# REVERSIBLE INACTIVATION OF RAT LIVER SERINE DEHYDRATASE BY ITS SUBSTRATES

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## 1. Introduction

L-Serine (threonine) dehydratase of mammalian liver can catalyse the deamination of serine and threonine [1]; however, the sheep liver enzyme exhibits good activity only with threonine, since with serine it is rapidly inactivated in the course of the reaction [2]. A similar inactivation by serine has been reported for threonine dehydratases from *E. coli* [3] and yeast [4]. In this paper, evidence is presented which suggests that serine dehydratase of rat liver can be inactivated by any of its substrates *in vitro*, when assayed at the optimal alkaline pH in the absence of added pyridoxal phosphate. Inactivation can be prevented by physiological concentrations of potassium ions or by lowering the pH and substrate concentration to the physiological range, Substrate induced inactivation of the serine dehydratase of rat liver is readily reversed by addition of pyridoxal phosphate.

## 2. Material and methods

Livers from rats fed a 90% casein diet [5] for 5 or 7 days were homogenized in 4 volumes of 0.25 M sucrose, and the high speed supernatant (105,000 *g*) was used throughout. The standard assay mixture for serine dehydratase consisted in 0.05 M tris-HCl, pH 8.5, 1 mM dithioerythritol, 0.1 mM pyridoxal phosphate (PLP), 0.15 mM DPNH, 0.01 mg of lactate dehydrogenase (Boehringer) and various amounts of liver extracts. After preincubation for five minutes, the reaction was started by addition of 0.1 M substrate. All assays were carried out at room temperture (ca. 23°) in 1 cm light path cuvettes, in a final volume of 1 ml; changes in optical density at 340 mμ were recorded in a Cary 15 spectrophotometer.

## 3. Results and discussion

Serine dehydratase assay requires added PLP for optimal activity [6]. Fig. 1 shows that when PLP was omitted from the standard assay mixture, there was a progressive decline, with time, in the activity towards serine. A similar result was obtained with 0.1 M sodium borate, pH 8.3, instead of tris-HCl. Addition of PLP resulted in a rapid reactivation. In the presence of 0.1 M KCl, without PLP, the reaction proceeded linearly. Nevertheless, the addition of KCl in the course of the reaction did not reverse the inactivating effect of serine. With threonine as substrate, without added PLP, the reaction proceeded linearly although at a very low rate. Addition of PLP caused a considerable activation, up to the activity found with the entire assay mixture. When serine was added in the course of the reaction with threonine as substrate (broken line in fig. 1), the initial burst in activity towards serine was not observed, suggesting that the enzyme had been inactivated (modified) by threonine. Similar results were obtained with 0.01 M substrates.

At pH 7, with 0.1 M serine, the pattern found was similar to that obtained at pH 8.5. However, with 0.01 M serine (fig. 2), the reaction in the absence of added PLP and potassium ions was almost linear

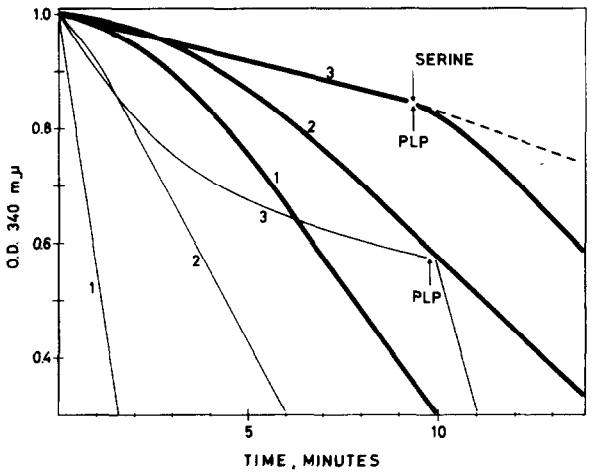


Fig. 1. Reversible inactivation of serine dehydratase by serine and theronine. Serine dehydratase was assayed as described in Methods with either 0.1 M serine (——) or 0.1 M threonine (——) in presence of 0.1 mM PLP [1]; without PLP, but with 0.1 M KCl [2]; and without PLP and KCl [3]. Additions of PLP or serine, for 0.1 mM and 0.1 M concentration respectively, are indicated by the arrows in the figure. The broken line stands for the result obtained after additon of serine.

