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Demonstration of Hepatic Triglyceride Lipase-like Activity in Ascites Fluid of Mice with Sarcoma 180

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Abstract—*The contents of apolipoproteins of plasma and ascites fluid of mice with Sarcoma* 180 *were measured. The apolipoprotein A-I contents of plasma decreased with development of the tumor. The apolipoprotein C-II and C-III contents of plasma reached a maximum on day* 7 *after tumor inoculation and then decreased. The apolipoprotein A-I content of the ascites fluid was lower than that of normal mouse plasma. In contrast, the apolipoprotein C-II and C-III contents of the ascites fluid were higher than those of normal mouse plasma. The ascites fluid of mice with Sarcoma* 180 *was found to contain at least two lipases. One had a pH optimum of* 5.5–7.0 *and was strongly inhibited by chlorpromazine. The other had an alkaline pH optimum and was inhibited only slightly by chlorpromazine. When the ascites fluid was applied to a heparin-Sepharose column* 40–45% *of the applied triglyceride lipase activity was retained on the column, which was eluted with* 0.75 M NaCl. *This fraction was inhibited by heat-inactivated* (56°C, 10 min) *human serum, and was relatively resistant to* 1 M NaCl. *These results suggest that one of the lipolytic enzymes present in the ascites fluid of mice with Sarcoma* 180 *is hepatic triglyceride lipase.*

INTRODUCTION

FREE fatty acids are required by tumor cells and most of these fatty acids are supplied by the host [1]. At least some of them are derived from circulating lipoproteins. Brenneman *et al.* [2] reported that the ascites fluid formed with Ehrlich ascites tumor contains large amounts of very low-density lipoprotein (VLDL), which may serve as a vehicle for transport of free fatty acids to the tumor. The source of VLDL in the ascites fluid is probably blood plasma [2, 3]. There is no report of lipase in Ehrlich ascites tumor. However, Kralovic *et al.* [4] found that the ascites fluid of rats with Walker 256 carcinoma contains lipase activity. They examined the pH-dependence of this activity, but they did not characterize it further.

At least two lipolytic enzymes are known to be involved in the metabolism of lipoproteins present in the bloodstream. One is hepatic triglyceride lipase (hepatic TGL), which is synthesized in liver parenchymal cells. Hepatic TGL has an alkaline pH optimum, is relatively resistant to 1 M NaCl and does not require an apolipoprotein cofactor [5–7]. The other is lipoprotein lipase (LPL), which is synthesized in extrahepatic tissues, mainly adipose tissue and heart. LPL has an alkaline pH optimum, is inhibited completely by 1 M NaCl and requires an apolipoprotein cofactor for maximal activity [8–12]. These enzymes can be separated easily by heparin-Sepharose column chromatography [7, 13].

This study indicates that there are at least two TGLs in the ascites fluid of mice with Sarcoma 180 and that the properties of one of them are similar to those of hepatic TGL.

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MATERIALS AND METHODS

Materials

Glycerol tri[1-^{14}C]oleate (30–60 mCi/mmol) and ACS-II were purchased from Amersham, Arlington Heights, IL. Triolein and chlorpromazine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO. Bovine albumin

(fraction V from bovine plasma) was obtained from Wako Pure Chemicals, Osaka, Japan. Heparin-Sepharose CL-6B was obtained from Pharmacia Fine Chemicals, Sweden. Apo A-I, Apo A-II, Apo C-II, Apo C-III and Apo E plates were obtained from Daiichi Pure Chemicals Co., Tokyo, Japan. Other chemicals were products of Wako (Japan).

Tumor

Sarcoma 180 cells were inoculated intraperitoneally into male ICR/JCL mice (5 weeks old) as described previously [14].

Collection of plasma and ascites fluid

Mice were fed *ad libitum* and were killed by decapitation and bled between 9:00 and 9:30 a.m. The blood and ascites fluid were centrifuged at 1000 **g** for 10 min at 4°C.

