Studies on Inactivation of Lipoprotein Lipase: Role of the Dimer to Monomer Dissociation†

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ABSTRACT: Sedimentation equilibrium analysis demonstrated that preparations of bovine lipoprotein lipase contain a complex mixture of dimers and higher oligomers of enzyme protein. Enzyme activity profiles from sedimentation equilibrium as well as from gel filtration indicated that activity is associated almost exclusively with the dimer fraction. To explore if the enzyme could be dissociated into active monomers, 0.75 M guanidinium chloride was used. Sedimentation velocity measurements demonstrated that this treatment led to dissociation of the lipase protein into monomers. Concomitant with dissociation, there was an irreversible loss of catalytic activity and a moderate change in secondary structure as detected by circular dichroism. The rate of inactivation increased with decreasing concentrations of active lipase, but addition of inactive lipase protein did not slow down the inactivation. This indicates that reversible interactions between active species precede the irreversible loss of activity. The implication is that dissociation initially leads to a monomer form which is in reversible equilibrium with the active dimer, but which decays rapidly into an inactive form, and is therefore not detected as a stable component in the system.

Lipoprotein lipase has a central role in the metabolism of plasma lipoproteins. Studies of the enzyme molecule have revealed several interactions which may have important roles for the enzyme's function in vivo. Its catalytic activity is influenced by the physical state of the lipid substrate as well as by a specific activator protein, apolipoprotein CII [reviewed in Olivecrona & Bengtsson (1984), Quinn et al. (1983), and Smith et al. (1984)]. This suggests that the enzyme has a lipid binding site and a site for interaction with activator protein. Proper cooperation between these sites and the enzyme's active site is required for full catalytic activity. This conclusion is supported by studies on the kinetic properties of a proteolytically "nicked" lipoprotein lipase (Bengtsson & Olivecrona, 1981; Olivecrona et al., 1982). In addition to these sites, the enzyme has a site for interaction with polyanions [reviewed in Olivecrona & Bengtsson (1984), Quinn et al. (1983), and Smith et al. (1984)]. Binding of ligands to this site does not seem to directly affect the catalytic function (Olivecrona & Bengtsson, 1984). It has been suggested that in vivo this site binds to endothelial cell heparin sulfate and thereby anchors the enzyme to the capillary wall (Olivecrona et al., 1977). The enzyme can also bind long-chain fatty acids. This binding impedes or abolishes all of the above listed interactions, i.e., binding to lipid (Bengtsson & Olivecrona, 1980b), to activator protein (Bengtsson & Olivecrona, 1978a), and also to polyanions (Olivecrona & Bengtsson, 1984). It has been suggested that binding of fatty acids mediates the strong product inhibition of the enzyme (Olivecrona & Bengtsson, 1984; Bengtsson & Olivecrona, 1980b).

To gain further insight into the mechanisms and functions of these interactions, physical studies on enzyme–ligand systems are needed. However, at present only little is known about the physical state of the enzyme itself (Chung & Scanu, 1977; Fielding et al., 1974; Inverius & Östlund-Lindqvist, 1976; Olivecrona et al., 1982), and nothing is known about the molecular transitions it may undergo. Particularly troublesome is the fact that the enzyme tends to rapidly lose its catalytic activity in buffer solution (Olivecrona & Bengtsson, 1984), indicating that it may undergo structural changes even in the absence of added ligands. Therefore, we have undertaken to define the physical state of active lipoprotein lipase and its molecular transitions. In particular, we have explored whether the monomeric form of the enzyme is catalytically active.

Materials and Methods

Lipoprotein lipase was prepared from unpasteurized bovine milk by affinity chromatography on heparin–Sepharose as described previously (Bengtsson & Olivecrona, 1977). The enzyme (0.2–0.7 mg/mL) was stored in 1.5 M sodium chloride–10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, at −70 °C and was used within 1 month. A slow loss of activity is always observed upon storage of lipoprotein lipase. The rate of inactivation varies between preparations. The preparations used in these studies exhibited a single band on gel electrophoresis in sodium dodecyl sulfate.

