Molecular Weight of Beef Heart Lactate Dehydrogenase†

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ABSTRACT: The subunit molecular weights of lactate dehydrogenase from beef heart and dogfish muscle have been investigated by high-speed sedimentation equilibrium in 6 M guanidine hydrochloride at pH 7 and 2, dodecyl sulfate–polyacrylamide gel electrophoresis, and gel filtration on a calibrated agarose column in 6 M guanidine hydrochloride. Molecular weights of near 35,000 were obtained by all three methods. Smaller molecular weight species (between 22,000 and less than 11,000) have been observed inconsistently in some experiments; these are due most likely to degradation induced by some contaminant in one of the lots of the commercially obtained guanidine.

Lactate dehydrogenase is usually regarded as a tetramer of 142,000 molecular weight (Jaenicke and Knof, 1968) composed of four subunits of molecular weight near 35,000 (Appella and Markert, 1961). The four-subunit composition of this enzyme has been substantiated by observations using various biochemical techniques. Four moles of N-terminal acetylalanine per mole of beef heart lactate dehydrogenase has been observed (Brummel *et al.*, 1971) as well as four residues of C-terminal leucine per mole of the beef heart enzyme (Appella and Zito, 1968; Stegink *et al.*, 1971). Dodecyl sulfate–polyacrylamide gel electrophoresis has shown a molecular weight of 36,000 (Weber and Osborn, 1969) and membrane osmometry in 6 M guanidine hydrochloride containing 0.5 M 2-mercaptoethanol yielded 36,180 ± 800 (Castellino and Barker, 1968). Perhaps the most unequivocal evidence of the four-subunit hypothesis has been the elucidation of the dogfish muscle lactate dehydrogenase structure at 2.8-Å resolution by X-ray diffraction which has clearly shown this enzyme to be a tetramer (Adams *et al.*, 1970).

There have been literature observations at variance with the four-subunit hypothesis, however. More than five isozymic bands have been separated by starch gel electrophoresis (Koen, 1967; Houssais, 1966; Costello and Kaplan, 1963), although more recent work on extracts of mouse tissue seem to indicate that the bands in amounts greater than that predicted by the tetrameric hypothesis may be due to the binding of small molecules to free sulfhydryls (Dudman and Zerner, 1969; Dudman, 1969). Tryptic digest peptides of beef heart lactate dehydrogenase in amounts fewer than expected from amino acid composition have been observed (Appella, 1964).

Millar and coworkers (1969) have investigated the number of subunits comprising beef heart lactate dehydrogenase by sedimentation equilibrium measurements in concentrated guanidine hydrochloride solutions. They observed a molecular weight of about 35,000 for the enzyme in either 6.2 or 7 M guanidine hydrochloride (pH 7) in agreement with the tetrameric hypothesis. However, after exposure of the enzyme to 6.2 M guanidine hydrochloride (pH 2), they reported a molecular weight of 18,000 and concluded that the molecule was not fully dissociated in the guanidine at pH 7 and was in fact an octamer composed of two nonidentical chains. Apparent equilibrium constants were determined for the association of lactate dehydrogenase subunits at pH 2 in the absence of guanidine hydrochloride assuming identical subunits.

Since various solution properties of a protein depend on a knowledge of the number of subunits, we decided to reexamine this question. The molecular weights of the enzyme from beef heart and dogfish muscle have been examined in 6 M guanidine hydrochloride at pH 7 and 2 by high-speed sedimentation equilibrium, dodecyl sulfate–polyacrylamide gel electrophoresis, and gel filtration on a calibrated agarose column in 6 M guanidine hydrochloride. Molecular weights close to 35,000 have been obtained by all three methods, in good agreement with the values predicted by the tetramer hypothesis. Smaller molecular weight species have been observed in a few experiments; these have been attributed most likely to degradation induced by some contaminant in one lot of the commercially obtained guanidine.

