Purification and Characterization of Lipoprotein Lipase from Pig Adipose Tissue*

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SUMMARY

Lipoprotein lipase was purified from acetone powders of pig adipose tissue. Extraction of acetone powders with 1.2 M NaCl in 0.005 M sodium-barbital buffer, pH 7.4, or heparin (200 units per ml) in distilled water, was 6 times as effective as extraction with 0.025 M NH₂OH·NH₄Cl buffer, pH 8.6, the commonly used extractant for lipoprotein lipase. At pH values below 7.5, over 85% of the activity extracted into 1.2 M NaCl could be recovered after 4 hours. The partially purified enzyme at later stages was stabilized by the inclusion of 20% glycerol in the buffers. Most of the purification was accomplished by affinity chromatography on Sepharose 4B columns containing covalently bound heparin. At this step, the preparation was purified 600-fold. This purified enzyme binds reversibly to columns containing concanavalin A covalently bound to Sepharose. Lipolytic activity was eluted from concanavalin A-Sepharose column with 0.2 M α-methyl-D-mannoside, 1.0 M NaCl and 0.005 M sodium-barbital, pH 7.0. At this stage, the enzyme was purified 2100-fold. Isoelectric focusing yielded a single major peak of activity with an isoelectric point of 4.0. Minimum molecular weight determination by gel filtration in buffers containing 1.0 M NaCl and by disc gel electrophoresis in sodium dodecyl sulfate yielded values of 62,000 and 60,000, respectively. The crude enzyme, and that eluted from heparin-Sepharose columns, did not show stimulation by heparin, whereas that obtained after isoelectric focusing exhibited a 60 to 100% stimulation at 22 μg of heparin per ml. Activation by diazoyed serum was dependent on the stage of purification. The crude enzyme showed a 20-fold stimulation by serum but showed some activity in its absence; that purified by isoelectric focusing exhibited a 20-fold stimulation by serum but was dependent on the stage of purification. The purified enzyme at later stages was stabilized by the inclusion of 20% glycerol in the buffers. Most of the purification was accomplished by affinity chromatography on Sepharose 4B columns containing covalently bound heparin by a method similar to that developed recently for the purification of milk lipase (5) and of a postheparin plasma lipolytic enzyme (6). Affinity chromatography was also conducted on agarose columns containing covalently bound concanavalin A. The known inhibitors and activators of the crude adipose tissue lipoprotein lipase were re-evaluated with the highly purified enzyme.

MATERIALS AND METHODS

Lipoprotein lipase activity was assayed in duplicate with a synthetic [14C]triolein substrate emulsified in the presence of gum arabic. Triolein containing [14C]triolein in all three positions was purchased from DHOM Products, North Hollywood, California. The assay system contained the following components in a total volume of 0.5 ml: 0.76 μmole of [14C]triolein (0.13 μCi per μmole of triolein); 2.5 mg of gum arabic; 5 mg of albumin; 0.06 ml of normal dialyzed pig serum; 0.1 μmole of Tris buffer; 0.05 μmole of NaCl; 5 μmoles of CaCl₂; and 0 to 0.05 ml of enzyme preparation. In kinetic studies of the effect of substrate concentration on enzyme activity and in activity measurements of enzyme purified by isoelectric focusing, the [14C]triolein specific activity was increased to 0.53 μCi per μmole of triolein. Assays were conducted at pH 8.6 at 30°C. Enzyme activity was linear with time up to 60 min and proportional to amount of enzyme added. Labeled fatty acids released were separated from the substrate by the liquid-liquid partition system of Belfrage and Vaughan (7). Variation in NaCl molarity (0 to 1.0 M) and in pH (6 to 10) did not affect the partition coefficient of [14C]oleic acid.

Sources of Enzyme Activity—Swine, fed ad libitum and weighing between 15 and 80 kg, were anesthetized with pentobarbital and exsanguinated via a catheter placed in the carotid artery. Subcutaneous adipose tissue was dissected from the cervical, dorsal, and lumbar regions, and stored at −20°C. Acetone powders were prepared as previously described (8). Lipase activity in acetone powders stored at −20°C was stable for up to 2 weeks. Acetone powders were extracted with the following solutions:

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0.025 M NH₄OH-NH₄Cl, pH 8.6; various concentrations of NaCl in 0.005 M sodium-barbital, pH 7.4; 1 mg glucose; and various concentrations of heparin (Sigma Chemicals, St. Louis, Missouri) in glass-distilled water. Unless otherwise specified, 1 ml of extracting solution was used per 50 mg of acetone powder.

