Yoshida, M., and Ukita, T. (1965), *J. Biochem.*, *58*, 191.

Yoshida, M., and Ukita, T. (1966), *Biochim. Biophys. Acta* *123*, 214.

Molecular Weight and Subunit Structure of Yeast Enolase*

Kenneth G. Mann,† Francis J. Castellino,‡ and Paul A. Hargrave

ABSTRACT: The native and subunit molecular weights of yeast enolase have been reinvestigated by sedimentation equilibrium analysis, gel filtration in guanidinium chloride, and gel electrophoresis in sodium dodecyl sulfate. The values obtained in this work with three different and highly purified enolase preparations are 88,000 daltons for the native (dimeric) enzyme and 44,000 daltons for the subunits. These new values are considerably higher than the published ones (67,000 and 34,000 daltons) and it will be necessary to reevaluate the published molecular properties of yeast enolase.

A molecular weight of 67,000 daltons has been generally accepted for yeast enolase for the last 20 years. This value has been arrived at by a number of different methods: mercury analysis of the crystalline Hg–enzyme and sulfur analysis assuming 1 mole of Hg^{2+} and 8 moles of S per mole of enzyme (Warburg and Christian, 1941), quantitative amino-terminal analysis (Malmström *et al.*, 1959; Brewer *et al.*, 1970), light-scattering measurements (Bucher, 1947), sedimentation–diffusion, and sedimentation equilibrium measurements (Bergold, 1946; Brewer and Weber, 1968; Gawronski and Westhead, 1969), all giving molecular weight values in the range from 64,000 to 68,000 daltons. Early unsuccessful attempts to effect subunit dissociation together with the evidence from the chemical analyses led to the conclusion that the enzyme consisted of a single polypeptide chain of molecular weight 67,000 daltons. More recently, the existence of subunits was clearly demonstrated by the observed reduction in molecular weight to 34,000 daltons when the enzyme was exposed to high concentration of KCl or KBr in the absence of Mg^{2+} (Brewer and Weber, 1968; Gawronski and Westhead, 1969).

In our studies of the subunit structure and chemical properties of yeast enolase, we accumulated a number of quantitative data which were incompatible with a molecular weight of 67,000 daltons and consequently decided to reinvestigate the molecular weight of both the native enzyme and its subunits. The results of this investigation are reported here and lead to a revised molecular weight of 85,000–90,000 daltons for yeast enolase.

The existence of multiple molecular forms of yeast enolase has been well documented (Malmström, 1957; Westhead and McLain, 1964). It is, therefore, conceivable that variations in results from different laboratories and at different times are due to different sources of yeast or to differences in isolation procedures leading to the purification of different enolase isozymes. These possible variations were checked to a limited extent in this work by comparing three different preparations of yeast enolase, two obtained from separate batches of Fleischmann bakers yeast (Pekin, Ill.) at an interval of 6 months and one obtained from brewer's yeast (Carling Brewing Co., Atlanta, Ga.).[1] The purity and specific activity measurements and some of the molecular weight studies were carried out for these three enzymes in parallel experiments. The three preparations are identified as P, Q, and R.

Experimental Section

Activity Assay. Enolase activity was measured by the direct spectrophotometric assay of phosphoenolpyruvate production monitored at 230 nm at 30° (Westhead, 1966). The substrate, barium D-glycerate 2-phosphate (Sigma), was converted into and used as the water-soluble cyclohexylammonium salt.

Purification of Enzyme. Enolase was prepared according to the method described by Westhead (1966). The final product of each individual preparation was subjected to chromatography on TEAE-cellulose[2] (Westhead and McLain, 1964) to separate different active enolase species, and the subsequent work was done only with the so called A isozyme (the first major peak eluted from the TEAE-cellulose column). The purity of each, preparation was checked by specific activity measurements, disc gel electrophoresis, and isoelectric focusing.

Physical Methods. Polyacrylamide gel electrophoresis was

* From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27706, and the Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received May 29, 1970.* Supported in part by U. S. Public Health Service Research grants (AM-04576, HE-06400, and GM-15053), Postdoctoral Fellowships (GM-34119 (K. G. M.) and AM-34008 (F. J. C.)), and Predoctoral Fellowship (GM-33541 (P. A. H.)).

† Present address: Department of Biochemistry, University of Minnesota, St. Paul, Minn. 55101; to whom to address correspondence.

‡ Present address: Department of Chemistry, University of Notre Dame, Notre Dame, Ind. 46556.