Assays. Lipoprotein lipase activity was measured by using tri[^{3}H]oleoylglycerol-labeled Intralipid as the substrate (a kind gift from AB Vitrum, Stockholm, Sweden). The final incubation contained in a total volume of 200 μL 0.8 mg of triacylglycerol, 20 μmol of sodium chloride, 30 μmol of Tris-HCl, 20 μL of heat-inactivated rat serum, 50 μg of heparin (AB Vitrum, Stockholm, Sweden), and 12 mg of bovine serum albumin (Sigma, St. Louis, MO). The pH was 8.5, and incubations were performed at 25 °C. The released fatty acids were extracted by the method of Belfrage & Vaughan (1969) but with 0.5 M carbonate buffer. Control experiments showed that the amounts of guanidinium chloride (GdmCl) in the final assay did not affect enzyme activity. The soluble substrate *p*-nitrophenyl butyrate (Sigma, St. Louis, MO) was dissolved

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in acetone (100 μmol/mL) and then diluted into 25 mM Tris-HCl buffer, pH 7.4, to a concentration of 0.5 μmol/mL just prior to use. The release of *p*-nitrophenol was followed at 405 nm with a Beckman 25 spectrophotometer. The release was linear with time for at least 5 min. Additional details are given in the figure legends.

Circular Dichroism. Circular dichroic measurements were performed with a Cary 61 spectropolarimeter equipped with a thermostated cell holder and standardized with *d*-10-camphorsulfonic acid. Data were collected and processed automatically with a computer-based data acquisition system.[1] Spectra were obtained from 270 to 200 nm, and after subtraction of appropriate blanks, mean residue ellipticities were calculated according to the equation:

$$[\theta]_\lambda = \frac{115(\theta)_{obsd}}{10lc} \quad (1)$$

where $(\theta)_{obsd}$ is the observed ellipticity, 115 is the mean residue molecular weight of the polypeptide component of bovine lipoprotein lipase, l is the path length in centimeters, and c is the protein concentration in grams per milliliter. The temperature was maintained at 8 °C throughout each study. Additional details are given in the figure legends.

Ultracentrifugation. Sedimentation velocity measurements were performed by using a Beckman Model E ultracentrifuge at 10 °C. Data were collected at 4-min intervals at 280 nm with the photoelectric ultraviolet scanner. The sedimenting boundary was symmetrical for all runs, and points of inflection were determined graphically at each time point. The logarithm of the radial position of the boundary as a function of time was fit to the following equation to obtain the least-squares values of A, B, C, and D:

$$\ln r = \frac{A + Bt + Ct^2}{1 + Dt} \quad (2)$$

where r is the radial distance from the center of rotation in centimeters and t is the time in seconds. Sedimentation coefficients as a function of time were then obtained analytically with the equation:

$$s = \frac{1}{\omega^2}\left[\frac{B + 2Ct}{1 + Dt} - \frac{(A + Bt + Ct^2)D}{(1 + Dt)^2}\right] \quad (3)$$

Sedimentation equilibrium measurements were performed by using a Beckman Model E ultracentrifuge equipped with a temperature-control system and an ultraviolet photoelectric scanner. Data were collected automatically with a computer-based data acquisition system.[1] Each sample was dialyzed exhaustively against the appropriate buffer at 4 °C, and the temperature was maintained at 10 °C during centrifugation. Double-sector cells with quartz windows and charcoal-filled epon centerpieces were centrifuged in an Ang-Ti rotor. Each cell contained 150 μL of sample and 160 μL of buffer. Absorbancy measurements prior to centrifugation combined with scans of the cell at 280 nm at time zero when the rotor reached speed and base-line determinations at 48 000 allowed extinction coefficients to be calculated for each run. Equilibrium was established by comparing scans, 280 nm, taken at 4-h intervals after approximately 24 h of centrifugation.

Apparent weight-average molecular weights (M_w^{app}) were obtained as described previously (Osborne et al., 1977). Briefly, ln c vs. r^2 data were fit to the following equation by using a nonlinear least-squares computer program (MLAB) developed at NIH:

$$Y(x) = \frac{A + Bx + Cx^2}{1 + Dx} \quad (4)$$

where Y corresponds to ln c and x corresponds to r^2. M_w^{app} as a function of concentration or radius was then generated analytically with

$$M_w^{app} = \frac{2RT}{\omega^2(1 - \bar{v}\rho)}\left[\frac{B + 2Cx}{1 + Dx} - \frac{(A + Bx + Cx^2)D}{(1 + Dx)^2}\right] \quad (5)$$

where R is the gas constant, T is the absolute temperature, ω is the angular velocity, $\bar{v}$ is the partial specific volume [0.725 for bovine lipoprotein lipase (Olivecrona et al., 1982)], and ρ is the solvent density.

