IDENTIFICATION OF ONE OF THE L-SERINE DEHYDRATASE ISOENZYMES FROM RAT LIVER AS L-HOMOSERINE DEHYDRATASE

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Summary: The higher molecular weight L-serine dehydratase was purified from rat liver and fractionated into two active components by Sephadex G-200 gel chromatography. On the basis of molecular weight, ultracentrifugal pattern and substrate specificity of the major component, it was concluded that this component is identical to L-homoserine dehydratase. Some misinterpretations which have been derived from the work on L-serine dehydratase in animal tissues are pointed out.

We have previously reported on the presence of two forms of L-serine dehydratase in rat liver. These are different from each other in molecular weight and heat stability (1). The lower molecular weight enzyme (SDH-A) has been isolated and highly purified after inducing it by starvation of the animals under the influence of cortisone (2) or by a high casein hydrolysate administration (2 - 5). The level of the higher molecular weight enzyme (SDH-B) remains hereby almost unchanged. Recently Pitot et al. (6) isolated the two types of this enzyme from rat liver.

In the present work the higher molecular weight enzyme was purified to an extent completely free from SDH-A, and furthermore, it was fractionated into two components. We could show that one component of SDH-B is identical to L-homoserine dehydratase which is known to show a relatively broad substrate specificity for a variety of related compounds including L-serine and L-threonine (7. 8. 9).

MATERIALS AND METHODS

For the purification and subfractionation of SDH-B, 30 female rats (Sprague-Dawley; Ivanovas, Kisslegg/Allgäu; weighing 110 - 130 g) were fed a protein-free diet (protein content less than 1 %) for 5 days in order to minimize the activity of SDH-A (1). The extraction of the enzyme from rat liver and the assay of L-serine dehydratase were the same as previously described (1, 2). The activity of yeast alcohol dehydrogenase (Boehringer & Söhne, M. W. 150,000), used as internal marker for the estimation of relative molecular weight by the sucrose gradient centrifugation (10), was determined by the method of Racker (11). Gel filtration on a Sephadex G-100 or G-200 column was carried out using a buffer consisting of 0.033 M potassium phosphate (pH 7.9), 10^{-4}M EDTA and 10^{-5}M pyridoxal 5'-phosphate (buffer I).

RESULTS

The crude extract was heated at 55° for 15 min under constant stirring and cooled in an ice-water bath. No significant loss in SDH-B activity was observed during the heat treatment. After centrifugation at 27,000 g for 30 min, the supernatant was subjected to fractionation with solid $(NH_4)_2SO_4$. The fraction precipitated between 35 - 60 % saturation was collected. As measured by the gel filtration profile on a Sephadex G-100 column, the small amount of SDH-A activity which had been in the crude extract was completely removed by the above treatments. The supernatant of the 35 - 60 % fraction was brought to 80 % saturation with $(NH_4)_2SO_4$ and the precipitate was collected too. Gel filtration on a Sephadex G-200 column indicated the presence of two active components in the 35 - 60 % $(NH_4)_2SO_4$ fraction (B-1 and B-2) and one component in the 60 - 80 % $(NH_4)_2SO_4$ fraction (Fig. 1). Both

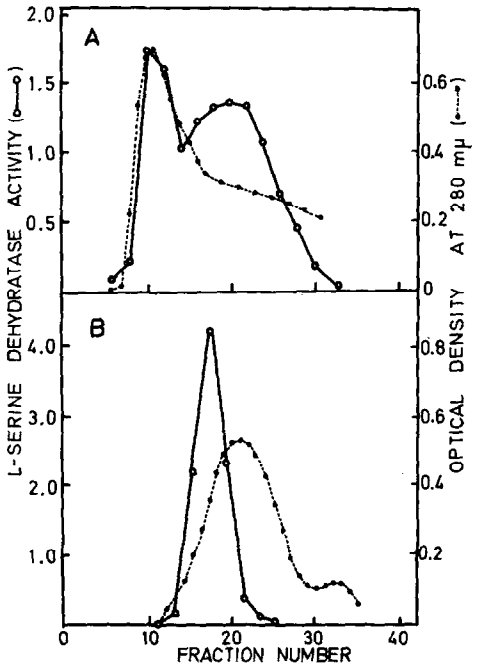

Fig. 1. Gel filtration of the enzyme fractions precipitated with 35 - 60 % (A) and 60 - 80 % saturation (B) of $(NH_4)_2SO_4$. 7.6 units of 35 - 60 % $(NH_4)_2SO_4$ fraction were placed on a Sephadex G-200 column (1.8 x 50 cm) which had been equilibrated with buffer I. By elution with the same buffer fractions of 5 ml were collected. Aliquots from each fraction were subjected to the enzyme assay (o—o) and UV absorption at 280 m/u (•--•).

fractions were further purified by gel filtration on a Sephadex G-200 column (2.5 x 95 cm). The slowly migrating component out of the 35 - 60 % fraction (B-2) and the material of the 60 - 80 % fraction - already identified as B-2 - were combined and precipitated with $(NH_4)_2SO_4$ to 80 % saturation. About a 130-fold purification was achieved by these processes with a yield of 32 %. The molecular weight of this major enzyme (B-2) was estimated by sucrose gradient centrifugation and found to be approximately 182,000 (mean value of duplicate runs). The three components having L-serine dehydratase activity (SDH-A, B-1 and B-2) could be separated from each other also by DEAE-cellulose chromatography. It was confirmed by thin-layer chromatography that

pyruvate is formed from L-serine by the action of all three fractions. As presented in Fig. 2, the distribution of L-homoserine dehydrating activity of B-2 among the sucrose gradient coincided completely with that of L-serine dehydratase activity.