[1] We are greatful to Dr. J. M. Brewer for providing us with this purified enolase and thus enabling us to carry out the comparative work.

[2] Abbreviations used are: TEAE, triethylaminoethyl; Gu·HCl, guanidinium chloride.

carried out at pH 8.9 (Davis, 1964), at pH 4.5 (Reisfeld *et al.*, 1962), and in 6.25 M urea–0.9 M acetic acid (Panyim and Chalkley, 1969). Protein (50 μg) in 3–10 μl was applied to each gel. Isoelectric focusing on 6-cm polyacrylamide gel columns was carried out essentially according to Dale and Latner's (1968) method, except that experiments were conducted for 4 hr at 350 V, at 4°, as described by Wrigley (1968). After the gels had been washed several times with trichloroacetic acid, they were stained with Amido Black (Leaback and Rutter, 1968) and destained with 7% acetic acid.

Molecular weights were determined by different methods. Both the native enzyme and its subunits were studied by sedimentation equilibrium, and the subunit molecular weights were also determined by sodium dodecyl sulfate electrophoresis and gel filtration in 6 M Gu·HCl.

Ultracentrifuge studies were performed with the Spinco Model E ultracentrifuge equipped with Rayleigh interference optics. The molecular weights of yeast enolase were estimated using both the high-speed sedimentation equilibrium technique of Yphantis (1964) and the conventional low-speed sedimentation equilibrium method. Samples for study were dissolved in the appropriate solvent and dialyzed for at least 24 hr prior to ultracentrifugal analysis. All sedimentation equilibrium experiments were conducted at pH 6.0. Solvent pH was adjusted by the addition of 1 M HCl or 1 M NaOH. In studies designed to promote dissociation to the ultimate subunit, enolase was reduced with 0.1 M 2-mercaptoethanol in 6 M Gu·HCl (pH 8.6) prior to dialysis.

Sedimentation equilibrium studies were performed at 25° using approximately 3-mm solution columns in double-sector cells equipped with sapphire windows. In all cases, the attainment of equilibrium was determined directly by measuring fringe shifts at given radial positions on successive photographs. In high-speed experiments, water blanks were run to correct for window distortion.

In low-speed experiments, the protein concentration at the meniscus, C_m, was calculated using the appropriate value for the initial concentration, C_0, and the conservation of mass expression

$$C_m = C_0 - \frac{r_b^2(C_b - C_m) - \frac{r_b}{r_m} r^2 dc}{r_b^2 - r_m^2}$$

where the term r denotes the radical position, and the subscripts b and m denote the cell bottom and meniscus, respectively. The term C_0 was determined in two fashions. The first of these involved the conventional synthetic boundary run performed after the final equilibrium photograph was taken. The second procedure involved calculation of C_0 in fringes by means of the absorbance of the protein solution at 280 nm, the extinction coefficient ($E^{1\%}_{280}$ 0.891; Warburg and Christian, 1941), and the protein refractive index increment of 4.1 fringes/mg per ml (Babul and Stellwagen, 1969). Excellent agreement was obtained between the C_0 values determined directly and those calculated from the absorbance of enolase solutions.

Molecular weights were calculated for all sedimentation equilibrium data using the expression

$$M_{wr} = \frac{2RT\frac{(d \ln c)r}{dr^2}}{\omega^2(1 - \phi\rho)}$$

where M_{wr} is the weight-average molecular weight at any radial position r, ϕ' is the effective partial specific volume, ω is the rotor angular velocity, and ρ is the solvent density. The values for d ln c/dr^2 were obtained from plots of log fringe displacement *vs.* radial distance squared.

In the experiments performed at high speed in Gu·HCl and in the low-speed experiments plots of ln c *vs.* r^2 were linear, indicating homogeniety within experimental error. In these cases, a unique molecular weight is obtained throughout the cell and $M_{wr} = M$.

High-speed experiments performed on the native protein resulted in nonlinear ln c *vs.* r^2 plots. In these cases, point-average molecular weights were obtained. In order to obtain plots of molecular weight *vs.* concentration, fringe displacements were converted into protein concentrations using the refractive index increment of Babul and Stellwagen (1969).