Electrophoresis of lipoproteins

Electrophoresis of the lipoproteins of plasma and of the ascites fluid was carried out by the method of Feliste *et al.* [15] with slight modifications. A disc electrophoretic method in 3.85 and 7.6% acrylamide separation mixed gel permitted separation of lipoproteins on the same gel. The lipoproteins were prestained with Sudan black-B dissolved in ethylene glycol before electrophoresis.

Analyses of apolipoproteins

Apolipoproteins were analyzed by the single radial immunodiffusion method, using Apo A-I, Apo A-II, Apo C-II, Apo C-III and Apo E plates.

Heparin-Sepharose column chromatography

One volume of the ascites fluid was mixed with 1 vol. of 5 mM veronal buffer (pH 7.0) containing 0.4 M NaCl and applied to a heparin-Sepharose column. The TGL activity was cleansed by stepwise elution and assayed as described previously [14].

Measurement of triglyceride lipase activity

The TGL activity was measured with glycerol tri[1-^{14}C]oleate as described previously [14].

Protein determination

Protein was measured by the method of Lowry *et al.* [16].

Statistics

Data were analyzed by Student's *t* test.

RESULTS

Electrophoretic analyses of lipoproteins

Electrophoretic analyses of lipoproteins of plasma of normal mice and of plasma and ascites fluid of mice on day 10 after inoculation of Sarcoma 180 were carried out (Fig. 1). Normal mouse plasma contained a small amount of VLDL but a very large quantity of high-density lipoprotein (HDL). Low-density lipoprotein (LDL) was not observed under these conditions. Tumor-bearing mouse plasma contained much more VLDL and less HDL than normal mouse plasma. Of HDL fractions, the one that has the greatest anodal mobility was remarkably reduced. In tumor-bearing mouse plasma lipoproteins could be observed in the LDL region. The electrophoretic pattern of HDL in the ascites fluid was very similar to that in tumor-bearing mouse plasma. But the ascites fluid contained less VLDL than tumor-bearing mouse plasma.

Apolipoprotein contents of plasma and ascites fluid

The contents of apolipoproteins of plasma of normal and tumor-bearing mice and of the ascites fluid were measured (Table 1). The apolipoprotein A-I contents of plasma were decreased with development of the tumor. The apolipoprotein C-II contents of plasma reached a maximum on day 7 after tumor inoculation and then decreased. The apolipoprotein C-III content of plasma was also increased transiently. The apolipoprotein A-I content of the ascites fluid was lower than that of normal mouse plasma, the average content being 64.3% of that of normal mouse plasma. In contrast, the apolipoprotein C-II and C-III contents were higher than those of normal mouse plasma. Apolipoproteins A-II and E of plasma of normal and tumor-bearing mice and of the ascites fluid could not be detected by the present method using Apo A-II and Apo E plates.

Hepatic TGL-like activity in ascites fluid of mice with Sarcoma 180

The rate of hydrolysis of glycerol tri[1-^{14}C]oleate increased linearly with enzyme con-

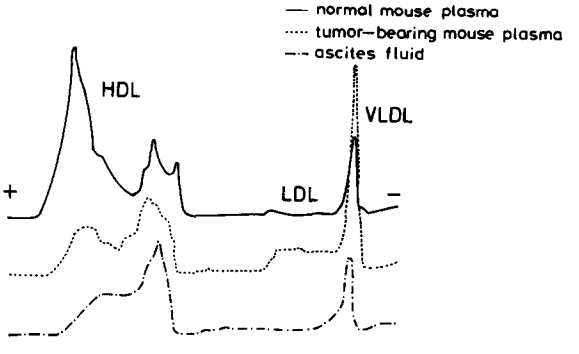

Fig. 1. Electrophoretic patterns of lipoproteins of plasma of normal and tumor-bearing mice and of the ascites fluid. The plasma and ascites fluid were stained with Sudan black-B prior to electrophoresis. Equal volumes of plasma, 30 µl, were electrophoresed in each of the three specimens.

