyceryl-tri-[1-¹⁴C]butyrate, 8.0 mCi/mmol) was custom synthesized by American Radiolabeled Chemicals, Inc., St.

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¹ The abbreviations used are: LPL, lipoprotein lipase; ELISA, enzyme-linked immunosorbent assay; LRP, LDL receptor-related protein; PL, pancreatic lipase; HL, hepatic lipase.

Louis, MO. Rat lymph chylomicrons, labeled *in vivo* in triacylglycerols by feeding ^{14}C -labeled oleic acid to cannulated rats, were kindly provided by Dr Roger Savonen, Department of Medical Biochemistry and Biophysics, Umeå University. The chylomicrons were recovered after ultracentrifugation as described previously (14). The concentrations of triacylglycerols in the preparations were measured using the Triglycerides/GB Kit (no. 450032) from Boehringer Mannheim. The 5D2 monoclonal antibody against bovine LPL was a generous gift from Dr. John Brunzell, Department of Medicine, University of Washington, Seattle, WA. Chicken antibodies against bovine LPL were made as described previously (15). Peroxidase-labeled rabbit anti mouse IgG (A2554) was from Sigma. Altered Sites mutagenesis kit was from Promega. Dulbecco's modified Eagle's medium was obtained from Life Technologies, Inc. pcDNA1 expression vector was from Invitrogen.

Site-directed Mutagenesis and Transient Expression in COS Cells—A 2.4-kilobase *Pst*I-*Xba*I fragment from a lipoprotein lipase clone (a kind gift of Dr. R. Lawn, Genentech Corp.) extending from nucleotides 1 to 2413, was cloned into the pSelect phagemid vector (Promega). Site-directed mutagenesis was performed with the Altered Sites site-directed mutagenesis kit from Promega. C-terminal deletion mutants were constructed by replacement of tryptophan codons with alanine codons using the following primers: M55A, 5'-ATCCATGGCGCCACG-GTAACA-3'; M114A, 5'-TTTATCAACGCCATGGAGGAG-3'; M382, 5'-AAGCTCAAAGCCAAGAGTGAT-3'; M390A, 5'-TACTTTAGCGCCT-CAGACTGG-3'; M393, 5'-TGGTCAGACGCCTGGAGCAGT-3'; M394A, 5'-TCAGACTGGGCCAGCAGTCCC-3'; M393/4A, 5'-TGGTCAGACG-CCGCCAGCAGTCCC-3'.

Following mutagenesis, the LPL cDNA was cloned into an eukaryotic expression vector (pcDNA1, Invitrogen). COS cells were cultured in 12-cm plates until 60–70% confluence was reached. Transfection was performed using the DEAE-dextran method as described previously (16). At 24 h post-transfection, heparin (0.25 unit/ml) was added. Media were collected 24 h later and stored at -70°C .

Isolation of LPL from Culture Media by Adsorption to Heparin-Sepharose—Media were collected 48 h after transfection and were immediately frozen at -70°C . Available amounts of each expression medium (50–240 ml) were passed over small columns of heparin-Sepharose (2 ml) at 4°C . To avoid the risk of contamination, a new gel was used for each LPL variant. The flow rate was 1 ml/min, and binding of LPL activity was almost complete. The columns were washed with 35 ml of 20 mM Tris-Cl, pH 7.4 containing 20% (w/v) glycerol and 1 mg of bovine serum albumin/ml. Elution was by linear gradients from 0 to 1.6 M NaCl in the same buffer (50 ml + 50 ml). Fractions of 5 ml were collected. They were immediately analyzed for activity against the sonicated Intralipid-based substrate to localize the peaks of active dimers, and were then frozen at -20°C . Recovery of LPL activity over the columns was between 60 and 70%, and recovery of LPL protein mass was usually around 75%. The recoveries were higher when higher amounts of starting material was available (volume of expression medium and expression level).

