MULTIPLE FORMS OF AMP DEAMINASE IN VARIOUS RAT TISSUES

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1. Introduction

The importance of AMP deaminase in the purine nucleotide cycle [1,2], interconversion of adenine, inosine, and guanine nucleotide [3–6], and stabilization of energy charge [7] in different rat tissues makes this enzyme an interesting subject for physico–chemical studies from the regulatory point of view.

In our previous report, it was shown that at least four characteristically distinct AMP deaminases were present in adult rat brain [8]. All of them display sigmoid kinetics with AMP, allosteric activation by ATP and alkali metal ions, and inhibition by GTP and inorganic phosphate.

In the present investigation, phosphocellulose column chromatography was used to separate the AMP deaminases of rat tissues. It will be shown that each tissue has a characteristic distribution of these multiple forms.

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2. Materials and methods

Nucleotides were obtained from Boehringer. Phosphocellulose was purchased from Brown Co. Other reagents used were of analytical reagent grade.

After Wister rats weighing 200–300 g were decapitated, liver, skeletal muscle and other tissues were rapidly removed and frozen. Tissue extracts were prepared by homogenizing in 5 vol of Buffer A, which consisted of 0.02 M potassium phosphate buffer (pH 7.0) and 0.1% 2-mercaptoethanol, with a Waring Blender for 3 min at 18 000 r.p.m. The extracts were centrifuged in a Hitachi 18 PR-3 centrifuge at 20 000 *g* for 30 min to obtain supernatants.

Column chromatography on phosphocellulose was used to separate the multiple forms of AMP deaminase. Each tissue extract was applied to a column (0.9 × 10 cm) of phosphocellulose equilibrated with Buffer A containing 0.1 M NaCl. Elution of the enzyme was accomplished by first washing the column with 30 ml of 0.1 M NaCl in Buffer A, and then using a 0.1 to 1.2 M NaCl linear gradient (200 ml) in the same buffer.

Enzyme activity was measured colorimetrically by estimating production of ammonia. Typical reaction mixture contained 5 mM AMP, 5 mM ATP, 20 mM potassium phosphate buffer (pH 7.0), 50 mM NaCl, 0.02% 2-mercaptoethanol, 0.05% bovine serum albumin in a final volume of 0.25 ml. The amount of ammonia was determined by the phenol–hypochlorite

reagent [9]. For the kinetic experiment, the reaction mixture of 0.25 ml contained 20 mM Tris–HCl buffer (pH 7.0), 0.05% bovine serum albumin, various concentrations of AMP and effectors, and enzyme free from 2-mercaptoethanol, and the amount of ammonia was estimated by Nessler's reagent. The reaction was usually carried out at 37°C for 10 min, and one unit of enzyme activity is defined as one μmole of ammonia yielded per min.

3. Results

The results of typical chromatographic patterns of AMP deaminase from rat tissues are shown in figs. 1 and 2. Each type of tissue was found to have qualitatively as well as quantitatively distinct patterns of multiple forms.

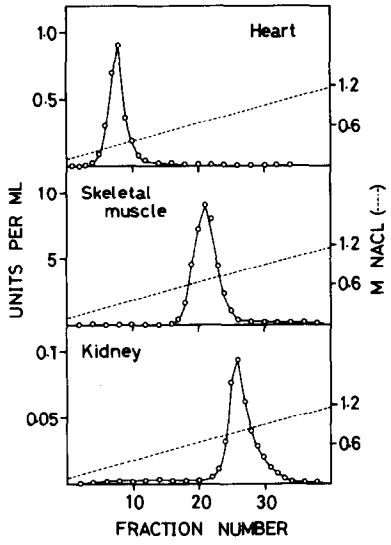

Fig. 1. Phosphocellulose column chromatography of AMP deaminase from extracts of heart, skeletal muscle, and kidney. A frozen preparation, stored at −20°C, was blended for 3 min in 5 vol of cold Buffer A with Waring Blender at 18 000 rpm. The homogenate was centrifuged at 20 000 *g* for 30 min. The supernatant was saved and applied to a phosphocellulose column (0.9 × 10 cm) in the cold. The column was washed with 30 ml of Buffer A containing 0.1 M NaCl. Elution was carried out with a linear NaCl gradient, from 0.1 to 1.2 M, in Buffer A. The reservoir and mixing bottle each contained 200 ml of appropriate solution. Fractions, about 10 ml each, were collected and assayed for enzyme activity. In these particular experiments, heart (10 g), leg skeletal muscle (16.5 g), and kidney (22 g) were used.

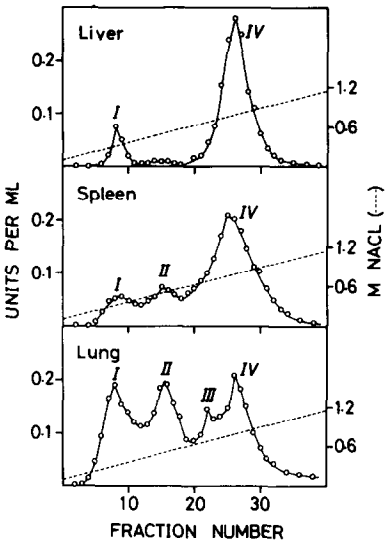

Fig. 2. Phosphocellulose chromatography of AMP deaminase from extracts of liver, spleen, and lung. The preparation of tissue extracts and chromatographic conditions were as in fig. 1. Liver (18 g), spleen (7 g), and lung (18 g) were used.