Materials and Methods

Guanidine hydrochloride, Ultra Pure grade (Heico, Inc., lots 217011, 217013, and 217016),[1] dinitrophenylglycine and Coomassie Brilliant Blue (Sigma Chemical Co.) were used without further purification. Sodium dodecyl sulfate (Sigma Chemical Co). was recrystallized twice from ethanol, and iodoacetate (Sigma Chemical Co.) was recrystallized once just before use from benzene–ligroin. Dithioerythritol (Cyclo Chemical Co.) and 2-mercaptoethanol (Calbiochem) were used without further purification. DEAE-Sephadex and Blue Dextran 2000 were obtained from Pharmacia Fine Chemicals. DEAE-cellulose (Sigma Chemical Co.) was washed with 0.1 N HCl and 0.1 N NaOH prior to use.

Preparation of the Enzymes. Lactate dehydrogenase was isolated from fresh beef hearts using modifications of the procedure developed by Pesce and coworkers (1964) for the

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[1] Mention of companies or products is for the convenience of the reader and does not constitute an endorsement by the U. S. Department of Agriculture.

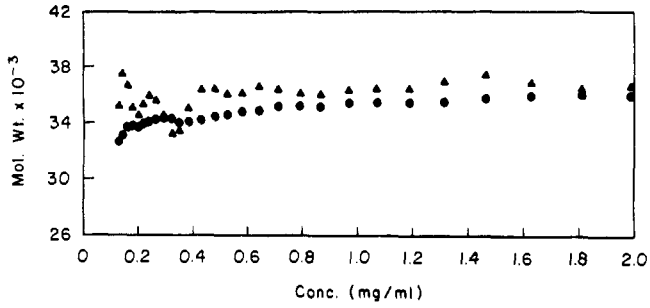

FIGURE 1: Dependence of the molecular weight averages on lactate dehydrogenase concentration in mg/ml, circles represent the point number-average molecular weights and triangles the point weight-average molecular weights. Conditions: 6 M guanidine hydrochloride, 0.01 M dithioerythritol, and 0.05 M KH_2PO_4 (pH 7.0), 36,000 rpm, 20°, initial concentration 0.64 mg/ml.

chicken heart enzyme. The chromatography on DEAE-cellulose was performed using a linear gradient of 0–0.2 M NaCl in 0.005 M Tris-chloride buffer (pH 7.0). Further purification was accomplished by ion-exchange chromatography on DEAE-Sephadex using a linear gradient of 0–0.2 M NaCl in 0.04 M phosphate buffer (pH 7.0). The main peak, corresponding to the H_4 fraction, was assayed for purity by disc gel electrophoresis at pH 9.5 (Zweig and Whittaker, 1967), and by gel electrofocusing (Wrigley, 1968) using an ampholyte range of pH 3–10. A single band was observed by both techniques for the H_4 fraction. The purified isozyme was stored in saturated ammonium sulfate at 4° until needed.

Beef heart lactate dehydrogenase was also purchased from a commercial source (Worthington Biochemical Corp., lots OHA, 1AB). The enzyme was purified by ion-exchange chromatography on DEAE-Sephadex as described above. Three peaks were observed, the largest two of which had considerable lactate dehydrogenase activity. The major peak (H_4) was assayed for purity as above and stored in saturated ammonium sulfate at 4°.

Dogfish muscle lactate dehydrogenase was prepared from frozen muscle using the procedure of Pesce and coworkers (1967). In addition, it was found necessary to chromatograph the enzyme on CM-cellulose using a linear gradient of 0–0.05 M NaCl in 0.005 M phosphate buffer (pH 7.0) (Shen and Wassarman, 1970).

Molecular Weight Determinations. For a typical experiment, a sample was withdrawn from the ammonium sulfate solution and dialyzed against 0.005 M Tris-chloride buffer (pH 8.0) at 6°. After being freed of ammonium sulfate, the sample was filtered through a Millipore filter (AAWPO1300) and the concentration determined from the absorbance at 280 nm using an absorptivity value of 1.50 l./(g cm) (Pesce *et al.*, 1964). The concentration was adjusted to the desired value with buffer and the sample was mixed with an equal volume of the buffered guanidine hydrochloride solution at the desired pH. The sample was then dialyzed against the guanidine solution until an aliquot was withdrawn for molecular weight analysis. The concentration of the enzyme in guanidine solution was determined from the absorbance at 280 nm using the absorptivity value of 1.455 l./(g cm) (Appella and Markert, 1961) for beef heart lactate dehydrogenase in 5 M guanidine hydrochloride.

