PURIFICATION OF MONOACYLGLYCEROL HYDROLASE FROM HUMAN POST-HEPARIN PLASMA

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1. Introduction

Post-heparin plasma contains triacylglycerol lipase (TGL), monoacylglycerol hydrolase (MGH) and phospholipase activities. On the basis of several criteria, these enzymes are considered to be different from one another [1–3]. In order to study the detailed properties of these enzymes, it is necessary to purify the enzyme from others. Nilsson-Ehle and Belfrage [4] reported a 7.2-fold purification of MGH in post-heparin plasma by removal of TGL–substrate complex, precipitation at low pH and low ionic strength, and molecular exclusion chromatography. In the present study, we have described the purification of MGH in human post-heparin plasma by the removal of TGL-substrate complex and affinity chromatography on Sepharose 4B column containing covalently bound heparin.

2. Materials and methods

2.1. *Materials*

[^{3}H]Glyceryl monooleate was prepared from [^{3}H]-glyceryl trioleate, obtained from the Radiochemical Centre, Amersham, by pancreatic lipase (Sigma Chemical Co.), purified by thin-layer chromatography, and was better than 98% pure. Unlabeled monoolein was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, purified by silicic acid column chromatography, and was better than 95% pure. Triton X-100 and fatty acid-poor bovine serum albumin (BSA) were obtained from Sigma Chemical Co., St. Louis, and were used without further purification. Glycerol was from Wako Pure Chemical Industries, Ltd., Osaka. All the other chemicals employed were of reagent grade.

2.2. *Source of enzyme*

Human post-heparin plasma was obtained from healthy young donors who had fasted overnight. Blood was collected in tubes containing 1/20 vol of 0.25 M sodium citrate 10 min after intravenous administration of sodium heparin of 10 units/kg of body weight. All samples were kept on ice and plasma was separated by centrifugation at 5000 *g* and 4°C. Plasma samples were then either stored at −20°C or used immediately.

2.3. *Enzyme assay*

Monoacylglycerol hydrolase activity assays were carried out by the method previously reported [5] using [^{3}H]glyceryl monooleate emulsified in the presence of Triton X-100 as substrate.

Triacylglycerol lipase activity was assayed with [^{3}H]glyceryl trioleate substrate emulsified with Triton X-100 and fatty acid-poor BSA in the presence of 0.15 M NaCl and fasted plasma as previously described [6].

2.4. *Enzyme purification*

Post-heparin plasma was incubated with 0.1 vol of 20% Intralipid for 8 min at 37°C, and the resulting TGL–substrate complex removed by flotation in the ultracentrifuge at 60 000 *g* for 1 h at 4°C. The infranatant, which still contained some TGL activity, was transferred to new tubes and the same procedure repeated two more times. The final infranatant was diluted 10 times with 0.005 M sodium barbital buffer, pH 7.4, containing 0.15 M NaCl. The sample was applied on a Sepharose-4B column (2.0 × 12.0 cm) containing covalently bound heparin [7]. The column was equilibrated with 0.15 M NaCl in 0.005 M sodium

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barbital buffer, pH 7.4. After washing with the same buffer, elution was conducted with a linear NaCl gradient (0.5 ml per min) from 0.15 M to 2.0 M NaCl in 0.005 M sodium barbital buffer, pH 7.4. The total volume of gradient was 200 ml and fractions of 5 ml were collected.

2.5. *Other procedures*

Protein was determined by the method of Lowry et al. [8] using BSA as a standard. Chloride concentrations were determined with a Evans chloridemeter.

3. Results and discussion

As shown in table 1, the specific activity for MGH of human post-heparin plasma is about 12 times that of TGL. Although Nilsson-Ehle and Belfrage [4] reported no measurable TGL activity in their final infranatant after five treatments with Intralipid, the present results showed that approx. 18% of TGL activity remained in the final infranatant. After affinity chromatography, however, almost no measurable TGL activity was detected in the combined fraction. Recent evidences from several laboratories have indicated the presence of protamine- or salt-resistant lipase and protamine- or salt-inactivated lipase in post-heparin plasma [9,10]. Ehnholm et al. [10] reported that 40–45% of the salt-resistant lipase and 5–10% of the salt-inactivated lipase activity remained in the final infranatant after two treatments with Intralipid. They suggested that both lipases were able to bind to Intralipid although the affinity of the saltresistant lipase was lower.

As described by Greten et al. [11], MGH activity eluted from heparin-Sepharose column with that of TGL (fig.1). Then, it is necessary to separate these two enzyme activities. Recently TGL in post-heparin plasma was purified by affinity chromatography on heparin-Sepharose. These preparations were supposed to contain MGH activity which was more stable than TGL. The present results show that the final specific activity of MGH is 5737 nmol glycerol liberated per mg protein per min, representing approx. 535-fold purification from post-heparin plasma.

