Molecular Weight and Subunits of Serine Transhydroxymethylase†

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ABSTRACT: Rabbit liver serine transhydroxymethylase has a molecular weight of 215,000 ± 11,000 in aqueous solutions as determined by the methods of sedimentation equilibrium in the ultracentrifuge, membrane osmometry, and chromatography in Sephadex G-200. In 6 M quanidine hydrochloride in the presence of 2-mercaptoethanol the molecular weight of serine transhydroxymethylase is 47,700 ± 2500. From the electrophoretic mobility is sodium dodecyl sulfate–polyacrylamide gels, the molecular weight of the polypeptide chains of the enzyme is 53,000 ± 5300. It is concluded that native rabbit liver serine transhydroxymethylase consists of four subunits each with a molecular weight of 47,700 ± 5000.

Serine transhydroxymethylase (EC 2.1.2.1) is a pyridoxal phosphate containing enzyme catalyzing the interconversion of glycine and serine and, at least for the rabbit liver enzyme, it can also induce cleavage of the carbon–carbon bond of several α,β-hydroxyamino acids (Schirch and Diller, 1971). Tetrahydrofolate is a coenzyme in some of these reactions.

The molecular weight of rabbit liver serine transhydroxymethylase was reported as 330,000 by Schirch and Mason (1963). On this basis the authors also reported 6 moles of pyridoxal phosphate bound per mole of enzyme. On the other hand, more recently Fujioka (1969) reported a molecular weight of 175,000 and 4 moles of bound pyridoxal phosphate for rabbit liver serine transhydroxymethylase. However, most of the research with serine transhydroxymethylase uses the bound pyridoxal phosphate chromophore as a reporter of events at the active site in enzyme–substrate interactions (Schirch and Mason, 1963; Schirch and Jenkins, 1964a,b). Thus it becomes of importance to clarify the current discrepancy in the reported molecular weight, the number of subunits of the enzyme, and the relationship of the number of moles of pyridoxal phosphate bound per mole of enzyme subunit.

We have undertaken a study of the molecular weight and subunits by a variety of physical methods. This information is essential as a prerequisite to further studies on the function of serine transhydroxymethylase.

Experimental Section

Enzyme. Serine transhydroxymethylase was purified from rabbit livers by the method of Schirch and Mason (1963). Studies were performed with enzyme preparations with a 280:430 mμ absorbance ratio of 7.5. These preparations showed as a single protein band on starch gel electrophoresis.

Chemicals. Guanidine hydrochloride was Spectral grade quality from Mann Research Laboratories and was twice crystallized from ethanol. All protein standards were from Sigma Chemical Co.

Osmometry. Osmotic pressures were measured with a Hewlett-Packard high-speed membrane osmometer. The molecular weight of the enzyme was determined at 5 ± 0.2° using membranes with molecular weight retention values of 20,000 (Hewlett-Packard Co., type B-19). The number-average molecular weight of the enzyme was determined by

$$M_n = \frac{RT}{\left(\frac{\pi}{C}\right)_{c=0}}$$

The gas constant R is given as $84.7/\rho$ l. cm mole^{-1} deg^{-1}, where ρ is the density of the buffer used at a given temperature, T. π is the osmotic pressure values and c the protein concentration in grams per liter.

The second virial coefficient values, B, were calculated from the slope of the plots of π/c *vs.* C using

$$B = \frac{\Delta(\pi/c)}{\Delta C}\frac{1}{RT}$$

and a linear least-squares analysis of the data.

Sedimentation Equilibrium Studies. The short-column high-speed method of Yphantis (1964) was used in all sedimentation equilibrium studies.

The enzyme preparations were always dialyzed in the appropriate solvent for at least 24 hr prior to sedimentation analysis.

Sedimentation equilibrium studies were performed with a Spinco Model E analytical ultracentrifuge equipped with Rayleigh interference optics and a split-beam photoelectric scanning apparatus, at a temperature of 20°. Fluorocarbon FC-45 (0, 0.2 ml, Beckman) was used to provide a flat, transparent cell bottom using approximately 3-mm column heights.

Calculation of the molecular weights were performed as described by Yphantis (1964) using the format previously described by Castellino *et al.* (1970).