The molecular weight of the sample was determined by high-speed sedimentation equilibrium (Yphantis, 1964). The sedimentation experiments were conducted at 20° in a Spinco, Model E, analytical ultracentrifuge equipped with schlieren and interference optics, electronic speed control, and RTIC temperature control unit. For the equilibrium experiments, a Kel-F coated aluminum double-sector centerpiece with sapphire windows was used. The windows and centerpiece were coated with a 1:25 dilution of Desicote (Beckman Instruments, Inc.) in chloroform (Millar *et al.*, 1969). The light of an AH-6 mercury lamp was filtered with a Kodak wratten filter and a polarizing lens and the interference patterns were recorded on Kodak IIIG spectroscopic plates. A shutter was employed to allow a white light exposure of the air reference fringes to facilitate alignment of the plate on the microcomparator.

The concentration of lactate dehydrogenase at various radial positions was determined from the correspondence of fringe displacement to concentration using a double-sector, capillary-type, synthetic boundary cell. Assuming the refractive index increment, dn/dc, to be constant, the concentration of protein in milligrams per milliliter is a linear function of the fringe displacement in microns (μ), $c = kf$. The value of k in 6 M guanidine hydrochloride was determined to be 0.00102 mg/ml-μ.

Using the white light fringes, the interference plate was aligned on a Nikon 6C microcomparator. The vertical displacement of the average of three light fringes with radial distance was determined and punched onto a paper tape. This was read into a Wang 370 programmable electronic calculator and, using a program developed by Aune (Aune and Timasheff, 1971), both the number- and weight-average molecular weights at each radial position were obtained. The value of the partial specific volume, $\bar{v}$, used was the same as that used by Millar and coworkers (1969), namely, 0.740, as determined by Appella and Markert (1961) for beef heart lactate dehydrogenase at 20.2° in 5 M guanidine hydrochloride. The $\bar{v}$ was corrected neither for preferential interaction with guanidine hydrochloride, nor for charge effects on the molecular weight at pH 2. Neither correction would affect the conclusions of this paper.[2]

Molecular weights were also determined by dodecyl sulfate–polyacrylamide gel electrophoresis using the procedure of Weber and Osborn (1969). A 10% gel was used and the electrophoresis was performed at 8 mA/gel for 5 hr. Before staining, the position of the marker dye was indicated by insertion of a small copper wire through the center of the band. The gels were then stained with Coomassie Brilliant Blue for 2–4 hr and destained by diffusion. After the gels had been cleared, the mobility was determined as the ratio of the position of the dye band to that of the protein band.

Molecular weights were also obtained by gel filtration on a calibrated agarose column in 6 M guanidine hydrochloride (Fish *et al.*, 1969). Prior to application to the column, the

[2] Appella and Markert (1961) have measured the interaction of guanidine hydrochloride with lactate dehydrogenase by equilibrium dialysis and found it to be 0.04 g/g of protein. Introducing this into the equation: $\phi' = \bar{v}_2^0 - (\xi_3^0/\rho^0)(1 - \bar{v}_3\rho^0)$ (Casassa and Eisenberg, 1964; Reisler and Eisenberg, 1969) (where ρ^0 is the solvent density, $\bar{v}_2^0$ and $\bar{v}_3$ are the partial specific volumes of protein and guanidine hydrochloride, respectively, and ξ_3^0 is the extent of preferential interaction of the guanidine hydrochloride with protein) results in a value of ϕ', the apparent partial specific volume of LDH corrected for solvent interactions, of 0.736. This would give an error of about 3% in molecular weight.