The partial specific volume for yeast enolase has been reported by Bergold (1946) to be 0.735 cc/g. This value of $\bar{v}$ has been used for ϕ' in the calculation of the molecular weight of the native protein in dilute salt solutions. The choice of ϕ' in concentrated Gu·HCl is somewhat ambiguous. Hade and Tanford (1967) have shown that owing to preferential guanidinium ion binding, ϕ' may be decreased as much as 0.01 cc/g in 6 M Gu·HCl. With this in mind, we have calculated molecular weights in 6 M Gu·HCl in two fashions. The first of these is to assume minimal preferential interaction, and set ϕ' equal to $\bar{v}$ or 0.735 cc/g. The second assumes a maximal change in $\bar{v}$ of 0.01 cc/g and a ϕ' of 0.725 cc/g.

Gel filtration in 6 M Gu·HCl was performed on 4 and 6% agarose (Sepharose 4B and 6B, Pharmacia) columns according to the method of Fish *et al.* (1969). Protein samples for study were reduced with 0.1 M 2-mercaptoethanol in 6 M Gu·HCl (pH 8.6) and carboxymethylated with a slight excess of iodoacetic acid at the same pH. Protein elution positions were monitored spectrophotometrically by absorbance at 280 nm. Apparent molecular weights were estimated from a calibration curve of log molecular weight *vs.* distribution coefficients, K_d, obtained with proteins of known molecular weight (Fish *et al.*, 1969).

Sodium dodecyl sulfate electrophoresis was performed by the method of Shapiro *et al.* (1967; Shapiro and Maizel, 1969) as described by Weber and Osborn (1969). The reference proteins were all from commercial sources. "Electrophoresis grade" acrylamide (Bio-Rad Laboratories) was used to make gels with 5–8% cross-linking. The 10-cm long gels were prepared in 0.5 × 12 cm glass tubes in the cold by layering the chilled acrylamide solution over a 1-cm layer of 40% sucrose. This method was found to give a very smooth and flat gel surface, which was subsequently used as the gel top for sample application. Each protein (1–2 mg/ml) was incubated at 37° for 3 hr or heated in a boiling water bath for 10–15 min in 0.01 M sodium phosphate buffer (pH 7.0), containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. Samples were then dialyzed against phosphate buffer containing 0.1% sodium dodecyl sulfate and 0.1% mercaptoethanol overnight. The final solution applied to the gel contained 10–50 μg of each protein, brom phenol blue (as marker), and 10% sucrose in 20–50 μl. The sample was applied to the gel top under a layer of buffer, most runs being performed with the phosphate concentration of the gel buffer reduced to half that used by Weber and Osborn (1969). After electrophoresis the gels were stained with coomassie blue and destained electrophoretically.

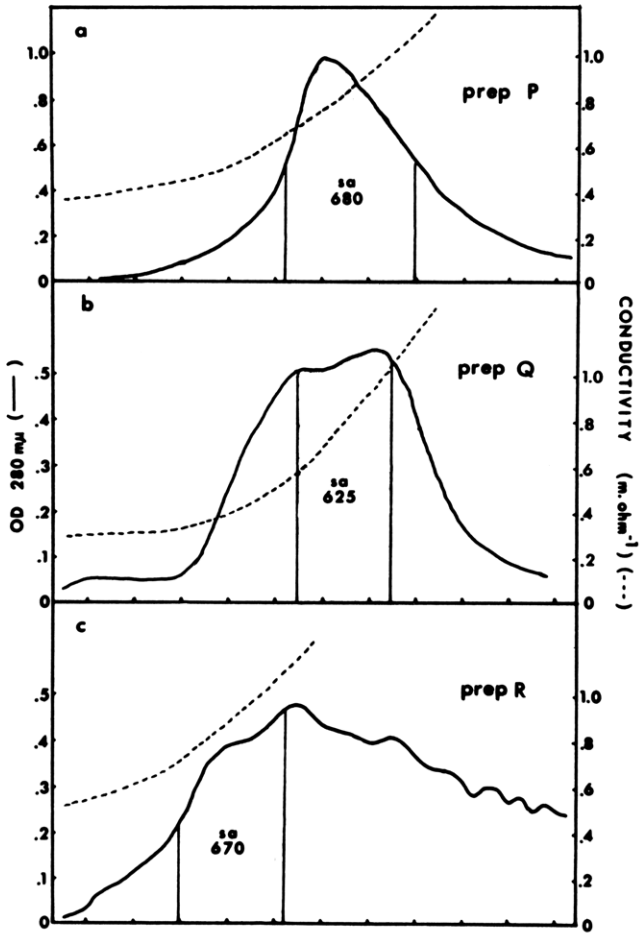

FIGURE 1: TEAE-cellulose chromatography of yeast enolase at pH 8.65, 4°, with a Tris-HCl three-chamber gradient (Westhead and McLain, 1964). Buffer reservoirs were 80 ml, column dimensions 1.8 × 12 cm. The column was run at 1.7 ml/min and 2-min fractions were collected. Fractions of conductivity approximately 0.6–1.1 mmhos were pooled for assay, lyophilized, and used in further studies. Preparations P and Q had been prefractionated on a larger scale previously. Shaded portions represent the pooled fractions (specific activity shown) used for further study. The specific activity of chemically pure yeast enolase is 620 (ΔOD_{230}/min per OD_{280} of enzyme) (Westhead, 1966).