Sephadex G-200 Gel Filtration. The molecular weight of the enzyme was estimated by chromatography in a 2.5 × 73 cm column of Sephadex G-200 equilibrated with 0.1 M KCl in potassium phosphate buffer (0.05 M, pH 6.85) and EDTA (0.001 M). The molecular weight was estimated from

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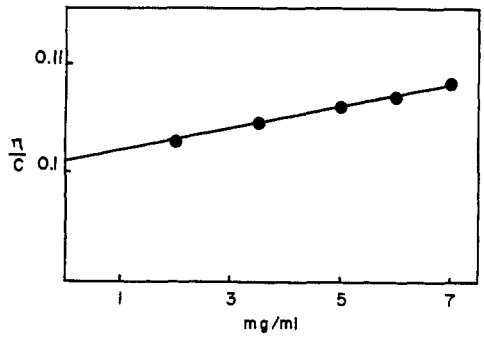

FIGURE 1: Plot of π/C *vs.* C for serine transhydroxymethylase in 0.01 M KCl–0.1 M potassium phosphate buffer (pH 6.8) at 5°.

a plot of logarithm of molecular weight against elution volume (Andrews, 1965).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Each protein (0.5 mg) was dissolved in 1 ml of 0.01 M sodium phosphate buffer (pH 7.0), 1% in 2-mercaptoethanol, and incubated at 37° for 2 hr. The solutions were then dialyzed overnight against two changes of 0.1% sodium dodecyl sulfate, 0.1% 2-mercaptoethanol, and 0.005% bromophenol blue all in the same phosphate buffer. Polyacrylamide gels (10%), containing 0.1% sodium dodecyl sulfate and 0.1% 2-mercaptoethanol, were used in the electrophoresis following the procedure of Weber and Osborn (1969). After electrophoresis the gels were stained with coomassie blue.

Results

Osmometry. The averaged data for three different experiments are plotted in Figure 1. Values of $(\pi/C)_{c=0}$ were obtained by linear least-squares analysis of the data plotted. From these data a number molecular weight of 211,500 ± 5200 was calculated for serine transhydroxymethylase.

Sedimentation Equilibrium. NATIVE MOLECULAR WEIGHT. Plots of ln C *vs.* r^2 for serine transhydroxymethylase in buffer are shown in Figure 2 for those measurements where the concentration gradient was followed from the Rayleigh fringe separations. Each point is the difference between the average of readings made on five fringes and the average of readings made on five fringes of the water blank. The material was homogeneous with respect to molecular weight as evidenced from the linearity of the plots in Figure 2. The molecular weight of the native enzyme was thus determined at 215,000 ± 11,000.

Since this enzyme possesses a prosthetic group, pyridoxal phosphate, with absorbance at 430 mμ, other measurements were made at this wavelength using the photoelectric scanning apparatus. These data also resulted in linear plots of ln C *vs.* r^2. In this case the absorbance gradient measured in the scanner chart served as an index of concentration. The molecular weight by this method was 240,000 ± 24,000 for native serine transhydroxymethylase.

SUBUNIT MOLECULAR WEIGHT. Sedimentation equilibrium centrifugation in the presence of a subunit dissociating agent such as 6 M Gdn·HCl[1] resulted in data which produced a linear relationship of plots of ln C *vs.* r^2. The value of the molecular weight of the subunits of serine transhydroxymethylase in 6 M Gdn·HCl in the presence of 2-mercaptoethanol is 47,700 ± 2500 using the partial specific volume ($\bar{v}$) value of the native enzyme calculated from the amino acid composition (0.734 ml/g).

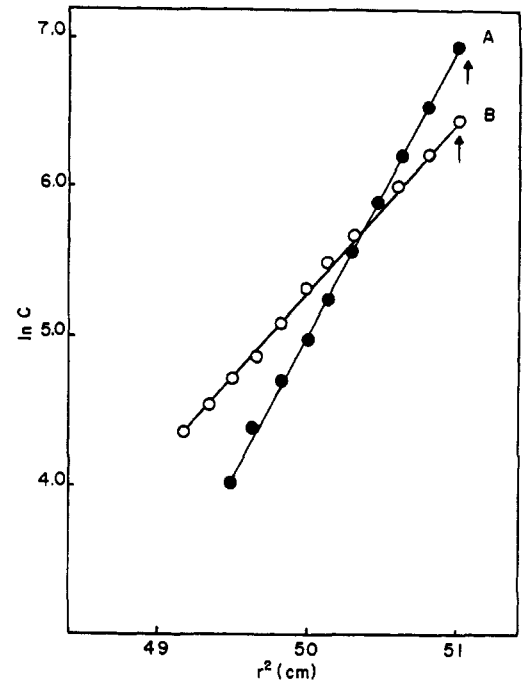

FIGURE 2: Plots of ln C *vs.* r^2 of serine transhydroxymethylase in 0.05 M potassium phosphate buffer (pH 7.3)–0.1 M KCl at 25°. (A) 13,000 rpm and (B) 11,000 rpm. The arrows indicate the bottom of the cell.