In fig. 1, chromatographic patterns of rat heart, skeletal muscle, and kidney are shown. In these tissues, extracts yielded only one peak of activity on chromatography, although their elution positions were apparently different. Heart enzyme was eluted with activity peak at 0.3 M NaCl, skeletal muscle enzyme at 0.65 M NaCl, and kidney enzyme at 0.8 M NaCl.

As shown in fig. 2, liver, spleen, and lung extracts demonstrated multiple peaks of AMP deaminase activity within each single tissue, although quantitative proportions of multiple peaks differed in these various tissues. Fig. 2 also shows that the activity peaks of the deaminases from various tissues were fractionated at the same NaCl concentration. For brevity, the peaks are hereafter designated as Type I, II, III, and IV [8]. Type I is the enzyme form that was eluted with an activity peak at 0.3 M NaCl, Type II at 0.5 M NaCl, Type III at 0.65 M NaCl, and Type IV at 0.8 M NaCl.

The effects of hydrogen ion concentration on the activity of AMP deaminases in various tissues were compared over the pH range of 4.5 to 9.5. The activities of different enzyme forms were not similar to one another. The typical experimental results obtained

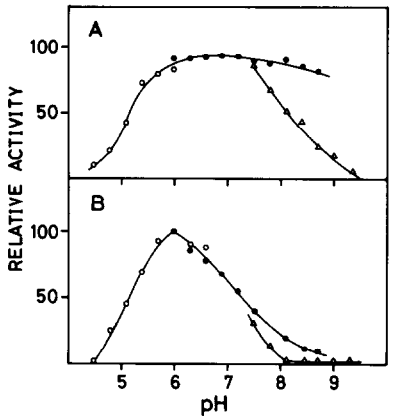

Fig. 3. Effect of pH on heart and kidney AMP deaminase activities. Enzyme activity was determined in 20 mM citrate phosphate (○), 20 mM potassium phosphate (●), and 20 mM borate (△) buffers at different pH. (A) heart enzyme; (B) kidney enzyme.

with partially purified heart and kidney enzymes were shown in fig. 3. With heart enzyme the optimum was broad (fig. 3A), while a sharp optimum at pH 6.0 was observed with kidney enzyme (fig. 3B). Skeletal muscle enzyme showed an optimum pH profile similar to that of kidney enzyme. In tissues such as liver, spleen, or lung, where more than two forms of AMP deaminase existed, Type I, which was eluted fastest from phosphocellulose, showed an optimum pH quite similar to that of heart enzyme, and Type IV, the last peak, similar to that of kidney enzyme.

In a preliminary kinetic experiment, it was observed that all AMP deaminases exhibited a cooperative effect towards AMP in the absence of activators, such as ATP and alkali metal ions. In the presence of 50 mM NaCl, the enzymatic response to AMP was transformed into a hyperbolic curve and apparent K_m values for AMP decreased in all types of deaminase. As generally accepted in this enzyme, however, the maximum velocities did not change both in the absence and in the presence of activators. The K_m values of various AMP deaminase forms for AMP in the absence and presence of 50 mM NaCl are summarized in table 1. Heart enzyme and Type I in tissues, such as spleen or lung, showed apparently smaller K_m values both in the absence and in the presence of activators than those for kidney, skeletal muscle, and Type IV enzymes.

Table 1
Apparent K_m values for AMP in the absence and presence of 50 mM NaCl

Enzymes		K_m values for AMP (mM) without NaCl	With 50 mM NaCl
Heart		6	1
Skeletal muscle		25	2–3
Kidney		30–40	6–9
Liver	IV	41	9
Spleen	I	6	1–2
	IV	31	7
Lung	I	6	1–2
	IV	30	6

The active fractions under each peak in figs. 1 and 2 were pooled, concentrated by ultrafiltration, and used as enzyme source. Reaction mixtures (0.25 ml) were set up, as described in Materials and methods.

4. Discussion

The data presented here indicated that distinct forms of AMP deaminase occur in various rat tissues. The column chromatographic separation indicated that the distribution of multiple forms in different tissues varies quantitatively as well as qualitatively. For example, heart, skeletal muscle, and kidney contain qualitatively distinct forms. On the other hand, although lung contains the same AMP deaminase forms as does spleen, it has much greater proportions of Type I and II than does spleen. The predominant form of AMP deaminase in liver is Type IV. While multiple forms of the enzyme exist in liver, spleen, and lung, only one form, specific to each tissue, is found in heart, skeletal muscle, and kidney. Previously, we reported that in rat brain AMP deaminase existed in multiple forms, which differed from one another both in their elution profiles from phosphocellulose column, and their K_m values for AMP in the absence and presence of activators [8]. From the present studies, along with previous results in brain, it is likely that heart, skeletal muscle, and kidney enzymes correspond, respectively, to Type I, III, and IV in brain, lung, spleen, and liver, since each of these corresponding enzymes shares a similar elution profile, a similar pH optimum, and similar K_m values for AMP both in the absence and in the presence of activators. The definitive conclusion, however, must await an immunological study.

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