The molecular weight of the active species of bovine lipoprotein lipase was determined by sedimentation equilibrium measurements in a swinging bucket rotor (Beckman SW50L). Teflon inserts were used to accommodate the 5 × 41 mm Ultra-Clear tubes used in the experiment. The buffer used was 0.5 M ammonium sulfate, 0.2 M sodium chloride, 0.0001 M sodium azide, 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.01 M Tris (pH 7.4) containing 10 mg/mL bovine serum albumin (BSA). The BSA was added to stabilize the resulting gradient, which was stable for ~1 h after the rotor was stopped. The sample volume was 250 μL maintained at 10 °C. The rotor was "overspeeded" at 20 000 rpm for 24 h to decrease the time required to establish equilibrium which was usually about 100 h for these studies. The final rotor speed was 10 000 rpm. Fractions were taken from the top of the centrifuge tube by using a Hamilton syringe, and enzyme activity was measured after appropriate dilution. Protein concentrations were measured in each fraction according to the method described by Bradford (1976). The corresponding distances from the center of rotation were calculated, and the molecular weight of catalytically active lipoprotein lipase was obtained by using eq 5 above.

Results

To keep lipoprotein lipase in solution at sufficiently high concentrations to evaluate its physical properties, a buffer with high salt concentration is needed (Olivecrona & Bengtsson, 1984). In previous studies, 1.5 M sodium chloride at neutral pH was used (Olivecrona et al., 1982). We have now found that enzyme stability is better in ammonium sulfate than in sodium chloride only. In the present studies, we have therefore used a buffer with 0.5 M ammonium sulfate and 0.2 M sodium chloride (see legend to Figure 1 for complete composition). In this buffer (buffer A), concentrated solutions of lipoprotein lipase retained their catalytical activity for several days at 4 °C.

We have demonstrated previously by sedimentation equilibrium measurements in 6 M guanidinium chloride (GdmCl) that the monomeric molecular weight of bovine lipoprotein lipase is 41 700 (Olivecrona et al., 1982). Sedimentation equilibrium measurements in the absence of denaturant (buffer A) at several different rotor speeds and initial concentrations demonstrate the presence of species with molecular weights as high as 180 000 (data not shown). However, the system was quite complex since the data obtained at different rotor speeds did not overlap one another; at any given total concentration of protein, the apparent weight-average molecular weight decreased with rotor speed. This could have been due to slowly reversible or irreversible oligomer formation (Osborne et al., 1982) or reversible interactions with pressure effects (Formisano et al., 1978), with increasing pressure causing

[1] R. Tate, A. Schultz, and J. C. Osborne, Jr., unpublished results.

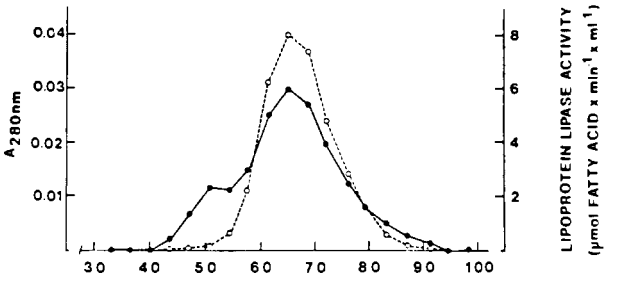

FIGURE 1: Gel filtration of lipoprotein lipase. A column of Sephadex G-150 (600 × 15 mm i.d) was equilibrated with 0.5 M $(NH_4)_2SO_4$, 0.2 M NaCl, 10 mM Tris-HCl, 1 mM sodium azide, and 1 mM EDTA, pH 7.4 at 10 °C. One milliliter of lipoprotein lipase (0.5 mg/mL) in the same buffer was applied to the column. The flow rate was about 10 mL/h. Fractions of 3.6 mL were collected in weighed tubes. The weight of each fraction was determined by a second weighing of the tube. Lipase activity was determined in the Intralipid assay. Recovery of lipase activity was 85%. (●) A_{280}; (○) lipase activity. The standards used for calibration were human IgG (M_r 150 000), tranferrin (M_r 89 000), bovine serum albumin (M_r 67 000), ovalbumin (M_r 43 000), α-chymotrypsinogen (M_r 23 000), and myoglobin (M_r 18 000). The logarithm of the molecular weight of the standards was a linear function of elution volume; active lipase had an apparent molecular weight of 110 000.