Assays for LPL Activity—Four different assays were used. One was described previously and is based on Intralipid into which ^3H -labeled trioleoylglycerol is incorporated by sonication (17). This assay was used for analyses of column fractions. In another assay the only difference was that the emulsion was custom prepared at Pharmacia Hospital Care, where a small batch of Intralipid[®] 10% was made also containing ^3H -labeled trioleoylglycerol. The advantage of this emulsion is that it is stable for years if properly stored, and it is therefore better suited for comparative kinetic studies than the sonicated emulsion. A third assay used rat chylomicrons as substrate. Both the second and the third assay contained (in a total volume of 200 μl): triacylglycerols from Intralipid or from chylomicrons corresponding to 1 mg/ml unless otherwise stated, 0.15 M Tris-Cl, 50 mg of bovine serum albumin/ml, 15 IU heparin/ml, 0.15 M NaCl at pH 7.4. The fourth assay with tributyrin glycerol contained (in a total volume of 200 μl): 0.15 M Tris-Cl, 0.15 M NaCl, 15 IU heparin/ml, 2.0 mg of bovine serum albumin/ml at pH 8.5. For the assays against long-chain triacylglycerols, the fatty acids were extracted based on the method of Dole as described (17). For the tributyrin assay, the released fatty acids were extracted according to the method of Belfrage and Vaughan (18).

All activity measurements were made within 2 weeks after purification of the LPL dimers. During this time no appreciable loss of catalytic activity occurred as compared with the initial assay before storage of the fractions at -20°C . Comparative analyses of activities against the three different substrates were made on the same day and on material that had only been thawed once.

Immunoassays for LPL Mass—LPL protein in culture media and in column fractions was measured by two different enzyme-linked immu-

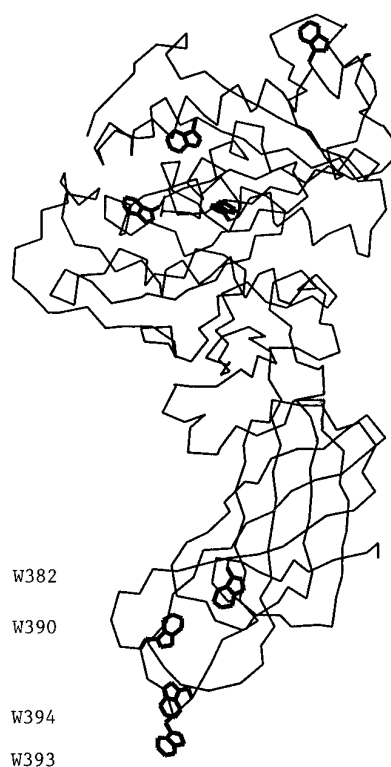


FIG. 1. Distribution of tryptophan residues in LPL. The model of van Tilbeurgh *et al.* was used (3). Side chains of the tryptophans are represented as double rings on the backbone of the protein. There are two tryptophan-rich areas: the region surrounding the active site in the N-terminal (upper) domain and the exposed loop between the stacked β -sheets in the C-terminal (lower) domain.

noassays (ELISAs). In both affinity-purified chicken IgG (chicken 225) was used for capture of the antigen. Monoclonal 5D2 antibodies were used together with peroxidase-labeled rabbit anti-mouse IgG for one ELISA as described previously (19), while affinity-purified and peroxidase-labeled IgG from chicken 224 were used for detection in the second ELISA (20). For each measurement three dilutions of each sample were analyzed, and usually a mean value from these three measurements was used. Purified human LPL from post-heparin plasma was used as standard in both assays.

Calculations—For curve fitting by nonlinear regression and calculations of values for V_{\max} and K_m , the program FigP (BIOSOFT, Cambridge, United Kingdom) was used.

RESULTS

Expression of Mutant and Wild-type LPL—Fig. 1 shows a model of LPL based on the structure of pancreatic lipase (3). Inspection of the model, in which tryptophan residues are highlighted, reveals that there are two regions which are enriched in tryptophan residues. One is located in the N-terminal domain, around the catalytic site, while the another is located in the C-terminal domain, close to and in an exposed surface loop. For the present study the following tryptophan residues in the C-terminal domain were replaced by alanine residues: Trp³⁸², Trp³⁹⁰, Trp³⁹³, and Trp³⁹⁴. A double mutant, W393A/W394A, was also constructed. In addition, two of the residues in the N-terminal domain, Trp⁵⁵ and Trp¹¹⁴, were replaced by alanine residues. From the model it appeared as if all the targeted tryptophan residues were exposed on the surface of the molecule. Both wild-type LPL and the mutants were transiently expressed in COS cells. The expression levels varied from 26 ng/ml culture medium to 596 ng/ml (Table I). The lowest expression levels were obtained with mutant W114A, and the highest were obtained with wild-type LPL.