**Enzyme Purification**—Except in the isoelectric focusing studies, all buffers used during purification contained 20% glycerol, 0.005 M sodium-barbital, 20% glycerol, pH 7.0. The suspension was centrifuged at 30,000 × g for 10 min and the supernatant fraction was dialyzed against 40 volumes of 0.001 M sodium-barbital, pH 7.0, for 4 hours with two changes of buffer.

The dialyzed sample was turbid due to protein precipitation. After 48 hours, the supernatant and protein determined after solubilization in a small volume. Details of the procedure will be published elsewhere.  

**RESULTS**

**Lipoprotein Lipase from Adipose Tissue Acetone Powders**—In order to test the efficiency of extraction of lipoprotein lipase by various solutions, acetone powders of swine adipose tissue were sequentially extracted (1 ml/50 mg of acetone powder), twice with 0.025 M NH₄OH-NH₄Cl and then with either 1.5 M NaCl-0.005 M sodium-barbital, pH 7.4, or a heparin solution in water (200 units per ml). The results of this experiment (Table I) demonstrate clearly that considerable residual lipoprotein lipase activity is still present in acetone powders extracted twice with the commonly used NH₄OH-NH₄Cl extractant. The buffered NaCl solutions and the heparin solution extracted, respectively, 2- and 5-fold more lipoprotein lipase activity than the first NH₄OH-NH₄Cl extraction. Furthermore, the specific activity of the enzyme extracted by NaCl was considerably higher than that of the enzyme extracted by NH₄OH-NH₄Cl. Heparin solutions appeared to be even more selective as extractants than NaCl solutions, yielding enzyme with more than three times the specific activity. As shown in Table I, the lipase activity extracted with either NaCl or heparin was serum-dependent and showed marked inhibition when assayed in the presence of 1 M NaCl, properties similar to those of the enzyme extracted with NH₄OH-NH₄Cl. Prostaminate sulfate (0.3 mg per ml of assay system) added at zero time, i.e. without preincubation, consistently inhibited the enzyme extracted by NaCl or by heparin but had a lesser effect on that extracted initially by NH₄OH-NH₄Cl. In some experiments, the NH₄OH-NH₄Cl extracts actually showed increased activity in the presence of prostaminate sulfate. Preincubation of enzyme with prostaminate sulfate, however, led to inhibition of all preparations.

Graded levels of NaCl and of heparin were tested for their effectiveness in extracting lipoprotein lipase activity relative to that of NH₄OH-NH₄Cl. As shown in Fig. 1, extraction of lipoprotein lipase was maximal at 200 units per ml. Extraction by NaCl increased progressively with the concentration used up to 1.2 M, where it plateaued at a level 30% below that reached with heparin extraction. At their optimal concentrations, NaCl and heparin extract six and seven times more enzyme activity, respectively, than a single NH₄OH-NH₄Cl extraction. All enzymatic assays were conducted in the presence of the same final 39 mM EDTA (pH 8.6) concentration.

1 A. Bensadoun, D. Weinstein, and D. Steinberg, unpublished data.
Sequential extraction of lipoprotein lipase from acetone powders with various extracting agents

The complete system is described under "Materials and Methods." Protamine sulfate was added to provide a final concentration of 0.3 mg per ml of assay mixture. NaCl when used as an inhibitor was added to supply a 1 M concentration in the final assay mixture. Six aliquots of the same acetone powder preparation were extracted twice with 0.025 M NH₄OH-NH₄Cl, pH 8.6 (Extractions 1 and 2). Three were then extracted with 1.5 M NaCl, 0.005 M sodium-Verona1 buffer, pH 7.4 (Extraction 3a), and the remaining 3 samples with heparin, 200 units per ml. All enzymatic assays were conducted in the presence of the same heparin (17 units per ml) and NaCl (0.1 M) concentrations. Enzyme activities are expressed as micromole of fatty acid released per ml of enzyme extract per hour at 30°.

