DISSOCIATION OF CYSTATHIONASE

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SUMMARY

The molecular weight of cystathionase from rat liver was determined by light scattering. In the absence of denaturing agents the enzyme has approximately a molecular weight of 160,000 daltons. In the presence of SDS (0.1%) the enzyme dissociates into subunits of approximately 20,000 daltons; the dissociation was demonstrated by two independent methods, SDS-polyacrylamide gel electrophoresis and equilibrium sedimentation. It is proposed that the enzyme cystathionase is composed of 8 subunits.

INTRODUCTION

The enzyme cystathionase from rat liver has been purified by Matsuo and Greenberg (1). These authors also performed sedimentation and diffusion measurements designed to determine the molecular weight of the crystalline enzyme. A molecular weight of 170,000, determined from sedimentation and diffusion values extrapolated to zero protein concentration, has been reported (2). The purpose of the present communication is to demonstrate that the enzyme cystathionase from rat liver dissociates into subunits of 20,000 molecular weight in the presence of the denaturing agent,

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sodium dodecyl sulfate (SDS). It is proposed that the enzyme is composed of eight subunits of similar molecular weight.

EXPERIMENTAL PROCEDURE

Cystathionase was prepared by the procedure of Matsuo and Greenberg (2). This preparation exhibited an absorption band at a wavelength of (420 nm) which is characteristic of Pyridoxal-5-Phosphate bound to the protein (3). The Pyridoxal-5-P content of the enzyme, as determined by the procedure of Wada and Snell (4), varied between 3.2 and 3.8 moles of Pyridoxal-5-P per mole of protein (molecular weight = 160,000).

The number of reactive sulphydryl groups was determined spectrophotometrically with DTNB, as described by Ellman (5). The reaction of three sulphydryl groups per molecule of protein leads to a complete loss of cystathionase activity (6).

Light scattering measurements were performed in a Brice-Phoenix light scattering photometer (Series 2000) (7). Refractive index measurements were performed at 25° C with a Brice-Phoenix differential refractometer calibrated with solutions of KCl of known concentrations. Ultracentrifugation studies were conducted in the Spinco Analytical ultracentrifuge (Model E) equipped with a rotor temperature indicator control unit set at 20°. Sedimentation coefficients were determined from the position of the Schlieren peak recorded on a Kodak Spectrophotometric Plate 11G. The molecular weight of the SDS protein complex was determined with the high speed equilibrium sedimentation method of Yphantis (8). Changes in concentration were determined with the Rayleigh interference system. Molecular weights were calculated with the aid of Equation [1]:

$$M = 2RT\ (d\ln C/dx^2)/(1-e\bar{v})\ w^2 \qquad [1]$$

RESULTS AND DISCUSSION

The technique of light scattering was used to determine the molecular weight of cystathionase at pH 7.4. Prior to the light scattering measurements, the samples of enzyme were clarified by repeated direct filtration through type G-S millipore filters into the square cells. Scattering ratios were determined at 25° C with incident light (546 nm) from a mercury lamp.

The light scattering results were analyzed with the following equation:

$$\frac{H \cdot c}{\tau} = \frac{1}{\bar{M}w} + 2\,B \cdot C \cdot \qquad [2]$$

For Equation [2], τ is the turbidity, c is the concentration in g/ml., $\bar{M}w$ is the weight average molecular weight, H is a factor derived from the Rayleight scattering law (7), and B is the second viral coefficient.

The results obtained with several samples of cystathionase are shown in Fig. 1. It should be noted that with a decrease in the concentration of protein, the plot of $\frac{H \cdot c}{\tau}$ vs. c approaches the ordinate to a value that corresponds to a molecular weight of 160,000. At protein concentrations larger than 1.0 mg/ml., however, the light scattering measurements yield values for the

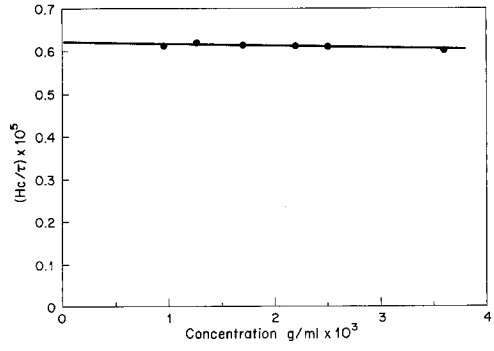

Fig. 1. Plot of $H \cdot c/\tau$ vs. concentration was derived from the light scattering measurements of cystathionase. Results were obtained in Phosphate buffer, I = 0.1 at pH 7.4 and 25°C.

molecular weight that fluctuate between 160,000-170,000, as illustrated in Fig. 1. Since the measurements were performed at protein concentrations higher than 1 mg/ml., the size of cystathionase was investigated at concentrations that approach those used in enzymatic assays. Gel filtration experiments were carried out with a Sephadex G-200 column (70 cm x 1 cm) equilibrated with 0.1 M phosphate buffer (pH 7.4) (9). The elution profile of cystathionase was nearly identical to that displayed by the markers yeast alcohol dehydrogenase and Bovine γ-globulin. Thus, it appears that cystathionase is not dissociated at concentrations lower than 1 mg/ml. Similar results were obtained using phosphate buffers of pH 6.5 and pH 7.8.

