EVIDENCE FOR THE PRESENCE OF TWO FORMS OF L-SERINE DEHYDRATASE IN RAT LIVER

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L-serine dehydrates (L-serine hydrolyase (deaminating), EC 4.2.1.13) has been highly purified from rat liver after inducing the enzyme either by feeding the animals with a high-protein diet [1–4] or by fasting them under simultaneous administration of cortisone [4]. As reported by Schmidinger and Kröger [5] L-serine dehydratase activity rapidly decreases in rat liver if the animals are fed with a protein-free diet. However, the enzyme level does not reach zero, even after giving the diet for 7 days. The enzyme activity increases again, as soon as the rats are fasted or put on a diet of casein hydrolysate.

The present paper deals with the demonstration of two forms of L-serine dehydratase from rat liver. The enzymes are different from each other concerning their molecular weight and heat stability.

Five female rats (Sprague-Dawley strain; Ivanovas, Kisslegg/Allgäu; weighing 110–130 g) were put on a protein-free diet (protein content less than 1%) for 7 days and thereafter starved for 38 hr in order to get induction of the enzyme. The animals were then killed and their livers removed. These were homogenized (Ultra-Turrax) for 40 sec in 4 volumes of 0.14 M KCl containing 10^{-4} M EDTA and 10^{-5} M PALP. The homogenates were centrifuged (105,000 × *g*; 40 min) and the supernatants were combined. This crude extract is defined as the "induced" enzyme preparation. If the rats, on the other hand, were not starved after getting the protein-free diet for 7 days – thus no induction took place – the supernatants mentioned above result in an extract called the "non-induced" enzyme preparation. The assay for L-serine dehydratase was carried out according to Schmidinger and Kröger [5], except for the incubation period which was extended to one hour. The activity of the enzyme is expressed as μmoles pyruvate formed per hour under the experimental condition.

The induced and non-induced enzymes were incubated at 55° for the times indicated and subsequently cooled in ice. After centrifugation (12,000 × *g*; 10 min) the supernatants were subjected to the determination of enzyme activity. As can be seen in fig. 1, the activity of the non-induced enzyme remains almost constant during the 10 min heat treatment, whereas the induced enzyme loses 65% of its activity. For this reason it was very likely that the rat liver

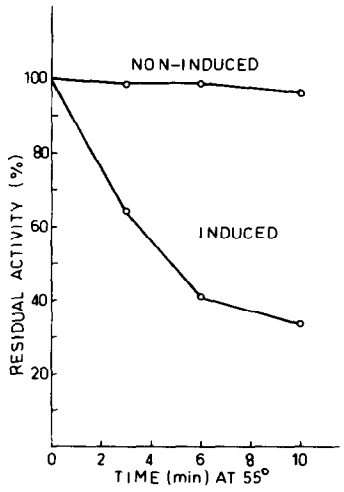

Fig. 1. Heat treatment of non-induced and induced L-serine dehydratases from rat liver. The enzyme preparations were incubated at 55° for the times indicated. Non-induced: 29.6 mg protein/ml, specific activity 0.087 μmoles pyruvate/hr/mg protein. Induced: 34.2 mg protein/ml, specific activity 0.565 μmoles pyruvate/hr/mg protein.

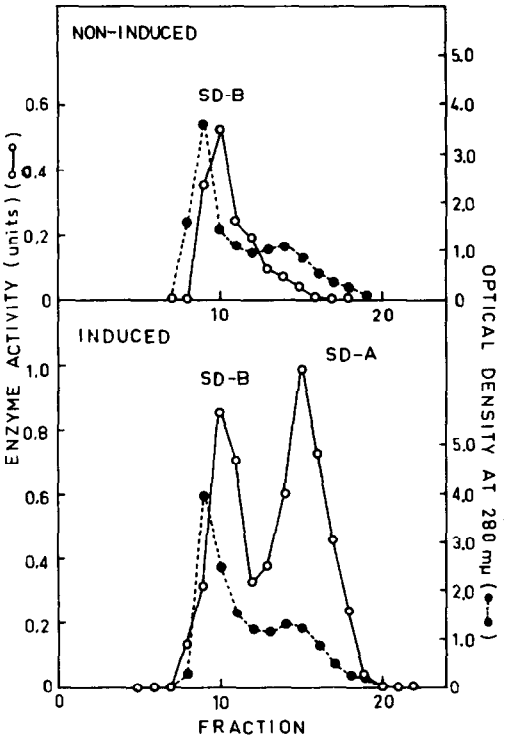

Fig. 2. Gel filtration of non-induced and induced L-serine dehydratase. 1.5 ml of each enzyme preparation was put into a Sephadex G-100 column (1.8 X 45 cm), which had been equilibrated with 33 mM potassium phosphate buffer (pH 7.8) containing 10^{-4} M EDTA and 10^{-5} M PALP. Elution was done with the same buffer (flow rate 25 ml/hr) and 5 ml fractions were collected. Protein content and specific activity of the preparations, see fig. 1.

