rapid communication

Identification of a heparin-binding domain in the distal carboxyl-terminal region of lipoprotein lipase by site-directed mutagenesis

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Abstract **The interaction of lipoprotein lipase (LPL) with heparan sulfate proteoglycans plays an important role in the metabolism and catalytic function of the enzyme. We have used site-directed mutagenesis to replace the basic residues contained in a discontinuous charge cluster (residues Lys 321, Arg 405, Arg 407, Lys 409, Lys 415, and Lys 416) of avian LPL with asparagine. The mutant proteins were expressed in Chinese hamster ovary cells and their affinity for heparin was evaluated by heparin-Sepharose chromatography. Mutation of residues Lys 321, Arg 405, Arg 407, Lys 409, and Lys 416 resulted in a decrease in affinity for heparin. The triple mutant LPL(R405N, R407N, K409N) possessed almost no high-affinity binding. The LPL mutants showed enzymatic activities ranging between 50–100% of that seen for wild-type LPL demonstrating that the overall structure of the enzyme was not significantly altered by the mutations. Mutation of previously identified heparin-binding regions of LPL results in a relatively small decrease in heparin-binding affinity, as compared with mutations in this carboxyl-terminal region, indicating that Lys 321, Arg 405, Arg 407, Lys 409, and Lys 416 constitute the major heparin-binding domain in LPL.—**Sendak, R. A., and A. Bensadoun. **Identification of a heparin-binding domain in the distal carboxyl-terminal region of lipoprotein lipase by site-directed mutagenesis. *J. Lipid Res.* 1998.** 39: **1310–1315.**

Supplementary key words heparin-Sepharose chromatography • Chinese hamster ovary cells • discontinuous binding region

Lipoprotein lipase (LPL), an enzyme of central importance in lipid metabolism, hydrolyzes triglycerides in circulating lipoproteins (for reviews, see 1 and 2). The interaction of LPL with heparan sulfate proteoglycans (HSPGs) is vital to the function of this important enzyme. HSPGs anchor LPL at its site of action in capillaries and arteries where the hydrolysis of dietary triglycerides takes place (3). In addition, the binding of newly synthesized LPL to cell surface HSPGs may provide a control point in the regulation of enzyme efflux; binding of LPL to HSPGs is followed either by release of LPL into the medium or internalization and subsequent degradation (or recycling) of the enzyme (4). Furthermore, it has been shown that the presence of LPL enhances the binding of triglyceride-rich lipoproteins to cell surface heparan sulfate (5); this interaction may facilitate subsequent cell-surface events such as receptor binding and uptake of the lipoproteins.

In order to understand the basis for the interaction of LPL with HSPGs, the identification of the specific sites within LPL that confer affinity for heparin has been of interest. Through the use of site-directed mutagenesis, several amino acid residues within LPL have been shown to be important in the binding of LPL to heparin. Mutation of residues 281–284 in avian LPL, and the corresponding residues, 279–282, in human LPL was found to reduce the affinity of LPL for heparin (6–8). In addition, Lys 296 and Arg 297 were found to contribute to the binding of human LPL to heparin (7). The mutation of these residues resulted in a reduction in affinity for heparin; however, the site-directed mutants retained considerable heparin-binding ability, suggesting the presence of at least one other high-affinity heparin-binding site within LPL. Moreover, experiments involving the construction of chimeric lipases also indicate the presence of an additional heparin-binding region in the carboxyl-terminus of the protein (9–11).

The crystal structure of pancreatic lipase has been used as a model to predict the tertiary structure of human LPL (12). The generation of this model has made possible the identification of potential clusters of positive charge that may constitute heparin-binding regions. Three of the four predicted clusters are continuous and have been studied by site-directed mutagenesis (6–8). The fourth cluster is discontinuous, containing residues Lys 321, Arg 405, Arg 407, Lys 409, Lys 415, and Lys 416 (avian numbering), and

Abbreviations: LPL, lipoprotein lipase; HSPG, heparan sulfate proteoglycan; CHO cells, Chinese hamster ovary cells; ELISA, enzyme-linked immunosorbent assay; LRP, low density lipoprotein receptor-related protein.