While the exact net charge of lactate dehydrogenase at pH 2 is not known, an estimate of the maximal value of this effect would result in an increase of the reported molecular weights in pH 2 guanidine by 1600 (Huston *et al.*, 1972).

proteins were reduced under nitrogen for 5 hr in 6 M guanidine hydrochloride containing 0.2 M Tris-chloride buffer, 0.01 M EDTA, and 0.1 M 2-mercaptoethanol (pH 8.6). Iodoacetate was then added and the reaction proceeded for 30 min in the absence of light. The sample was then dialyzed against distilled water and lyophilized. The carboxymethylated sample was dissolved in the guanidine solution, dialyzed against 6 M guanidine hydrochloride for 24 hr, and applied to a 0.9 × 100 cm column. The effluent was monitored at 280 nm with an LKB Uvicord II column monitor. Blue Dextran, which had been chromatographed on Sephadex G-200, superfine, in 0.5 M NaCl to remove low molecular weight contaminants, was used to determine the void volume of the column and dinitrophenylglycine was used in measure the volume accessible to the solvent.

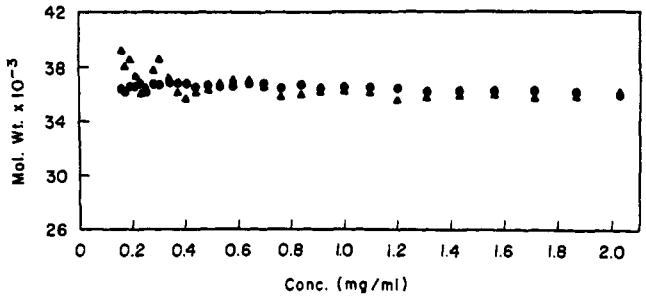

FIGURE 2: Dependence of the molecular weight averages on lactate dehydrogenase concentration in mg/ml, circles represent the point number-average molecular weights and triangles the point weight-average molecular weights. Conditions: 6 M guanidine hydrochloride, 0.01 M dithioerythritol, and 0.1 M H_3PO_4 (pH 2.0), 36,000 rpm, 20°, initial concentration 0.49 mg/ml.

Results and Discussion

Molecular Weight at pH 7 in 6 M Guanidine Hydrochloride. When beef heart lactate dehydrogenase was dialyzed against 6 M guanidine hydrochloride containing 0.01 M dithioerythritol–0.05 M KH_2PO_4 (pH 7.0) for 23 hr at 25°, a molecular weight distribution was obtained by high-speed sedimentation equilibrium (conditions: 36,000 rpm, 20°) as shown in Figure 1. In Figure 1, the calculated point number- and weight-average molecular weights are shown as a function of protein concentration. A least-squares analysis of the reciprocal of the point molecular weights as a function of concentration yielded a value of 33,700 for the number-average and 35,200 for the weight-average molecular weight when extrapolated to infinite dilution. The similarity of the weight- and number-average molecular weights, as well as the small concentration dependence would indicate that, under these conditions, the enzyme is fairly monodisperse and the subunit molecular weight is near 35,000. This is in good agreement with the results of Millar and coworkers (1969) who also observed a molecular weight of 35,000 at pH 7 in guanidine hydrochloride.

Molecular Weight at pH 2 in 6 M Guanidine Hydrochloride. When the beef heart enzyme was dialyzed *vs.* 6 M guanidine hydrochloride containing 0.1 M H_3PO_4–0.01 M dithioerythritol (pH 2.0) for 28 hr at 25°, high-speed sedimentation equilibrium at 36,000 rpm and 20° yielded molecular weights similar to those obtained for the enzyme in guanidine hydrochloride at pH 7. In Figure 2, the plots of the point number- and weight-average molecular weights as a function of concentration show essentially no difference between them and no concentration dependence. A least-squares analysis of the reciprocal of the molecular weights *vs.* the concentration, yielded a value of 32,400 for the number-average and 35,100 for the weight-average molecular weight extrapolated to infinite dilution. It should be pointed out that these conditions are similar to those used by Millar and coworkers (1969) when they reported molecular weights of 18,000, although their dialyses were performed at 4° for about 18 hr. In light of our inability to obtain the 18,000 molecular weight species under conditions which were nearly equivalent to those of Millar and coworkers (1969), we decided to investigate some of the possible causes for this.

