CRYSTALLIZATION AND CHARACTERISTICS OF SERINE DEHYDRATASE FROM RAT LIVER

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The identification of serine dehydratase (E.C.4.2.1.13) and cystathionine synthase (E.C.4.2.1.21) in higher animals is now under active investigation in a number of laboratories, since Greenberg et al. (1959, 1965) suggested that both enzyme reactions are catalyzed on a single protein.

Rose et al. (1939) showed that L-cystine had a sparing effect of L-methionine for growth of rats. In 1964, Kato et al. interpreted this in terms of repression of cystathionine synthase, which is known to be a key enzyme for cystine formation from methionine in higher animals, by the endproduct cystine. Later, it was demonstrated (Suda, 1967) that cystathionine synthase was repressed by dietary cystine, while serine dehydratase was not. It was also shown that serine dehydratase was induced by dietary serine or in a state of increased gluconeogenesis (Freeland et al., 1964, Ishikawa et al., 1965), while cystathionine synthase was not affected by these conditions. These findings suggest either that cystathionine synthase is entirely different from serine dehydratase or that if a single enzyme protein catalyzes both enzyme reactions, their regulation mechanisms differ.

To obtain further insight into this problem, it is necessary to prepare highly purified serine dehydratase. In the present work, we succeeded in crystallizing the enzyme from rat liver.

This preliminary communication reports the crystallization procedure and some characteristics of the enzyme.

RESULTS AND DISCUSSION

150 male Wistar rats, weighing 150 to 250 g, were used in one experiment. They were maintained on a high protein diet (91% casein) for 5 days before sacrifice to induce serine dehydratase. The purification procedure to step 4 was carried out according to the method of Kato et al. (1965), unless otherwise mentioned.

Step 1, crude extract. Rat livers were homogenized with 3 volumes of buffer A (0.05 M potassium phosphate buffer, pH 7.2, 0.15 M KCl, $1x10^{-3}$M EDTA). The homogenate was centrifuged for 20 minutes at 10,000 x g.

Step 2, ammonium sulfate fractionation. The fraction precipitated between 30 and 45% saturation of ammonium sulfate was dissolved in buffer A containing $1x10^{-3}$M β-mercaptoethanol and dialyzed for 4 hours against 4 changes of 20 volumes of the same buffer. Pyridoxal phosphate and dithiothreitol were added to the dialyzed enzyme preparation at final concentrations of $5x10^{-4}$M and $1x10^{-3}$M, respectively, and the solution was stored at -20°. Dithiothreitol was found to stabilize the enzyme activity effectively.

Step 3, 1st acetone fractionation. The fraction precipitated between 44 and 67% acetone was dissolved in buffer A containing $5x10^{-5}$M pyridoxal phosphate and $5x10^{-4}$M dithiothreitol.

Step 4, 2nd acetone fractionation. The 2nd acetone fractionation was performed as the first one. The fraction precipitated between 44 and 67% acetone was collected and dissolved in a minimal volume of buffer B (0.01 M potassium phosphate buffer, pH 7.8, $1x10^{-3}$M EDTA and $1x10^{-3}$M dithiothreitol) and the insoluble material was removed by brief centrifugation. The yellowish supernatant was applied on a column of Sephadex G-25 (6.2 x 60cm) which had been equilibrated with 0.01 M potassium phosphate buffer containing $1x10^{-3}$M EDTA to remove acetone. The eluate was adjusted to $1x10^{-3}$M dithiothreitol and stored at -20°.

Step 5, DEAE-cellulose column chromatography. The above fraction was applied to a DEAE-cellulose column, 3.5 x 40cm, which had been equilibrated with 0.01 M potassium phosphate buffer, pH 7.8, containing $1x10^{-3}$M EDTA. The column was

washed with 500 ml of the same buffer. The enzyme was eluted by linear gradient elution. The mixing chamber contained 1.5 l of 0.01 M potassium phosphate buffer, pH 7.8 - $1x10^{-3}$M EDTA and the reservoir contained an equal volume of 0.25 M potassium phosphate buffer, pH 7.8 - $1x10^{-3}$M EDTA. Fractions of 6 ml were collected in a fraction collector. The active fractions (tubes 20 to 36) were combined and adjusted to $1x10^{-3}$M dithiothreitol.