| Extraction conditions (at 0°) | | Assay conditions |
|-------------------------------|----------------------|-------------------|-------------------|
|                               | Complete system      | + Protamine       | + NaCl            | Serum             |
|                               | µmole/ml/hr          |                   |                   |                   |
| 1. 0.025 M NH₄OH-NH₄Cl, pH 8.6 | 3.6 ± 0.2            | 3.4 ± 0.1         | 1.2 ± 0.1         | 0.3 ± 0.01        |
|                               | (0.95 ± 0.05)        |                   |                   |                   |
| 2. 0.025 M NH₄OH-NH₄Cl, pH 8.6 | 1.5 ± 0.1            |                   |                   |                   |
|                               | (3.5 ± 0.23)         |                   |                   |                   |
| 3a. NaCl 1.5 M 0.005 M Na-veronal, pH 7.4 | 7.3 ± 0.4 | 4.0 ± 0.3 | 1.4 ± 0.1 | 0.4 ± 0.1 |
|                               | (7.5 ± 0.41)         |                   |                   |                   |
| 3b. Heparin (200 units/ml)    | 19.3 ± 3.2           | 15.6 ± 3.0        | 4.9 ± 0.2         | 0.8 ± 0.1         |
|                               | (29.3 ± 4.86)        |                   |                   |                   |

* The figure in parentheses represents the specific activity of the enzyme extract (micromole of fatty acid released per hour per mg of protein).

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Concentrations of heparin (17 units per ml) and NaCl (0.1 M). Thus, the results reflect exclusively the effectiveness of heparin and NaCl on extraction of enzyme activity. Moreover, heparin has essentially no effect on the activity of the crude swine enzyme at concentrations up to 20 units per ml. Glucose (1 M) and methyl-a-D-glucopyranoside (1 M) extracted only traces of lipoprotein lipase activity from swine acetone powders.

Purification of Swine Adipose Tissue Lipoprotein Lipase—The results of a representative purification carried out on 14.5 g of acetone powder are presented in Table II. Dialysis of the crude acetone powder extract was accompanied by precipitation of some protein but this contained no enzyme activity. Recovery of enzyme activity after dialysis varied from 80 to 137%, (107% in the example shown in Table II). Raising the NaCl concentration of the dialyzed solution before applying it to the heparin-Sepharose column decreased the mass of protein adsorbed onto the column and allowed loading of up to 250 ml of extract without significant loss of activity. Fig. 2 presents the elution pattern of lipoprotein lipase from a heparin-Sepharose column. The enzyme activity was eluted as a single peak at a salt concentration of 1.1 M. The specific activity of the pooled material in this peak was more than 600 times that of the original extract.

Lipoprotein lipase purified through heparin-Sepharose chromatography was then applied to a concanavalin A column. The protein not bound to the column contained only traces of activity. Again, only negligible amounts of activity were eluted during washing of the column with 1 M NaCl. Fifty-seven per cent of the applied lipase activity was recovered in a single sharp peak by elution with 0.2 M α-methyl-D-mannoside, 1 M NaCl, 0.005 M sodium-barbital, pH 7.0. Over 80% of the recovered activity was eluted in 14 ml. The specific activity of the pooled

![Fig. 1. Effect of increasing concentrations of NaCl and heparin on the lipoprotein lipase activity extracted from porcine acetone powder. Over hundred-milligram aliquots of the same acetone powder preparation were extracted for 1 hour at 0° with graded levels of NaCl in 0.005 M sodium-barbital, pH 7.4, or increasing concentrations of heparin in water. Except for the samples extracted with 1 unit and 1000 units of heparin, each point represents the mean plus or minus S.E. of four determinations analyzed in duplicate. Lipoprotein lipase activity is expressed as a percentage of the activity extracted with 0.025 M NH₄OH-NH₄Cl, pH 8.6.](http://www.jbc.org/)

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material representing the entire peak (30 ml) was 3.4 times that of the enzyme applied and more than 2000 times that of the original extract.

