Effects of Dextran Sulfate on Stabilization of Milk Lipoprotein Lipase and VLDL Triglyceride Hydrolysis in Vitro

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MATSUOKA, N., SAITO, Y. and YOSHIDA, S. *Effects of Dextran Sulfate on Stabilization of Milk Lipoprotein Lipase and VLDL Triglyceride Hydrolysis in Vitro.* Tohoku J. exp. Med., 1986, **149** (1), 61-66 — The effects of dextran sulfate (DS), which has various molecular numbers, on hydrolysis of very low density lipoprotein triglyceride (VLDL-TG) by bovine milk lipoprotein lipase (LPL) and the stability of LPL were studied. VLDL-TG hydrolysis was increased by the addition of DS; DS caused linear increase in the Vmax for VLDL-TG with increase in its sulfate content, but did not change the Km value for VLDL-TG. DS also stabilized LPL, but this effect was not dependent on its sulfate content. These results suggest that the mechanism of action of DS in LPL stabilization may be different from that in enhancement of VLDL hydrolysis. — lipoprotein lipase; dextran sulfate; very low density lipoproteins

Dextran sulfate (DS) is reported to cause liberation of lipoprotein lipase (LPL) from endothelial cells into the blood stream in vivo (Cohen and Tudhope 1956) and to increase hydrolysis of very low density lipoproteins (VLDL) by milk LPL (Matsuoka et al. 1982). DS consists of components of different molecular weight and different sulfate content (Yamada et al. 1961), and the components of different molecular weight are reported to have different effects on fibrinolysis (Yamada and Kuzuya 1962). The relationship between the molecular weight of DS and its effects on LPL is unknown. In the present study, we examined the relationship between the molecular weight of DS and its effects on stabilization of purified bovine milk LPL and on enhancement of hydrolysis of VLDL-triglyceride (TG) by LPL.

MATERIALS AND METHODS

Materials

Heparin sodium salt (porcine intestinal mucosa, 169.9 USP units/mg), fatty acid-free bovine serum albumin (BSA), dextran and trioleoyl glycerol were purchased from Sigma.

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TABLE 1. *Various dextran sulfate and their sulfate content*

Dextran sulfate	M.W.	Sulfate content(%)	Inherent viscosity
I	1190	3.0	0.0272
II	1320	5.6	0.0414
III	1370	9.0	0.0379
IV	1920	15.9	0.0324

Dextran sulfates were kindly provided by Kowa Co., Ltd. (Tokyo). The dextran sulfates used are shown in Table 1. Tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol (50 mCi/mmole) was purchased from Japan Isotope Association. Lipoprotein lipase was purified from bovine skimmed milk by affinity chromatography on heparin-Sepharose (Iverius 1971) as described previously (Matsuoka et al. 1980).

Apolipoprotein C-II was a gift from Prof. Jackson (Cincinnati, OH, USA). It had been isolated from VLDL obtained from the plasma of fasting normal individuals (Jackson et al. 1977).

Assay of lipoprotein lipase activity

LPL was assayed by measuring release of [$1\text{-}^{14}\text{C}$]-labeled fatty acids from tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol as described by Nilsson-Ehle et al. (1976). The standard assay mixture contained 0.378 μmole of trioleoyl glycerol, 2 nCi of tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol emulsified with 0.02% Triton X-100, 2% fatty acid free bovine serum albumin (BSA), 2.0 μg of apolipoprotein C-II and 50 mM Tris-HCl, pH 8.6, in a final volume of 0.25 ml. Incubation was carried out for 30 min at 37°C.

Preparation of tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol labeled VLDL

Very low density lipoproteins ($d < 1.006$) were isolated from normal human serum by ultracentrifugation. The once obtained VLDL by ultracentrifugation of human serum were dialysed against a buffer containing 0.9% NaCl, 10 mM Tris-HCl pH 7.4 and were ultracentrifuged again at $105,000 \times g$ for 22 hr, and the supernatant fractions were used for VLDL. Tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol was incorporated into normal human VLDL using dimethylsulfoxide, according to the method of Fielding (1979).

VLDL-triglyceride (VLDL-TG) hydrolysing activity

The standard assay mixture contained 0.20 μmole of tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol labeled VLDL (S.A. 0.37 $\mu\text{Ci}/\text{mole}$, 6.26 mg/ml of triglyceride, 2.73 mg/ml of total cholesterol, 2.25 mg/ml of phospholipid), 2% BSA, 0.1 M Tris-HCl (pH 8.2) and 20 ng of LPL, in a final volume of 0.25 ml. Incubation was carried out for 60 min at 37°C. The [$1\text{-}^{14}\text{C}$]-labeled fatty acids released from tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol-labeled VLDL were measured by the method of Nilsson-Ehle et al. (1976).