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is in the carboxyl-terminus of the protein. Recently we identified a monoclonal antibody to avian LPL that is capable of inhibiting the binding of LPL to primary avian adipocytes, heparin, and heparan sulfate (13). The epitope of this antibody contains Arg 405 and is in close proximity to this fourth predicted charge cluster, providing evidence that cluster four contributes to the binding of LPL to heparin.

Our previous report demonstrated that the epitope of an antibody to LPL that inhibited heparin-binding overlapped with the predicted charge cluster four. Due to the relatively large size of an Fab fragment, as compared to that of a heparin-binding domain, further characterization was needed to further define the residues involved in the heparin-binding of LPL. In this study, we have made and characterized site-directed mutants to test whether cluster 4 does, in fact, contribute to the affinity of LPL for heparin. We used heparin-Sepharose chromatography to evaluate whether the mutations affected the ability of LPL to bind heparin. Our results show that residues within this cluster constitute the major heparin-binding region of LPL.

MATERIALS AND METHODS

Molecular biology reagents

Restriction endonucleases, Taq DNA polymerase, and other molecular biology reagents were purchased from New England Biolabs (Beverly, MA) or Gibco BRL (Gaithersburg, MD). Oligonucleotides used for mutagenesis were synthesized by the Cornell Biotechnology Facilty.

Site-directed mutagenesis

The avian LPL cDNA, previously described by Cooper et al. (14), was excised from pGEM-3Z (Promega) by digestion with Cfr10 I and Sal I. The fragment was cloned into the Xma I and Xho I sites of pGEX-4T-2 (Pharmacia) to create pGEX-4T-2/LPL. PCR, alone or in combination with the overlap extension method (15), was used to generate nucleotide substitutions using pGEX-4T-2/LPL as a template for the mutagenesis. PCR products were cloned, screened by restriction digest, and sequenced completely. Subsequently, fragments containing the appropriate mutation(s) were recloned into the plasmid pRc/CMV/LPL, a previously described construct (6) in which the LPL cDNA has been inserted into pRc/CMV (Invitrogen). The construction and cloning into pGEX-4T-2/LPL of LPL(R405N, R407N, K409N), LPL(R405N), LPL(R407N), LPL(K409N), and LPL(K415N, K416N) was previously described (13). Fragments containing the mutations were subcloned into pRc/CMV/LPL utilizing the BstE II site within LPL and the Not I site in the multiple cloning region of both vectors. LPL(K415N) and LPL(K416N) were constructed using the forward primers 5′ GGACTCCATTTGCAT TCACCATTCAGCGCGTACGTGTGAAGTCAGGCGAAACTCA GAATAAGGTGGTATTCTGT 3′ and 5′ TGGACTCCATTTGC ATTCACCATTCAGCGCGTACGTGTGAAGTCAGGCGAAACTC AGAAAAATGTGGTATTCTGT 3′ (mutated residues underlined), respectively, which contain silent mutations that introduce a BsiW I site that was used subsequently for restriction screening. The reverse primer was the pGEX 3′ sequencing primer (5′ CCGGGAGCTGCATGTGTCAGAGG 3′) (Pharmacia). The PCR product was cloned into pGEX-4T-2/LPL using the Bsm I and Bgl II sites within the LPL cDNA. The mutations were subcloned into pRc/CMV/LPL using the Afl II and BstE II sites. LPL (K321N) was constructed using the reverse primer 5′ AGAGAT CAGGAATGGCTGGTCTACCTTGGTCACATTTGTCTTCCC AAAGAAATGTATATTGACCTGATAATG 3′ which contains a silent mutation to create a Bbs I site that was used subsequently for screening. The oligonucleotide 5′ GATGTGGAT CAGCTGGTG 3′ which binds to nucleotides 905 through 922 of the cLPL cDNA was used as the forward primer and the fragment was cloned using the Stu I and Acc I sites within the LPL cDNA. The mutation was subsequently cloned into pRc/CMV/LPL using the Afl II and BstE II sites within the LPL cDNA.