Much broader activity peaks were obtained when elution was conducted with linear gradients of NaCl (0 to 1 M) and α-methyl-d-mannoside (0 to 1 M) with a total volume of 60 ml. Methyl-α-D-glucopyranoside, when tested either in a step elution (0.2 M or 1.0 M) or as a gradient, produced a broader activity peak, although the total activity recovered was similar to that observed with α-methyl-d-mannoside. Both the presence of α-methyl-d-mannoside and high NaCl molarity (1 M) are necessary for elution of the enzyme. No significant elution of activity occurred in the presence of 0.2 M α-methyl-d-mannoside with NaCl molarities of 0.15 or 0.5 M. The lipoprotein lipase preparation obtained at this stage was weakly stimulated (12 to 25%) by the presence of heparin (4 units per ml) in the medium.

**Isoelectric Focusing**—A preliminary study in the pH range 3 to 10 yielded a single peak of activity. Subsequent analyses were conducted in the 3 to 6 pH range. Enzyme preparations purified by heparin-Sepharose chromatography or sequentially by both heparin-Sepharose and concanavalin A-Sepharose chromatography produced a single activity peak (Fig. 3) with a pI of 4.0 to 4.5. The lipoprotein lipase preparation obtained at this stage was weakly stimulated (12 to 25%) by the presence of heparin (4 units per ml) in the medium.

**Immunoelectrophoresis**—Samples of the purified enzyme after heparin-Sepharose chromatography or sequentially by both heparin-Sepharose and concanavalin A-Sepharose chromatography yielded a single major band. The lipoprotein lipase preparation obtained at this stage was weakly stimulated (12 to 25%) by the presence of heparin (4 units per ml) in the medium.

**Protein**

| Step | Protein | Specific Activity | Recovery | Over-all Recovery | Purification |
|------|---------|------------------|----------|-------------------|-------------|
|      | mg      | pmol/mg protein/hr | %        |                    | fold       |
| Extraction of 14.5 g of acetone powder | 908      | 1.6              | 100      |                   |             |
| Dialyzed extract | 825      | 1.9              | 107      |                   |             |
| Heparin-Sepharose chromatography | 735      | 1.9              | 100      |                   |             |
| Applied | 649      | 2.9              | 100      |                   |             |
| Unadsorbed protein | 649      | 2.9              | 100      |                   |             |
| Washing | 85       | 8                | 100      |                   |             |
| Activity recovered | 2.91    | 60               | 990      | 94.6              | 69          |
| Tube 21 to 46 | 2.91    | 60               | 990      | 94.6              | 69          |
| Tube 31 to 42 | 0.91    | 60               | 990      | 94.6              | 69          |
| Tube 3 to 20 | 0.10    | 36               | 3417     | 57                | 39          |

* Recovery or loss for a given chromatographic step.

**Enzyme activity**

![Fig. 3](http://www.jbc.org/) **Fig. 3.** Isoelectric focusing of porcine adipose tissue lipoprotein lipase. An aqueous extract (250 ml) of acetone powder prepared as described under "Materials and Methods" was applied to a column (10 × 1.5 cm) equilibrated with 0.5 M NaCl, 0.005 M sodium-barbital, pH 7.0. The column was developed with a linear NaCl gradient between 0.5 and 1.5 M NaCl in 20% glycerol, 0.005 M sodium-barbital, pH 7.0, using a total volume of 260 ml. Enzyme activity is expressed as micromole of fatty acid liberated per ml of enzyme solution per hour.

**Table 11**

**Purification of swine adipose tissue lipoprotein lipase**

| Step | Protein | Specific Activity | Recovery | Over-all Recovery | Purification |
|------|---------|------------------|----------|-------------------|-------------|
|      | mg      | pmol/mg protein/hr | %        |                    | fold       |
| Extraction of 14.5 g of acetone powder | 908      | 1.6              | 100      |                   |             |
| Dialyzed extract | 825      | 1.9              | 107      |                   |             |
| Heparin-Sepharose chromatography | 735      | 1.9              | 100      |                   |             |
| Applied | 649      | 2.9              | 100      |                   |             |
| Unadsorbed protein | 649      | 2.9              | 100      |                   |             |
| Washing | 85       | 8                | 100      |                   |             |
| Activity recovered | 2.91    | 60               | 990      | 94.6              | 69          |
| Tube 21 to 46 | 2.91    | 60               | 990      | 94.6              | 69          |
| Tube 31 to 42 | 0.91    | 60               | 990      | 94.6              | 69          |
| Tube 3 to 20 | 0.10    | 36               | 3417     | 57                | 39          |

* a) Recovery or loss for a given chromatographic step.

