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Properties of Salt-Resistant Lipase and Lipoprotein Lipase Purified from Human Post-Heparin Plasma

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Lipoprotein lipase and salt-resistant lipase were isolated from human post-heparin plasma. The proteins of human post-heparin-plasma lipoprotein lipase and salt-resistant lipase were identified and demonstrated to be immunologically different. Significant differences between the two enzymes in their relative amino acid composition were demonstrated, which indicates that the two enzymes are different proteins. When analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, the enzymes seemed to have monomer molecular weights similar to that of lipoprotein lipase purified from bovine milk.

The presence in post-heparin plasma (hereafter called simply 'plasma') of at least two lipase activities, the salt-resistant lipase and the lipoprotein lipase (EC 3.1.1.3), has been reported by Fielding (1972), Greten *et al.* (1972), La Rosa *et al.* (1972), Assman *et al.* (1973) and Ehnholm *et al.* (1975*b*). The salt-resistant lipase activity was assumed to originate in the liver (Greten *et al.*, 1974; Ehnholm *et al.*, 1977) and has also been called 'hepatic lipase' (Augustin *et al.*, 1976). The physiological role of this lipase is unknown. Lipoprotein lipase is supposed to be located at the capillary surface where lipoprotein triacylglycerols are hydrolysed (Robinson, 1970).

In contrast with lipoprotein lipase, the salt-resistant lipase hydrolyses emulsified triacylglycerols in media that contain high salt concentrations (1 M-NaCl), and the enzyme does not require the presence of apolipoprotein C-II (apo C-II) (La Rosa *et al.*, 1970; Greten *et al.*, 1972). Antibodies prepared against salt-resistant lipase have no effect on the lipoprotein lipase activity (Ehnholm *et al.*, 1975*a*).

The present investigation demonstrates that the two human plasma lipases, salt-resistant lipase and lipoprotein lipase (Östlund-Lindqvist & Boberg, 1977) differ both in their immunological properties and in their amino acid composition.

Materials and Methods

Materials

Fresh post-heparin plasma was obtained from healthy students (with their informed consent). Sepharose CL-6B was purchased from Pharmacia Fine Chemicals A.B., Uppsala, Sweden. Tri[9,10(n)-^{3}H]oleoylglycerol was purchased from Dr. Lennart Krabisch, Department of Medical Chemistry 4, University of Lund, Lund, Sweden. All other chemicals used were of analytical grade.

Abbreviations used: SDS, sodium dodecyl sulphate; IgG, immunoglobulin G.

Purification of salt-resistant lipase and lipoprotein lipase from human post-heparin plasma and lipoprotein lipase from bovine milk

Salt-resistant lipase and lipoprotein lipase were purified from human post-heparin plasma by affinity chromatography on conventional heparin–Sepharose (Iverius, 1971; Iverius & Östlund-Lindqvist, 1976), and on heparin–Sepharose with low affinity for antithrombin as described by Östlund-Lindqvist & Boberg (1977). The fractions containing the activities of salt-resistant lipase and of lipoprotein lipase were dialysed against 3.6 M-$(NH_4)_2SO_4$/10 mM-sodium phosphate buffer, pH 6.5. The resultant protein precipitates were dissolved in 50% (v/v) glycerol, 10 mM-sodium phosphate buffer, pH 7.5, and stored at −20°C (Iverius & Östlund-Lindqvist, 1976; Östlund-Lindqvist & Boberg, 1977). The 'plasma' lipases used in the inhibition studies (Figs. 2*a* and 2*b* below) were isolated by the additional purification steps described by Östlund-Lindqvist & Boberg (1977). Lipoprotein lipase from bovine milk was isolated to complete purity as described by Iverius & Östlund-Lindqvist (1976).

