On the separation of adenylate kinase of the water-soluble liver fraction from nucleoside monophosphate kinase

It has been previously shown by the senior author[1] that mammalian liver tissue contains a water-soluble adenylate kinase (ADP phosphomutase) which differs in some respects from the adenylate kinase of muscle (myokinase). In view of the recent discovery of nucleoside monophosphate kinases[2-6] the question arises as to the specificity of the water-soluble hepatic adenylate kinase.

Yeast nucleoside monophosphate kinase has been shown[2] to be different from adenylate kinase. The former enzyme has also been detected in various liver preparations[3-5] but the question remained open whether or not this enzyme is distinct from adenylate kinase.

It has been shown in an earlier paper[7] that the adenylate kinase and nucleoside monophosphate kinase of aqueous extracts from rabbit liver are different entities insofar as their response to changes in the pH of the medium and to heating is concerned. The present publication reports the separation of the two enzymes by ethanol fractionation of dialysed aqueous liver extracts of rabbit liver prepared as described previously[1]. The ethanolic precipitates were dissolved in water, reprecipitated with ethanol, dried *in vacuo* in the cold, dissolved in a definite volume of water and then used in the experiment.

To assay the activity of adenylate kinase or nucleoside monophosphate kinase aqueous solutions of the ethanol fractions were incubated 30 min with the respective substrates (0.5 to 1.5 μM), phosphate buffer (pH 7.6) and 0.005 M Mg^{++}. Protein was precipitated with $HClO_4$, the excess of the acid being removed as the insoluble potassium salt. The reaction products were estimated in the supernatant by paper chromatography in two solvent systems (ethanol–ammonium acetate, pH 3.8[8] and isobutyric acid–NH_3–ethylenediaminetetraacetate[9] followed by elution of the spots and spectrophotometric estimation of the absorbancies of the eluates. In some experiments the reaction products were analyzed by fractional elution from chromatographic columns with anion-exchange resins (AW-16 or Amberlite IR-400)[10].

Preliminary fractionation of the enzyme extract with ethanol showed that adenylate kinase activity was found in the 0–20 % ethanol fraction while that of nucleoside monophosphate kinase is in the 40–50 % ethanol fraction. In this way, practically complete separation of the enzymes can be achieved almost without mutual contamination. In contrast to experiments on crude extracts[7], the ethanol fractions did not exhibit any apparent nucleoside tri- or di-phosphatase activity and hence did not require any additional treatment.

Fig. 1 illustrates the determination of adenylate kinase activity (substrates: ATP + AMP) in ethanol fractions of the aqueous extract of liver, and Fig. 2 the determination of nucleoside monophosphate kinase activity with ATP + UMP as substrates. It is clear that the adenylate kinase reaction can be detected in fraction 1 (0–20 % ethanol) but not in fraction 2 (40–50 % ethanol), whereas the nucleoside monophosphate kinase reaction takes place in fraction 2 but not in fraction 1. A quite similar distribution of activity in the ethanol fractions has been found for the nucleo-

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine-5-phosphate; UTP, uridine triphosphate; UMP, uridine-5-phosphate; GMP, guanosine-5-phosphate; ITP, inosine triphosphate; IMP, inosine-5-phosphate.

side monophosphate kinase with ATP–GMP, UTP–AMP and ITP–AMP as substrates. The reaction in fraction 2 was always negative with the following substrate pairs: UTP–UMP, ITP–IMP, ITP GMP and ITP UMP.

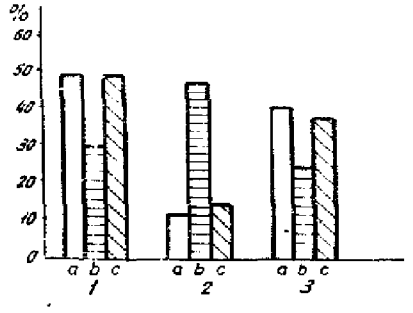

Fig. 1. The activity of adenylate kinase in ethanol-precipitated fractions from aqueous extract of rabbit liver. The results are given in % of total E_{222} for each assay. The reaction products were fractionated on a column of Amberlite IR-400. a, control; b, 0–20% ethanol fraction, c, 40–50% ethanol fraction. 1, AMP; 2, ADP; 3, ATP.

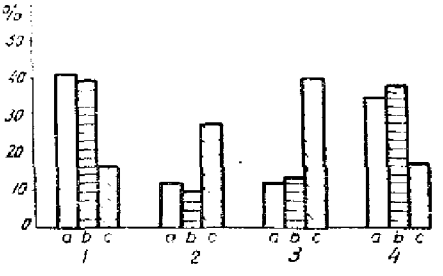

Fig. 2. The activity of nucleoside monophosphate kinase in ethanol-precipitated fractions from aqueous extracts of rabbit liver. Symbols are the same as in Fig. 1, except for substrates: 1, UMP; 2, UDP; 3, ADP; 4, ATP.

It thus appears that adenylate kinase of the water-soluble liver fraction catalyses phosphate transfer only between adenine nucleotides.

Nucleoside monophosphate kinase appears to catalyse phosphate transfer from nucleoside triphosphate to nucleoside monophosphate under the following conditions: (1) The nucleotides of each pair should contain different bases. No reaction can be obtained between nucleotides with similar bases (adenine nucleotides included). (2) In every nucleotide pair one of the nucleotides (the phosphate donor or acceptor) should be an adenine nucleotide.

That an adenine nucleotide is necessary for nucleoside monophosphate kinase has been shown earlier[3,6]. This is confirmed by the present data with regard to the water-soluble liver enzyme.

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