Source of the Enzyme. One possible cause of a decreased molecular weight could be contamination with a proteolytic enzyme. Millar and coworkers (1969) used beef heart lactate dehydrogenase from Worthington Biochemical Corp. without further purification. By ion-exchange chromatography on DEAE-Sephadex, we could separate the Worthington enzyme into three p[illegible], the smallest of which lacked any lactate dehydrogenase activity. When the Worthington enzyme was used without any purification in molecular weight determination experiments in pH 2 guanidine hydrochloride, a molecular weight close to 36,000 was obtained (Table I, run 829). Hence, the effect of the contaminating species was not to decrease the molecular weight. The other runs listed in Table I using beef heart enzyme were all performed using a single preparation of the enzyme which had been isolated and purified from fresh beef hearts as described in the Materials and Methods section.

Length of Exposure to the Denaturant. In order to see if prolonged exposure of the enzyme to guanidine hydrochloride could lead to a decrease in the molecular weight, samples were withdrawn at various times of dialysis and the molecular weight was determined by high-speed sedimentation equilibrium and by dodecyl sulfate–polyacrylamide gel electrophoresis. The molecular weights obtained by the sedimentation experiments for several of the runs are listed in Table I. The number-average ($\overline{M}_n$) and the weight-average ($\overline{M}_w$) molecular weights given are values of intercepts of least-squares plots of the reciprocal of the molecular weight as a function of concentration. In two experiments, runs 818 and 811 (Table I), a considerable reduction of the molecular weight was observed. The results of exposure of the beef heart enzyme to 6 M guanidine hydrochloride containing 0.1 M 2-mercaptoethanol–0.1 M H_3PO_4 (pH 2.0) at 25° for 84 hr (run 811) are shown in Figure 3a,b. The plot of the ln f *vs.* r^2 (Figure 3a) displays a great deal of curvature indicative of a paucidisperse system. The dashed line, corresponding to the slope calculated for an 18,000 molecular weight species, is seen to pass through several of the values obtained in the region with small fringe displacement (96–118 μ). The more sensitive indicator of molecular weight, the point weight- and number-average molecular weights as a function of concentration (Figure 3b) also yielded small molecular weights, although not values as low as 18,000 for the weight-average molecular weights. A value of 18,000 was used as the molecular weight in the program (Aune and Timasheff, 1971)[3] to calculate the point-average number-average molecular weights; therefore, the values obtained near the meniscus

[3] The Aune and Timasheff program for calculating the number-average molecular weights requires that an estimated value be assigned to the molecular weight extrapolated to zero concentration. Thus, in this experiment, 18,000 should be considered as a maximal value of the extrapolated number-average molecular weight.

TABLE I: Molecular Weight Determinations in 6 M Guanidine Hydrochloride.

Run No.	Enzyme Source	Conditions	Length of Dialysis (hr)	Temp of Dialysis (°C)	$\overline{M}_n(c \rightarrow 0)^a$	$\overline{M}_w(c \rightarrow 0)^a$
817	Beef H_4	Gdn·HCl[b] (lot 217011)–0.01 M DTE–0.05 M KH_2PO_4 (pH 7.0)	23	25	33,700	35,200
821	Beef H_4	Gdn·HCl (lot 217011)–0.01 M DTE–0.1 M H_3PO_4 (pH 2.0)	28	25	34,400	35,100
829	Worthington beef heart	Gdn·HCl (lot 217011)–0.01 M DTE–0.1 M H_3PO_4 (pH 2.0)	36	25	35,800	36,200
810	Beef H_4	Gdn·HCl (lot 217011)–0.1 M 2-ME–0.1 M H_3PO_4 (pH 2.0)	48	5	36,400	36,600
818	Beef H_4	Gdn·HCl (lot 217013)–0.01 M DTE–0.05 M NaH_2PO_4 (pH 7.0)	48	25	20,000	25,000
811	Beef H_4	Gdn·HCl (lot 217013)–0.1 M 2-ME–0.1 M H_3PO_4 (pH 2.0)	84	25	17,000	26,000
881	Beef H_4	Gdn·HCl (lot 217016)–0.01 M DTE–0.05 M NaH_2PO_4 (pH 7.0)	528	25	36,900	37,000
882	Beef H_4	Gdn·HCl (lot 217016)–0.1 M H_3PO_4–0.01 M DTE (pH 2.0)	120	25	33,000	33,400
855	Dogfish M_4	Gdn·HCl (lot 217016)–0.01 M DTE–0.05 M NaH_2PO_4 (pH 7.0)	28	25	35,000	36,000
909	Dogfish M_4	Gdn·HCl (lot 217016)–0.01 M DTE–0.1 M H_3PO_4 (pH 2.0)	456	25	33,500	33,900