extract contained two forms of enzyme. In order to separate these enzymes they were subjected to gel filtration on a Sephadex G-100 column. 1.5 ml of each enzyme preparation was put onto the column (1.8 X 45 cm), which had been equilibrated with 33 mM potassium phosphate buffer (pH 7.8) containing 10^{-4} M EDTA and 10^{-5} M PALP. The elution was performed with the same buffer and five ml fractions were collected for the enzyme assay and the measurement of optical density at 280 mμ. The results are presented in fig. 2: the non-induced enzyme consists of one active component (SD-B). Filtration of this enzyme preparation through a Sephadex G-100 column (calibrated with cytochrome *c*, soy bean trypsin inhibitor, bovine serum albumin and catalase [6] yields

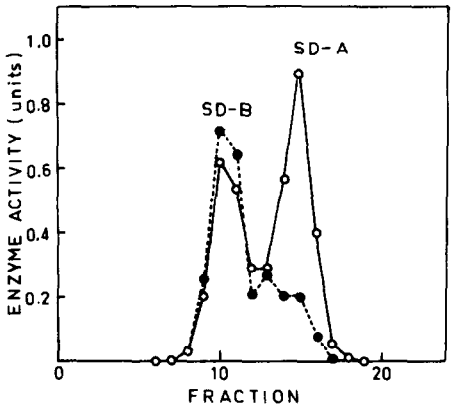

Fig. 3. Heat treatment of induced L-serine dehydratase from rat liver. Gel filtration was carried out as described in fig. 2. After gel filtration the fractions were incubated at 55° for 8 min; enzyme activity was determined before and after the heat treatment. Protein content of the preparation and specific activity are 35.2 mg/ml and 0.685, respectively. o—o activity before heat treatment; ●—● activity after heat treatment.

an elution volume for SD-B which corresponds to a molecular weight of approximately 80,000. The same gel filtration of the induced enzyme, on the other hand, results in an additional peak (SD-A) which has a lower molecular weight. This preparation contains about 60% SD-A and 40% SD-B calculated on the basis of the enzyme activity. The elution volume of SD-A led to a value of 42,000 for its molecular weight. This is in perfect agreement with the previously reported molecular weight which was determined for L-serine dehydratase purified from livers of starved cortisone-treated rats [4].

Fig. 3 illustrates that the enzyme form SD-B is much more stable to heat than SD-A. The induced enzyme was filtered through Sephadex G-100, and the fractions were tested for enzyme activity before and after they were incubated at 55° for 8 min. In contrast to SD-B, SD-A is readily destroyed by heat. The rather high heat inactivation of the induced enzyme, demonstrated in fig. 1, may be explained in terms of the difference between the heat stability of SD-A and SD-B present in the preparation. SD-A is easily induced by starvation or by application of a high-protein diet, but no remarkable increase of SD-B can be observed by these treatments. If L-serine dehydratase is induced up to its maximum either by

starving the rats for 50 hr under the administration of cortisone or by feeding them with casein hydrolysate for 4 days, the resulting preparations contain SD-B in an amount less than 1% of SD-A. For this reason it was difficult to find this extremely small content of SD-B in the enzyme preparation after maximal induction.

Two types L-serine (L-threonine) dehydratase, the biosynthetic and the biodegradative one, have been found in *E. coli* [7,8]. The biosynthetic enzyme, which is formed constitutively, is inhibited by isoleucine as an allosteric effector. The biodegradative enzyme can be repressed by glucose catabolites and is activated by some nucleotides such as AMP and ADP. These compounds affect the association-dissociation equilibrium of the biodegradative L-serine (L-threonine) dehydratase obtained from *E. coli* [9,10] and *Clostridium tetanomorphum* [11]. In the present work it is shown that the crude enzyme extract from rat liver contains the L-serine dehydratase in two forms (SD-A and SD-B). They are different from each other in molecular weight, heat stability and inducibility. With respect to the molecular weight the two forms SD-A (42,000) and SD-B (80,000) are clearly different from the L-serine dehydratase purified from rat liver by Nagabhushanam and Greenberg (20,000) [1] or by Nakagawa et al. (63,500) [2]. It is yet uncertain, whether the reason for the different enzyme forms can be seen in the association-dissociation equilibrium.

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