dissociation of oligomeric lipoprotein lipase. When the apparent weight-average molecular weight at a single rotor speed using two different initial concentrations of protein was compared as a function of concentration, the M_w^{app} obtained with the lower initial concentration of protein crossed that found with the higher initial concentration of protein. Since the pressure at any given point in an equilibrium concentration profile increases with decreasing initial concentration of protein, the nonoverlapping M_w^{app} profiles are not simply due to pressure effects in the ultracentrifuge. Therefore, oligomer dissociation was not rapidly reversible under these conditions. It should be emphasized at this point that the M_w^{app} profiles were obtained at equilibrium in the ultracentrifuge. Thus, there were no observable time-dependent changes in molecular weight over the 4-h interval used to verify equilibrium, and rates of molecular interconversions must have been much faster or much slower than 4 h. If all the molecular interconversions were much slower than 4 h, i.e., the enzyme was irreversibly aggregated, then molecular weights obtained at a single rotor speed by using several different initial concentrations of protein would be a function of radial position only and independent of the actual concentration of protein at the position in the cell (Osborne et al., 1982). In other words, in this case, data plotted as molecular weight vs. radius would overlap one another; this is not the case for lipoprotein lipase under these conditions. The combined results from sedimentation equilibrium experiments are consistent with the presence of both reversibly and irreversibly associated oligomers in purified lipoprotein lipase preparations.

While sedimentation equilibrium allows determination of molecular weight based on first principles, it does not differentiate between active and inactive species. On gel filtration, active lipase eluted with an apparent molecular weight of 110 000 (Figure 1). Some inactive material eluted before the active peak close to or with the void volume of the column. Some variation in the amount of this aggregated material between preparations has been observed, and in some cases, this material has displayed a low but significant activity. There was also some trailing of inactive material at the end of the active peak. It has been reported previously that lipoprotein lipase is asymmetrical in shape (Inverius & Östlund-Lindqvist, 1976). If this were the case, the active species may correspond to dimeric lipase which has a molecular weight of approxi-

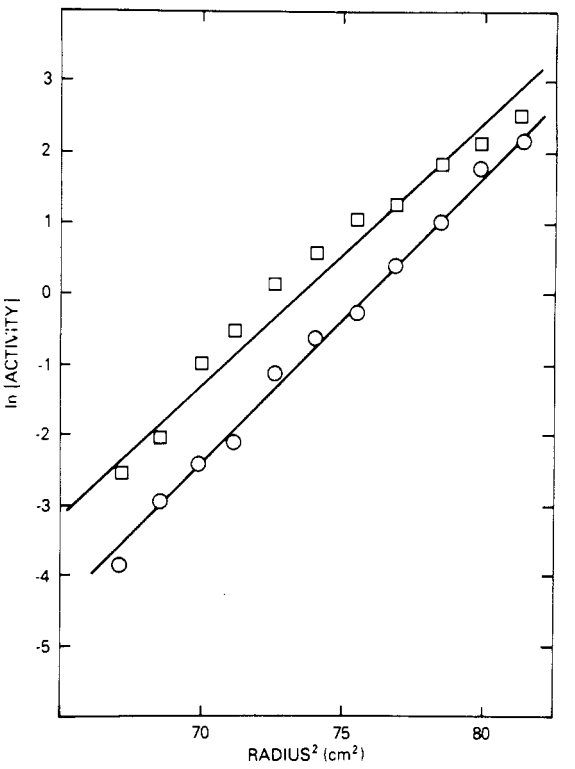

FIGURE 2: Determination of the molecular weight of catalytically active lipoprotein lipase by sedimentation equilibrium ultracentrifugation. The buffer used was 0.5 M $(NH_4)_2SO_4$, 0.2 M NaCl, 1 mM sodium azide, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4. The temperature was maintained at 10 °C. The initial concentrations of enzyme were 0.01 and 0.25 mg/mL for the circles and squares, respectively. Lipase activity was determined in the Intralipid assay. The recovery of activity over the 100-h run was 60% for the lower and 16% for the higher initial concentration of enzyme. As activity was usually stable for this period of time, the loss of activity presumably corresponds to enzyme pelleted to the bottom of the centrifuge tube. The rotor speed was 10 000 rpm. Additional details are given under Materials and Methods.

mately 83 000. This was investigated directly by measuring the activity profile of a lipoprotein lipase solution which had been centrifuged to equilibrium in a preparative ultracentrifuge (see Materials and Methods for details). The buffer used contained 10 mg/mL bovine serum albumin, and the activity gradient generated by centrifugation was stable for approximately 1 h after the rotor was stopped. Molecular weights were calculated from the slope of plots of lipase activity vs. radius squared by using eq 5. Representative data are shown in Figure 2. At both low (0.010 mg/mL) and high (0.25 mg/mL) initial concentration of enzyme, the results were consistent with the dimer being the predominant active form of bovine lipoprotein lipase, i.e., M_r 76 000 and 69 000, respectively. It is emphasized that these results do not depend upon the shape of the active enzyme molecule.