RESULTS AND DISCUSSION

Tissues contain DS of various molecular numbers (Yamada et al. 1961), but relationship between different molecular numbers of DS and their physiological effects are not well known. We have previously reported that DS increases the hydrolysis of VLDL-TG by LPL (Matsuoka et al. 1982). In this series of experiments, we compared their effects on hydrolysis of VLDL by LPL with those on

Fig. 1. A : Effects of DSs on hydrolysis of VLDL-TG at various concentrations of VLDL-TG. The incubation mixture consisted of the indicated concentration of tri [1^{14} C] oleoyl glycerol-labeled VLDL, 2% BSA, 0.1 M Tris-HCl, pH 8.2, and 20 ng of LPL, with or without DS (10 ng/ml) in a final volume of 0.25 ml. B : Lineweaver-Burk double reciprocal plots of VLDL-TG hydrolysis by LPL. Lineweaver-Burk plots ($1/v$ vs $1/s$) were constructed from the data in Fig. 1A.

stability of LPL. At first, the effects of various DSs on the hydrolysis of VLDL-TG were studied at various concentrations of VLDL-TG. Hydrolysis of VLDL-TG increased with an increase in the amount of individual DS added to incubation mixture as shown in Fig. 1A. To understand the mechanism of enhancement of hydrolysis of VLDL-TG, Lineweaver-Burk double reciprocal plots were made from Fig. 1A and shown in Fig. 1B, and kinetic parameters were examined. The DS did not change the apparent K_m value (0.5 mM) for VLDL-TG, but changed the V_{max} value for VLDL-TG by LPL, which was largest with DS-IV (7.8 released FFA nmole/mg prot/hr). To understand the activation mechanism of LPL-catalyzed VLDL-TG hydrolysis by DS, the relationship between V_{max} value for VLDL-TG hydrolysis and sulfate content of DS used in the experiments of Fig. 1B are shown in Fig. 2A. The V_{max} value increased with an increase in the sulfate content of DS and the relationship between V_{max} value and sulfate content of DS was linear. These results suggest that the sulfate

Fig. 2. A : Relationship between the change of Vmax value for VLDL-TG hydrolysis by various DSs and sulfate contents DSs. These values were constructed from Fig. 1B.
 B : Relationship between the stabilizing effect of various DSs on LPL activity, and sulfate contents of DSs. The values were constructed from Table 1 at the preincubating condition at 37°C for 2 hr.

moiety of DS plays an important role in enhancing the effect of DS on the hydrolysis of VLDL by LPL.

In the next series of experiments, the effect of DS on the stability of LPL was examined. Enzyme activity was assayed after the LPL was preincubated at 37°C for indicated times. As shown in Table 2, LPL was inactivated by the incubation. In the treatment at 37°C for 1 hr or 2 hr, DS-I and II did not stabilize the LPL activity, DS-III and IV prevented the loss of LPL activity to almost the same extent. At least, DS-IV was not better than DS-III. The relation of stabilizing effect of various DSs and sulfate content of DSs was shown in Fig. 2B. They were not in a linear relationship. These results indicated that the effects of DSs on LPL stabilization were not dependent on the sulfate content in contrast with the enhancing effects of DSs on VLDL-TG hydrolysis as shown in Fig. 2A. Clarke et al. (1983) recently reported that the types of complex produced by the combination of lipoprotein lipase with heparin were dependent on the molecular weight of heparin (Mr 6600-18400). Therefore, the stabilization of LPL by DSs may relate to the formation of complex with LPL and DS. The results obtained in the present experiments suggest that the mechanism of LPL stabilization by DS

TABLE 2. *Effects of DSs on LPL stability*

Pextran sulfate	37°C					
	1 hr			2 hr		
None	35.9	±	7.9	11.7	±	5.2
I	29.5	±	1.3	13.4	±	4.4
II	20.9	±	4.0	6.2	±	2.5
III	70.3	±	9.4	68.6	±	5.3
IV	69.6	±	6.6	53.2	±	7.4

Mixtures of 0.4 μg of LPL, 1 mg of BSA with or without various DSs (0.2 $\mu\text{g}/\text{ml}$) and 0.1 M Tris-HCl, pH 8.2, were preincubated at 37°C for indicated times. After preincubation 0.05 ml of the mixtures were added to LPL activity assay mixture in order to measure the enzyme activity. These assay mixtures contained 0.378 μmole of tri [$1\text{-}^{14}\text{C}$] oleoyl glycerol emulsified with 0.02% Triton X-100, 2% BSA, 2.0 μg of apolipoprotein C-II and 50 mM Tris HCl, pH 8.6 in a final volume of 0.25 ml. Values represent the percentage of initial activity.

is different from that of its effect on an increase in VLDL-TG, hydrolysis. But, DS-III and IV had a greater stabilizing effect than DS-I and II. Therefore, the stabilizing effect of DS may be partly involved in the enhancing effect of DS on VLDL-TG hydrolysis. Experiments are now in progress to clarify the mechanism of action of DS on the action of LPL.

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