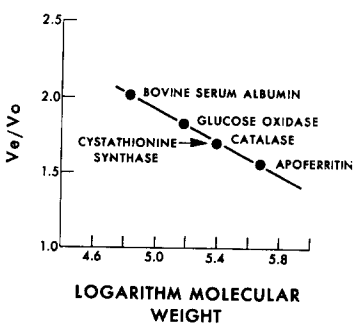
SHORT COMMUNICATIONS

BBA 63470

Studies of cystathionine synthase of rat liver: Dissociation into two components by sodium dodecyl sulfate disc electrophoresis

Cystathionine synthase catalyzes the condensation of homocysteine and serine to cystathionine which is an important intermediate in the transsulfuration pathway from methionine to cysteine in mammals.

Cystathionine synthase was first purified from rat liver and reported to be the same enzyme as serine dehydratase[1]. Later reports provided evidence that cystathionine synthase was a different enzyme from serine dehydratase[2–4]. The procedure for the purification of cystathionine synthase is the same as that described in another publication[5].



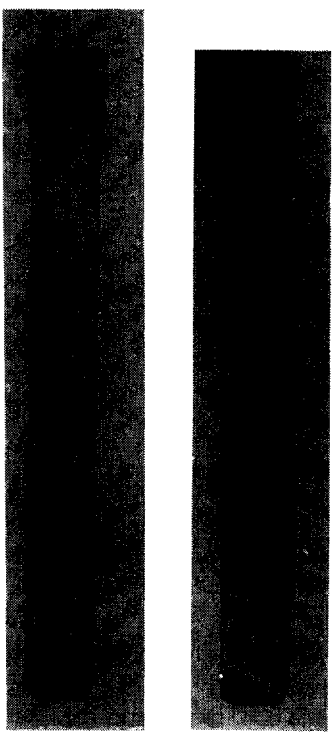


Fig. 1. Sepharose-6B column (1.1 cm × 44 cm) was equilibrated with 0.1 M potassium phosphate buffer, pH 7.5. Eluate was collected in 1.8-ml fractions at a flow rate of 9.0 ml/h.

Fig. 2. Purified cystathionine synthase (1 mg/ml) was incubated in 1% sodium dodecyl sulfate —0.1 M sodium phosphate buffer, pH 7.1, at 37° for 3 h, with or without 1% 2-mercaptoethanol. The sample was then dialyzed for 16 h against 0.1% sodium dodecyl sulfate–0.01 M sodium phosphate buffer, pH 7.1, with or without 0.1% 2-mercaptoethanol. Electrophoresis was carried out at 5 mA per tube for 2 h in 65 mm 5% polyacrylamide gel containing 0.1 M sodium phosphate buffer, pH 7.1, with 0.1% sodium dodecyl sulfate. Gel was fixed with 20% sulfosalicylic acid and then stained with 0.25% Amido black in 7% acetic acid. Top is the cathode and bottom the anode. (A) Enzyme (22 μg) treated with 2-mercaptoethanol. (B) Without 2-mercaptoethanol.

The high purity of the enzyme preparation is indicated by the elution profile of the purified enzyme (specific activity, 487) rechromatographed on a small Sepharose-6B column shown in Fig. 4 of ref. 5 and the single migrating boundary obtained on acrylamide gel disc electrophoresis at pH 8.5 in Fig. 5 of ref. 5.

The molecular weight of the purified cystathionine synthase was determined using a Sepharose-6B column (1.1 cm × 44 cm). As shown in Fig. 1, the ratio of elution volume to void volume determined by Blue Dextran 2000 was plotted against the logarithm of the molecular weights of certain known proteins, *i.e.* bovine serum albumin (68 000), glucose oxidase (150 000), catalase (250 000) and apoferritin (480 000). The molecular weight of cystathionine synthase was estimated to be approx. 250 000. This value is in good agreement with that reported by NAKAGAWA AND KIMURA[6].