To explore if the lipase could be dissociated to an active monomeric species, we used low concentrations of guanidinium chloride. With 0.4 M or less GdmCl added, a solution of 30 μg of lipoprotein lipase per milliliter in buffer A lost less than 10% of its catalytic activity in 2 h at 8 °C. With 0.5 M GdmCl added, about 20% of the activity was lost in 2 h. With 0.75 M GdmCl, this figure increased to about 90%, and with 1 M GdmCl, 50% of the activity was lost in 20 min (data not shown). From such experiments, it was decided to use 0.75 M GdmCl which caused a time-dependent loss of enzyme activity with an experimentally convenient rate.

The rate of inactivation in 0.75 M GdmCl was strongly dependent on temperature (data not shown). At or below 15

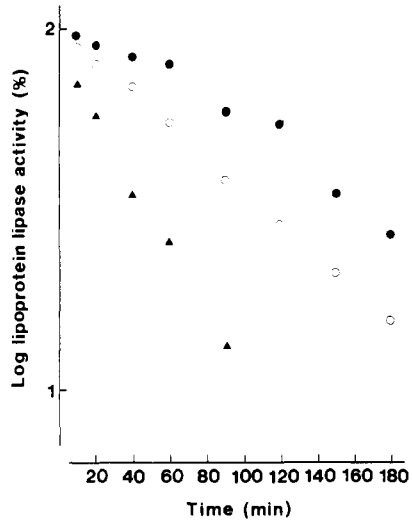

FIGURE 3: Effect of the concentration of lipoprotein lipase on its inactivation by GdmCl. Lipoprotein lipase in 0.5 M $(NH_4)_2SO_4$, 0.2 M NaCl, and 10 mM Tris-HCl, pH 7.4 (0.35 mg/mL), was diluted (●) 1:2, (○) 1:5, and (▲) 1:50 in this buffer also containing GdmCl to give a final concentration of 0.75 M. Bovine serum albumin was added to give the same protein concentration in all samples. The samples were kept at 8 °C. Lipase activity was measured (Intralipid assay) at the indicated times and is expressed as the percent of activity at time 0 min in samples diluted without GdmCl. Samples in the absence of GdmCl lost less than 10% of their activity during the course of the experiment.

Table I: Effect of Proteins on Inactivation of Lipoprotein Lipase in 0.75 M GdmCl[a]

protein added	concn (mg/mL)	$t_{1/2}$ (h) control	$t_{1/2}$ (h) GdmCl
none		21	1.4
BSA	1	17	1.4
BSA	10	61	1.8
β-lactoglobulin	1	24	1.1
inactive LPL	0.3	∞	1.1

[a] Lipoproein lipase (LPL) (20 μg/mL) was incubated at +10 °C in 0.5 M ammonium sulfate, 0.2 M NaCl, and 10 mM Tris-HCl, pH 7.4, in the presence of 0.75 M guanidinium chloride. Samples (5 μL) were removed at a series of time intervals, and remaining enzyme activity was measured (using the Intralipid assay). The inactivation followed first-order kinetics. The table shows the corresponding half-times in hours. The addition of other proteins to the incubation mixture had little effect on lipase inactivation. The inactive lipoprotein lipase added in one experiment had been incubated with 0.75 M guanidinium chloride at 25 °C for 3 h. It displayed no enzyme activity.

°C, there was little or no loss of enzyme activity in 5 min. At 25 °C, 50% of the activity was lost in 5 min, and at 37 °C, there was complete loss of activity in 5 min. The rate of inactivation was also dependent on the concentration of enzyme (Figure 3). The lower the lipase concentration, the more rapid was the inactivation. Addition of other proteins to the medium, e.g., albumin, had only small effects on the rate of inactivation (Table I). Thus, the change in rate with lipase concentration was not due simply to nonspecific absorption processes. Addition of inactive lipase, even in relatively high concentrations, did not markedly change the rate of inactivation (Table I). Thus, the concentration which determines the progress of inactivation is not that of total lipase protein but only the concentration of catalytically active forms of the lipase. At low concentrations of lipase, the inactivation appeared to follow first-order kinetics, but at higher enzyme concentration, the process was more complex (Figure 3).