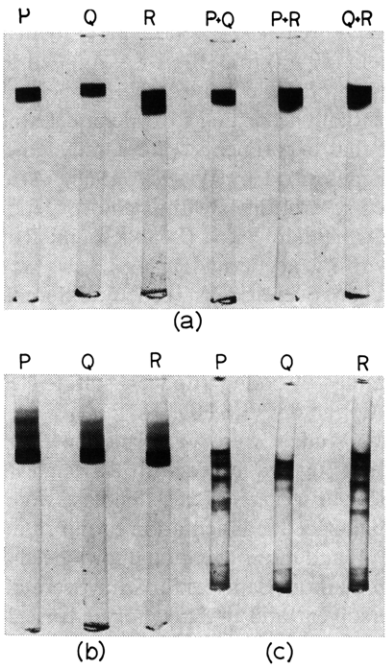

FIGURE 2: (a) Polyacrylamide gel electrophoresis at pH 8.9. (b) Polyacrylamide gel electrophoresis at pH 4.3. (c) Electrofocusing in polyacrylamide gel, pH range 6 (bottom) to 8 (top). (Bands near top of gel P are due to an aged hemoglobin mixture added to the sample.) Each gel contained 50 μg of enolase or 25 μg of each component in the mixtures.

Results

Some of the properties of the three enolase preparations used in this work are given in Figures 1 and 2. Figure 1 shows part of the TEAE-cellulose elution profile and indicates the fractions which were pooled for further studies and also gives their respective specific activity. It should be noted that the side fractions of the major broad peak contain enolase of very similar specific activity. Figure 2 shows the homogeneity of the three preparations in disc gel electrophoresis and isoelectric focusing. Samples P and Q appear homogeneous at pH 8.9, while sample R consists of two distinct bands. At pH 4.3 all samples run as a single major component with a few per cent impurities. Since it is known that yeast enolase is labile below pH 5, it is quite likely that the slower moving minor bands represent inactive aggregated material. Gel electrophoresis in urea–acetic acid (Panyim and Chalkley, 1969) gave single homogeneous bands. The isoelectric focusing results in Figure 2c are much more problematic. The main feature of this figure is the unique pattern of each individual preparation. This phenomenon has been found to be quite general for yeast enolase, and no matter how carefully selected and pure a fraction is by disc gel electrophoresis, it always resolves into multiple isoelectric species in this system. There is no indication that the unique isoelectric differences of different enolase samples are reflected in any of the other molecular properties studied (activity, native and subunit molecular weight, carboxy- and amino-terminal analysis (Hargrave and Wold, 1969)).

Subunit Molecular Weight. It has been well established that yeast enolase is a solvent-dependent associating–dissociating system (Brewer and Weber, 1968; Gawronski and Westhead, 1969). For this reason we have approached the problem of the molecular weight and subunit structure of this protein starting with the subunit molecular weight.

Figure 3 presents the graphical data for two high-speed sedimentation equilibrium experiments performed on a 0.01% solution of reduced yeast enolase in 6 M Gu·HCl–0.1 M 2-mercaptoethanol (pH 6.0) at 37,020 and 43,040 rpm. Both plots are linear to the bottom of the cell, and assuming that ϕ' equals $\bar{v}$, yield molecular weights of 45,800 and 45,300, respectively. If one assumes a maximum degree of noncovalent guanidinium ion binding to the protein (Hade and Tanford, 1967), ϕ' would be 0.725 cc/g. In this case, the computed values would be 42,700 and 42,300. Even within the limits of uncertainty in ϕ', these values represent a significant difference from those reported in the past (Gawronski and Westhead, 1969; Brewer *et al.*, 1970).

