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Effects of substitutions of glycine and asparagine for serine[132] on activity and binding of human lipoprotein lipase to very low density lipoproteins

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For studying the role of Ser[132] in the putative catalytic site of human lipoprotein lipase (LPL), mutant LPL cDNAs expressing LPLs with amino acid substitutions of Gly or Asn for Ser[132] were obtained by site-directed mutagenesis, and were expressed in COS-1 cells. Considerable amounts of LPL enzyme protein mass were detected in the culture medium of COS-1 cells transfected with wild-type LPL, LPL-Gly[132], or LPL-Asn[132]. LPL-Gly[132] hydrolyzed Triton X-100-triolein and tributyrin as effectively as wild-type LPL, whereas LPL-Asn[132] showed no activity. LPL-Asn[132] bound to very low density lipoproteins as effectively as wild-type LPL.

Lipoprotein lipase; Catalytic site; Very low density lipoprotein

1. INTRODUCTION

Lipoprotein lipase (EC 3.1.1.34) (LPL) is present on the surface of endothelial cells [1] and catalyzes the hydrolysis of triglyceride in circulating chylomicrons and very low density lipoproteins (VLDL) [2]. The structural basis of the catalytic activity of LPL is still not fully understood.

With regard to the catalytic site, it is reported that lipases have the amino acid sequence glycine-X-serine-X-glycine which is homologous to that of serine proteases [3] and the nucleophilic residue serine might be involved in the cleavage of ester bonds. Winkler et al. [4] concluded from an X-ray crystallographic study that Ser[152] in human pancreatic lipase is the crucial residue in the catalytic triad. In human LPL, serine[132] is located in the motif glycine-X-serine-X-glycine [5]. To clarify the role of this serine[132] of human LPL, we replaced it by other amino acid residues by site-directed mutagenesis, expressed the wild-type and mutant enzymes in COS-1 cells and examined for their catalytic activities.

Abbreviations: LPL, lipoprotein lipase; Ser, serine; Gly, glycine; Asn, asparagine; VLDL, very low density lipoproteins; Triton X-100-triolein, triolein emulsified with Triton X-100; Ser[132], Ser at amino acid residue of 132; LPL-Gly[132], LPL with substitution of Gly for Ser[132].

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2. MATERIALS AND METHODS

2.1. *Site-directed mutagenesis of lipoprotein lipase cDNA*

Human LPL cDNA (LPL35) was kindly provided by Dr. Michael C. Schotz [5]. LPL35 was suncloned into pBluescript II SK$^-$ and used as a template for site-directed mutagenesis. Mutagenesis of LPL cDNA was carried out as described by Kunkel et al. [6] using a site-directed mutagenesis kit (Mutan-K; from Takara Shuzo Co.). Oligonucleotides for mutagenesis (5′-CCATCTCTTGGGATAC-GGCTTGGAGCCCATGCTGCTGCC-3′ for Ser[132] to Gly, and 5′-CCATCTCTTGGGATACAACTTGGAGCCCATGCTGCTGC-C-3′ for Ser[132] to Asn conversion) were 5′-phosphorylated and annealed with single-stranded template containing deoxyuracil. Mutants were verified by dideoxynucleotide sequencing as shown in Fig. 1.

2.2. *Expression of wild-type and mutant LPLs in COS-1 cells*

Wild-type and the mutant LPL cDNAs were inserted into the *Hind*III and *Eco*RI sites of expression vector, pKCR. These LPL cDNAs were transfected to COS-1 cells according to the method of Selden [7] using DEAE dextran. The cells were cultured in Waymouth's MAB 87/3 medium (Gibco) at 37°C for 72 h and the culture medium was used for assay of LPL enzyme protein and activity.

2.3. *Determination of lipoprotein lipase protein*

The concentration of LPL protein was determined by a modification of sandwich enzyme-linked immunosorbent assay [8] using monoclonal antibody against human LPL (Washington Research Foundation) and polyclonal antibody against bovine milk LPL raised in chickens.

2.4. *Determination of enzyme activities of LPL*

LPL activities and esterase activities of wild-type and mutant LPLs were determined using tri[1-^{14}C]oleoyl glycerol emulsified with Triton X-100 and tri[1-^{14}C]butyryl glycerol, respectively, as substrates [9,10].