For the above experiments, the enzyme activity was determined with a triacylglycerol emulsion as substrate, and with

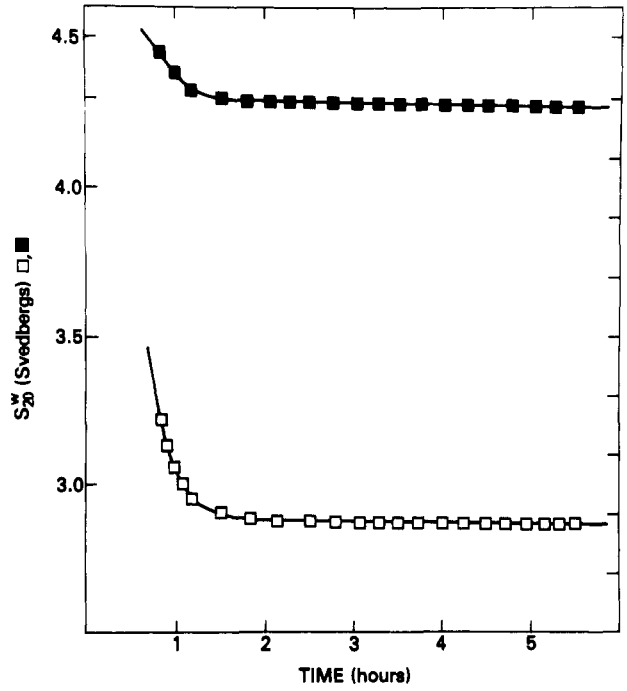

FIGURE 4: Sedimentation coefficient of lipoprotein lipase in the presence (□) and absence (■) of 0.75 M GdmCl. Buffer of the same composition as in Figure 2 was used. The temperature was maintained at 10 °C. At time zero, an appropriate volume of stock 6 M GdmCl in the above buffer was added to lipase (open squares) to give a final GdmCl concentration of 0.75 M. The protein concentrations were 0.36 mg/mL (■, □). Sedimentation coefficients were computed as described under Materials and Methods and have not been corrected to standard conditions. The corrected $s_{20,w}$ values for monomer and dimer are 3.40 and 4.90 S.

activator protein present. In this system, the activity is dependent on cooperation between several sites on the enzyme molecule, i.e., the active site, as well as the sites for binding to lipid–water interfaces and for interaction with activator protein [reviewed in Olivecrona & Bengtsson (1984), Quinn et al. (1983), and Smith et al. (1984)]. The activity against the soluble substrate *p*-nitrophenyl butyrate was lost at the same rate as that observed with the triacylglycerol emulsion (data not shown). Thus, the inactivation appeared to hit the active site directly and was not secondary to some other change in the enzyme molecule.

Sedimentation velocity measurements in the presence and absence of 0.75 M GdmCl are illustrated in Figure 4. It took about 50 min to assemble the cell and reach rotor speed, so evaluation of sedimentation coefficients was limited to times greater than about 1 h. In the absence of GdmCl, there was an initial decrease in *s* which probably corresponds to sedimentation of aggregated lipase. The plateau value of 4.3 S is in the range one would expect for dimeric lipase. In the presence of GdmCl, the sedimentation coefficient decreased to approximately 2.9 S. Additional measurements indicate that this *s* value was constant for at least 48 h and sedimentation equilibrium measurements within 48 h of treatment with 0.75 M GdmCl showed that this *s* value corresponded to a molecular weight of 39 000 for monomeric lipase. These data thus demonstrate that 0.75 M GdmCl dissociates the lipase into monomer. Attempts to demonstrate lipase activity in the monomer species were unsuccessful. Enzyme was centrifuged, by using a swinging bucket rotor, in the presence and absence of 0.75 M GdmCl. After 4 h, when dissociation in the presence of GdmCl would be appreciable, the rotor was stopped, each sample was fractionated, and the fractions were assayed for protein content and lipase activity. Sedimentation profiles

