OPPOSITE EFFECTS OF POTASSIUM IONS ON THE AFFINITIES OF RAT LIVER SERINE DEHYDRATASE FOR COENZYME AND SUBSTRATE

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1. Introduction

Potassium ions have been shown to affect serine dehydratase activity in several ways. They can prevent the substrate-induced inactivation which occurs at alkaline pH when the activity is assayed without added pyridoxal-5′-phosphate (PLP) [1]. They also protect the enzyme against inactivation by elementary sulfur and *p*-chloromercuriphenylsulfonate [2]. In addition, the presence of 0.1 M KCl has been shown to decrease markedly the resolution by dialysis of serine dehydratase [1], suggesting a strong effect of this cation on the apparent affinity of the enzyme for PLP. In this paper we present kinetic evidence which further supports this suggestion. In particular it is shown that K^+ ions can specifically lower some fifty times the apparent K_m of serine dehydratase apoenzyme for PLP. On the other hand, K^+ ions can inhibit serine dehydratase holoenzyme in a competitive form with regard to substrate, although this seems to be an unspecific effect, apparently related to their contribution to a high ionic strength of the medium.

2. Materials and methods

Serine dehydratase activity was assayed at room temperature in high speed liver supernatant as previously described [1]. Serine dehydratase apoenzyme was prepared by dialysis of rat liver extract against 0.05 M tris HCl pH 8.5, overnight. This treatment allows removal of more than 95% of the PLP in serine dehydratase, as ascertained by assays carried out with and without added PLP.

3. Results and discussion

Reconstitution of the holoenzyme at low concentrations of PLP is greatly favoured by the presence of physiological concentrations of K^+ ions. Preincubation of the apoenzyme with different concentrations of PLP in the presence of 0.1 M KCl, as indicated in fig. 1, lowered the apparent K_m for PLP from a value of 50 μM in the absence of K^+ ions to 1 μM in their presence.

The specificity of this effect was studied by comparing the ability of KCl, NaCl and NH_4Cl to activate the reconstitution of serine dehydratase holoenzyme at a low concentration of PLP as indicated in fig. 2. The results of this experiment were that no activation took place with NaCl at concentrations ranging between 10 and 50 mM. On the contrary, either KCl or NH_4Cl activated 3 to 4 times in the same conditions. These results clearly indicate that there is no involvement of unspecific effects due to ionic strength of the medium on the K^+-dependent activation of serine dehydratase and that the Cl^- anion is not an activator. The similitude of the K^+ and NH_4^+ effects suggests that this activation is related to the size of the cation. From the data in fig. 2 an activation constant for K^+ ions of ca. 6 mM has been obtained.

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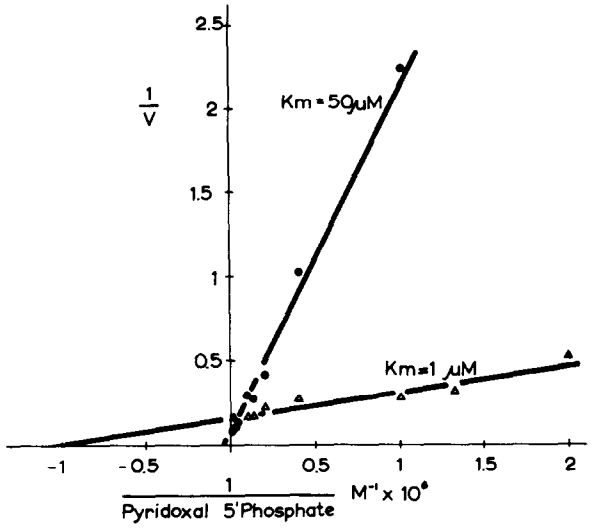

Fig. 1. Effect of potassium ions on the affinity of serine dehydratase apoenzyme for PLP. Aliquots of a dialyzed liver extract were preincubated for 1 hr at 37° with increasing concentrations of PLP in the assay mixture minus substrate (•) or in the same mixture plus 0.1 M KCl (△). After addition of 0.1 M serine, final concentration, the changes in absorbance were recorded at room temperature.

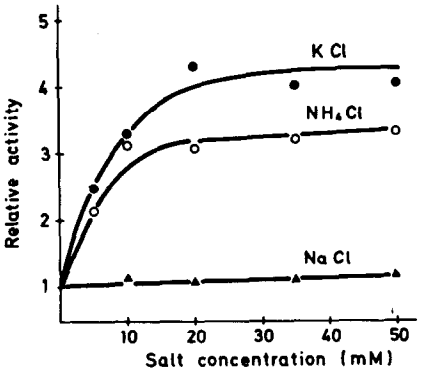

Fig. 2. Differential effects of cations on serine dehydratase reconstitution. Samples of a dialyzed liver extract were preincubated at room temperature with 1 μM PLP and salt additions as indicated, in the assay mixture minus serine. After 5 min the reaction was started with serine for 0.1 M final concentration.