Step 6, 2nd ammonium sulfate fractionation. The pooled active fractions were concentrated with ammonium sulfate (50% saturation) and the precipitate was dissolved in a small volume of 0.025 M potassium phosphate buffer, pH 7.2, containing $1x10^{-3}$M EDTA and $1x10^{-3}$M dithiothreitol. The insoluble material was centrifuged down and the precipitate was extracted twice with the same buffer.

Step 7, crystallization. A faint birefringence began to appear when the above supernatant combined with the washings was allowed to stand for approximately 5 hours at 4°. This birefringence increased with time. Microscopic examination showed that the birefringence was due to crystal formation. Typical needle shaped crystals are shown in Fig. 1. The enzyme preparation at this step could be stored at 4° for at least 2 months without appreciable loss of activity.

Table 1 gives a summary of the purification of serine dehydratase. As seen in the table, approximately 1,250 fold purification was achieved at the crystallization step. It was found that there was only one band on disc electrophoresis of the crystalline material, while there were three distinct bands on electrophoresis of the mother liquor. Recrystallization of the enzyme with ammonium sulfate, however, gave no further increase in specific activity.

Crystalline enzyme was shown to be homogenous by ultracentrifugation. The molecular weight was calculated to be 63,500 by the sedimentation equilibrium, assuming that the specific volume of the enzyme protein was 0.72. The sedimentation constant was determined to be 4.19 at protein concentrations of 5.58, 7.42 and 10.00 mg/ml on the basis of extrapolation to zero concentration. These values are different from those of Nagabhushanam and Greenberg (1965) (M.W.=21,000, $S_{20,w}$=2.50). The main difference between the two purification procedures is in

Table 1

Summary of Purification of Serine Dehydratase from Rat Liver

Fraction	Volume (ml)	Total units	Total protein (mg)	Specific activity	Yield (%)	SDH* / TDH
Crude extract	3,360	42,600	344,000	0.124	100	2.25
1st $(NH_4)_2SO_4$ Fr.	1,230	30,600	67,500	0.453	71.8	2.34
1st acetone Fr.	600	27,500	8,400	3.26	64.7	2.19
2nd acetone Fr.	400	16,000	2,240	7.01	37.6	1.67
DEAE cellulose column	370	8,200	278	29.5	19.3	1.45
2nd $(NH_4)_2SO_4$ Fr.	13.2	10,300	172	60.7	24.2	1.40
Crystallization	2.4	8,200	52.8	155.0	19.3	1.42

* SDH, serine dehydratase; TDH, threonine dehydratase.

Serine dehydratase was assayed in a system containing (μmole): serine (100), pyridoxal phosphate (0.05), EDTA (1), potassium phosphate buffer, pH 8.0 (100) and enzyme in a final volume of 1 ml. Incubations were carried out for 5 minutes at 37°. The reaction was stopped by addition of 0.5 ml of 10% TCA. The amount of pyruvate formed was determined with deproteinized supernatant by a modification of the method of Sayer and Greenberg (1960).

The assay method for threonine dehydratase was essentially the same as that for serine dehydratase except that serine was replaced by threonine.

One unit of enzyme was defined as the amount which catalyzes the formation of 1 μmole of pyruvate or α-ketobutyrate per minute under the assay conditions described above. Specific activity was defined as units per mg of protein.

Protein concentrations were determined according to the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

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the acetone fractionation steps. The problem of whether the differences observed are due to a conformational change of the enzyme protein during purification awaits further investigations.