Table 1. Apolipoprotein contents of plasma and ascites fluid

	Normal	Days after tumor inoculation 4	7	10
Plasma				
Apolipoprotein A-I (mg/dl)	418.7 ± 26.1*	494.6 ± 36.7	395.4 ± 97.6	353.8 ± 20.4†
Apolipoprotein C-II (mg/dl)	3.77 ± 0.15	5.11 ± 0.24§	7.13 ± 0. 27§	5.19 ± 0.57†
Apolipoprotein C-III (mg/dl)	3.11 ± 0.19	3.87 ± 0.13†	4.11 ± 0.21‡	3.67 ± 0.03†
Ascites fluid				
Apolipoprotein A-I (mg/dl)	—	256.3 ± 44.7§	276.8 ± 31.1§	274.2 ± 6.7§
Apolipoprotein C-II (mg/dl)	—	5.88 ± 0.25§	6.84 ± 0.34§	6.05 ± 0.56§
Apolipoprotein C-III (mg/dl)	—	4.33 ± 0.22§	4.33 ± 0.22§	3.89 ± 0.34

*Mean ± S.E. ($n = 3$ mice).
Significant difference from the contents of normal mouse plasma: †$P < 0.05$, ‡$P < 0.02$, §$P < 0.01$.

centration using up to 0.2 ml of ascites fluid as enzyme source (Fig. 2) and the enzyme activity was linear for 1 hr (data not shown), suggesting that TGL is present in the ascites fluid.

The pH dependence of the TGL activity of the ascites fluid was evaluated in the absence and presence of chlorpromazine (Fig. 3). In the absence of chlorpromazine, the main peak of TGL activity was between pH 5.0 and 10.0. On addition of chlorpromazine the TGL activity between pH 5.5 and 7.0 was strongly inhibited, whereas that between pH 7.5 and 9.5 was inhibited by 15–20%.

A typical chromatogram of the elution of TGL activity in the ascites fluid from a heparin-Sepharose column is shown in Fig. 4. Of the applied TGL activity, 40–45% was retained on the column, and on stepwise elution of the heparin-Sepharose only one TGL activity was eluted with 0.75 M NaCl. This TGL activity was inhibited by 30.2 ± 5.6% by addition of 10% heat-inactivated human serum to the assay mixture (Fig. 4). This TGL activity was relatively resistant to 1 M NaCl (TGL activity in the absence and presence of 1 M NaCl, 46.6 and 35.5 μmol/mg protein, respectively) and had an alkaline pH optimum (Fig. 5). These results indicate that an alkaline TGL in the ascites fluid has properties similar to those of hepatic TGL.

Effects of whole serum and ascites fluid on hepatic TGL-like activity

The effects of heat-inactivated whole sera of normal and tumor-bearing mice and of heat-

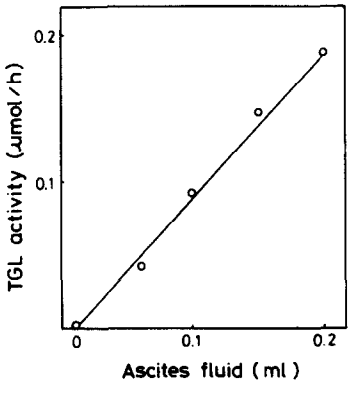

Fig. 2. TGL activity in the ascites fluid of mice with Sarcoma 180. Reaction mixtures in a total volume of 0.503 ml *contained* 0.036 ml *of substrate emulsion and the indicated volume of the ascites fluid. Incubation was carried out at* 37°C *for* 60 min *in the absence of heat-inactivated human serum. Details were as described previously* [14].

