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The recovery of dysfunctional lipoprotein lipase (Asp204-Glu) activity by modification of substrate

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Abstract

Functional deficiency of lipoprotein lipase (LPL) was found in a patient with severe hypertriglyceridemia. The patient was 39-year-old man with a plasma triglyceride level of 2032 mg/dl, and suffered from recurrent pancreatitis. His post heparin plasma LPL mass was almost normal, but the LPL activity was remarkably decreased. Gene analysis showed that homozygote missense mutation (204 Asp (GAC)-Glu (GAG)) exists in exon 5 of LPL gene. The patient LPL purified from post heparin plasma scarcely hydrolyzed VLDL-triglyceride and also triolein emulsified with Triton X-100 or phosphatidylcholine. When phosphatidylethenolamine, phosphatidylserine and cardiolipin were used as an emulsifier for triolein, triolein-hydrolyzing activity of the patient's LPL was observed and was much higher than that of wild-type LPL. Mutant LPL gene (Asp204-Glu) was made by site-direct mutagenesis and was transfected to COS-1 cell. The expressed LPL (Asp204-Glu) also showed the same properties. These results suggested that the LPL (Asp204-Glu) is a functional deficiency, and the activity could be recovered by using acidic phospholipids as an emulsifier.

Keywords: Lipoprotein lipase; LPL (Asp204-Glu); Functional defect; Substrate condition

1. Introduction

Lipoprotein lipase (LPL) hydrolyses triglycerides in chyromicrons or very low-density lipoproteins (VLDL). Dysfunction of this enzyme causes severe hypertriglyceridemia [1,2]. One of the causes of the LPL dysfunction was LPL gene mutation. The decreased enzyme activity could be occurred by genetic defect in LPL gene, leading to inactive protein [3–5] or a defect of enzyme protein itself [6,7].

As for a structure of LPL, the presence of a lipid interface-recognition site (IRS) and catalytic site was proposed [8–12], as with the case of pancreatic phospholipase A2 defined by DeHaas and his co-workers [13]. And, the catalytic sequence is proposed as follows; the enzyme recognizes the interface of triglyceride-rich lipoproteins or lipid emulsion with the IRS and the concealed catalytic site reveals. Then, revealed catalytic site handles the hydrolysis of ester bond of triglyceride with the aid of apolipoprotein C-II [10].

Kobayashi et al. previously reported a case with functional deficient LPL (Ser447-Ter). The patient's LPL activity changed depending on the substrate condition [7] and found that medium-chain triglyceride-emulsified triolein was hydrolyzed by Ser447-Ter, and they proposed that LPL (Ser447-Ter) is a functional deficiency with impaired substrate recognition site [8]. In their case, hypertriglyceridemia was improved by medium-chain triglyceride administration [9].

Several authors found a truncated enzyme (LPL204Asp-Glu), which C–G transversion at nucleotide 867 in exon 5 of LPL gene, converts the aspartate 204 codon (GAC) to a glutamate codon (GAG), in hypertriglyceridemias and reported that LPL mass was almost normal, but enzymatic activity was remarkably decreased [6,14]. And they concluded that Asp 204-Glu was inactive form.

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In this paper, we analyzed the enzymatic activity of the LPL mutation (Asp204-Glu), in order to clarify the possibility of the recovery of the enzymatic activity.

2. Subject and the methods

2.1. Case history of a subject

The patient was a 39-year-old man. He had suffered recurrent pancreatitis, with severe hypertriglyceridemia. His serum triglyceride levels were 2032 mg/dl, total cholesterol level was 230 mg/dl and HDL-cholesterol level was 16 mg/dl. Plasma concentration of apolipoprotein C-II was 6.0 mg/dl. His height was 177-cm levels. And body weight was 61.0 kg. He showed no hepatosplenomegaly, eruptive xanthoma, and also no cardiovascular disease. He had no factors increasing serum triglyceride levels, such as obesity, diabetes mellitus, or heavy alcohol intake. He was treated with diet composed of low fat 1800 kcal (protein:fat:carbohydrate 25:15:70)/day, and also was administered bezafibrate. But, these treatment were rarely able to reduce fasting plasma triglyceride levels < 1000 mg/dl, but could prevent the attack of pancreatitis.