* b) Extracting buffer was equal to 1.2 M NaCl, 0.005 M sodium-barbital, 20% glycerol, pH 7.0.

for 2 hours, only a very small fraction of the protein entered the gel and multiple fine bands were observed. The major band observed after treatment of the protein samples at 90° (Fig. 4) was shown by disc gel scanning to account for over 95% of the
total proteins stained by Coomassie blue. Its mobility indicated a molecular weight of 60,000 (average of two estimations on two enzyme preparations run on two occasions with three replicates each, 58,000 and 63,000). Mobility of the reference proteins (transferrin, albumin, H chain of γ-globulin, ovalbumin, and L chain of γ-globulin) was not changed by treatment at the higher temperature.

Gel Filtration—Gel filtration chromatography on Sephadex G-150 was conducted in the presence of 1 M NaCl, 20% glycerol, 0.005 M sodium-barbital buffer, pH 7.0. In solutions of low ionic strength both the crude enzyme and the purified enzyme (after heparin-Sepharose or concanavalin A-Sepharose steps) bind tightly to Sephadex G-150. Sodium chloride at concentrations of 0.15 or 0.5 M failed to elute the enzyme. However, in the presence of 1 M NaCl in the buffer, the crude enzyme produced a single activity peak (\(K_v = 0.209\)) corresponding to a molecular weight of 62,000 (reference proteins: transferrin, serum albumin, and ovalbumin).

Enzyme Stability—Stability of the crude enzyme extract (in absence of glycerol) was studied by extracting aliquots of the same acetone powder pool for 1 hour with 1.2 M NaCl in 0.005 M sodium-barbital buffers at pH 6.5, 7.0, 7.5, 8.0, and 8.6. The enzyme activities of these extracts, held at 0°, were then assayed immediately and at various time intervals for 4 hours. Over the range studied, pH did not affect the total amount or specific activity of the enzyme extracted. The enzyme activity was remarkably stable at pH 6.5 and 7.0 (less than 10% loss in 4 hours). However, at pH values above 7.5 there was a progressive loss of activity, especially marked at pH 8.6 (30% loss in 4 hours). Storage for 24 hours at 0° resulted in loss of almost 90% of the activity at pH 8.6, whereas almost 50% was retained at pH 6.5.

Stability of the partially purified enzyme (heparin-Sepharose step) in 1.0 M NaCl at 0-3° at pH 7.0 (0.005 M sodium-barbital) was also studied. Over 40% of the activity was lost during the 1st hour, after which activity was lost more slowly but exponentially for the next 4 hours, with a half-life of approximately 3.5 hours (Fig. 5). However, the purified enzyme in the presence of 1.0 M NaCl could be stabilized by the addition of glycerol to a final concentration of 20%. As shown in Fig. 5, 80% of the original activity was retained after 3 hours in the presence of 20% glycerol.

The purified enzyme could be frozen at -70° with no loss of activity on storage for several days in the presence of bovine serum albumin (1 mg per ml) or glycerol (50%) (Table III).

Stimulation of Lipoprotein Lipase by Serum Activator—The crude enzyme (acetone powder extracted with NaCl solutions) and that obtained after concanavalin A-Sepharose chromatography of lipoprotein lipase purified by heparin-Sepharose and concanavalin A-Sepharose chromatography. Stain, Coomassie blue. Origin at top (cathode). The three faint bands below the major band have mobilities identical to those of the three bands obtained with "blank" eluates and with crystalline concanavalin A (see "Materials and Methods").
Lipoprotein lipase, at 30°C, yielded a linear rate of release of fatty acids from gum arabic-stabilized triolein emulsions for at least 10 days. The enzyme activities are expressed as percentages of that present at time of storage.