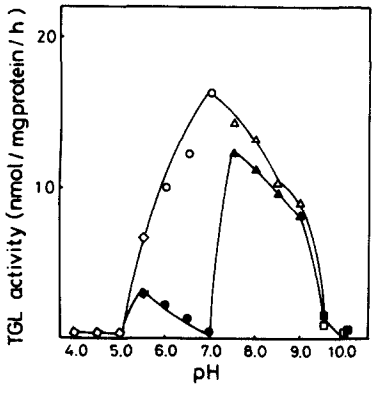

Fig. 3. pH dependence of TGL activity of the ascites fluid of mice with Sarcoma 180. *Fresh ascites fluid* (0.2 ml) *was assayed as described for Fig. 2 at the indicated pH in the presence (closed symbols) and absence (open symbols) of 500* μM *chlorpromazine,* ◆, ◇, *pH* 4.0–5.5, 0.2 M *acetate buffer;,* ●, ○, *pH* 6.0–7.0, 0.2 M *potassium phosphate buffer;* ▲, △, *pH* 7.5–9.0, 0.2 M *Tris-HCl buffer;* ■, □, *pH* 9.5 *and* 1.00, 0.2 M Na_2CO_3-$NaHCO_3$ *buffer.*

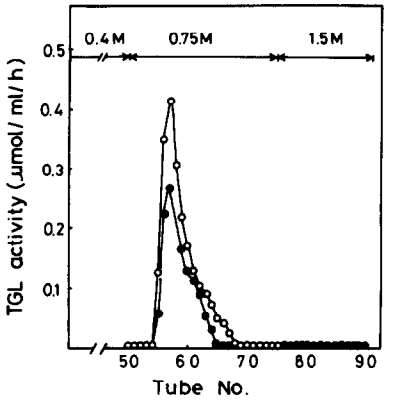

Fig. 4. Heparin-Sepharose column chromatography of the ascites fluid of mice with Sarcoma 180. The ascites fluid (150 ml) was mixed with 150 ml of 5 mM veronal buffer (pH 7.0) containing 0.4 M NaCl. This mixture was applied to a heparin-Sepharose column and the TGL activity was eluted and assayed in the presence (●) and absence (○) of heat-inactivated human serum as described previously [14]. Fractions of 4.8 ml of eluate were collected.

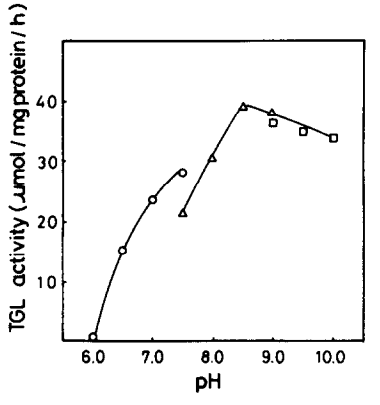

Fig. 5. pH dependence of TGL activity eluted from the heparin-Sepharose column. The TGL activity eluted from the heparin-Sepharose column with 0.75 M NaCl was measured at the indicated pH as described for Fig. 2. ○, pH 6.0-7.5, 0.2 M potassium phosphate buffer; △, pH 7.5-9.0, 0.2 M Tris-HCl buffer; □, pH 9.0-10.0, 0.2 M Na_2CO_3-$NaHCO_3$ buffer.

inactivated ascites fluid on the hepatic TGL-like activity are shown in Fig. 6. The hepatic TGL-like activity was concomitantly inhibited with the volumes of sera in the assay mixture. Inhibition by serum of tumor-bearing mice was similar to that by serum of normal mice. Inhibition of the activity by addition of the ascites fluid was less than that by addition of sera. The hepatic TGL activity obtained from heparin perfusate of normal mice also inhibited in a similar manner (data not shown).

DISCUSSION

The present study demonstrates that the ascites fluid of mice with Sarcoma 180 contained at least two TGLs. One is a chlorpromazine-sensitive acidic TGL. Jensen *et al.* [17] reported that

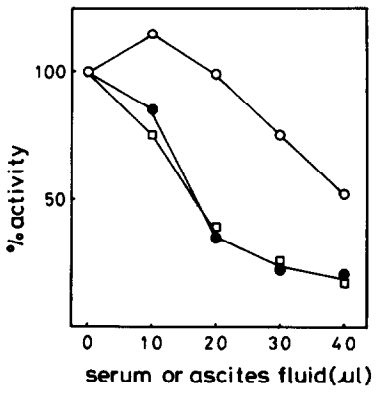

Fig. 6. Effects of heat-inactivated sera and ascites fluid on hepatic TGL-like activity. The hepatic TGL-like activity was measured in the presence of the indicated volumes of heat-inactivated sera or ascites fluid as described for Fig. 2. The TGL-like activity in the absence of sera or ascites fluid was 58.7 μmol/mg protein. (□) Plasma of normal mice; (●) plasma of tumor-bearing mice; (○) ascites fluid of mice on day 10 after tumor inoculation.