The final purification of the 'plasma' lipases was carried out by SDS/polyacrylamide-gel electrophoresis. The SDS-containing discontinuous buffer system by Neville (1971) was used. The gels used for amino acid analyses were stained for 12 h with 0.25% (w/v) Coomassie Blue R250 in acetic acid/methanol/water (7:5:43, by vol.) and were destained in acetic acid/methanol/water (7:25:68, by vol.) (Tornqvist

& Belfrage, 1976) before the protein bands were excised.

Antibodies against salt-resistant lipase from post-heparin plasma and against lipoprotein lipase from bovine milk

The protein band corresponding to salt-resistant lipase on the SDS/polyacrylamide gel was made visible by 1M-KCl (Nelles & Bamburg, 1976), cut out of the gel, dialysed against distilled water for 24h and homogenized. The homogenate was emulsified with Freund's complete adjuvant and injected into one lymph node on the back side of each thigh of a rabbit.

Three booster doses with incomplete adjuvant were given by the same route, at intervals of 2 weeks. The antibodies against highly purified lipoprotein lipase from bovine milk (Iverius & Östlund-Lindqvist, 1976) were raised in rabbits as described by Östlund-Lindqvist & Boberg (1977). The IgG fractions were isolated (Levy & Sober, 1960). The IgG fraction of serum obtained from non-immunized rabbits was used as the control.

Immunodiffusion and immunoelectrophoresis

Double immunodiffusion (Ouchterlony, 1958) and immunoelectrophoresis (Grabar & Williams, 1953) were carried out in 1% (w/v) agarose gel containing 0.04M-sodium veronal buffer, pH 8.6, and 0.01% (w/v) Nonidet P-40 (Iverius & Östlund-Lindqvist, 1976).

Protein determinations

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, or alternatively was measured by A_{280} (Little & Donahue, 1968).

Amino acid analysis

Stained bands of enzyme protein and protein-free gel from the SDS/polyacrylamide-gel electrophoresis were excised, destained further in 7% (v/v) acetic acid and freeze-dried (Houston, 1971; Tornqvist & Belfrage, 1976). The freeze-dried material was hydrolysed in 6M-HCl containing 1% (w/v) phenol at 110°C for 24h. The solvent was removed by evaporation. The residue was dissolved in 1ml of distilled water, filtered through a Millipore filter (3μm pore size), freeze-dried and finally dissolved in the loading buffer. Amino acid analyses on the sample and on the control were carried out as described by Spackman *et al.* (1958) with a Beckman 121M analyser. It was not possible to determine the basic amino acids owing to the high concentrations of ammonia in the samples.

Enzyme assays

The assay medium for determination of lipoprotein lipase activity consisted of Tris/HCl buffer, pH 8.5, at physiological ionic strength (I 0.15), human blood serum (20%, v/v), bovine serum albumin (5.5%, w/v) and a 20% (v/v) emulsion of soya-bean oil/egg phosphatidylcholine (50:3, w/w) radioactively labelled with tri[9,10(n)-^{3}H]oleoylglycerol (1.3 Ci/mol) (0.25%, v/v) (Östlund-Lindqvist & Boberg, 1977).

The salt-resistant lipase activity was assayed in a medium similar to that used for lipoprotein lipase. However, blood serum was omitted and 1:1 (w/w) emulsion of tri[9,10(n)-^{3}H]oleoylglycerol (1.7 Ci/mol)/gum arabic was used as the substrate (10mg of trioleoylglycerol/ml of medium) (Bensadoun *et al.*, 1974).

The enzyme solution was mixed with increasing amounts of the IgG fractions in a total volume of 0.2ml. After preincubation at 0°C for 1h, lipase activity was assayed in a total volume of 0.5ml for 30min at 37°C for lipoprotein lipase and at 28°C for salt-resistant lipase as described by Östlund-Lindqvist & Boberg (1977).