Heparin-Sepharose Chromatography of Wild-type and Mutated LPL Variants—To allow comparison of specific catalytic

activities of the different mutants, we purified and concentrated LPL dimers from the culture media by chromatography on heparin-Sepharose (Fig. 2). Fractions from the columns were analyzed for LPL activity and LPL mass, to localize the peaks of active dimers eluting later in the gradients well separated from inactive LPL monomers, which eluted in an earlier peak. Some of the substitutions affected the reactivity with the 5D2 monoclonal antibody, and we therefore made parallel assays using a polyclonal antibody for detection as well, as was described previously (20). For most mutants the monomer/dimer ratio was slightly altered as compared with wild-type LPL. Interestingly, mutant W382A appeared to be more stable than the others, since it gave mostly dimers (Fig. 2B). Pilot experiments showed that all mutants except W55A and W114A

had catalytic activity. Separation of the expression medium for W114A revealed that it contained only inactive LPL monomers (not shown). Therefore mutants W55A and W114A were not further studied.

Reactivity with the Monoclonal Antibody 5D2—It is clear from Fig. 2 (C and D) that W390A and for W393A/W394A reacted poorly with the monoclonal 5D2, while the immunoreactivity of wild-type LPL and W382A was similar in the two ELISAs (Fig. 2 and Table II). This is interesting since the substituted residues are outside the proposed epitope (residues 396–405) for 5D2 (21). Additionally, mutants W393A and W394A showed decreased reactivity to 5D2 as compared with the polyclonal chicken IgG (Table II). Therefore, in the experiments described below, only data from the polyclonal ELISA were used for calculations of specific catalytic activities.

Catalytic Activity against Long-chain Triacylglycerols—Since we knew that some of the mutations were likely to interfere with the lipid-binding ability of LPL, and thus with the apparent substrate affinity, comparisons of the catalytic efficiencies were made as a function of substrate concentration in assays with Intralipid^R and with the rat chylomicrons. Replacements of residues Trp³⁹⁰, Trp³⁹³, and Trp³⁹⁴ by alanine dramatically decreased the catalytic efficiency of LPL. The double mutant W393A/W394A was the least active both with the synthetic lipid emulsion (Intralipid) and with rat lymph chylomicrons. In contrast, with both substrates the specific activity of mutant W382A was higher than that of wild-type LPL. Mutant W382A was about 30% more active against both

TABLE I
Expression levels of LPL variants in COS cells as determined with the polyclonal ELISA

The media were collected 48h after transfection, and the listed numbers represent different preparations.

LPL variant	LPL mass
	ng/ml medium
Wild type	228, 301, 417, 319, 596, 252
W382A	245
W390A	267, 207, 324, 252
W393A	287, 135
W394A	313, 354, 396, 316, 140
W393A/W394A	198, 364, 183
W114A	26