[a] Values for number and weight averages at infinite dilution were obtained by a least squares of the reciprocal of the molecular weights *vs.* the concentration extrapolated to zero concentration. [b] Gdn·HCl = guanidine hydrochloride; DTE = dithioerythritol; 2-ME = 2-mercaptoethanol.

are prejudiced by this assignment. The number- and weight-average molecular weights are seen to be quite different, the number-average being considerably smaller throughout the cell. Such a distribution would be expected from the appearance of smaller molecular weight species. The concentration dependence observed by us is somewhat different from that obtained by Millar *et al.* (1969). In subsequent experiments reported below, we have found that exposure of the enzyme to this guanidine hydrochloride solution resulted in rather nonspecific cleavage of the enzyme and so variation in the concentration dependence could be expected. The fact that the molecular weight of the enzyme had also decreased substantially after exposure of the enzyme to 6 M guanidine hydrochloride (pH 7) at 25° for 48 hr (run 818, Table I) suggested that the effect was due less to pH than to exposure to the guanidine hydrochloride. The beef heart enzyme used in all the experimental determinations except run 829 (Table I) was from a single preparation of the enzyme, which would seen to rule out some contaminant initially present in the enzyme preparation causing the splitting. While it is not possible to completely rule out bacterial contamination as a cause of splitting, the fact that the decreased molecular weights could only be obtained when one lot of the guanidine hydrochloride was used would seem to render this possibility unlikely unless the guanidine itself was contaminated with some proteolytic agent. In subsequent experiments using another lot of guanidine hydrochloride, we were unable to obtain such decreased molecular weights. Even exposure of the enzyme to the guanidine solutions at pH 2 for 120 hr at 25° or pH 7 for 528 hr at 25° (runs 882 and 881, respectively, Table I) showed no evidence of splitting into fragments of small molecular weight.

In order to examine whether the decreased molecular weight was a function of the lot of guanidine hydrochloride used, experiments were carried out in which aliquots of the enzyme were examined by dodecyl sulfate–polyacrylamide gel electrophoresis after various lengths of exposure to the different lots of guanidine. After exposure of the enzyme to a 6 M guanidine hydrochloride (lot 217016) solution, pH 2 for 320 hr at 25°, only one band with a molecular weight of near 35,000 was observed. However, exposure of the beef heart enzyme to a 6 M guanidine hydrochloride solution (pH 2) made from lot 217013, *i.e.*, the same lot as that which had previously resulted in molecular weight decreases (runs 811