2.2. Serum lipid analysis

Venous blood was collected after an overnight fasting. Serum was obtained after centrifugation at $1500 \times g$ for 10 min at room temperature. Serum total cholesterol, triglyceride and phospholipid were measured by enzymatical methods using autoanalyser HITACHI H-7150. Very low-density lipoprotein (VLDL) ($d = 1.006$) was isolated by ultra centrifugation essentially according to the methods of Havel et al. [15].

2.3. Lipoprotein lipase gene analysis

Genomic DNA from the patient was isolated from peripheral whole blood by the sucrose lysis procedure as described previously. After DNA extraction, LPL gene amplification was performed by polymerase chain reaction (PCR). DNA (0.2–0.5 μg) was amplified in a 50 μl reaction mixture containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 250 μM each of dATP, dTTP, dCTP and dGTP, 0.25 μM of each primer, and 1.25 units Ex Taq polymerase (Takara Syuzo Co. Ltd., Shiga, Japan). The amplification cycle was performed on a Robo-cycler gradient 40 Thermal Cycler (Staratagene, CA, USA; Nippon Genetics, Tokyo, Japan) and entailed 3 min of denaturation at 95 °C followed by 35 cycles of 0.5 min at 95 °C, 1.5 min at 55 °C, and 1 min at 73 °C. This was followed by 3 min of extension at 73 °C. The sequence of oligonucleotide primers used for PCR primer was derived from the described previously. Primers were synthesized by standard β-cyanoethyl phosphoramidite chemistry using a model 381A Synthesizer (Applied Biosystems Inc., Foster City, CA) and were purified by reverse-phase chromatography cartridges (OPC Applied Bio System Inc.) as described previously [16].

Direct sequencing of amplified DNA was performed by dideoxy methods with Thermo Sequenase DNA polymerase (Amersham Pharmacia Biotech Inc., USA) after PCR products purification. Sequencing was performed on a Gene Rapid DNA sequencer (Amersham Pharmacia Biotech Inc., USA).

2.4. Measurement of lipoprotein lipase activity in post heparin plasma

The patient fasted overnight, and after 30 units of heparin/kg body weight was injected intravenously and the blood was collected 10 min later. Lipoprotein lipase activities were measured by a modification of the methods of Krauss et al. [17]. Hepatic triglyceride lipase activity was measured in the presence of 1 M NaCl, and lipoprotein lipase activity was calculated by subtracting hepatic lipase activity from total lipase activity in the presence of apolipoprotein C II and 0.14 M NaCl. For total lipase activity, The reaction mixture contained 0.33 mg of triolein, 5 mg of fatty acid-free bovine serum albumin, 0.02% Triton X-100, 25 μl of HDL-cholesterol (5 mg protein/ml) as a sense of apo CM, and 0.14 M NaCl, and an appropriate amount of post heparin plasma in a final volume of 0.25 ml of 0.1 M Tris–HCl (pH 8.4). After incubation for 60 min at 37 °C, the enzyme reaction was terminated by the addition of diisoproprylfluorophosphate (DIF). The lipase activity was calculated by measuring released free fatty acid concentration in the reaction mixture using enzymatic assay kit NescautoNEFA-V2 (Azwell Inc., Osaka, Japan). LPL activity was expressed as micromole fatty acid released in 60 min at 37 °C.

2.5. Purification of lipoprotein lipase from post heparin plasma

Post heparin plasma from the patient and normal individuals was obtained 10 min after intravenous administration of 30 units of sodium heparin/kg body weight. The plasma (10 ml) was applied to a column of anti LPL antibody-conjugated Sepharose 4B (3 ml) equilibrated with 100 mM Na, K phosphate buffer (pH 7.2) containing 1 M NaCl and 30% glycerol. The column was then washed first with 100 mM Na, K phosphate buffer (pH 7.2). Then, material was eluted with the 100 mM Na, K phosphate buffer containing 3 M NaSCN and 30% glycerol (pH 7.2), and the eluate was collected. Glycerol was added to this eluate to stabilize lipoprotein lipase. The LPL protein in the elute from column of anti LPL antibody-conjugated Sepharose 4B was measured by ELISA method using LPL ELISA, Daiichi Pure Chemicals Ltd., Tokyo, Japan (DAIICHI), most high-concentration fraction was used for examination.