Cell culture and protein expression

Chinese hamster ovary cells (CHO-K1) were obtained from American Type Culture Collection (CCL 61). CHO cells were maintained in a humidified, 5% CO_2 atmosphere at 37°C in Ham's F12 medium (Gibco BRL) supplemented with 5% fetal bovine serum, 2 mm l-glutamine, and 10 mm HEPES, pH 7.2. CHO cells were stably transfected with pRc/CMV/LPL wild-type and mutant DNA using LIPOFECTAMINE™ (Gibco BRL) according to the manufacturer's protocol. Cells were cultured on 60-mm dishes and were transfected at 80–90% confluency. Cells were incubated in serum-free medium with two levels of DNA (2 and 4 μg/dish) and one level of LIPOFECTAMINE™ (10 μl/dish) for 2 h after which the medium was aspirated and complete medium was added. Twelve hours later, the transfected cells were split into 75-cm^2 flasks. One day later, medium was aspirated and selection medium containing 0.5 mg/ml Geneticin (G418) (Gibco BRL) was added to the flasks. Upon reaching confluency (1–2 days), the cells were split 1:3 or 1:5. Cells were cultured in selection medium until the control cells (no DNA) died. One day prior to use for heparin-Sepharose chromatography, regular medium was added to the cells. Transfected cells were frozen at −140°C in medium containing an additional 5% fetal bovine serum and 8% glycerol.

Medium collection

Cells were cultured in 75-cm^2 flasks for heparin-Sepharose chromatography and activity measurements. At 70–80% confluency, the medium was changed and the cells were used the following day. For heparin-Sepharose chromatography, 4.5 ml of fresh medium was added to each of 3 flasks followed by a 5-h incubation. The medium was removed and centrifuged at 15,000 rpm for 30 min at 4°C in a Sorvall SS-34 rotor. For activity measurements, cells were incubated with 4.5 ml of medium containing 10 units of heparin (Sigma) per ml for 5 h.

LPL mass and activity measurements

A sensitive sandwich ELISA (4) was used to assay for LPL mass. The lipolytic activity of LPL was determined as described previously (16). The specific activity of the prepared substrate was 500,000 cpm/μeq fatty acid. Two aliquots of the medium, 100 μl and 300 μl, were assayed to ensure that the activity was in the linear range of the assay. To correct for the activity of the endogenous lipase of CHO cells, samples were incubated for 45 min at 4°C with 2.5 μl of either control serum or avian LPL antiserum (titer of 19,000 μeq fatty acid/h/ml). This level of antiserum was sufficient to inhibit the activity of avian LPL more than 100-fold, but did not interfere with the activity of the endogenous CHO lipase (6).

Heparin-Sepharose chromatography

Affinity chromatography was carried out using an FPLC system (Pharmacia) at 4°C. A 1-ml HiTrap heparin-Sepharose column (Pharmacia) was equilibrated in 0.5% BSA, 30% glycerol, 0.1 m NaCl, 10 mm phosphate, pH 7.0. Media samples were loaded at a flow rate of 0.5 ml/min using a peristaltic pump. The column

was washed with 5 ml of equilibration buffer and then eluted with a 40-ml linear salt gradient from 0.1 m to 2.0 m NaCl at a flow rate of 0.5 ml/min. One-ml fractions were assayed for LPL by ELISA and for conductivity using a conductivity meter (Radiometer, Copenhagen). The previously described mutant 5G (6), in which the residues Arg 281, Lys 282, and Arg 284 (cluster 1, (12)) have been mutated to Gln, was also run on the column as a means for comparison to the cluster 4 mutants.