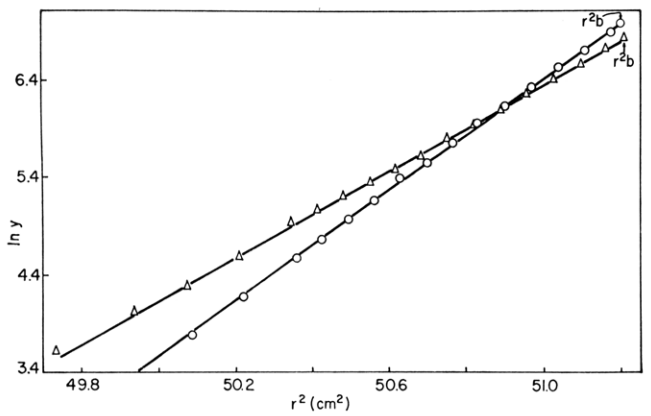

FIGURE 3: Sedimentation equilibrium data for a 0.01% solution of reduced yeast enolase in 6 M Gu·HCl–0.1 M 2-mercaptoethanol (pH 6.0, 25°). Data for two experiments at 42,040 rpm (○) and 37,020 rpm (△) are plotted in terms of ln fringe displacement (Y) *vs.* radial distance squared (r^2).

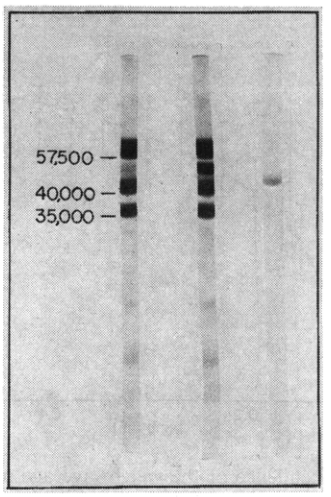

FIGURE 4: Polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Gel A contained, from top to bottom, catalase, aldolase, and lactate dehydrogenase. Gel B contained the same proteins (the subunit molecular weights of the protein markers given in the figure are taken from Klotz and Darnall (1969)) and yeast enolase. Gel C (a different experiment) contained enolase only. No differences were observed with the three enolase preparations, when run individually or in the same gel with marker proteins.

The apparent molecular weight of yeast enolase was also evaluated by gel filtration in 6 M Gu·HCl according to the method of Fish *et al.* (1969). Molecular weights derived by this technique are, in reality, a reflection of the volume of a linear randomly coiled polypeptide chain, rather than the mass. However, since the volume of a linear random coil is a measure of the number of monomer units in the chain, an apparent molecular weight can be determined. Thus the observation of an apparent molecular weight from gel filtration studies consistent with the true mass is indicative of linear random coil dimensions.

Gel filtration experiments were performed on 4 and 6% agarose columns prepared and operated in 6 M Gu·HCl with reduced and carboxymethylated yeast enolase. The distribution coefficient observed for yeast enolase eluted from the 6% agarose column was consistent with an apparent molecular weight of 43,000 ± 3000. A similar experiment performed with a 4% agarose column gave an apparent molecular weight of 40,000 ± 5000.[3] Both of these values are in agreement with the mass data obtained from sedimentation equilibrium. In order to look for differences in subunit molecular weight of the three different preparations, a 1:1:1 mixture of the three yeast enolases were reduced, carboxymethylated, and subjected to gel filtration in 6 M Gu·HCl on 6% agarose. The mixture of the three enolases was eluted as a single component of apparent molecular 45,000 ± 3000. In the same experiment, cytochrome *c*, which was mixed with the applied enolase sample as an internal standard, was eluted at a position corresponding to a molecular weight of 12,300.

Figure 4 shows typical sodium dodecyl sulfate–electrophoresis patterns for enolase and protein standards. Again, no significant difference between the three preparations was apparent, and when compared to individual reference proteins, subunit molecular weights obtained from some 20 separate runs fell in the range from 47,500 to 50,000.

[3] The error limits cited for 4% agarose are due to a less precise calibration than for the 6% agarose column. For this reason, the data from the 4% agarose column are not included in the average value cited in Table I.