### Table III

| Additions                      | Relative enzyme activity<sup>a</sup> |
|-------------------------------|-------------------------------------|
|                               | -20<sup>b</sup> | -70<sup>b</sup> |
|                               | %               |
| None                          | 9               | 37               |
| Albumin (1 mg/ml)             | 95              | 99               |
| Glycerol (60%)                | 54              | 97               |
| Dimethylsulfoxide (10%)       | 35              | 61               |
| Heparin (1 unit/ml)           | 5               |                  |

<sup>a</sup> Lipoprotein lipase activity of enzyme preparations stored for 10 days. The enzyme activities are expressed as percentages of that present at time of storage.

### Table IV

| Stage of purification<sup>a</sup> | Complete system<sup>b</sup> | No serum present | Stimulation<sup>c</sup> |
|----------------------------------|----------------------------|-----------------|-------------------------|
|                                   | µmole/ml/hr                | µmole/ml/hr     | µmole/ml/hr             |
| NaCl extraction                  | 7.3 ± 0.4                  | 0.4 ± 0.1       | 18                      |
| After Concanavalin              | 6.50                       | 0.71            | 9                       |
| After Sepharose                 | 7.12                       | 0.68            | 10                      |
| A-Sepharose                     | 0.29                       | 0.01            | 29                      |
| After isoelectric focusing      | 0.26                       | 0.01            | 26                      |
|                                  | 0.35                       | N.D.<sup>c</sup> |                         |
|                                  | 0.32                       |                 |                         |

<sup>a</sup> The values given for the NaCl extraction (1.5 x NaCl-0.005 M sodium-Veronal buffer, pH 7.4) represent the average ± S.E. of three determinations on the same pooled sample. Other values represent enzyme activities on separate enzyme preparations.

<sup>b</sup> The complete assay system contained the following components in a total volume of 0.5 ml: [14C]triolein substrate, gum arabic, CaCl<sub>2</sub>, albumin, Tris buffer, 0.05 ml of dialyzed serum and 0.05 ml of enzyme.

<sup>c</sup> N.D., not detectable (less than 0.01 µmole per ml per hour).

For these preparations after isoelectric focusing, the standard assay system was modified to increase sensitivity by increasing incubation time to 1 hour, substrate specific activity to 0.53 µCi per µmole, and enzyme volume to 0.2 ml.

### Figure 6

Properties of porcine adipose tissue lipoprotein lipase purified by heparin-Sepharose chromatography with respect to pH of assay, substrate concentration, CaCl<sub>2</sub> concentration, NaCl molarity, incubation time, and heparin concentration in assay. Enzyme activity is expressed as micromole of fatty acid liberated per ml of enzyme solution per hour. Different enzyme preparations were employed with specific activities varying between 900 and 1200 µmoles per mg of protein per hour.

90 min. The pH activity curve showed a single, fairly sharp peak at about pH 8.8. With triolein substrate stabilized with gum arabic, maximal velocity was attained at 1 mM triolein. When the ionic strength in the assay medium was modified by addition of NaCl an optimum at 0.08 M was observed. Higher salt concentrations caused progressive inhibition, maximal at 0.6 M when the enzyme was 80% inhibited. All enzyme determinations were carried out without prior preincubation at the NaCl molarity being tested. The heparin-Sepharose purified enzyme, in contrast to the enzyme further purified by isoelectric focusing, was not significantly stimulated by heparin to 50 µg per ml (9 units per ml). At high levels of heparin (100 µg per ml) there was a small but consistent inhibition.

The effects of protamine sulfate are illustrated in Fig. 7. The enzyme purified by heparin-Sepharose chromatography showed no inhibition at 125 µg per ml of protamine sulfate and was only inhibited by 22% at the highest concentration tested (1000 µg per ml). In contrast, the crude preparation obtained by extraction of an acetone powder with 1.2 x NaCl was inhibited 50% at 333 µg per ml of protamine sulfate and 74% at 1000 µg per ml. Data for a crude extract prepared from an acetone powder of rat adipose tissue are presented for comparison. Even 100 µg per ml of protamine sulfate inhibited the rat enzyme by 70%. When the crude pig enzyme was preincubated with protamine sulfate for 1 hour at 30°C, the inhibition was considerably greater, as shown in Fig. 7. Inhibition was greater than 80% with 300 µg per ml of protamine sulfate.