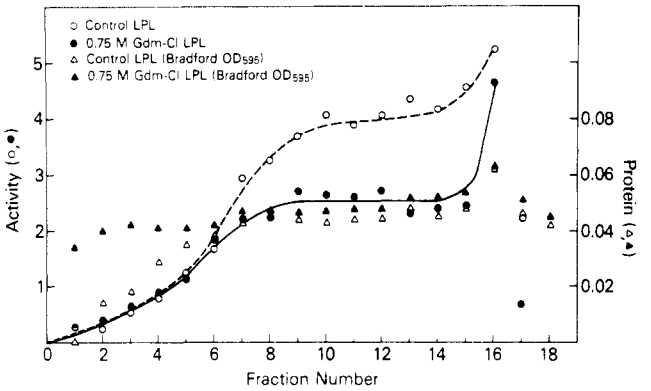

FIGURE 5: Sedimentation velocity of lipoprotein lipase based on enzyme activity. Samples were centrifuged in a swinging bucket rotor (SW 41-Ti) at 28 000 rpm for 4 h (4 °C) and then fractionated from the meniscus with a Hamilton syringe. Teflon inserts were used to allow small sample volumes (250 μL). Sucrose (2.5% by weight) was added to the buffer, of the same composition as in Figure 2, in order to stabilize the gradient during fractionation. Samples in the presence (closed symbols) and absence (open symbols) of 0.75 M GdmCl were quantitated for protein (▲, △) and activity (●, ○).

based on activity were the same in the presence and absence of GdmCl and corresponded to dimer (Figure 5). Profiles based on protein were different; higher protein concentrations near the meniscus in the GdmCl-treated sample indicate substantial monomer formation.

We have also measured the effects of GdmCl on the secondary structure of lipoprotein lipase. There was no immediate effect of GdmCl concentrations below 1 M and a monotonic decrease in structure at higher concentrations (data not shown). At 4 M GdmCl, the CD spectrum of lipoprotein lipase resembled that of a random coil. When the GdmCl concentration of a sample exposed to 4 M GdmCl was decreased by dialysis, the enzyme regained some, but not all, structure. This indicates that the process was not fully reversible. In agreement with this, we have not been able to regain lipase activity by removal of GdmCl under a variety of conditions of buffer composition, temperature, and rate of decrease in GdmCl concentration.

Even though there was no marked immediate change in the CD spectrum by the lower concentrations of GdmCl, there was a progressive time-dependent change. After 16 h, the change had reached an apparent plateau; there was no further change during the next 24 h. The change in structure occurred at a similar rate as the loss of enzyme activity (Figure 6). As was found for the loss of enzyme activity, the loss of structure occurred more rapidly at lower enzyme concentrations (Figure 6).

Discussion

In the present paper, we demonstrate by sedimentation equilibrium experiments using activity measurements that dimer is the predominant active species of bovine lipoprotein lipase. The results were obtained from first principles and do not depend upon assumptions about the shape of the lipase molecule. On gel filtration, active lipoprotein lipase eluted with an apparent molecular weight of ~110 000. Since the enzyme is asymmetrical (Iverius & Östlund-Lindqvist, 1976), the active species on gel filtration probably also corresponds to the dimer.

The behavior of total lipoprotein lipase protein in the ultracentrifuge is more complex than that observed by using activity measurements alone. Iverius & Östlund-Lindqvist (1976) found it impossible to carry out sedimentation equilibrium analysis because of enzyme aggregation. They were,

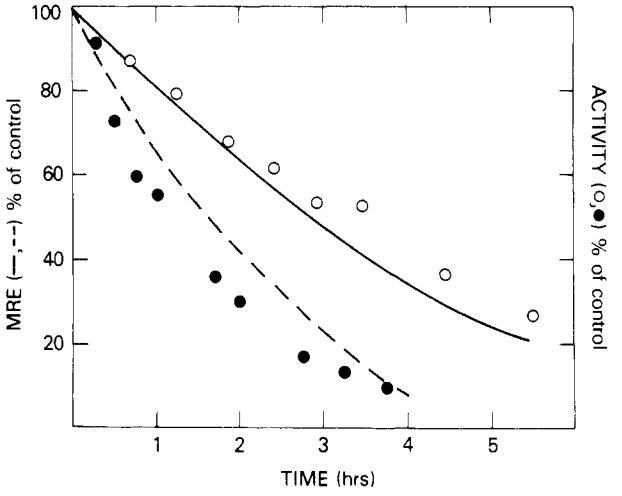

FIGURE 6: Correlation between the loss of enzyme activity and the change in mean residue ellipticity. Circular dichroic spectra were obtained from 270 to 200 nM at a series of times after the samples were made 0.75 M in GdmCl. The spectra at time zero for all lipase concentrations studied were the same as that reported previously (Olivecrona et al., 1982). The buffer used was 0.5 M $(NH_4)_2SO_4$, 0.2 M NaCl, 1 mM sodium azide, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4. There was an initial time-dependent decrease in the ellipticity which reached a plateau within 24 h (θ_{215} = 7800). The difference between this plateau at 215 mm and the original value was taken as 100%. The ellipticity at intermediate times is expressed in relation to this. Enzyme activity was measured in the Intralipid assay. Two concentrations of enzyme protein were studied: 0.32 (○) and 0.05 (●) mg/mL.