Native Molecular Weight. High-speed sedimentation equilibrium experiments were performed with 0.01% yeast enolase in 0.1 M $MgCl_2$–0.1 M NaCl (pH 6.0, 25°). Figure 5 presents the graphical data from these experiments. Both plots are curved, indicating a nonuniform distribution of molecular weight throughout the cell. Since the data on the subunit molecular weight of the protein have already established that the subunits are homogeneous with respect to mass, the molecular weight distribution can only be interpreted as a concentration dependent equilibrium process. For the experiment performed in 0.1 M NaCl, the values of M_w at the foot of the concentration gradient and at the cell bottom were 48,900 and 70,900, respectively. For the experiment performed in 0.1 M $MgCl_2$ values of 57,800 and 82,300 were obtained at the leading edge and cell bottom. The concentration dependence of the molecu-

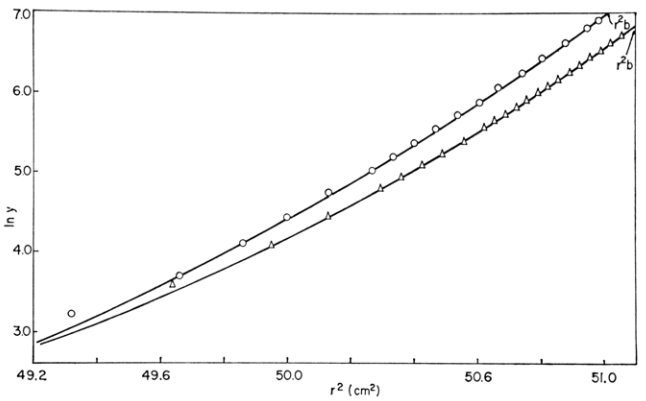

FIGURE 5: High-speed sedimentation equilibrium data for 0.01% yeast enolase solutions in 0.1 M NaCl, pH 6.0, 25,980 rpm, 25° (○), and 0.1 M $MgCl_2$, pH 6.0, 24,000 rpm, 25° (△). The data are plotted in terms of ln fringe displacement (Y) *vs.* radial distance squared, r^2.

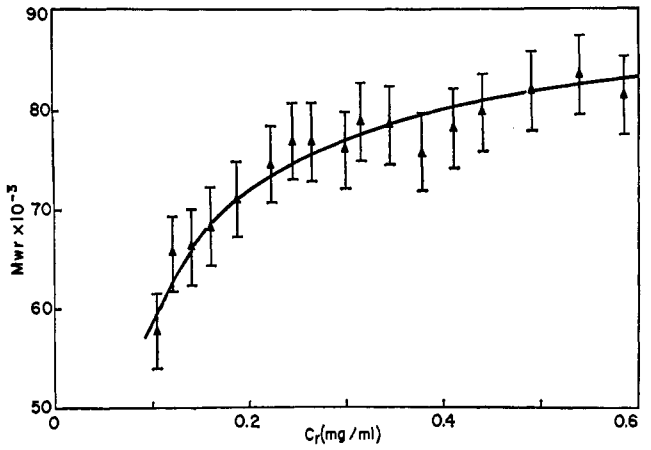

FIGURE 6: The dependence of the weight-average molecular weight M_{wr}, of yeast enolase in 0.1 M $MgCl_2$, pH 6.0, 25°, with protein concentration, C_r, in terms of milligrams per milliliter. The point slopes used to calculate M_{wr} were obtained from the data of Figure 2. Fringe displacements were converted into absolute concentration units as described in text. The error bars represent the determined value, ±4000.

lar weight observed in 0.1 M $MgCl_2$ is presented in Figure 6. It can be seen that at concentrations below approximately 0.5 mg/ml, the protein is dissociated to an appreciable extent.

The conventional low-speed sedimentation equilibrium technique was chosen in order to extend the observable concentration region to a point where the molecular weight would be independent of concentration. Figure 7 (circles) presents the equilibrium distribution of a 1.642-mg/ml solution of yeast enolase in terms of ln fringe number, f, *vs.* r^2. The experiment was performed at 10,000 rpm in 0.1 M $MgCl_2$ (pH 6.0, 25°). The plot is curved at values of ln f below 1.7, and linear above this value. The concentration dependence of the local weight-average molecular weight is given in Table I. In order to obtain equally spaced points for least-squares analysis at given radial positions, values of ln f and r^2 were obtained from

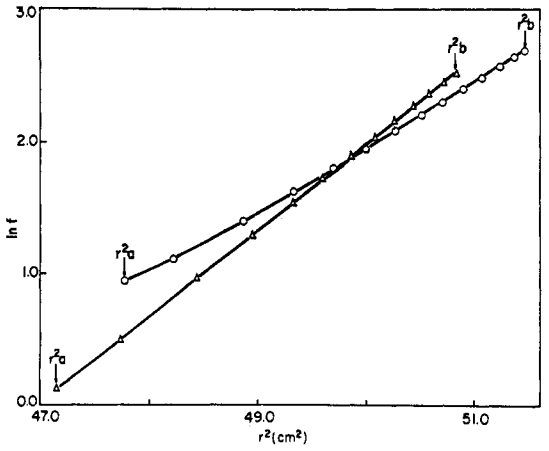

FIGURE 7: Low-speed sedimentation equilibrium data for yeast enolase in 0.1 M $MgCl_2$, pH 6.0, at 25° (○) and 20° (△). Data are plotted in terms of ln fringe number (f) *vs.* radial distance squared (r^2). The position of the meniscus (r^2_a) and cell bottom (r^2_b) are different owing to the use of different amounts of fluorocarbon in each case.