Results

Purification of salt-resistant lipase and lipoprotein lipase from human post-heparin plasma by SDS/polyacrylamide-gel electrophoresis

The enzymes were partially purified by affinity chromatography (as described in the Materials and Methods section). The partially purified enzyme fractions were dissolved (0.5–1.0mg/ml) in 10mM-sodium phosphate buffer, pH 7.5, containing 1% (w/v) SDS and 1% (v/v) β-mercaptoethanol for 2h at 37°C. Enzyme sample (50μl) was mixed with 5μl of Bromophenol Blue (0.05%), β-mercaptoethanol (5μl) and glycerol (about 5μl) before being applied to the SDS/polyacrylamide gel. The electrophoretic separation was carried out in 11% (w/v) polyacrylamide gel at 20°C with current of 1.5mA/gel. The separation was stopped when the Bromophenol Blue tracer reached the lower end of the gel. The two 'plasma' lipases, salt-resistant lipase and lipoprotein lipase, showed one major protein band that seemed to have a mobility to that of lipoprotein lipase from bovine milk (Fig. 1). In addition, several minor protein bands were observed. The arrows in Fig. 1 indicate the major protein bands, which were tentatively identified as containing the enzyme proteins.

Identification of salt-resistant lipase and lipoprotein lipase from human post-heparin plasma and lipoprotein lipase from bovine milk purified by SDS/polyacrylamide-gel electrophoresis

The presumptive salt-resistant lipase was isolated and antibodies were raised against it. These antibodies inhibited the salt-resistant lipase activity of the partially purified enzyme fraction (Fig. 2*a*). Similarly, the lipoprotein lipase activity of human 'plasma' and of bovine milk were inhibited by the

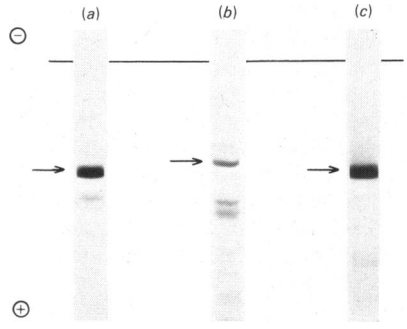

Fig. 1. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of fractions of (a) salt-resistant lipase from human post-heparin plasma (15 μg), (b) lipoprotein lipase from human post-heparin plasma (5 μg), and (c) lipoprotein lipase from bovine milk (30 μg)*

The gels were stained with Amido Black. The arrows indicate the major protein bands of each enzyme fraction. These bands are the 'tentative' enzyme proteins.

antibodies raised against highly purified bovine milk lipoprotein lipase (Figs. 2*b* and 2*c*). The inhibition of the 'plasma' lipoprotein lipase and of the bovine milk lipoprotein lipase was 85% at IgG concentrations of 0.2 and 0.03 mg/ml respectively. This probably means that the bovine milk lipoprotein lipase and the 'plasma' lipoprotein lipase possess slightly differing compositions, which also is shown by the partial identity demonstrated by immunodiffusion (see below). The antibodies against salt-resistant lipase did not differ significantly in their effect on lipoprotein lipase activities from the behaviour of the control IgG fraction. Neither did the antibodies against the bovine milk lipoprotein lipase significantly differ from the control IgG fraction in their effect on the salt-resistant lipase activity.

The presumptive 'plasma' lipoprotein lipase obtained from the SDS-containing gel was identified by double immunodiffusion as demonstrated in Fig. 3. The protein indicated by the arrow in Fig. 1(*b*) was made visible by means of 1 M-KCl (Nelles & Bamburg, 1976) and excised from the gel. The slice was placed into agarose gel containing 0.01% Nonidet P-40. As Fig. 3 shows, one precipitation line was obtained between the human 'plasma' lipoprotein lipase protein and the IgG fraction against bovine milk lipoprotein lipase and between highly purified bovine milk lipoprotein lipase, excised from the SDS-containing gel, and the same IgG fraction. There was partial identity between these two lines.

Double immunodiffusion as well as immunoelectrophoresis showed only one precipitation line between the IgG fraction raised against the tentative salt-resistant lipase protein and the enzyme fraction.