### Effects of Polypeptides Isolated from Human Very Low Density Lipoproteins

—The activation of the purified lipoprotein lipase by an apolipoprotein prepared from human very low density lipoprotein...
lipoproteins (12) (apoLp-Glu) was investigated. No serum was added in these experiments (Fig. 8). The activity in the absence of activator was subtracted from that determined with various levels of activator, as suggested by Bier and Havel (13). The data obtained were found to fit Michaelis-Menten kinetics with an apparent $K_m$ of 3.3 µg/ml (average of two values: 3.6 and 3.1). High concentrations of apoLp-Glu up to 50 µg/ml were not inhibitory. The lipoprotein lipase activity measured in the presence of 30 µg of human apoLp-Glu was not significantly different from that measured with porcine serum.

A partially purified lipoprotein lipase preparation (heparin-Sepharose step) gave an activity of 11.56 ± 0.36 nmole per ml of enzyme per hour (mean of three observations ± S.E.) in the presence of 0.1 ml of porcine serum per ml of assay and 10.33 ± 0.11 when the serum was replaced by human apoLp-Glu. A second polypeptide isolated from human very low density lipoprotein, apoLp-Ala, was inhibitory in the presence of whole serum in the assay system. Fifty percent inhibition of lipolytic activity was observed at 35 µg per ml (Fig. 8). ApoLp-Ala was an equally potent inhibitor of swine lipoprotein lipase and gave 50% inhibition at 36 µg per ml. Human hemoglobin and heart cytochrome c at a level of 35 µg per ml did not affect lipoprotein lipase activity when added to the serum-containing assay system.

DISCUSSION

The present results show that extraction of acetone powders of adipose tissue with either high concentrations of NaCl or heparin considerably enhances the yield of lipoprotein lipase. Yields were six times higher than those obtained with the classical method of NH$_4$OH-NaCl extraction (8). The lipolytic activity extracted with either NaCl or heparin exhibited the expected properties of adipose tissue lipoprotein lipase, namely, serum dependence and inhibition by high concentrations of NaCl. Solutions of high ionic strength have not previously been used for extraction of lipase, probably because of the well established inhibitory effect of high salt concentrations on lipoprotein lipase. However, as shown in the present study, the enzyme is not irreversibly inhibited by 1.0 M NaCl provided it is handled appropriately. By keeping the pH near 7, storing at low temperature and incorporating 20% glycerol in the buffer solutions, the enzyme retains activity for many hours. Of course, the NaCl concentration in the assay must be reduced by dilution.

The failure of previous investigators to identify significant lipoprotein lipase activity in acetone powders of adipose tissue of some mammalian species (3, 4) may very well be traced to the inefficiency of NH$_4$OH extraction. Previous physiologic studies of lipoprotein lipase levels, using NH$_4^+$ extraction, may require re-evaluation. If the small fraction of lipoprotein lipase extracted by NH$_4^+$ is a constant fraction of the total the results would be valid, but if the physiologic variables studied influence extractability, the results may not reflect changes in the true tissue content of enzyme.

Heparin-Sepharose column chromatography has been utilized
previously in the purification of bovine milk lipase (5) and post-
heparin serum lipase (6). Egelrud (15) has more recently
demonstrated the reversible binding of chicken adipose tissue
lipoprotein lipase to heparin Sepharose; however, no quantitative
data on yields and no further characterization of purified adipose
tissue lipoprotein lipase has been previously reported.

In the present study, the heparin-Sepharose chromatography
gave a purification of approximately 600-fold. Enzyme activity
was eluted along with the tail of a protein peak and use of a
less steep gradient might further improve purification in this
step.

Concanavalin A covalently linked to agarose was utilized to
achieve further purification. Under the conditions used, all of
the enzyme activity was retained during loading of the column.
Elution of the enzyme occurred when the eluting buffer con-
tained both 1 M methyl-α-D-mannoside and 1 M NaCl. The
presence of 1 M NaCl alone in the medium was not sufficient
to elute enzyme activity. Concanavalin A is a phytomannoside
which binds to specific carbohydrate residues of glycoproteins (16).
The tight but reversible binding of the enzyme to concanavalin A suggests that the porcine adipose tissue
lipoprotein lipase is itself a glycoprotein or that it is associated
with a tightly bound carbohydrate moiety.