The purified cystathionine synthase was treated with sodium dodecyl sulfate with or without added 2-mercaptoethanol, and acrylamide gel disc electrophoresis was run. As shown in Fig. 2, two distinct bands are recognized. The molecular weights of the fast- and slow-moving components were determined according to the method of SHAPIRO *et al.*[7]. The reliability of this method has been confirmed by WEBER AND OSBORN[8]. Estimation of the relative molecular weight was performed by referring to known proteins, namely, cytochrome c (12 000), ovalbumin (43 000) and bovine serum albumin (68 000). A linear relationship was obtained between the logarithm of the molecular weight and relative migration (Fig. 3). The molecular weights of the

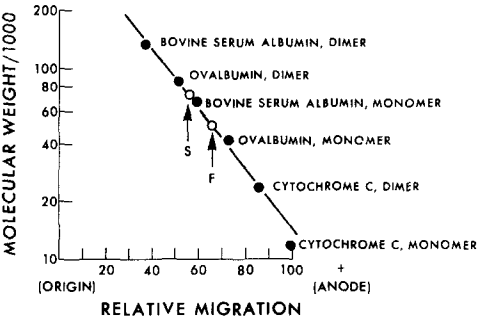


Fig. 3. Cystathionine synthase was treated as mentioned in Fig. 2. Reference proteins were run at the same time.

fast- and slow-moving components were estimated to be approx. 51 000 and 73 000, respectively. Since the molecular weight of the native enzyme was found to be about 250 000, it appears reasonable to calculate that cystathionine synthase consists of four subunits, *i.e.* two identical fast-moving components (2 × 51 000 = 102 000) and two slow-moving components (2 × 73 000 = 146 000). 2-Mercaptoethanol had no effect on the dissociation of the cystathionine synthase into two different components, since treatment of the native enzyme with sodium dodecyl sulfate *plus* 2-mercaptoethanol gave the same result as that with sodium dodecyl sulfate alone (Fig. 2).

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Stereochemistry of glyoxylate oxidation by NAD and mammalian lactate dehydrogenase

The NAD-dependent oxidation and reduction of glyoxylate to oxalate and glycolate, respectively, are catalyzed by L-lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27). It has been proposed that both reactions occur in the same active site(s) and involve common functional catalytic groups of the enzyme[1]. If the same active sites, including binding and catalytic groups, are utilized for both reactions, the transfer of hydride ion to and from the NAD coenzyme should take place on the same side of the pyridine ring in glyoxylate oxidation and in glyoxylate reduction. In reduction reactions involving L-lactate dehydrogenase and NADH, the hydride ion is transferred from the A side[2] of the dihydropyridine ring[3,4]. In the present investigation it is shown that glyoxylate oxidation by L-lactate dehydrogenase involves transfer of a hydride ion from glyoxylate to the A side of the 4 position of the pyridine ring of NAD^+.

Glyoxylate*, containing ^3H on the aldehyde carbon, was prepared in ^3H_2O (5 mC) from oxalic acid and magnesium[5], and the glyoxylate was separated from oxalate by ion-exchange chromatography[1]. The specific activity of the [^{3}H]glyoxylate was 7000 disint./min per μmole. [^{3}H]NADH was obtained by oxidation of 40 μmoles of [^{3}H]glyoxylate with 40 μmoles of NAD^+ by 2.5 mg of pig heart lactate dehydrogenase at pH 11 (ref. 1). Oxalate was precipitated with $BaCl_2$, and barium [^{3}H]NADH was then precipitated with alcohol[6]. The specific activity of the [^{3}H]NADH was 5700 disint./min per μmole.

Reaction mixtures containing glycolate and NAD^+, formed from glyoxylate and [^{3}H]NADH, were streaked onto Whatman No. 3 MM chromatography paper. The paper was developed by ascending chromatography in ethanol–conc. NH_4OH–water (80:5:15, by vol.). The R_F for glycolate, determined by migration of [^{14}C]glycolate along one edge of the paper, was 0.44, and that for NAD^+, determined by ultraviolet visualization, was 0.04. The appropriate portions of the paper were cut out and eluted

* Glyoxylate is O^1HCCOO^- and tritiated glyoxylate is O^3HCCOO^-.