RESULTS

Eight site-directed mutants were generated and expressed in CHO cells (**Table 1**). In order to minimize the effect of the amino acid substitutions on the structure of LPL, the mutated basic residues were changed to asparagine. Heparin-Sepharose chromatography was used to measure the affinity of the mutants for heparin. Heparin-Sepharose chromatography of LPL has been used previously in several studies to determine the relative affinities of heparin-binding mutants of the enzyme for heparin (6–8). Two peaks are typically seen in a heparin-Sepharose chromatogram; peak 1, which elutes at low salt, and peak 2, which elutes at higher salt (6, 17, 18). The position of the peaks in a heparin-Sepharose chromatogram reflect the affinity of the protein for heparin-Sepharose. Peak 1 consists of monomeric partially or fully denatured protein (17) and arises from the non-specific interactions between residues of LPL and the heparin-Sepharose. Due to the nature of this peak, its position remains constant with mutation of individual residues. Peak 2, on the other hand, arises from the specific interaction between heparin-binding sites of active, dimeric LPL, and heparin-Sepharose (6, 17, 18). Therefore, the position of this peak is expected to shift to a lower salt concentration upon mutation of any residue(s) that form a heparin-binding site in the wild-type enzyme. The shift of peak 2 to lower salt concentration (as compared to peak 2 of wild-type enzyme) reflects a loss in affinity of the mutant for heparin; a lower ionic strength is needed to disrupt the heparin–LPL interaction because it is weaker than the heparin–LPL interaction seen for wild-type enzyme. In this study, as with the previous heparin-binding studies of LPL (6–8) it is the position of the second peak in the heparin-Sepharose elution profile that is of interest.

Avian LPL purified from chicken adipose tissue and wild-type LPL expressed in CHO cells showed the same heparin-Sepharose elution profile. Media samples from cells expressing each mutant were run in triplicate to verify the reproducibility of the elution profile. The salt concentration of the individual fractions was determined using conductivity. The conductivity of five gradients selected randomly from the 33 gradients required to analyze the constructs was measured. The average standard deviation of the measurements was found to be 0.046 m NaCl. Based on this value, a difference of more than two fractions in the peak elution position is statistically significant ($P < 0.05$).

Initially, two mutants were made in which multiple residues were substituted; LPL(R405N, R407N, K409N), and LPL(K415N, K416N). A reduction in heparin-binding ability, as judged by heparin-Sepharose chromatography, of these mutants was found (**Table 2**); subsequently mutants containing single substitutions of all of the residues were constructed. The averages of three runs of wild-type LPL, LPL(K321N), and LPL(R405N, R407N, K409N) are shown in **Fig. 1**. Wild-type LPL eluted at two different salt concentrations (Fig. 1, Table 2); 0.39 m (peak 1) and 1.19 m NaCl (peak 2). The position (fraction number and salt concentration) and relative mass of peaks 1 and 2 for each of the mutants are shown in Table 2. All of the mutants containing a single amino acid substitution, with the exception of LPL(K415N), demonstrated a reduced hep-

TABLE 1. Nucleotide and amino acid sequences of heparin-binding mutants of avian LPL