however, able to monitor the initial sedimentation velocity. These results were interpreted in terms of an asymmetrical molecule with a molecular weight of ~100 000.[2] For the present studies, we have used a buffer with higher salt concentration than that used by Iverius & Östlund-Lindqvist (1976). Under our conditions, the enzyme is stable, and it was possible to obtain reproducible sedimentation equilibrium data. These data are consistent with the dimer as a major species. In addition, our analysis demonstrates the presence of irreversibly, as well as reversibly, associated higher oligomeric species. The results of gel filtration also indicated the presence of oligomeric forms of the lipase. In this case, the higher oligomers eluting in or near the void volume of the column displayed little or no enzyme activity.

Treatment with 0.75 M GdmCl was found to dissociate the oligomers of lipoprotein lipase to the monomeric state. Dissociation resulted in an irreversible loss of enzyme activity and was paralleled by a moderate change in secondary structure. The fractional rate of inactivation and of refolding increased with decreasing concentrations of lipase protein. These changes were not due to nonspecific absorption processes since the rates were not much affected by the presence of other proteins such as bovine serum albumin. Moreover, the loss of secondary structure paralleled the irreversible loss of activity at all concentrations investigated. These combined results are consistent with the following scheme of lipase organization. (1) The dimer (M_r 83 400) of lipase is catalytically active. This was measured directly in the preparative centrifuge. (2) Inactive species of lipase exist in solution. This was demonstrated directly by sedimentation equilibrium and gel filtration experiments. (3) There is a reversible interaction between active or activatable species of lipase in solution. Reversible inter-

[2] The frictional coefficients based on sedimentation equilibrium and velocity measurements obtained in the present study are 1.30 and 1.43 for monomer and dimer species, respectively.

actions were observed upon analytical ultracentrifugation. In addition, the rate of inactivation in 0.75 M GdmCl increased with decreasing concentration of active lipase. If there were no molecular transitions between active species, the rate of inactivation would be independent of concentration. The implication is that dissociation leads initially to a monomer form which is in reversible equilibrium with the active dimer, but which decays rapidly into an inactive form, and is therefore not detected as a stable component in the system. (4) The inactive forms can reassociate to higher molecular weight aggregates but do not regain catalytic activity. This is based on the observation than when the GdmCl was removed from samples which had been irreversibly inactivated in GdmCl, oligomers were again formed from the inactive monomer. This reassociation was apparent both in ultracentrifugation studies and during gel filtration but was not analyzed in detail.

Lipoprotein lipase is known to be unstable in buffer, particularly at low concentrations of lipase protein (Olivecrona & Bengtsson, 1984). It is possible that inactivation takes place by the same pathway as indicated here for the inactivation by low concentrations of GdmCl. Lipase prepared in different laboratories by slightly different methods and stored for varying lengths of time must be expected to differ in state of aggregation and content of inactive species. This, as well as the possibility that lipase itself may undergo the molecular transitions described in the present paper, must be taken into account when interpreting studies on molecular interactions between lipase and other macromolecules. Aggregates can be removed from lipase preparations by gel chromatography as in Figure 1. Most of the inactive species can also be removed by rechromatography on heparin–Sepharose.

Binding to lipid (Bengtsson & Olivecrona, 1980a), to certain detergents (Baginsky & Brown, 1977; Bengtsson & Olivecrona, 1979b), and to heparin (Inverius et al., 1972; Bengtsson & Olivecrona, 1985) protects the enzyme from inactivation. Whether these ligands act by binding to dimeric lipase and impede its dissociation to monomer or act through some other mechanism requires further studies. The process of inactivation is of potential physiological importance, since it is known that the activity of the lipase at its physiological site of action can decrease rapidly (Cryer, 1981). Whether this occurs by actual loss of enzyme protein or through a conformational transition is not known.

Registry No. GdmCl, 50-01-1; lipoprotein lipase, 9004-02-8.

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