NOTE

Lipolytic Activities other than Lipoprotein Lipase in Mouse Mammary Gland

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Abstract

The lipolytic activities with acid pH optimum were extracted from the acetone powders of the pregnant and lactating mouse mammary glands. Since the activities were strongly inhibited by human serum and albumin which were required for lipoprotein lipase assay, they were considered to be ascribable to lipase(s) other than lipoprotein lipase. Despite the fact that the amount of fatty acid released by the extract prepared from the glands in pregnancy (P-extract) was proportional to the incubation time, the release of the fatty acid by the extract from those in lactation (L-extract) ceased during incubation for a short time. The cessation, however, was avoided by preincubating the L-extract at 50°C for 10 min before assay. In the presence of L-extract, the lipolytic activity in the P-extract was also almost completely lost after a short incubation. From these results, the existence of a heat labile inactivator of the lipolytic activities in lactating mouse mammary glands was suggested.

While many investigations revealed that lipoprotein lipase (LPL) activity of mammary glands remarkably increased after parturition (McBride and Korn, 1963; Robinson, 1963; Falconer and Fiddler, 1970; Hamosh *et al.*, 1970), little attention has been paid to the possible existence of other kinds of lipases in the glands. Among these studies on LPL, McBride and Korn (1963) suggested the possibility of the presence of the other lipases in the mammary gland of the pregnant guinea pig. Recently, we found high lipolytic activities ascribed to the activities of lipases other than lipoprotein lipase in the mammary gland of both pregnant and lactating mouse, and suggested

that this unknown lipase may play an important role in the decomposition of the tryglyceride occurs in the glands at or about the time of parturition (Anzai *et al.*, 1979). The preliminary experiments revealed that the unknown lipase and LPL could be separately extracted from acetone powders of the glands by changing the extraction buffer. Some properties of the enzyme(s) were therefor compared with those of LPL.

Materials and Methods

Chemicals

Bovine serum albumin (fraction V) and other chemicals were of the highest purity grade and were purchased from Wako Chemicals (Tokyo). Fatty acid free olive oil was prepared as described by Dirstin *et al.* (1968) according to the method of Vogel and Zieve (1963). Oil emulsion (20%, v/v) was prepared by sonifying the fatty acid free olive oil in a 5% gum arabic solution at 50 watts/10 ml with a Branson sonifier.

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Abbreviations: EDTA; ethylenediamine tetraacetate
LPL; lipoprotein lipase MES; 2-(n-morpholino)
ethane sulfonic acid Tris; Tris (hydroxymethyl)
aminomethane.

Measurement of lipolytic activities and assays

Acetone dried powders were prepared from the inguinal 4 mammary glands of ddy mice on the 20th day of pregnancy and the 3rd day of lactation by the routine method. The powders were homogenized with 5 volumes of buffer-I, which was composed of 100 mM MES-Tris (pH 6.0), 1 mM EDTA, 6 mM MgCl_2 , 2.5 mM dithiothreitol, 0.25 M sucrose and 30% glycerol, then stirred for 30 min at 0°C and centrifuged at 10,000 g for 25 min. The supernatant was used as an enzyme source. In some experiments, the 2nd extraction was made on the remaining precipitates with buffer-II, composed of 50 mM Tris-HCl (pH 8.0) and 1 M ethyleneglycol, which was known to be suitable for the extraction of LPL (Girfunkel, *et al.*, 1976). Since the assay method of Dirstine *et al.* (1968), which had been employed in our previous studies (Anzai *et al.*, 1979), was reported to be unsuitable for the measurement of fatty acids in the presence of high protein concentration (Hayase and Tappel, 1970), the following assay method was used in the present experiment. The reaction emulsion contained 0.1 ml of the oil emulsion, 0.05 ml of 1 M MES-Tris (pH 5.0-7.5) or Tris-MES (pH 7.5-9.5) buffer of various pH and 0.15 ml of water. Occasionally, 0.1 ml of human serum and 0.05 ml 20% bovine serum albumin solution was added in stead of the water. The mixture was pre-incubated at 37°C for 30 min, the reaction was started by the addition of 0.2 ml the enzyme extract, so that final volume of the reaction emulsion was 0.5 ml. Incubation was carried out at 37°C for 45 min with continuous shaking (100 strokes/min) except where other-wise stated. After the incubation, 2.5 ml of Dole's reagent (Dole, 1956) was added to the reaction emulsion which was then mixed with a voltex mixer and the lower aqueous phase was eliminated. The remaining heptane phase was washed once with 2 ml of water and briefly centrifuged at 1,000 g. To the 1 ml of heptane phase, 2 ml of heptane-chloroform mixture (2 : 3) and Dumcombe's copper reagent (Dumcombe, 1963) were added, vigorously shaken and briefly centrifuged. Then, 1 ml of 0.5% sodium dithiodicarbamate in *sec*-butanol was added to 2 ml of the chloroform-heptane phase, and OD at 440 nm was measured. By using this method, about 85% of the oleic acid (0-2 μ moles) exogenously added to the reaction emulsion was able to be recovered consistently over the pH range from 5 to 9. The extraction rate was not affected by the presence of human serum and/or albumin.