Sephadex G-200 Gel Filtration. Serine transhydroxymethylase was mixed with several proteins and chromatographed on a Sephadex column. The proteins were monitored by absorption at 280 mμ. The molecular weight, using this technique, was estimated to be 220,000 ± 22,000.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. A plot of the electrophoretic mobility of several different polypeptide chains used as molecular weight markers against the logarithm of their molecular weights resulted in a straight line relationship which was used to calculate the molecular weight of the unknown serine transhydroxymethylase. This enzyme always migrated the same distance in the electrophoretic field as the subunit of glutamic dehydrogenase and gave a single band. Therefore, with this method we assign 53,000 ± 5300 as the molecular weight of the subunit of serine transhydroxymethylase.

Discussion

The present series of investigations indicates that the molecular weight of rabbit liver serine transhydroxymethylase is 215,000 ± 10,000 and that it is composed of four polypeptide chains.

Analysis of studies performed under dissociating and denaturing conditions, sedimentation equilibrium in Gdn·HCl and sodium dodecyl sulfate polyacrylamide gels, resulted in an estimated molecular weight of the subunit of 50,000 ± 5000. Uncertainty of the apparent partial specific volume value in the sedimentation equilibrium experiments is the major difficulty in obtaining accurate molecular weight in systems containing high concentrations of salts (Cassasa

[1] Abbreviations used are: Gdn·HCl, guanidine hydrochloride; ME, 2-mercaptoethanol.

and Eisenberg, 1961). Reithel and Sakura (1963) found little change in $\bar{v}$ for several proteins in 6 M Gdn·HCl. On the other hand, even assuming as large a decrease in the apparent $\bar{v}$ of 0.01 ml/g in 6 M Gdn·HCl as proposed by Hade and Tanford (1967), the molecular weight of the subunit would be 44,525 ± 2000, a value which does not compromise the number of subunits obtained. The results in sodium dodecyl sulfate–polyacrylamide, of course, contain a larger percentage of error but, taken together with the ultracentrifuge data, indicate that all four polypeptide chains are of identical molecular weight.

A comparison of the results from the various methods used to determine the molecular weight of the native holoenzyme and of the dissociated subunit is shown in Table I.

TABLE I: The Molecular Weights of Native and Dissociated Serine Transhydroxymethylase.

Method	Dissociating Medium	Mol Wt
Osmometry	None[a]	211,500 ± 5200
Sedimentation equilibrium (Rayleigh optics)	None[a]	215,000 ± 11,000
	Gdn·HCl–ME[b]	44,525 ± 2000
	Gdn·HCl–ME[c]	47,700 ± 2500
Sedimentation equilibrium (absorbance at 430 mμ)	None[a]	240,000 ± 24,000
Sephadex G-200 chromatography	None[a]	230,000 ± 23,000
Sodium dodecyl sulfate–polyacrylamide electrophoresis	SDS[d]–ME	53,000 ± 5300

[a] Buffer only. [b] For $\bar{v}$ = 0.724 ml/g. [c] For $\bar{v}$ = 0.734 ml/g. [d] SDS = sodium dodecyl sulfate.

The native molecular weight obtained by osmometry is in agreement with that obtained by ultracentrifugation. Membrane osmometry has been shown to be a good reproducible method (Castellino and Barker, 1968) and provides a number-average weight which is in close agreement with the weight-average molecular weight determined in the ultracentrifuge. The values of the second virial coefficient, B, calculated from the osmometry data are a function of the actual volume occupied by the protein. The calculated value (4.8×10^{-5} cm^3 mole g^{-2}) is in the range of the value expected of a native protein of this size (Tanford, 1966). This physical parameter, thus, agrees with the observation that under the experimental conditions used in the osmometry measurements the enzyme retained full catalytic activity. This would indicate that the physical measurements were made on non-denatured protein.

Membrane osmometry can also be carried out in protein denaturing solvents like Gdn·HCl (Castellino and Barker, 1968; Feliss and Martinez-Carrion, 1970). However, since this method requires large amounts of protein, measurements were not attempted in the presence of Gdn·HCl.

The fact that the sedimentation equilibrium data values for the native enzyme agree whether using Rayleigh optics or the absorbance values from the scanner at 430 mμ is significant since the enzyme contains pyridoxal phosphate as a chromophore. It is also known that not all pyridoxal phosphate binding sites are occupied as the enzyme catalytic activity can be stimulated by addition of pyridoxal phosphate (Schirch and Mason, 1963). Therefore, the sedimentation equilibrium results utilizing the two optical methods indicate that the partially saturated molecules possess the same molecular weight as the bulk of the protein. In other words, molecules with different amounts of bound pyridoxal phosphate do not dissociate or aggregate.

Since there are 4 moles of pyridoxal phosphate bound per mole of enzyme (L. Schirch, personal communication), it appears that there is one molecule of pyridoxal phosphate bound per polypeptide chain in serine transhydroxymethylase and that the molecular weight of the native enzyme is 215,000 ± 11,000.

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