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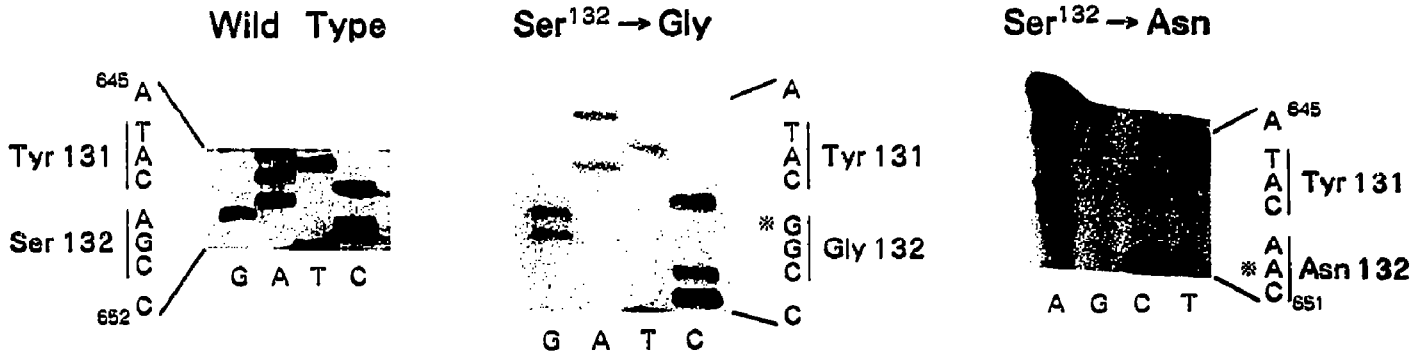

Fig. 1. Nucleotide sequence in the region of tyrosine[131] and serine[132] in wild-type LPL, LPL-Gly[132] and LPL-Asn[132].

2.5. *Determination of binding of LPL to VLDL*

For determination of binding of LPL to VLDL, 10 ng of LPLs in 300 μl of the culture medium of the transfected COS-1 cells was incubated with 100 μl of VLDL (500 mg/dl triglyceride) at 37°C for 10 min. The reaction solutions were centrifuged at 150,000 × *g* for 8 h. The bound LPL to VLDL was recovered in the supernatant, and unbound LPL was recovered in the bottom fraction. Those LPL mass was measured by the sandwich enzyme-linked immunosorbent assay.

3. RESULTS AND DISCUSSION

Antigen cross-reacting with monoclonal antibody against human LPL was detected in culture medium of COS-1 cells transfected with wild-type LPL cDNA as shown in Fig. 2. The medium also showed triolein hydrolyzing activity which was increased 6-fold by addition of apolipoprotein C-II (data not shown). The material with triolein hydrolyzing activity bound to heparin-Sepharose and its activity was inhibited by 1 M NaCl (data not shown). In the culture medium of COS-1 cells transfected with pKCR vector, LPL mass and activity were not observed (data not shown). Then, we concluded that wild-type LPL was expressed in COS-1 cells. The specific activity of lipase in the medium was 10.6 nmol/ ng/h in the presence of apolipoprotein C-II.

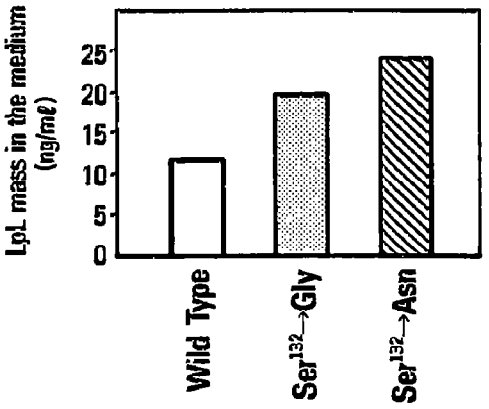

Fig. 2. Concentration of LPL in the culture media of COS-1 cells transfected with wild-type LPL cDNA and mutant LPL cDNAs with substitutions of glycine or asparagine for serine[132].

Then, we examined the concentrations and triolein hydrolyzing activities of mutant LPLs in which Ser[132] was replaced by Gly or Asn in the media of COS-1 cells (Fig. 2). The concentrations of mutant LPLs with substitution of Gly or Asn for Ser[132] in the medium were almost the same as that of the wild-type enzyme.