The Km value for serine was estimated to be 7×10^{-2}M and that for pyridoxal phosphate was 3×10^{-7}M after the coenzyme had been removed by dialysis against cysteine. The content of pyridoxal phosphate was determined by the method of Wada et al. (1961) as 2 moles per mole of the enzyme. No effect of nucleotides

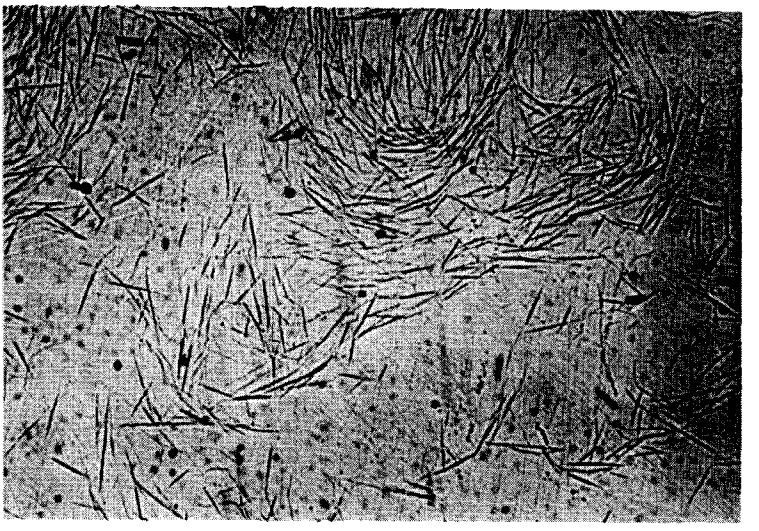

Fig. 1. Crystalline serine dehydratase from rat liver (x300).

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has so far been observed.

Goldstein et al. (1962) reported that the ratio of serine dehydratase to threonine dehydratase was constant (1.5) during the purification, suggesting that both reactions are catalyzed on the same enzyme protein. As shown in Table 1, it was confirmed that the ratio was almost constant during the purification. The antibody against serine dehydratase not only neutralized serine dehydratase activity but also threonine dehydratase activity.

Mudd et al. (1965) reported threonine dehydratase activity in a man lacking cystathionine synthase activity. Brown et al. (1966) also described the separation of serine dehydratase and cystathionine synthase in rat liver using a hydroxylapatite column. In connection with the above findings, it is worth noticing that neutralizing antibody against crystalline serine dehydratase did not inhibit cystathionine synthase activity in crude extract.

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REFERENCES

Brown, F. C., Mallady, J. and Roszell, J. A., J. Biol. Chem., 241, 5220 (1966).
Freeland, R. A. and Avery, E. H., J. Biol. Chem., 239, 3357 (1964).
Goldstein, L., Knox, W. E. and Behrman, E. J., J. Biol. Chem., 237, 2855 (1962).
Ishikawa, E., Ninagawa, T. and Suda, M., J. Biochem. (Tokyo), 57, 506 (1965).
Kato, A., Matsuzawa, T., Suda, M., Nakagawa, H. and Ishizuka, J., J. Biochem. (Tokyo), 55, 401 (1964).
Kato, A. Ogura, M. and Suda, M., J. Biochem. (Tokyo), 59, 40 (1966).
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Rand, R. J., J. Biol. Chem., 193, 265 (1953).
Mudd, S. H., Finkelstein, J. D., Irreverre, F. and Laster, L., Biochem. Biophys. Res. Commun., 19, 665 (1965).
Nagabhushanam, A. and Greenberg, D. M., J. Biol. Chem., 240, 3002 (1965).
Sayer, F. W. and Greenberg, D. M., J. Biol. Chem., 220, 787 (1956).
Selim, A. S. M., and Greenberg, D. M., J. Biol. Chem., 234, 1474 (1959).
Suda, M., Advances in Enzyme Regulation, 5, (1967), in press.
Wada, H. and Snell, E. E., J. Biol. Chem., 236, 2089 (1961).