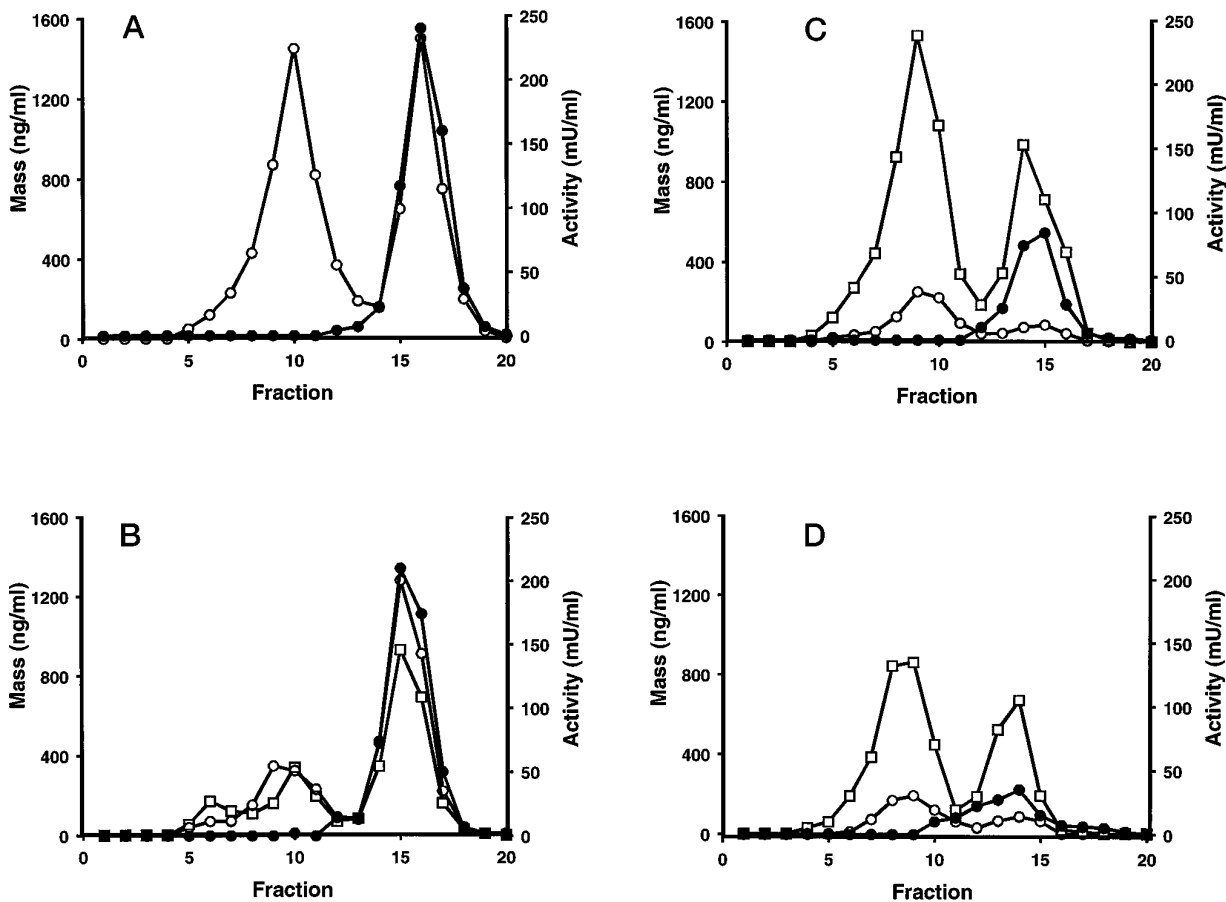


FIG. 2. Purification of the expressed wild-type and mutant LPL variants on heparin-Sepharose. The expression media were applied on a heparin-Sepharose column and eluted by a gradient of NaCl as described under "Experimental Procedures." The activity (●) was measured using Intralipid^R as substrate. The LPL mass in the fractions was determined using ELISA with the monoclonal antibody 5D2 (○) or with the polyclonal chicken antibody (□). Panels A, B, C, and D represent purification of wild-type LPL and mutants W382A, W390A, and W393A/W394A, respectively. For the wild-type enzyme (A), the mass was, in this experiment, only determined with the 5D2 assay. Other experiments showed that it reacted equally well in the polyclonal ELISA.

Intralipid^R and chylomicrons than wild-type LPL. The mutants W390A, W393A, and W394A had lower activity with Intralipid^R as substrate than with chylomicrons. This was true also for the double mutant W393A/W394A, which had only 6% of the activity against the synthetic emulsion as compared with wild-type LPL.

Catalytic Activity against Short-chain Triacylglycerols—It was previously known that the activity of LPL against short-chain triacylglycerols, like tributrylglycerol, was not affected by truncation of the lipase in the C-terminal domain (9). It was concluded that the truncation affected the lipid-binding ability of LPL or its ability to orient itself at a lipid/water interface. We therefore investigated here whether replacement of tryptophans for alanine residues in the C-terminal domain of LPL had any effect on the activity against tributrylglycerol (Table III). This substrate is much more soluble than the long-chain triacylglycerols and can therefore be used at non-aggregating substrate concentrations (here we used 0.09 mM). At higher concentrations (>0.4 mM at 37 °C) tributrylglycerol forms aggregates, but presumably with other interfacial properties than those of long-chain triacylglycerols and phospholipids.

At 0.09 mM tributryl glycerol, all mutants were more than 70% active compared with wild-type LPL and the activity for the double mutant W393A/W394A was more than 85% of that of the wild-type (Table III). Thus, the low catalytic activities with the long-chain triacylglycerol substrates were not due to general loss of catalytic function.