Nucleotide amino acid	Sequence	Construct
—^{1166}AAG^{1168}—	—^{1418}AGA GTC AGA GTG AAG TCA GGC GAA ACT CAG AAA AAG^{1453}	Wild-type LPL
^{321}**K**	^{405}**R V R V K S G E T Q K K**^{416}	
—^{1166}AA**T**^{1168}—	—^{1418}AGA GTC AGA GTG AAG TCA GGC GAA ACT CAG AAA AAG^{1453}	LPL(K321N)
N	**R V R V K S G E T Q K K**	
—^{1166}AAG^{1168}—	—^{1418}A**AC** GTC AGA GTG AAG TCA GGC GAA ACT CAG AAA AAG^{1453}	LPL(R405N)
K	**N V R V K S G E T Q K K**	
—^{1166}AAG^{1168}—	—^{1418}AGA GTC A**AC** GTG AAG TCA GGC GAA ACT CAG AAA AAG^{1453}	LPL(R407N)
K	**R V N V K S G E T Q K K**	
—^{1166}AAG^{1168}—	—^{1418}AGA GTC AGA GTG AA**T** TCA GGC GAA ACT CAG AAA AAG^{1453}	LPL(K409N)
K	**R V R V N S G E T Q K K**	
—^{1166}AAG^{1168}—	—^{1418}A**AC** GTC A**AC** GTG AA**C** TCA GGC GAA ACT CAG AAA AAG^{1453}	LPL(R405N, R407N, K409N)
K	**N V N V N S G E T Q K K**	
—^{1166}AAG^{1168}—	—^{1418}**C**GC GTA **C**GT GTG AAG TCA GGC GAA ACT CAG AA**T** AAG^{1453}	LPL(K415N)
K	**R V R V K S G E T Q N K**	
—^{1166}AAG^{1168}—	—^{1418}**C**GC GTA **C**GT GTG AAG TCA GGC GAA ACT CAG AAA AA**T**^{1453}	LPL(K416N)
K	**R V R V K S G E T Q K N**	
—^{1166}AAG^{1168}—	—^{1418}AGA GTC AGA GTG AAG TCA GGC GAA ACT CAG AA**T** AA**T**^{1453}	LPL(K415N, K416N)
K	**R V R V K S G E T Q N N**	

Mutated nucleotides and amino acids are underlined.

TABLE 2. Heparin-Sepharose elution data of wild-type and mutant LPL

Construct	Peak 1			Peak 2		
	Elution position			Elution position		
	Fraction	NaCl	Mass	Fraction	NaCl	Mass
		M	*relative units*		*M*	*relative units*
LPL(K321N)	8	0.34	0.34	21	1.00	0.66
LPL(R405N)	—	—	0	19	0.91	1.00
LPL(R407N)	9	0.39	0.24	18	0.87	0.76
LPL(K409N)	9	0.39	0.25	19	0.91	0.75
LPL(R405N, R407N, K409N)	9	0.39	0.64	13	0.57	0.36
LPL(K415N)	9	0.39	0.29	25	1.19	0.71
LPL(K416N)	—	—	0	19	0.91	1.00
LPL(K415N, K416N)	—	—	0	19	0.91	1.00
LPL 5G(R281Q, K282Q, R284Q)	9	0.39	0.43	19	0.91	0.57
Wild-type LPL	9	0.39	0.17	25	1.19	0.83

Affinity chromatography on heparin-Sepharose was carried out on media from CHO cells expressing wild-type and mutant LPL as described in "Materials and Methods." For each mutant the data from three individual runs were averaged and the salt concentration for the fraction of peak elution was determined by conductivity as described in "Results." A difference of more than two fractions in the position of the peak elution is statistically significant. The total amount of lipase that eluted from the column was normalized to a value of 1.0 and the relative amount of immunoreactive material in each peak is given.

arin-binding affinity as reflected by the position of peak 2 relative to that of peak 2 of wild-type enzyme. Peak 2 of wild-type LPL eluted at fraction 25 (1.19 m NaCl) while peak 2 of the single-substitution mutants (except LPL (K415N)) eluted at fractions 18–21 (0.87–1.00 m NaCl); these shifts in position were found to be statistically significant. Peak 2 of LPL(R405N, R407N, K409N) eluted at fraction 13, demonstrating a major loss in heparin-binding ability of this mutant.