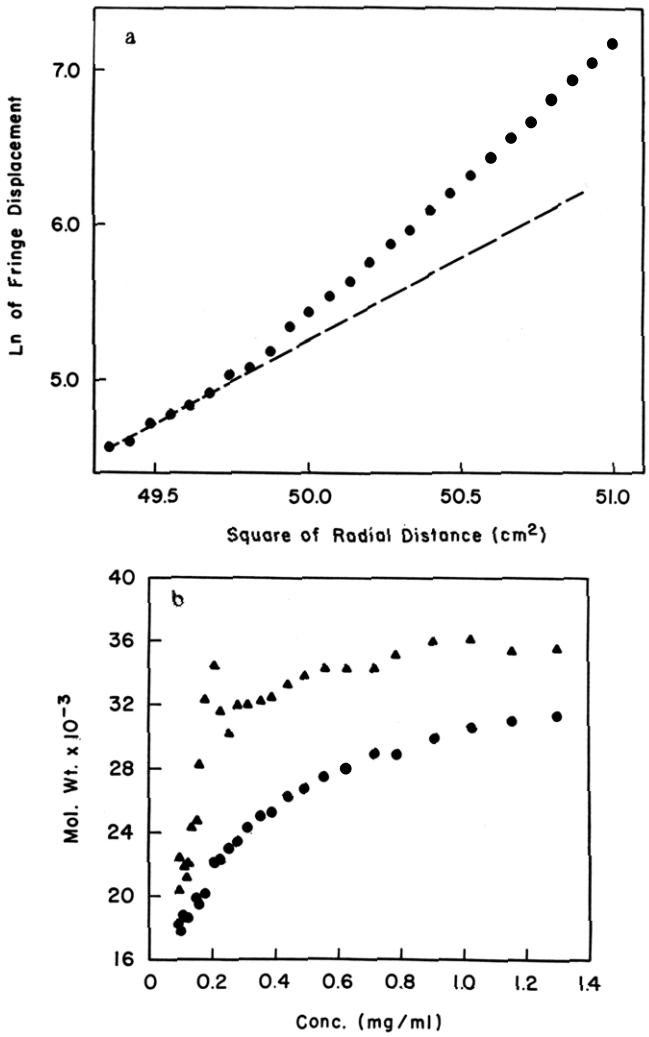

FIGURE 3: (a) Dependence of the natural logarithm of the fringe displacement (in microns) on the square of the radial distance. Conditions: 6 M guanidine hydrochloride, 0.1 M 2-mercaptoethanol, and 0.1 M H_3PO_4 (pH 2.0), 40,000 rpm, 20°, initial concentration 0.57 mg/ml. The dashed line corresponds to a molecular weight of 18,000. (b) Dependence of the molecular weight averages on lactate dehydrogenase concentration in mg/ml calculated from the data of Figure 3a, circles represent the point number-average molecular weights and triangles the point weight-average molecular weights.

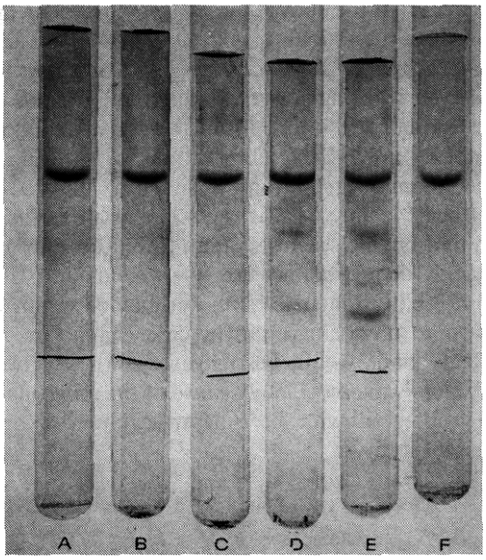

FIGURE 4: Results of dodecyl sulfate–polyacrylamide gel electrophoresis on samples taken at various times during the incubation of beef heart lactate dehydrogenase in 6 M guanidine hydrochloride, 0.01 M dithioerythritol, and 0.1 M H_3PO_4 (pH 2.0). (A) 52 hr, (B) 76 hr, (C) 103 hr, (D) 247 hr, (E) 528 hr, (F) 320 hr. Samples A–E were incubated in guanidine hydrochloride from lot 217013; sample F in lot 217016. Samples A–E were run using equivalent amounts of protein (6.5 μg of protein applied to each gel). The concentration of sample F applied to the gel was 5 μg of protein.

and 818), yielded smaller molecular weight fragments after prolonged incubation at 25°. The results of dodecyl sulfate–polyacrylamide gel electrophoresis of several samples taken at various times are shown in Figure 4. In the samples taken during the first 103-hr incubation (gels A–C, Figure 4), there is only one band with a molecular weight of 35,000. However, in the sample taken at 247 hr (gel D, Figure 4) at least two minor bands are in evidence in addition to the main band. The sample taken at 528 hr displays six minor bands in addition to the main band. The molecular weights of the three heavier of the minor bands were estimated to be 22,000, 14,000, and 11,000. The molecular weight of the still faster migrating bands could not be established since they were too low to estimate from the standard curve established for the dodecyl sulfate–polyacrylamide gels. They are, however, lower than 11,000. It could be concluded from this experiment that prolonged exposure to guanidine hydrochloride at pH 2 made from lot 217013 resulted in the splitting of the enzyme into several fragments, none of them of 18,000 molecular weight. The difference in time of appearance of the smaller molecular weight species as observed by sedimentation equilibrium *vs.* dodecyl sulfate gel electrophoresis is probably due to the smaller amounts of the degradation products not being detected by the electrophoresis during the shorter times of dialysis. Inclusion of Versene into the gels did not alter the pattern observed, ruling out the possibility of a metal ion bridging of smaller fragments.