At 2.5 mM tributrylglycerol, the pattern changed somewhat and the double mutant W393A/W394A had the lowest specific activity, which was 58% of the specific activity of wild-type LPL (Table III). Interestingly, also with tributrylglycerol, the mutant W382A was more active than wild-type LPL (about 15%). We cannot rule out the possibility that this was due to an underestimation in the mass measurement of W382A. It is notable that in this system the specific activities of wild-type LPL, even with the higher substrate concentration, are only a

few percent of that with emulsions of long-chain triacylglycerols. This is not the case when tributrylglycerol is emulsified with gum arabic and the released fatty acids are determined by titration (9). That type of assay is, however, not sensitive enough for the amounts of lipase used here. A summary of the relative activities of all the mutants against the different substrates is shown in Fig. 3.

Comparison of the Apparent Affinities for Lipid/Water Interfaces—The apparent K_m values of lipases for substrate emulsions are thought to reflect the affinity of the lipase for the lipid/water interface, rather than its affinity for individual substrate molecules at the interface (22). The substrate saturation curves (data not shown) were used for calculation of apparent K_m values for chylomicrons and for Intralipid^R, respectively (Table III). With Intralipid^R there were only small differences for the different LPL variants. Thus, the large effect on the specific catalytic activity of, e.g., W393A/W394A could not be explained by a change in the apparent affinity for the emulsion droplets. With chylomicrons the K_m values for wild-type LPL and for the mutant W382A were similar and comparable with those obtained with Intralipid^R. With the other mutants there was a 5–8-fold increase in the K_m values as compared with wild-type LPL, indicating that the abilities of these mutants to bind to the chylomicron surface was reduced.

DISCUSSION

Tryptophan residues are well suited to mediate interactions of proteins with lipid/water interfaces due to their amphipathic properties (11). Their structure with a large hydrophobic double ring and a nitrogen atom able form hydrogen bonds allows both hydrophobic and hydrophilic interactions. It is known that tryptophan residues play an important role in the stabilization and conformation of transmembrane helices in several integral membrane proteins (11).

Molecular modeling of LPL on the structure of pancreatic lipase reveals that there are two regions of exposed tryptophan residues (3). One is located in the N-terminal domain, around the catalytic site. Because of their location, these residues might contribute to the interaction of LPL with lipid/water interfaces (23). The second region is located in the C-terminal domain close to and in an exposed loop. This loop is sensitive to chymotrypsin (24). Cleavage of the peptide bonds between Phe³⁸⁸-Ser³⁸⁹ and Trp³⁹⁰-Ser³⁹¹ generates a truncated form of LPL, in which catalytic activity against relatively soluble substrates is retained while activity against chylomicrons is abolished (9). According to the model of van Tilbeurgh *et al.* (3), this exposed loop is located in the same plane as the part of the N-terminal domain that is likely to bind to lipid. There is evidence that the two domains cooperate for proper lipid binding and orientation of LPL at lipid/water interfaces (4, 23). Disturbances of the structure of the lid covering the entrance to the active site, either by cleavage with trypsin (24) or by site-

TABLE II

Effects of substitutions of tryptophan residues in LPL on the reactivity with the 5D2 monoclonal antibody

The reactivity in the 5D2 ELISA is expressed as percent of the reactivity in the polyclonal ELISA. Values are taken from peak fractions of the chromatographies of expression media on heparin-Sepharose (Fig. 2, and data not shown).

LPL variant	Reactivity with monoclonal 5D2	
	LPL monomer	LPL dimer
	(%)	
Wild type	100	100
W382A	109	120
W390A	22	14
W393A	80	78
W394A	43	17
W393A/W394A	20	11

TABLE III

Specific activities and apparent K_m values of wild-type and mutant LPL variants against different lipid substrates

The LPL variants were purified by chromatography on heparin-Sepharose (as in Fig. 2) and fractions containing dimeric LPL were assayed for mass and activity as described under "Experimental Procedures." The catalytic constants, V_{max} and K_m , for activity against Intralipid^R and against chylomicrons were calculated by nonlinear regression from curves spanning 6–8 different substrate concentrations. TB, tributrylglycerol.