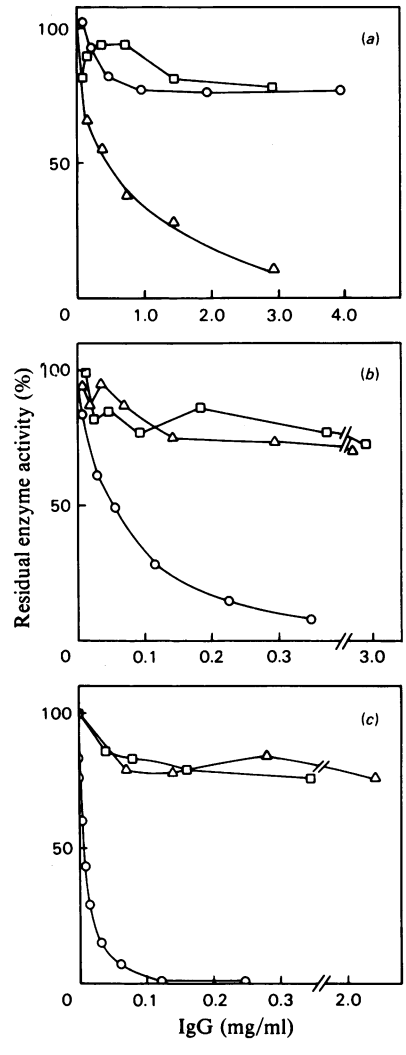

Fig. 2. *Effects of different antibodies on the activity of (a) salt-resistant lipase, (b) lipoprotein lipase from human post-heparin plasma and (c) lipoprotein lipase from bovine milk*

Antibodies against purified salt-resistant lipase (△), purified bovine milk lipoprotein lipase (○) and control rabbit antibodies (□) were incubated with the enzymes as described in the Materials and Methods section. An enzyme activity of 100% represents: for (*a*), 0.013; for (*b*), 0.04; and for (*c*), 0.05 μmol of fatty acid released/min per ml of assay medium.

No precipitation lines were observed between this IgG fraction and the lipoprotein lipase proteins nor between the IgG fraction raised against human antithrombin and the human 'plasma' lipase fractions (Fig. 3).

Amino acid analysis of salt-resistant lipase and lipoprotein lipase from human post-heparin plasma and of lipoprotein lipase from bovine milk

A portion (30–50 μg) of each protein preparation was applied to the SDS/polyacrylamide gel at the final purification step of the three lipases. The composition of 13 amino acids in five replicates of each enzyme protein was determined. Parallel to each determination, one slice of SDS-containing gel was analysed as a control. Significant amounts of serine, glycine, glutamine and glutamic acid were recovered in these controls. It was not possible to determine the basic amino acids, owing to the high concentration of ammonia in the sample even after evaporation had been carried out.

Mean values ± S.D. of the relative amounts of the amino acids in the three enzymes were calculated, with valine as the reference amino acid (Table 1). The lowest variance of the method was obtained with valine.

A comparison between the relative amino acid composition of the three enzyme proteins was made by the two-tailed Student's t test (Snedecor, 1961). As Table 1 shows, salt-resistant lipase is different from human 'plasma' lipoprotein lipase in the following respects: a higher proportions of threonine, methionine, isoleucine and leucine ($P<0.001$), and a lower proportions of tyrosine ($P<0.001$), in relation to valine. In addition, the salt-resistant lipase and bovine milk lipoprotein lipase have different amino acid compositions (Table 1). Slight differences were also observed between the human and the bovine lipoprotein lipase protein moieties. The human enzyme has significantly higher proportions of serine ($P<0.01$) and alanine ($P<0.005$) and lower proportions of methionine ($P<0.005$) and tyrosine ($P<0.005$) in relation to valine.