Measurement of amount of protein

The amount of protein was measured by the method of Lowry *et al.* (1951). The extracts prepared from pregnant and lactating mammary glands as mentioned above contain ca. 7 and 10 μ g of protein/ml, respectively.

Results and Discussion

Figure 1-a shows pH profiles of the lipolytic activities of the extracts which were prepared with buffer-I from the acetone dried powders of mammary glands on 20th day of pregnant (P-extract) and 3rd day of lactating (L-extract) mice in the presence or absence of serum and albumin. Optimum pH of the lipolytic activity of these extracts was about 6. Serum and albumin added to the reaction emulsion negated the activities almost completely in the optimum pH range and no lipolytic activity was observed in either extract either in the presence or absence of serum and albumin at pH about 8. These findings suggest the possibility that the lipolytic activity found in the extracts might not be due to LPL, and LPL may not be extracted with buffer-I. In order to confirm the possibility, the pH profiles of the lipolytic activity of the extracts prepared with buffer-II from the remaining precipitates obtained after the extraction with buffer-I were determined. As shown in Fig. 1-b, optimum pH of the lipolytic activity in either pregnant or lactating mammary gland in the presence of serum and albumin was about 8 corresponding to the optimum pH of LPL, but no activity was observed in the absence of the serum proteins. Therefore, this finding indicates that LPL remains in the precipitates, i. e., the lipolytic activities with acid pH optimum and LPL can separately be extracted from the acetone powders of mammary glands by successive extraction with buffer-I and buffer-II. Higher LPL activity expressed in terms of the wet tissue weight basis was obtained in the extract from the lactating gland rather than from the pregnant gland as reported by McBride and Korn (1963), Robinson (1963), Falconer and Fiddler (1970) and Hamosh *et al.* (1970). At pH about 6, low lipolytic activity was found in extracts from either pregnant gland or those from lactating gland.

Fig. 1. (a) pH profiles of the lipolytic activities of the extracts prepared with buffer-I from the acetone powders of the mouse mammary glands in pregnancy (20th day, P-extract) and in lactation (3rd day, L-extract). The activities of the P-extract in the presence (■) and absence (□) of human serum and bovine serum albumin, and those of the L-extract in the presence (●) and absence (○) of these substances were measured at various pHs. The activities of the L-extract heated at 50°C for 10 min prior to the assay (HL-extract) were also measured in the absence of albumin (Δ). (b) pH profiles of the lipolytic activities of the extracts prepared with buffer-II from the remaining precipitates obtained after the extraction with buffer-I mentioned above. The activities of the extract prepared from pregnant glands in the presence (■) and absence (□) of serum and albumin, and those from lactating glands in the presence (●) and absence (○) of these substances were measured at various pHs.