LPL variant	Intralipid ^R		Chylomicrons		LPL activity	
	V_{max}	K_m	V_{max}	K_m	TB (0.09 mM)	TB (2.5 mM)
	units/mg	mg/ml	units/mg	mg/ml	units/mg	units/mg
Wild type	164 ± 11	0.7 ± 0.1	320 ± 19	0.4 ± 0.1	0.46	4.5
W382A	217 ± 22	1.4 ± 0.2	409 ± 29	0.7 ± 0.2	0.52	5.2
W390A	53 ± 8	5.6 ± 1.9	188 ± 42	1.0 ± 0.3	0.33	3.5
W393A	65 ± 9	3.4 ± 1.1	139 ± 27	1.0 ± 0.2	0.35	3.8
W394A	81 ± 10	3.2 ± 0.8	207 ± 38	1.1 ± 0.3	0.39	3.2
W393A/W394A	10 ± 2	4.1 ± 1.3	45 ± 8	0.9 ± 0.3	0.40	2.6

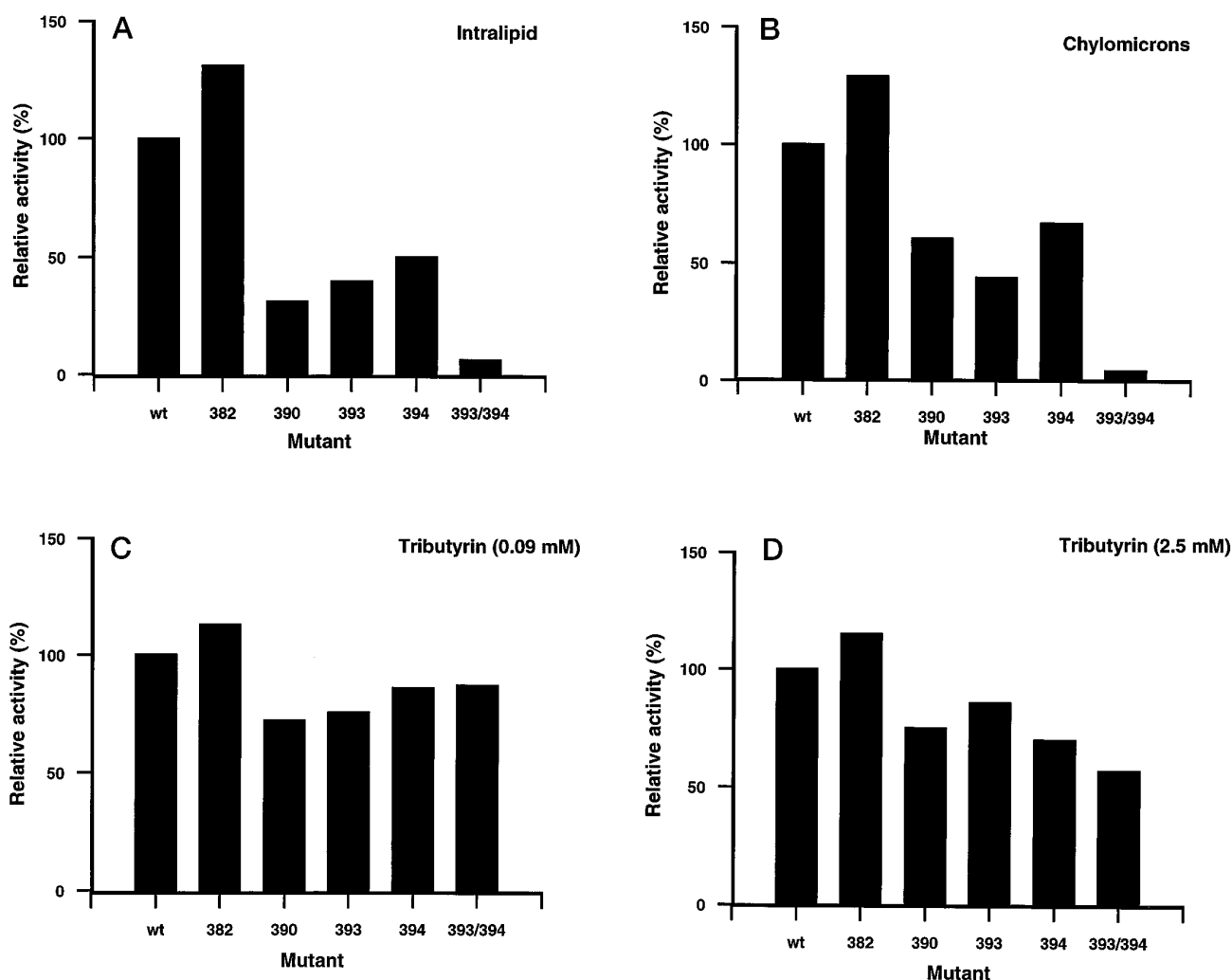


FIG. 3. Relative activities for the tryptophan mutants as measured with different lipid substrates. The following substrates were used: Intralipid^R (A), chylomicrons (B), tributyrilglycerol at low concentration (C), and tributyrilglycerol at high concentration (D). Relative activity is defined as the ratio of activities of an LPL mutant and the wild-type LPL, when determined under same conditions. In the case of Intralipid^R and chylomicrons, the V_{max} values from the fitted curves were used.

directed mutagenesis (25), have similar effects on the catalytic activity of LPL as disturbances of the structure in the C-terminal domain.

We found that the mutations of tryptophan residues in the N-terminal domain led to expression of inactive LPL. In the case of W114A, only low amounts of LPL monomers were expressed. The absence of activity of the W55A mutant is probably because this residue is in direct vicinity of the catalytic site, and, according to the model of LPL, it is involved in the formation of the oxyanion hole (3). The W114A is predicted to be part of an exposed helix, spanning residues 95–115. In this location, this residue is not likely to be involved in direct interactions at the catalytic site, but it could be necessary for the proper folding or for subunit interaction. The heparin affinity of the N-terminal mutants was decreased and comparable with the wild-type monomer. Previous experiences have shown that the conformation of active dimeric LPL is sensitive to changes in the N-terminal domain, and mutations here usually lead to expression of inactive monomeric enzyme (23).