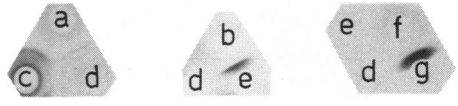

Fig. 3. *Immunodiffusion analysis with antibodies against bovine milk lipoprotein lipase (a), against salt-resistant lipase (b), and against human antithrombin (f) and pieces of SDS/polyacrylamide gel containing bovine milk lipoprotein lipase (c), human 'plasma' lipoprotein lipase (d), salt-resistant lipase (e) and human antithrombin (g)*

Discussion

The isolation by Östlund-Lindqvist & Boberg (1977) of salt-resistant lipase and lipoprotein lipase from human post-heparin plasma yielded quantities too small to identify and characterize the enzymes, and SDS/polyacrylamide-gel electrophoresis indicated the presence of impurities. In the present study SDS/polyacrylamide-gel electrophoresis was adopted as a final step for the purification of the enzymes. This technique inactivates the enzymes, which leads to difficulties in their identification on the SDS-containing gel. Identification of the enzyme proteins, which has not been done before, was achieved by raising specific antibodies against the pure enzyme proteins. The purity was determined by immuno-

Table 1. *Relative amino acid compositions of the lipases*

	Composition (relative to valine = 1)			
			Bovine milk lipoprotein lipase	
Amino acid	Human 'plasma' salt-resistant lipase*	Human 'plasma' lipoprotein lipase*	Present data*	Data of Iverius & Östlund-Lindqvist (1976)
Aspartic acid/asparagine	1.34±0.03	1.51±0.14	1.51±0.02†	1.43
Threonine	1.27±0.02	0.84±0.09†	0.80±0.06†	0.78
Serine	1.25±0.12	1.40±0.06	1.19±0.11	1.24
Glutamic acid/glutamine	2.02±0.09	1.63±0.05	1.28±0.13	1.23
Proline	0.86±0.03	0.77±0.08	0.71±0.03†	0.66
Glycine	1.33±0.06	1.15±0.09	1.11±0.18	1.07
Alanine	0.96±0.03	1.05±0.10	0.82±0.04†	0.82
Valine	1	1	1	1
Methionine	0.33±0.04	0.18±0.02†	0.24±0.02	0.24
Isoleucine	1.11±0.06	0.71±0.04†	0.69±0.02†	0.67
Leucine	1.58±0.02	0.99±0.08†	1.14±0.08†	1.00
Tyrosine	0.40±0.01	0.55±0.03†	0.63±0.02†	0.60
Phenylalanine	0.81±0.04	0.67±0.05	0.69±0.03†	0.72

* The mean values ± S.D. for five determinations are given.
† Statistical significance $P<0.001$ compared with salt-resistant lipase.

diffusion and immunoelectrophoresis. The antiserum against pure bovine milk lipoprotein lipase seemed to have a lower inhibitory effect on the activity of human 'plasma' lipoprotein lipase than it did on that of bovine milk lipoprotein lipase, a result that interpreted as being due to species differences.

The method of Houston (1971) and Tornqvist & Belfrage (1976) was used to determine the amino acids of stained protein bands of SDS-containing gels. The amino acid compostion of bovine milk lipoprotein lipase in SDS-containing gel was similar to that of highly purified lipoprotein lipase from bovine milk (Iverius & Östlund-Lindqvist, 1976), which confirmed the validity of this method. Significant differences in the relative amino acid compositions of salt-resistant lipase and the human 'plasma' lipoprotein lipase were demonstrated, which strongly indicates that the two enzymes are different proteins. This result disagrees with those of Augustin *et al.* (1978), who claimed that salt-resistant lipase and lipoprotein lipase from human 'plasma' have identical amino acid compositions and only differ in their carbohydrate contents. However, the purification procedure used by these authors probably does not separate the enzymes from antithrombin (Östlund-Lindqvist & Boberg, 1977), which is a glycoprotein with a molecular weight similar to that of the lipases.

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