The effect of K^+ on the affinity of serine dehydratase for PLP appears largely independent of the pH of the medium, since the same extent of activation was obtained when variable KCl concentrations were assayed for activation of serine dehydratase reconstitution in the pH range from 7.0 to 8.5. This result suggests that K^+ activation is not related to the occurrence of ionizable groups (i.e., at the PLP binding site).

In contrast with the markedly activatory effect of K^+ ions on the reconstitution of serine dehydratase, they can appreciably inhibit the activity of the holoenzyme when assayed with excess of added PLP. Fig. 3 shows that KCl competitively inhibits serine dehydratase activity with a K_i = 0.1 M. This inhibitory effect appears to be unspecific and probably related to the ionic strength of the medium, since NaCl and NH_4Cl as well as increasing concentrations of K^+ phosphate and tris-HCl buffers also inhibited at low substrate concentration (table 1). Table 2 shows the combined effects of pH and K^+ ions on the K_m and V_{max} of serine dehydratase. It is apparent that pH affects both parameters, whereas K^+ affects only the K_m. Changing from optimal conditions (pH 8.5, no K^+) to the more near physiological ones (pH 7.0, 0.1 M KCl) results in a lowering of near 50 times in the deaminating efficiency (V_{max}/K_m) of the enzyme. A result which reinforces the uncertainties about the extent of the operativity in vivo of the enzyme [5].

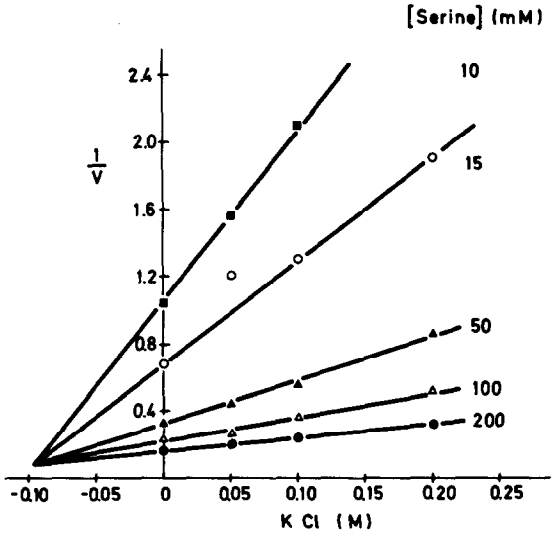

Fig. 3. Competitive inhibition of serine dehydratase activity by KCl. Serine dehydratase holoenzyme (non-dialyzed preparations) was assayed at different KCl and serine concentrations, in the presence of 0.1 mM PLP. The reciprocal of the resulting activities were plotted against KCl concentration [3].

Table 1
Effects of buffer concentration and salt additions on serine dehydratase activity.

Buffer (anion mM)	Salt addition (mM)	Activity
Experiment 1		
K phosphate (10)	–	100
(100)	–	39
Na phosphate (10)	–	99
(100)	–	36
Experiment 2		
Tris-HCl (10)	–	100
(50)	–	88
(50)	NH_4Cl (50)	65
(100)	–	67
(150)	–	43
(50)	KCl (100)	41

Assays were carried out at pH 8.5, with the indicated buffers and salt additions, in presence of 0.1 mM PLP, using a non-dialyzed liver extract. Serine for 0.01 M final concentration was added to start the reaction.

The results of this work support the conclusion raised in a previous work [2] about the marked dependence of serine dehydratase on an environment with a physiological concentration of K^+ ions. On the basis of this and previous results [1, 2] it is possible to differentiate two kinds of K^+ effects on serine dehydratase. One set, including protection against inactivation by substrate and sulphydryl poissons and the increase of the apparent affinity of the enzyme for PLP described in this work, which seems to be specifically related to K^+, presumably through conformational changes. The other one, inhibitory for enzyme activity, can be explained by an effect of the ionic strength of the medium on the apparent K_m of serine dehydratase for serine.

Table 2
Effects of KCl and pH on K_m and V_{max} for serine of rat liver serine dehydratase.

pH	KCl (M)	V_{max}	K_m
8.5	–	18	0.022
8.5	0.1	18	0.08
7.0	–	6.6	0.17
7.0	0.1	6.6	0.33

Assays were carried out with a non-dialyzed liver extract in 0.05 M tris-HCl at the indicated pH. The kinetic parameters were derived from double reciprocal plots [4].

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