Heparin-Sepharose chromatography of the C-terminal substitution mutants showed that all mutants were able to form active dimers, although the monomer/dimer ratio varied and was slightly lower for all than for wild-type LPL except for W382A. The reactivity with the 5D2 monoclonal antibody was significantly decreased for all C-terminal mutants, with the

exception of W382A. This may indicate that these exchanges cause small local conformational changes that are propagated into the 5D2 epitope, or that the mapped epitope on LPL for 5D2 (residues 396–405) is longer than previously reported (21).

Assays with long-chain triacylglycerols as substrate (Intralipid^R or chylomicrons) revealed that mutants W390A, W393A, and W394A and the double mutant W393A/W394A had significantly lower specific catalytic activities than the wild-type LPL. The double mutant exhibited the lowest activity, with only 6% left as compared with wild-type LPL. The activities obtained with rat chylomicrons, the physiological substrate of LPL, were in agreement with those obtained with the synthetic emulsion Intralipid^R. Overall, the specific activities for chylomicrons were about 2-fold higher than those for Intralipid^R, demonstrating that chylomicrons are more efficient as substrate. One explanation for this could be that the synthetic emulsion may contain excess phospholipids in the form of liposomes, which can compete for binding of LPL (26).

The relative activity of the W382A mutant as compared with wild-type LPL was in the same range for Intralipid^R as for tributyrilglycerol, suggesting that Trp³⁸² is not crucial in binding/docking to a lipid/water interface. The mutants W390A, W393A, and W394A and the double mutant W393A/W394A had considerably higher relative activity against tributyrilglycerol than against Intralipid^R and chylomicrons, demon-

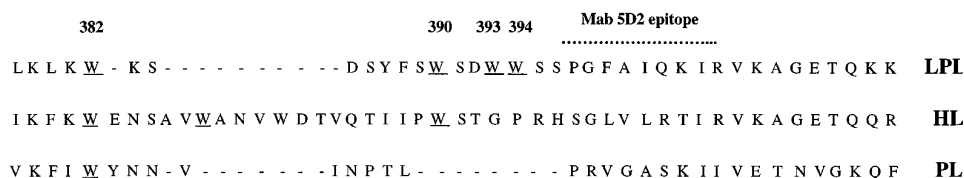


FIG. 4. Sequence comparison for the C-terminal parts of triglyceride lipases. Sequences around the loop region of the C-terminal domains are presented for human LPL, PL, and HL. Tryptophan residues are *underlined*. The previously published epitope in LPL for the monoclonal antibody 5D2 is indicated (21).

strating that these exchanges severely affected the binding/docking of LPL to emulsions of long-chain triacylglycerols. In all cases more than 70% of the activity remained against tributyrilglycerol, indicating that all the mutants retained most of their active site function.