Molecular weight estimation by gel filtration of the lipoprotein
lipase species present in 1 M NaCl gave a molecular weight
(62,000) similar to that obtained by disc gel electrophoresis
in the presence of 0.1% sodium dodecyl sulfate (58,000 to
63,000). Thus, the minimum molecular size determined for
this adipose tissue lipoprotein lipase is similar to that obtained
by Egelrud and Olivecrona (64,000) for the bovine milk lipase
(5). The similarity in poly peptide molecular size measured by
gel filtration in 1 M NaCl and by disc gel electrophoresis in the
presence of sodium dodecyl sulfate strongly suggests that 1 M
NaCl dissociates the enzyme to its monomeric form.

In the present study, the isolated lipoprotein lipase appeared
to be homogeneous in its affinity for heparin-Sepharose, and
yielded a single major peak on gel filtration on Sephadex G-150.
Isoelectric focusing also yielded a single peak of activity, either
when it followed heparin-Sepharose chromatography or when
it was used with preparations obtained after concanavalin
A-Sepharose chromatography. Two species of lipoprotein lipase
in extracts of acetone powders of rat adipose tissue have been
reported by Garfinkel et al. (17). These were separated by
agarose gel chromatography. These results cannot be directly
compared to the present findings since the former were obtained
with a different species and employed a different method of
acetone powder extraction, using low ionic strength buffer. It
should be noted that the present results, showing homogeneity
in the presence of 1.0 M NaCl, do not rule out the possibility of
multiple aggregate forms in solutions of lower ionic strength.

The present studies afforded the opportunity to compare the
properties of a highly purified adipose tissue lipoprotein lipase
with those of crude preparations. The porcine adipose tissue
lipoprotein lipase does not show stimulation by low levels of
heparin, either when added to assays of the crude extract or of
the heparin-Sepharose purified enzyme. Lipoprotein lipase
purified by isoelectric focusing, however, does show a marked
increase in activity in the presence of added heparin. The
purified enzyme was markedly inhibited by heparin when present
at levels above 100 µg per ml in the assay media as has been
reported for purified milk lipoprotein lipase (5). Protamine
sulfate added at zero time (without preincubation) inhibited
the purified enzyme much less than it did the enzyme in crude
extracts. Low concentrations (100 to 195 µg per ml) actually
increased activity slightly and inhibition was less than 15% at
300 µg per ml. The crude enzyme was more sensitive to inhibi-
tion but less so than crude enzyme prepared from acetone
powder of rat adipose tissue. These differences (between
purified and crude enzyme and between species) tended to be
eliminated by preincubation of the enzyme with the protamine
sulfate. The reasons for the differences are not clear but may
reflect in part differences in affinity of the enzyme preparations
for substrate i.e. in the presence of substrate the enzyme may
be partially protected from interaction with protamine sulfate.

The porcine adipose tissue lipoprotein lipase after heparin-
Sepharose chromatography exhibited lipolytic activity in the
absence of serum. The same behavior was reported for the
bovine milk lipoprotein lipase after the same purification step.
In the present study, isoelectric focusing yielded an enzyme
preparation which is completely dependent on serum activator.
This increase in the magnitude of stimulation by serum after
further purification suggests that traces of activator may be
tightly associated with lipoprotein lipase, co-chromatographing
through the two affinity steps but dissociating in the course
of isoelectric focusing. Of the three very low density lipoprotein
apolipoproteins which were studied, only apo-Lp-Glu could substitute
for serum as an activator, and this activation was as effective
as that produced by whole serum. ApoLp-Ala, when added to a
system containing serum, was inhibitory as has been previously
reported for milk lipoprotein lipase (18). A new and unexpected
finding was the potent inhibitory effect of apo-Lp-Ser in the
presence of serum in the assay system. At the same levels of
substrate and serum in the medium, apo-Lp-Ser was as potent
an inhibitor as apo-Lp-Ala. The physiological meaning of this
finding remains uncertain at this time but might be evaluated
by in vitro studies of the degradation of very low density lipoprotein
apolipoproteins by purified adipose tissue lipoprotein lipase.

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