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FAILURE OF PROTECTIVE ACTION OF SODIUM AND POTASSIUM IONS AGAINST HEAT INACTIVATION OF *ESCHERICHIA COLI* L-ASPARAGINASE

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SUMMARY

Two L-asparaginases (L-asparagine amidohydrolase, EC 3.5.1.1) isolated from *Escherichia coli* B were not protected from heat inactivation by sodium (or potassium) ions.

INTRODUCTION

It was shown that certain guinea pigs possessed a thermostable L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) in their sera, while others possessed a thermolabile form of the enzyme, and these two L-asparaginases were stabilized against heat inactivation by Na^+ and K^+ [1]. Roberts *et al.*[2] and Campbell *et al.*[3] demonstrated that *Escherichia coli* B L-asparaginase existed in two forms, one active and the other inactive against animal tumors. These two enzymes differ markedly by several criteria: solubility in ammonium sulfate solution, chromatographic behavior, enzyme activity as a function of pH, as well as the antitumor activity. Are these two *E. coli* L-asparaginases also protected from heat inactivation by Na^+ (or K^+), as well as guinea pig serum L-asparaginase? The present communication reports the effects of Na^+ and K^+ on the heat inactivation of two L-asparaginases isolated from *E. coli* B.

MATERIALS AND METHODS

Enzyme purification

Asparaginase was purified from *E. coli* B by the method of Roberts *et al.*[2], with some modifications.

Enzyme activity

L-Asparaginase assays were carried out according to the procedures described by Roberts *et al.*[2]. One international unit of activity is defined as that amount of enzyme which catalyzes the formation of 1 μmole of ammonia per min. Specific activity is expressed as units per mg of protein. The protein content of the samples was determined according to the method of Lowry *et al.*[4].

RESULTS AND DISCUSSION

Two L-asparaginase components are isolated from *E. coli* and these two enzymes differ markedly in their pH–activity profiles, their solubility, chromatographic behavior, their affinities for L-asparagine, their sensitivities to thermal inactivation, blood clearance and antitumor activity as described in earlier publications[2,3,5,6].

Several properties of two peaks of L-asparaginase activity separated by DEAE-cellulose column chromatography were examined, and subsequently were ascertained to be EC-2 and EC-1, respectively. Since the preparations of EC-2 and EC-1 were eluted from the column with a salt gradient and then concentrated, these enzyme preparations contained much salt. In order to remove much of the NaCl in the eluate, these preparations were dialyzed against 0.01 M NaCl. Specific activities of EC-2 and EC-1 were 3.4 I.U. per mg protein and 0.3 I.U. per mg protein, respectively.

Effect of Na^+ and K^+ on L-asparaginase EC-2

The activity of EC-2 was slightly (17.5% of initial asparaginase activity) lost after incubation for 30 min at 56 °C, but at 66 °C the enzyme activity was almost entirely (89.3%) destroyed. The thermolabile asparaginase activity in the guinea pig serum sample, which was entirely destroyed at 66 °C for 30 min, was completely (91.8%) stable even after heating in the presence of 0.75 M NaCl[1]. Accordingly, the protective effect of Na^+ (or K^+) against heat inactivation of EC-2 was examined.

TABLE I

HEAT STABILITY OF THE ACTIVITY OF EC-2 INCUBATED IN THE PRESENCE OF Na^+ (OR K^+)
Initial activity taken as 100%.

Salt	*Final concentration (M)*	*L-Asparaginase activity after incubation at 66 °C for 30 min (% of initial activity)*
None	–	10.7
NaCl	0.05	9.4
	0.25	21.8
	0.75	28.8
	1.0	23.2
	1.25	24.8
KCl	0.05	12.8
	0.25	24.0
	0.75	25.5
	1.0	24.8

NaCl (or KCl) at different concentrations was mixed with an equal volume of EC-2 preparation and the mixture was heated at 66 °C for 30 min, and then aliquots of the mixture were withdrawn and assayed for L-asparaginase activity. These results are shown in Table I. In the presence of a high concentration of Na^+, the activity was only slightly stable after heating (0.25 M, 21.8%; 0.75 M, 28.8%; 1.0 M, 23.2%; 1.25 M, 24.8%), whereas in the presence of a low concentration of Na^+, the activity was not protected from heat inactivation (0.05 M, 9.4%). K^+ also showed a similar effect on EC-2.

Effect of Na^+ and K^+ on L-asparaginase EC-1

After 30 min at 56 °C, 91.7% of the activity of EC-1, which coincided closely with the data reported by Schwartz *et al.*[5], was lost. Similarly, the activity of EC-2 was inactivated at 66 °C, whereas the inactivating temperature for EC-1 was 10 °C lower. Therefore, the protective effect of Na^+ (or K^+) during incubation at 56 °C on EC-1 was investigated. These results are indicated in Table II. The activity of EC-1 was also not protected from heat inactivation by Na^+ (or K^+). Thus Na^+ (or K^+) showed no significant protective effect on *E. coli* L-asparaginase, in contrast to the

TABLE II

HEAT STABILITY OF THE ACTIVITY OF EC-1 INCUBATED IN THE PRESENCE OF Na^+ (OR K^+)
Initial activity taken as 100%.

Salt	*Final concentration (M)*	*L-Asparaginase activity after incubation at 56 °C for 30 min (% of initial activity)*
None	–	8.3
NaCl	0.05	6.8
	0.25	8.7
	0.75	7.4
	1.0	6.7
KCl	0.05	6.8
	0.25	8.0
	0.75	7.3
	1.0	7.8

full protective effect of these ions on the activity of guinea pig serum L-asparaginase.

E. coli L-asparaginase (EC-2) and guinea pig serum L-asparaginase differ in their affinities for L-asparagine, molecular weights[7–9], the detection of the antitumor activity depending upon the method of testing[10] and blood clearance[6]. Therefore, a remarkable difference in the protective effect of Na^+ against heat inactivation between *E. coli* L-asparaginase and guinea pig serum L-asparaginase may be related to the difference in these properties of the two enzymes.

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