T A B L E I

Substrate specificity of SDH-A and B-2

Substrate	concentration (mM)	/u moles keto-acid formed/h[1] SDH-A[2]	B-2
L-Serine	100	63.0	2.3
L-Threonine	100	70.3	0.3
L-Homoserine	100	0.3	266
L-Cysteine	2	0.2	1.6
L-Cystine	2	0	2.4
L-Cystathionine	2	0	18.3

1) The incubation mixtures for the enzyme assay were the same as previously described (2), except that L-serine was replaced by the various substrates at the concentrations indicated. The enzymes used were 63.0 units SDH-A, and 2.26 units B-2.
2) Preparation of SDH-A was performed as previously described (2).

In Table 1 we presented the results on the substrate specificity of B-2; for comparison we also tested SDH-A, the L-serine dehydratase with the lower molecular weight. In contrast to SDH-A, the B-2 preparation dehydrated L-homoserine at a much higher rate than L-serine and L-threonine. L-Cystathionine, L-cystine and L-cysteine also served as substrates for B-2. It is highly probable, therefore, that B-2 is identical to L-homoserine dehydratase in rat liver (8).

It was found that the rapidly migrating minor component (B-1), which was observed in the gel filtration of the 35 - 60 % fraction,

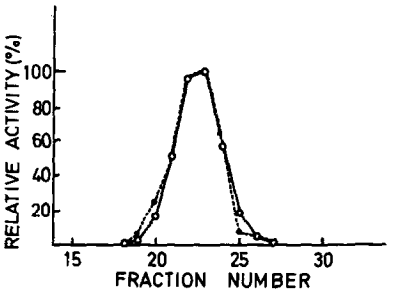

Fig. 2. Sucrose gradient centrifugation of the component B-2. One ml of B-2 (3.7 units, in buffer I) was placed on the sucrose gradient (30 ml, 5 - 20 % sucrose in buffer I) and centrifuged at 23,000 r.p.m. at 4° for 22 hrs using a swinging bucket type rotor No. 25.1. Fractions of 20 drops each were collected and assayed for L-serine dehydratase (o—o) and L-homoserine dehydratase (●--●).

does not show homoserine dehydratase activity and has a molecular weight of more than 200,000. This enzyme was relatively unstable and therefore not further characterized.

DISCUSSION

The data presented here strongly suggest that B-2 is identical to L-homoserine dehydratase, which was purified from rat liver by Matsuo and Greenberg (7) and Kato et al. (9). Its molecular weight has been found to be 180,000. It was reported that the purified enzyme dehydrates L-serine also at a rate of 2 % that of L-homoserine (8). The enzyme is active on L-cysteine, cystine and cystathionine too. It may be concluded, therefore, that a large part of the remaining L-serine dehydratase activity after a prolonged period of low-protein diet administration (more than 3 days) (1, 12, 13) is not due to "true" L-serine dehydratase but to homoserine dehydratase. Another evidence supporting this conclusion is that the antiserum against L-serine dehydratase (SDH-A) in rat liver does not inhibit both L-serine and L-homoserine dehydratase activities in the crude extract obtained from the rats which had been

fed the protein-free diet for 7 days (unpublished data). Fallon et al. (13) studied several properties of L-serine dehydratase from the liver of rats fed 2 % casein diet for 7 days. It is likely that they had dealed with L-homoserine dehydratase, since they reported the K_M for L-serine to be 275 mM. In the present study the K_M of B-2 was determined to be 250 mM. These K_M values are much higher than those reported with L-serine dehydratase (50 - 100 mM) (2, 3, 4, 14). Recently Pestaña (15) reported that a large difference exists between the K_M of L-serine dehydratase obtained from rats fed a high-protein diet and those fed glucose. Freedland and Avery (16) indicated that the extract from rat kidney, although quite low, contains L-serine dehydratase activity. Nakagawa and Kimura (17) also described the presence of the enzyme in rat kidney. Presence of L-cystathionase activity in rat kidney was reported by Finkelstein (18). Our studies with kidney of normally fed rats indicate that the extract is active on L-homoserine, and the L-serine dehydratase activity present is mostly due to B type. This was further confirmed by the fact that the antiserum against SDH-A inhibits only slightly the serine dehydratase activity in the crude extract from rat kidney.

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