chlorpromazine specifically inhibited lysosomal lipase activity and did not affect hepatic TGL and LPL. Thus at least a part of the TGL activity in the ascites fluid might be due to a lysosomal lipase. The partial chlorpromazine inhibition of TGL in the alkaline range suggests the presence of residual lysosomal lipase activity between pH 7.0 and 9.5. The other is a chlorpromazine-insensitive alkaline TGL, which has properties similar to those of hepatic TGL.

The role of the hepatic TGL-like activity in the ascites fluid of mice with Sarcoma 180 is still unknown.

Berry *et al.* [18] and Goldberg *et al.* [19] reported that the function of hepatic TGL appears to be similar to that of LPL in conversion of VLDL and intermediate-density lipoprotein (IDL) to LDL. In the present study we found that VLDL was present in the ascites fluid of mice with Sarcoma 180 (Fig. 1). This finding is consistent with the observation of Brenneman *et al.* [2] that the ascites fluid of mice with Ehrlich ascites tumor contains large amounts of cholesterol and triglycerides mostly in the form of VLDL. These observations suggest that the hepatic TGL-like activity in the ascites fluid of mice with Sarcoma 180 may hydrolyze VLDL to free fatty acids, which may account at least in part for the free fatty acids available to the tumor.

Hepatic TGL may also play a role in HDL metabolism [20-22]. In addition to VLDL, HDL is present in the ascites fluid of mice with Sarcoma 180 (Fig. 1), suggesting that the hepatic TGL-like activity may be involved in HDL metabolism in the ascites fluid.

The present study shows that mouse plasma on day 10 after tumor inoculation contained

lipoproteins in the LDL region (Fig. 1). Damen *et al.* [23] reported that the lipoprotein peak observed in the HDL region shifted into the LDL region on day 4 after the GRSL ascites tumor inoculation, at the time when the hepatic TGL activity was decreased. Jansen *et al.* [20] also reported a similar shift in rat plasma after intravenous administration of antibodies to hepatic TGL. Previously, we found that in the liver of mice on day 10 after tumor inoculation the hepatic TGL activity was very low [14]. These observations suggest that decrease of hepatic TGL leads to accumulation of lipids in HDL particles, which increase in size and decrease in density and mobilize in gel to the LDL region.

It is well known that hepatic TGL is inhibited by addition of whole serum to assay mixture [14, 24, 25]. Inhibition of the hepatic TGL-like activity by the ascites fluid was less than that by whole serum obtained from normal or tumor-bearing mice (Fig. 6). This may be due to the low apolipoprotein A-I content of the ascites fluid as compared with that of normal mouse plasma, since apolipoprotein A-I inhibits hepatic TGL [26]. On the other hand, despite the low apolipoprotein A-I content of plasma of mice on day 10 after tumor inoculation, inhibition of the hepatic TGL-like activity by plasma of tumor-bearing mice was similar to that by plasma of normal mice. This may be due to the high apolipoprotein C-III content of plasma of tumor-bearing mice because apolipoprotein C-III also inhibited the hepatic TGL activity [27].

The tissues in which the hepatic TGL-like activity in the ascites fluid of mice with Sarcoma 180 is synthesized are still unknown. It is unlikely that the hepatic TGL-like activity is a product from Sarcoma 180 cells. When Sarcoma 180 cells were homogenized and TGL was solubilized with Triton X-100, the solubilized TGL was not absorbed by the heparin-Sepharose [14].

The properties of TGL in the ascites fluid of mice with Sarcoma 180 which was not absorbed in the heparin-Sepharose column remain to be elucidated. A part of this activity may be due to lysosomal lipase.

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