TABLE I: Point-Weight-Average Molecular Weights, M_{wr}, Determined for the Low-Speed Sedimentation Equilibrium Experiment Performed on Yeast Enolase at 10,000 rpm in 0.1 M $MgCl_2$ at 25° (Figure 7, circles; 0).[a]

r^2 (cm²)	ln f	C (mg/ml)	M_{wr} (× 10^{-3})
48.2	1.105	0.736	72.0
48.4	1.190	0.801	74.0
48.6	1.281	0.878	76.1
48.8	1.373	0.963	78.5
49.0	1.467	1.058	80.6
49.2	1.562	1.163	81.9
49.4	1.663	1.287	82.5
49.6	1.762	1.421	82.4
49.8	1.860	1.570	84.4
50.0	1.959	1.730	86.6
50.2	2.060	1.910	87.1
50.4	2.159	2.110	86.9
50.6	2.258	2.330	86.5
50.8	2.357	2.580	87.4
51.0	2.456	2.840	88.6
51.2	2.556	3.142	87.8

[a] Values for M_{wr} were obtained by least-squares fit of equally spaced ln f points interpolated from the smooth curve fit of the data given in Figure 7.

a smooth curve drawn through the data presented in Figure 7 (circles). Owing to the relatively small fringe shift near the meniscus, the M_{wr} values obtained in this region are of poorer reliability than those obtained nearer the cell bottom.

From the data of Table I, it is observed that at concentrations higher than 1.73 mg/ml (r^2 = 50 cm²) the point-weight-average molecular weight is independent of concentration. Least-squares analysis of the observed fringe shift data for radial positions greater than 50 cm² gives a weight-average molecular weight of 87,300 (M_{wb}). The weight average at the meniscus was calculated in a similar fashion using the observed fringe shifts below 50 cm². The value for M_{wa} obtained in this fashion was 78,300. Combination of these data gave a value of M_z of 89,100.

An apparently linear relationship of ln f *vs.* r^2 was obtained at a slightly lower temperature. Figure 7 (triangles) presents the equilibrium data obtained at 11,000 rpm for a 1.15-mg/ml solutions of yeast enolase in 0.1 M $MgCl_2$ at 20°. The molecular weight calculated from these data was 89,400.

Discussion

The results of the molecular weight measurements for both the native enolase and its subunits are summarized in Table II. We chose to perform our subunit molecular weight studies in 6 M Gu·HCl since this solvent has been clearly demonstrated to destroy all noncovalent interactions in proteins (Tanford, 1968), and the effects of preferential interaction in this solvent have been carefully studied (Hade and Tanford, 1967; Castellino and Barker, 1968). There should therefore be no am-

biguity in the interpretation of the results obtained in this solvent, and the measurements both of the subunit mass by sedimentation equilibrium and of the hydrodynamic volume of the subunit linear random coil by gel filtration clearly establish that the subunit molecular weight of yeast enolase is 44,000 daltons rather than 34,000 daltons as previously reported. This suggests that the molecular properties of yeast enolase are very similar to enolases from other species (Winstead and Wold, 1965; Castellino and Barker, 1968; Cory and Wold, 1966).

The more empirical estimation of subunit molecular weight by sodium dodecyl sulfate electrophoresis gave somewhat higher values. The basic assumption in this method that all globular proteins interact with sodium dodecyl sulfate to the same extent, so that in the comparison of a series of proteins the amount of protein bound sodium dodecyl sulfate is a constant proportion of the total volume of the migrating protein. Weber and Osborn (1969) found empirically that the assumption has surprisingly general validity in their test of 40 different proteins. It is felt that the values obtained here (47,500–50,000) are sufficiently close to the 10% accuracy range suggested for this method to support the value arrived at by the more precise methods.