MGH activities in post-heparin plasma and in the infranatant after treatments with Intralipid were remarkably stable and they could be stored with no loss of activity for 24 h at 4°C. Stability of the purified enzyme after affinity chromatography at 4°C was also studied. Almost 40% of the activity was lost during the 1st hour, after which activity was lost more slowly for 24 h, with a half-life of approximately 11 h (fig.2); This enzyme is considered more stable than the purified adipose tissue TGL, which has a half-life of only 3.5 h [12]. The purified MGH could be stabilized by the addition of either glycerol to a final concentration of 20% or albumin of 1%. As shown in fig.2, 70 and 95% of the original activity were retained after 3 h in the presence of 20%

Table 1
Purification of monoacylglycerol hydrolase from human post-heparin plasma

	Protein	Monoacylglycerol hydrolase				Triacylglycerol lipase		Ratio MGH/TGL
	(mg)	Total Activity (unit)	Specific Activity (unit/mg)	Purification (fold)	Yield (%)	Total Activity (unit)	Yield (%)	
Post-heparin plasma (9 ml)	646.2	6931.8	10.7	1	100	572.4	100	12.1
Intralipid treatment	636.5	5864.3	9.2	0.9	84.6	107.0	18.7	54.8
Affinity chromatography								
Peak fraction (5 ml)	0.063	361.4	5736.5	535	5.2	[a]	[a]	
Combined fraction (15 ml)	0.225	946.8	4208.0	392	13.7	2.9	0.5	326.5

[a]Not measurable.

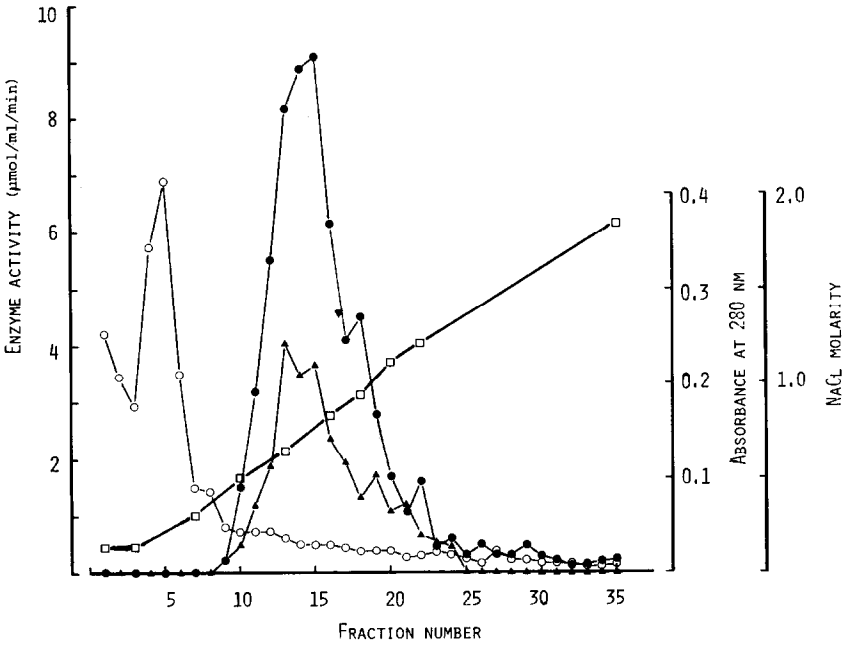
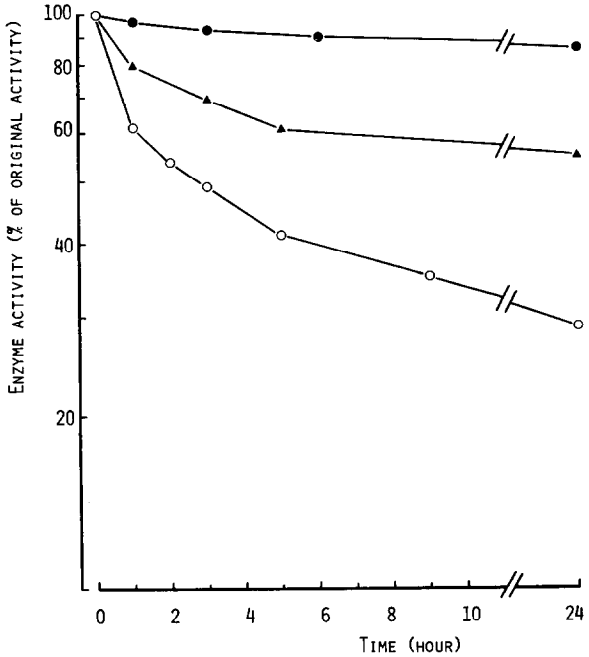


Fig.1. Affinity chromatography of human post-heparin plasma MGH on heparin-Sepharose. A plasma sample (9 ml) of the final infranatant of Intralipid treatment, which had been diluted before, was applied on the column. 100 ml of the starting buffer were then applied, eluting a large protein peak not shown on the figure. A gradient between 0.15 and 2.0 M NaCl with 0.005 M sodium barbital buffer, pH 7.4, was then started using a total volume of 200 ml. Fractions of 5 ml were collected. (○——○) Protein measured by absorbance at 280 nm. (●——●) MGH activity in the assay system described under Materials and methods without serum. (▲——▲) MGH activity in the assay system with 0.08 vol of serum. (□——□) NaCl molarity.



glycerol or 1% BSA, respectively. The results of Bensadoun et al. [12] and of the present study lead us to suppose that the affinity chromatography on heparin-Sepharose appears not only to purify the enzymes, but also to separate the stabilizing structure from the enzyme proteins.

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Fig.2. Stability of partially purified MGH from human post-heparin plasma (heparin-Sepharose step) at 4°C in the absence and presence either of 20% glycerol or 1% bovine serum albumin. (○——○) No addition. (▲——▲) 20% glycerol. (●——●) 1% bovine serum albumin.

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