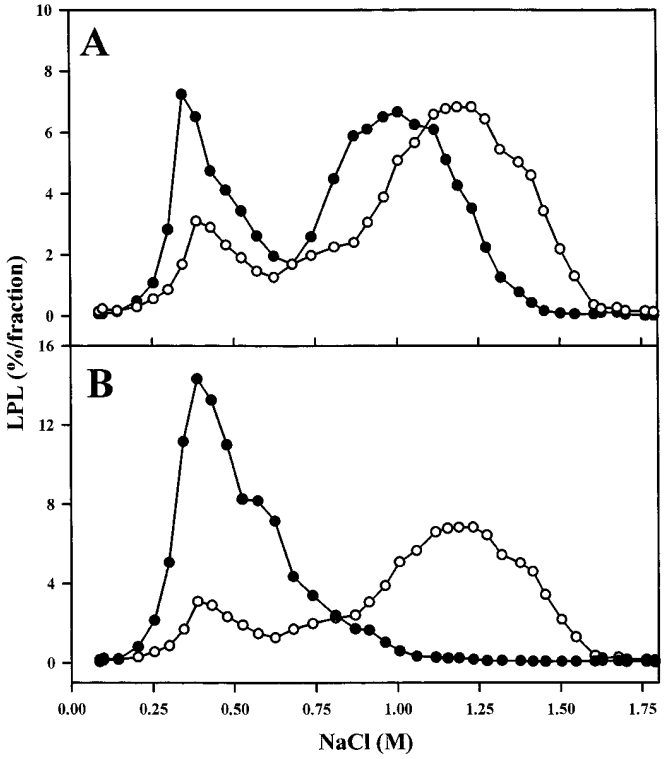

Fig. 1. Heparin-Sepharose chromatography of LPL(K321N) and wild-type LPL**.** Wild-type and mutant LPL were eluted from a heparin-Sepharose column using a linear gradient from 0.1 to 2.0 m NaCl as described in Materials and Methods. LPL is represented as a percent of the total mass that eluted from each run. The average of three elution curves of LPL(K321N) (●) and wild-type LPL (○) is shown (A) along with the elution curves of LPL(R405N, R407N, and K409N) (●) and wild-type LPL (○) (B).

All of the mutants that were constructed were found to be enzymatically active (**Table 3**). LPL(K415N), which elutes as wild-type LPL from the heparin-Sepharose column, had the same level of activity as wild-type. The other mutants, which showed a decreased affinity for heparin, were found to have a lower specific activity than wild-type enzyme. This is not surprising in light of the fact that HSPGs stabilize the enzymatic activity of LPL (19). The HSPGs present on the surface of CHO cells and the heparin in the medium during the 5-h incubation most likely stabilize LPL that possesses a high heparin-binding affinity more effectively than variants of LPL that bind heparin with lower affinity.

DISCUSSION

Site-directed mutagenesis was used to substitute the polar residue Asn for the basic residues in the proposed (12,

TABLE 3. Activity of heparin-binding mutants of avian LPL

Construct	Specific Activity
	μeq fatty acid/μg/h
LPL(K321N)	8.2 ± 0.9
LPL(R405N)	9.0 ± 0.8
LPL(R407N)	6.0 ± 2.5
LPL(K409N)	6.6 ± 1.1
LPL(R405N, R407N, K409N)	4.7 ± 0.1
LPL(K415N)	10.6 ± 0.9
LPL(K416N)	7.1 ± 1.2
LPL(K415N, K416N)	8.6 ± 1.2
Wild-type LPL	10.5 ± 0.5

The activity is given as the mean ± SD (n = 3).

13) heparin-binding region containing the residues Lys 321, Arg 405, Arg 407, Lys 409, Lys 415, and Lys 416 in avian LPL. These constructs were expressed in CHO cells and their heparin-binding ability was assessed by heparin-Sepharose chromatography. As shown in Table 2, all of the mutants containing a single amino acid substitution, with the exception of LPL(K415N), demonstrated a reduced heparin-binding affinity as reflected by the position of peak 2 relative to that of peak 2 of wild-type enzyme. This supports the conclusion that Lys 321, Arg 405, Arg 407, Lys 409, and Lys 416 are involved in the binding of LPL to heparin.