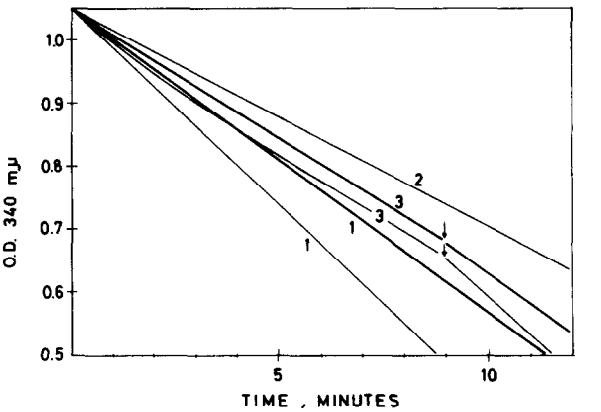


Fig. 2. Lack of substrate inactivation of serine dehydratase at physiological pH and substrate concentration. Serine dehydratase assay was carried out with 0.05 M tris-HCl pH 7 (instead of pH 8.5) and 0.01 M substrates. The symbols used (figures and lines) are the same as in fig. 1. Arrows indicate the addition of PLP for a 0.1 mM concentration.

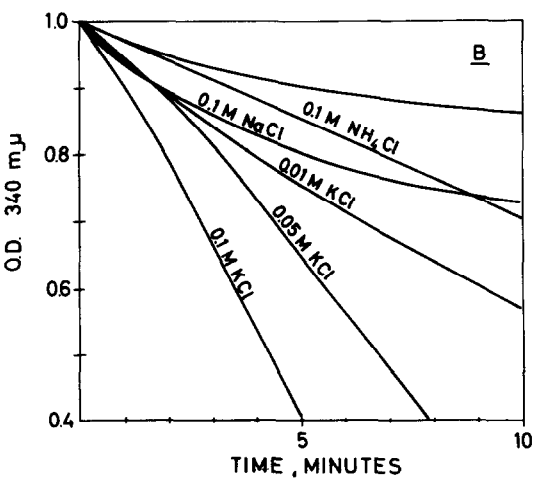


Fig. 3. Effect of several ions on serine dehydratase inactivation. Serine dehydratase was assayed with the standard assay mixture, but omitting PLP. Different ions concentrations, as indicated in the figure, were added to the assay mixture, before starting the reaction with 0.1 M serine.

(80% of the initial activity at 10 min), and addition of PLP resulted in only a slight activation. With 0.01 M threonine the reaction was linear and the activity was high, close to that obtained with serine, and no appreciable activation was obtained after addition of PLP.

It was of some interest to ascertain the protective role of potassium ions. In fig. 3 it can be seen that 0.1 M NaCl did not prevent the progessive inhibition by serine found at pH 8.5 in absence of PLP. 0.1 M $NH_4Cl$ in the same conditions did protect; however, KCl both protected and activated. From this figure and from data obtained with different concentrations of potassium (as potassium phosphate buffer, pH 8.5), the estimated minimal requirements of this ion for the reaction to proceed linearly are in the 0.025–0.05 M range. Potassium ions markedly affect the affinity of the serine dehydratase apoenzyme for PLP. Table 1 shows that dialysis (16–18 hr) of rat liver extracts against 0.01 M tris-HCl pH 8.5, caused more than 90% resolution. However, the presence of 0.1 M KCl in the dialysis medium almost completely protected the enzyme from resolution. This fact must be considered in estimations of the affinity of the enzyme for PLP, which, if carried out in the absence

Table 1
Resolution and reconstitution of serine dehydratase.

| | Activity (μmoles/min/ml) | |
|---|---|---|
| | Without added PLP | With 0.1 mM PLP |
| Undialysed control | 0.8 | 1.6 |
| Additons to the dialysis buffer | | |
| None | 0.035 | 1.2 |
| 0.01 M threonine | 0.045 | 1.4 |
| 0.01 M serine | 0.032 | 1.3 |
| 0.01 M serine + 0.1 M KCl | 0.62 | 1.5 |

2 ml fraction of a crude liver extract were dialysed overnight at 0°–4°C against 1 liter 0.01 M tris-HCl, pH 8.5, alone or with additions as indicated above. Aliquots of these dialysed extracts were assayed for serine dehydratase activity, (a) without added PLP, in a mixture containing 0.1 M potassium phosphate pH 8.5, instead of tris-HCl, and (b) with the standard assay mixture after preincubation with 0.1 mM PLP for 20 min at room temperature.

of potassium ions would give values considerably smaller than those to be expected in the physiological conditions prevailing *in situ*.

The results so far described indicate that serine dehydratase of rat liver can be inactivated by its substrates in certain conditions. Unlike sheep liver threonine dehydratase [7], this inactivation requires alkaline pH and absence of potassium ions or added PLP; moreover, threonine is a more efficient inactivator than serine in these conditions. In addition, the inactivation of the rat liver enzyme is readily reversed by PLP. At pH 7, and with substrate concentrations within the physiological range in liver, the rat enzyme seems to be insensitive to substrate induced inactivation.

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