Gel filtration of the H_4 enzyme on the calibrated agarose–6 M guanidine hydrochloride column yielded a single peak with a molecular weight of 35,500 ± 1000 in good agreement with the values predicted by the tetrameric hypothesis.

Dogfish Muscle Lactate Dehydrogenase. The dogfish muscle lactate dehydrogenase did not display any splitting to smaller molecular weight species upon prolonged exposure to guanidine hydrochloride at either pH 7 or pH 2 as observed by dodecyl sulfate–polyacrylamide gel electrophoresis. The molecular weight of the dogfish subunit measured by this technique was 36,000. However, the lot of commercial guanidine hydrochloride which caused splitting of the beef heart enzyme (lot 217013) had been exhausted before we could use it on the dogfish enzyme and splitting of the beef heart enzyme had not been observed in the other two lots of guanidine used.

The molecular weight of the dogfish enzyme was also determined by high-speed sedimentation equilibrium. After dialysis for 28 hr at 25° in 6 M guanidine hydrochloride–0.01 M dithioerythritol–0.05 M NaH_2PO_4 (pH 7.0) (run 855, Table I), very similar weight- and number-average molecular weights were obtained ($\overline{M}_n$ = 35,000; $\overline{M}_w$ = 36,000). Prolonged exposure (456 hr, 25°) to 6 M guanidine hydrochloride–0.01 M dithioerythritol–0.1 M H_3PO_4 (pH 2) (run 909, Table I) yielded similar weight- and number-average molecular weights ($\overline{M}_n$ = 33,500; $\overline{M}_w$ = 33,900) and displayed no evidence of any smaller molecular weight species.

We believe that the above data can be most easily explained in terms of a four-subunit enzyme, whether the source of the lactate dehydrogenase was beef heart or dogfish muscle. The observed splitting was most likely due to the presence of some contaminant in one lot of the commercial guanidine hydrochloride used. Such observations of splitting in concentrated guanidine solutions have been reported for pig and beef heart lactate dehydrogenase, as well as for β-lactoglobulin and bovine serum albumin (Jaenicke, 1969) and for spleen acid deoxyribonuclease (Townend and Bernardi, 1971). Since both β-lactoglobulin and bovine serum albumin are known to have single polypeptide chains, the splitting of covalent bonds would be obligatory in these two cases. Such splitting is the most likely cause of the molecular weight decreases observed with beef heart lactate dehydrogenase in the present study.

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Phosphorylation of the Membranous Protein of the Sarcoplasmic Reticulum Inhibition by Na^+ and K^+†

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ABSTRACT: Ca^{2+} activates the transfer of the γ-phosphate of ATP to a protein of the sarcoplasmic reticulum. Na^+ and K^+ strongly inhibit this reaction by competing with Ca^{2+} for its binding site. The degree of inhibition varies with ATP concentration and temperature. It is proposed that the binding of ATP to the membrane produces a conformational change of the Ca^{2+} binding site resulting in a modification of its specificity.

Fragmented sarcoplasmic reticulum isolated from skeletal muscle retains a highly efficient ATP-dependent Ca^{2+} transport system (Hasselbach and Makinose, 1961; Hasselbach, 1964). The active Ca^{2+} transport is mediated by a membrane-bound ATPase which is highly sensitive to change in free Ca^{2+} concentration on either side of the membrane. In the process of ATP hydrolysis, the γ-phosphate of ATP is covalently bound to a membrane protein (E). This phosphoprotein (E∼P) represents an intermediate product in the sequence of reactions leading to Ca^{2+} transport and phosphate

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