The triolein hydrolyzing activity in the medium of COS-1 cells transfected with mutant LPL cDNA with Gly in place of Ser[132] was nearly the same as that of wild-type LPL in the presence of apolipoprotein C-II (10.0 nmol/ng/h) as shown in Fig. 3. These results suggesting that substitution of Gly for Ser[132] did not impair the triolein hydrolyzing activity. In contrast, no triolein hydrolyzing activity was detected in the medium of COS-1 cells transfected with the mutant LPL cDNA with Asn in place of Ser[132] (Fig. 3.)

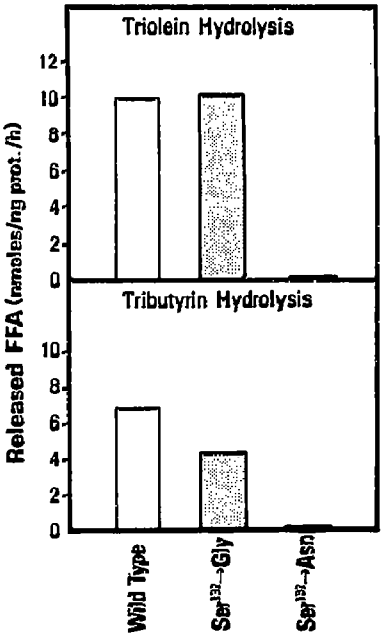

Fig. 3. Effect of substitution of glycine or asparagine for serine[132] on activity of LPL on Triton X-100-triolein in the presence of 5 μg/ml apolipoprotein C-II or tributyrin. LPL activity was not detected in the culture medium of cells transfected with pKCR vector only. Values are means for duplicate determinations.

Table I

Binding of wild-type LPL and mutant LPL (Ser^{132}→Asn) to VLDL

	Wild-type LPL	Mutant LPL	(Ser^{132}→Asn)
Total	9.56±1.24	10.00±1.68	N.S.
Unbound to VLDL	3.99±0.44	3.48±1.16	N.S.
Bound to VLDL	5.57±0.88	6.52±0.93	N.S.

Experiments were in triplicate. Values are mean ± SD (ng/tube). N.S. = non-significant.

The LPL reaction is thought to begin with binding to lipoprotein surface and then its catalytic site works on the ester bond [10,11]. When the LPLs were incubated with VLDL, and bound LPL to VLDL and unbound LPL were separated by ultracentrifugation as shown in table I, the amounts of unbound LPL of wild-type and LPL-Asn^{132} enzymes to VLDL, were almost the same. These results indicated that the binding ability of LPL-Asn^{132} to VLDL was not ruined. Namely, Ser^{132} did not seem to affect the lipid binding site.

Next, the effect of substitution of Asn for Ser^{132} on the catalytic site was studied, using a monomeric substrate, tributyrin. The rationale using monomeric substrate is that LPL can hydrolyze monomeric substrates even after loss of triolein hydrolyzing activity with trypsin treatment [11] and monomeric substrate is postulated to interact with catalytic site directly without interacting with lipid interface recognition site [10,11]. As shown in Fig. 3, tributyrin hydrolyzing activity was detected in the medium of COS-1 cells transfected with cDNAs of wild-type LPL (6.91 nmol/ng/h) and LPL-Gly^{132} (4.23 nmol/ng/h), but not in that of cells transfected with cDNAs of mutant LPL-Asn^{132}. These results suggested that the replacement of Ser^{132} to Asn impairs the catalytic site. Recently, Faustinella et al. [12] reported that three substitution mutants at Ser^{132}, namely, Ser→Thr, Ser→Ala and Ser→Asp, showed no triolein hydrolyzing activity. Thus they concluded that Ser^{132} is important for the catalytic activity of human LPL, being essential for formation of its catalytic site. In our study, we found substitution of asparagine for Ser^{132} abolished both the triolein and tributyrin hydrolyzing activity of human LPL. These findings were consistent with Faustinella's results. However, the substitution of glycine for $serine^{132}$ did not abolish hydrolyzing activity for triolein and tributyrin, indicating that the catalytic site may not be constituted only of Ser^{132}, but may also involve the surrounding amino acid residues or substituted Gly^{132}. Precise mechanism is still inclear. Further experiments are needed to clarify the role of Ser^{132} in human LPL function.

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