The increased specific activities against tributyrilglycerol under aggregating conditions (2.5 mM), as compared with activities under water-soluble conditions (0.09 mM), presumably demonstrate the effect of interfacial activation on the catalytic activity (22). The enhancement was about 10-fold for wild-type LPL and for all mutants, except for the double mutant. This indicates that the double mutant has reduced ability to benefit from interaction with a water/lipid interface. A similar tendency was also seen for the W394A mutant.

The calculated apparent K_m values of the LPL variants with the long-chain lipid substrates showed that the values were increased for all mutants as compared with wild-type LPL. While for Intralipid^R the apparent decrease in lipid affinity was moderate for all mutants, the apparent affinities for chylomicrons were lowered severalfold by replacement of the tryptophans. Our data indicate that binding/docking to chylomicrons is more difficult than binding to Intralipid^R. The moderate decrease in apparent lipid affinity (4–8-fold) compared with the marked decrease in maximal specific activities under saturating conditions for both Intralipid^R and for chylomicrons suggests that the major effect of the tryptophan substitutions was on the productive orientation of the lipase at the lipid/water interface rather than on the binding/docking to the interface. Williams *et al.* (8) found that replacement of Trp³⁹³ or Trp³⁹⁴ by Ala in C-terminal fragments of LPL spanning residues 313–348 completely abolished the ability of the fragments to bind to lipoproteins.

LPL is a member of the mammalian lipase gene family, consisting also of pancreatic lipase (PL) and hepatic lipase (HL) (1, 3). We compared the location of the tryptophans in the C-terminal domain of PL and HL with that of LPL (Fig. 4). This revealed that the tryptophan cluster involving residues 390–394 in LPL is not conserved in PL. Interestingly, the crystallographic studies of van Tilbeurgh *et al.* (27) on PL and colipase in the presence of lipid micelles showed that binding to lipid was mediated by the lid structure together with hydrophobic regions of colipase. The C-terminal region in PL, corresponding to residues 390–394 in LPL, interacted with colipase and not directly with the lipid. Inspection of the C-terminal domain of HL revealed that residues Trp³⁹³ and Trp³⁹⁴ were not conserved, while tryptophans corresponding to Trp³⁸² and Trp³⁹⁰ in LPL were also present in HL. In HL there is an insertion of 8 amino acid residues between residue Ser³⁸⁵ and Asp³⁸⁶ in LPL. This inserted part contains 2 tryptophan residues. Interestingly, activity against triolein for a chimera consisting of the N-terminal domain of LPL (residues 1–335) and the C-terminal domain of HL (residues 330–476) was significantly lower than activity of wild type LPL (28), demonstrating the importance of the C-terminal domain for activity toward insoluble substrates. For both HL and for LPL, the positions of the tryptophan residues in this region of the C-terminal domain are conserved

between different animal species. The apparent importance of the specific orientation of the LPL protein at the lipid/water interface implies that the C-terminal domain, aside from binding to lipid, may also influence substrate specificity. This was supported by a study of Davis *et al.* (29), who showed that exchanging the C-terminal domains of LPL and of HL resulted in altered specificities for phospholipids. Dugi *et al.* (30) have shown that the same applies also to differences in the lid structure of the N-terminal domain.

The effect of replacements for tryptophan on the lipid binding and catalytic properties of LPL were more prominent than effects of the same on the ability of LPL to mediate uptake of lipoproteins in cells, presumably via LRP. In a study by Krapp *et al.* (20), mutants W390A and W393A were shown to be as effective as wild-type LPL. This was also the case for a mutant in which residues 392 and 393 were replaced by those in HL. A reduced ability was shown only for an LPL variant in which residues 390–393 were deleted, but the deletion may have caused also conformational changes affecting other regions.

In summary we conclude that tryptophan residues in the C-terminal domain of LPL are involved in the binding of the enzyme to lipid/water interfaces. We postulate that, in addition to binding, the C-terminal domain is important for the specific orientation of the lipase at the interface and that this orientation may influence both the catalytic efficiency and the substrate specificity.

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