The shift in the elution position of peak 2 of the mutant 5G (cluster 1 completely mutated), as compared to peak 2 of wild-type enzyme, is six fractions (Table 2). This is equal to most of the shifts seen in the elution profiles of the individual cluster 4 mutants (Table 2) which show an average shift of six fractions (peak 2), while the shift of peak 2 of the mutant LPL(R405N, R407N, K409N) is 12 fractions. The construction of cluster 2 mutants (Lys 296 and Arg 297 of human LPL) has been previously reported (7). The shifts, from that of wild-type enzyme, seen in the elution profiles of the cluster 2 mutants are relatively small compared to those seen for the mutant LPL(R405N, R407N, K409N). This comparison of the magnitude of shift in elution position (relative to wild-type) suggests that the carboxyl-terminal heparin-binding region containing the residues Lys 321, Arg 405, Arg 407, Arg 409, and Lys 416 provides a larger contribution to the overall heparin-binding affinity of LPL than do the previously characterized residues contained in clusters 1 and 2.

Site-directed mutagenesis provides a unique way to study specific interactions of a protein; however, inherent in the technique is the possibility that the mutation causes a disruption in three-dimensional structure. Although this possibility cannot be ruled out in this study, it is unlikely for the following reasons. First, the substitution was a conservative one; positive residues (Lys and Arg) were replaced with a polar one (Asn). Second, the mutant LPL retained a high level of enzymatic activity (Table 3); even a relatively small disruption in structure would most likely lead to a significant loss of activity. Previous studies in which site-directed mutagenesis was used to identify heparin-binding regions of LPL reported similar or lower specific activities (6–8).

It is interesting to note that mutation of residues 405, 407, and 409 results in almost a complete loss of high affinity heparin-binding, as reflected by the major shift in the elution position of peak 2 (Fig. 1B, Table 2). As the other heparin-binding regions are intact in this mutant, this result suggests that there may be some cooperativity between heparin-binding sites of LPL. Three distinct regions of LPL (cluster 1, cluster 2, and cluster 4) on two identical monomeric subunits produce six regions of interaction that contribute to the heparin-binding of dimeric LPL. Within the monomer, these positive charge clusters are predicted to lie on one face of the protein at a distance of 10 to 16 Å from one another (12). A recent study indicated that the monomeric subunits of the LPL dimer are positioned in a head to tail conformation (20). Whether the heparin-binding faces of each subunit form a central "groove" in the dimeric form of the enzyme or are on outward facing sides of the dimer is not known. The binding of heparin produces no major conformational changes in LPL (21) suggesting that any potential cooperativity results from the three-dimensional positioning of these six sites in the native structure of the dimer. Further definition of the three-dimensional structure of dimeric LPL may provide insight into the nature of the interaction of these clusters with heparin.

The basic residues contained in cluster 4 are highly conserved among lipoprotein lipases of several different species. The residues are conserved to a lesser degree in hepatic lipase which possesses a lower affinity for heparin than does LPL. This suggests that cluster 4 residues may be responsible for the difference in heparin-binding affinity of the two lipases.

Several studies have indicated the presence of a heparin-binding region in the carboxyl-terminal domain of LPL. Chimera studies in which the carboxyl- and amino-terminal domains of hepatic and lipoprotein lipases were exchanged revealed that the carboxyl-terminal region of LPL possesses heparin-binding affinity (9–11). Another study revealed that the low density lipoprotein receptor-related protein (LRP) was capable of binding to residues 380–425 of bovine LPL and that this binding event was blocked by the presence of heparin (22). The identification of residues in cluster 4 by site-directed mutagenesis provides an explanation of the results obtained in these studies.

In summary, we have demonstrated that Lys 321, Arg 405, Arg 407, Lys 409, and Lys 416 form the major heparin-binding site of LPL. The mutation of the residues in this region appears to affect the affinity of LPL to a greater degree than the mutation of residues within previously characterized heparin-binding domains of LPL. The identification of the major heparin-binding region of LPL will facilitate future research on the mechanisms by which HSPG binding affects LPL activity, LPL turnover, and lipoprotein metabolism.

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