Similar pH profiles as shown in Fig. 1-b were obtained in the extracts prepared directly with buffer-II from the acetone powders of the mammary glands. Since only low lipolytic activities were observed at pH about 6 in the absence of serum and albumin even in these extracts, the lipolytic activities found in the present study may not be extracted with buffer-II or may be unstable at pH 8. The fact that very low activities were observed at pH 6 in the extracts prepared with buffer-I from the

remaining precipitates obtained after the extraction with buffer-II (unpublished data) supports the latter possibility.

Fig. 1-a show the pH profile of the L-extract which was preincubated at 50°C for 10 min prior to the assay (HL-extract) in the absence of serum and albumin. The profile was similar to the activity of the P-extract. In order to elucidate some characteristics of the newly found lipase(s) having optimum pH in the acid range, the following experiments were carried out. To the

Fig. 2. (a) Effect of the volumes of crude enzyme extracts on the fatty acid release. ■: P-extract. ●: L-extract. ○: HL-extract. (b) Time course changes in the release of fatty acid by P-, HL- and L-extracts. ■: P-extract (0.1 ml). ○: HL-extract (0.1 ml). ●: L-extract (0.1 ml). ▲: L-extract (0.2 ml). (c) Time course changes in the release of fatty acid by the mixture of P- and L-extracts. ■: P-extract (0.1 ml). ●: L-extract (0.1 ml). ▲: Mixture of P-(0.1 ml) and L-(0.1 ml) extracts. (d) Time course changes in the release of fatty acid by the mixture of HL- and L-extracts. ○: HL-extract (0.1 ml). ●: L-extract (0.1 ml). ▲: Mixture of HL-(0.1 ml) and L-(0.1 ml) extracts.

reaction emulsion, 0, 0.04, 0.08, 0.12, 0.16 and 0.2 ml of the extracts were added. Final volume was 0.5 ml in all cases, adjusted by the addition of a proper amount of extraction buffer, and the amounts of fatty acid released during 45 min incubation were measured (Fig. 2-a). The amount of fatty acid released by either P- or HL-extract was proportional to the volumes of the enzyme extract added. The amount released by L-extract, however, reached the maximum level when the reaction emulsion contained 0.12–0.16 ml of the enzyme extract, and it decreased when the reaction emulsion con-

tained larger a volume of the L-extract (0.20 ml). Fig. 2-b shows the results of the experiments in which the changes in lipolytic rates throughout the incubation period were examined in P- (0.1 ml), HL- (0.1 ml) and L- (0.1 ml, 0.2 ml) extracts, respectively. While the amount released by P- or HL-extract was proportional to the incubation time, that released by L-extract reached the plateau after incubation for a short time. 0.2 ml of L-extract released more fatty acid during the first 5 min incubation than 0.1 ml of the L-extract but reached a plateau more rapidly. The loss of lipolytic activity seemed

to be avoided by preincubating the L-extract at 50°C for 10 min before the assay, since the amount of fatty acid released by HL-extract was proportional to the incubation time. These results suggest the existence of a heat labile inactivator of the lipolytic activity in L-extract. In addition, the amount of fatty acid released by the mixture of P-(0.1 ml) and L-(0.1 ml) extracts (Fig. 2-c) or by the mixture of HL-(0.1 ml) and L-(0.1 ml) extracts (Fig. 2-d) increased during the first 5 min of incubation, but no further increase in the release related to the time of incubation occurred thereafter.

As shown in Fig. 1-a, the addition of albumin and serum to the reaction emulsion decreased the amount of fatty acid released by P- and L-extracts during 45 min incubation. Albumin alone also decreased the amount of fatty acid released by these extracts. The results support the earlier suggestion (Anzai *et al.*, 1979) that the lipolytic activity found in our studies differs from that of "hormone-sensitive lipase" which exists in other adipose tissues and the activity was increased by albumin (Rizack, 1961). The inhibition of lipase activity by albumin was also found in rat liver (Matsumura *et al.*, 1976).

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