Sedimentation equilibrium studies of native yeast enolase performed in other laboratories have been complicated both by solvent choice, and by the fact that the protein undergoes concentration-dependent association. The native molecular weight studies of both Brewer and Weber (1968) and Gawronski and Westhead (1969) were performed at high salt concentrations, and resulted in curved plots of ln c *vs.* r^2. The molecular weights were estimated from the limiting slope of the plotted concentration data at the cell bottom. Neither of these two reports demonstrated that a discrete aggregate of enolase subunits existed, but rather gave a maximum observed value under the conditions of solvent and protein concentration employed. We have empirically determined conditions of solvent, protein concentration, and temperature under which a single species (dimer) exists. Under conditions in which the protein is fully associated, a linear plot of ln c *vs.* r^2 is obtained. The molecular weight of 88,000 calculated from these data is consistent with a stable yeast enolase dimer composed of two subunits of 44,000 molecular weight. With this new molecular weight several quantitative data on the metal binding, the amino acid composition, and the identity of the two polypeptide chains need to be recalculated and reevaluated.

TABLE II

Solvent	Method of Determination	Mol Wt
6 M Gu·HCl	Sedimentation[a] equilibrium	45,600[b] 42,500[c]
6 M Gu·HCl	Gel filtration, 6% agarose	44,000
0.1 M $MgCl_2$	Sedimentation[d] equilibrium	87,300 (M_{wb}) 89,100 (M_z)
0.1 M $MgCl_2$	Sedimentation[e] equilibrium	89,400

[a] High-speed technique. [b] Assuming $\phi' = \bar{v}$ (0.735 cc/g). [c] Assuming ϕ' is decreased by 0.01 cc/g (0.725 cc/g). [d] Low-speed technique, 25°. [e] Low-speed technique, 20°.

Acknowledgment

The authors thank Drs. Charles Tanford, Robert Hill, and Finn Wold for their encouragement and advice during the course of this investigation, and for the use of their laboratory facilities.

References

Babul, J., and Stellwagen, E. (1969), *Anal. Biochem. 28*, 216.

Bergold, G. (1946), *Z. Naturforsch. 1*, 100.

Brewer, J. M., Fairwell, T., Travis, J., and Lovins, R. E. (1970), *Biochemistry 9*, 1011.

Brewer, J. M., and Weber, G. (1968), *Proc. Nat. Acad. Sci. U. S. 59*, 116.

Bucher, T. (1947), *Biochim. Biophys Acta 1*, 292.

Castellino, F. J., and Barker, R. (1968), *Biochemistry 7*, 2207.

Cory, R. P., and Wold, F. (1966), *Biochemistry 5*, 3131.

Dale, G., and Latner, L. (1968), *Lancet* 847.

Davis, B. J. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.

Fish, W. W., Mann, K. G., and Tanford, C. (1969), *J. Biol. Chem. 244*, 4989.

Gawronski, T. H., and Westhead, E. W. (1969), *Biochemistry 8*, 4261.

Hade, K., and Tanford, C. (1967), *J. Amer. Chem. Soc. 89*, 5034.

Hargrave, P. A., and Wold, F. (1969), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 28*, 857.

Klotz, I. M., and Darnall, D. W. (1969), *Science 166*, 126.

Leaback, D. H., and Rutter, A. C. (1968), *Biochem. Biophys. Res. Commun. 32*, 447.

Malmström, B. G. (1957), *Arch. Biochem. Biophys. 70*, 58.

Malmström, B. G., Kimmel, J. R., and Smith, E. L. (1959), *J. Biol. Chem. 34*, 1108.

Panyim, S., and Chalkley, R. (1969), *Arch. Biochem. Biophys. 130*, 337.

Reisfeld, R. A., Lewis, U. J., and Williams, D. E. (1962), *Nature 195*, 281.

Shapiro, A. L., and Maizel, Jr., J. V. (1969), *Anal. Biochem. 29*, 505.

Shapiro, A. L., Viñuela, E., and Maizel, Jr., J. V. (1967), *Biochem. Biophys. Res. Commun. 28*, 815.

Tanford, C. (1968), *Advan. Protein Chem. 23*, 121.

Warburg, O., and Christian, W. (1941), *Biochem. Z. 310*, 384.

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.

Westhead, E. W. (1966), *Methods Enzymol. 9*, 670.

Westhead, E. W., and McLain, G. (1964), *J. Biol. Chem. 239*, 2464.

Winstead, J. A., and Wold, F. (1965), *Biochemistry 4*, 2145.

Wrigley, C. (1968), *Sci. Tools 15*, 17.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.