On the other hand, a dissociation of the enzyme into subunits can be achieved by the addition of SDS. The dissociation was demonstrated by two different methods, SDS-polyacrylamide gel electrophoresis and equilibrium ultracentrifugation.

The molecular weight of the subunit was determined by SDS-polyacrylamide gel electrophoresis as described by Weber and Osborn (10). A sample of approximately 1-0 mg each of cystathionase and marker proteins was dissolved in 1.0 ml. of 0.01 M Barbital buffer (pH 7.4) containing SDS (0.1%). Each solution was incubated for 2 hours at 25°C. After dialysis of the solution against 0.01 M Barbital buffer (pH 7.4) containing SDS (0.1%), a sample of 50 µl each of the protein solution was applied on gel containing 5% acrylamide. Electrophoresis was performed at a constant current of 4mA/tube. After staining the gel with Amido Scwarz 10B, the molecular weight of the subunit was estimated from a calibration curve prepared from a simultaneous electrophoretic run of several protein markers.

Under this set of experimental conditions, only one band was detected after the denaturation of cystathionase with SDS (0.1%).

The relative migration of the subunit on SDS polyacrylamide gel disc electrophoresis was then compared with that of marker proteins. As shown in Fig. 2, the molecular weight of the subunit was estimated to be 2.2×10^4.

Further support for the contention that the enzyme cystathionase dissociates into subunits of approximately 20,000 molecular weight was derived from ultracentrifugation experiments. The sedimentation patterns obtained in the presence of SDS (0.1%), gave evidence for two sedimenting species; a sharp, slow moving peak ($S_{20,w} = 2.29$) and a fast moving component ($S_{20,w} = 8.79$) were observed. The rapidly sedimenting component represents less than 10% of the total protein content.

The apparent molecular weight of the SDS-enzyme complex was calculated from the slope of a plot of ln F (Fringe displacement in μ) vs. x^2 (square of the distance from the center of the

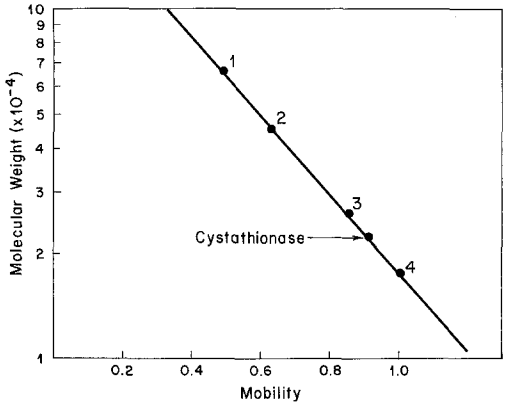

Fig. 2. Determination of the molecular weight of the subunit of cystathionase by SDS-polyacrylamide gel electrophoresis. The four marker proteins used were: 1) bovine serum albumin, 2) ovoalbumin, 3) chymotrypsinogen and 4) myoglobin.

rotor). The protein concentration was less than 1 mg/ml. As shown in Fig. 3, the plot of ln F vs. x^2 was virtually linear. A molecular weight of 18,200±1,400 was obtained for cystathionase in the presence of SDS (0.1%).

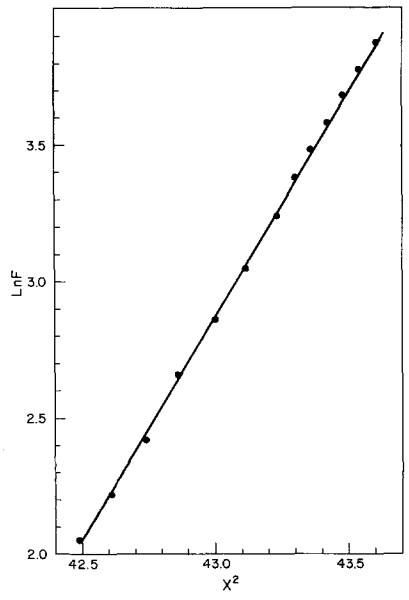

Fig. 3. Plot of ln F (Fringe displacement) vs. x^2 for cystathionase at a concentration of 0.05% in 0.1 M Phosphate buffer (pH 7.4), containing 0.1% SDS. The run was performed for 12 hours at 39,460 RPM (temperature 20°C).

These preliminary ultracentrifugation results agree with those obtained with the technique of SDS-polyacrylamide gel electrophoresis; cystathionase is an oligomer composed of 8 subunits with a molecular weight of approximately 20,000 daltons. It should be noted that there are more subunits than either Pyridoxal-5-P residues (3-4) or reactive sulphdryl groups (3) per mole of enzyme (160,000 molecular weight). With the available information, the subunits may be identical or it may be that two types of subunits (catalytic and regulatory) are present in the enzyme. This problem is currently being investigated in our laboratory.

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