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INTERACTION OF AMP DEAMINASE WITH RNA

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tRNA, 18 S and 28 S ribosomal RNAs were found to activate muscle AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) but inhibit liver and heart AMP deaminases. The macromolecular structures are essential for modulation of enzyme activity, since the effects of RNA disappeared after RNAase treatment. Sucrose density centrifugation experiments clearly demonstrated the binding of purified muscle AMP deaminase to tRNA, 18 S and 28 S RNAs. The binding is reversible and responsive to alterations of pH and KCl concentration. The binding was stable at pH 5.1–7.0 in 0.1 M KCl, but most of the enzyme dissociated at pH 7.5. KCl below 0.1 M concentration had no effect on dissociation of enzyme-RNA complex, but in 0.15 M KCl the complex was partially dissociated and in 0.2 M KCl most of the enzyme was released. Various nucleotides were also effective in dissociation of the enzyme from complex. The binding is saturable and the maximum number of muscle AMP deaminase molecules bound per mol 28 S RNA was calculated to be approx. 30. Liver and heart AMP deaminases were also found to interact with RNA.

Introduction

AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) catalyzes the deamination of AMP to form IMP and ammonia. The function of AMP deaminase in cellular metabolism remains obscure, but a number of physiological roles have been proposed. The enzyme may be responsible for the conversion of adenine nucleotide to inosine or guanine nucleotide [1–4], for stabilizing the adenylate energy charge [5,6], and for the reaction of the purine nucleotide cycle [7,8].

Chromatographic, kinetic, and immunological data strongly suggested the existence of three parental isozymes in rat [9–11]; isozyme A (muscle type), B (liver type) and C (heart type). All of them are allosteric enzymes being regulated by alkali metal ions, nucleoside di- or triphosphates and inorganic phosphate.

It has been reported that AMP deaminase interacts with the macromolecules, erythrocyte membrane [12,13] and myosin [14–16]. In this paper, we show that AMP deaminase activity can be regulated in the presence of RNAs and that purified rat AMP deaminase binds tightly to RNAs and the binding appears to be affected by the presence of certain ligands.

Materials and Methods

Nucleotides were obtained from Boehringer Mannheim. Ribonuclease T_1 and T_2 were the products of Sankyo Co. Ltd., Tokyo. Other reagents were commercial preparations of the highest purity available.

Isozymes A and B of AMP deaminase were prepared from rat leg muscle and liver, respectively, and brought to apparent homogeneity by the methods described previously [10]. Isozyme C was also purified from rat heart extracts as reported previously [9].

The deamination of AMP to IMP was measured by following the decrease in absorbance at 265 nm minus 300 nm on a 356 Hitachi two-wavelength

double-beam spectrophotometer at 25°C. Reaction mixtures typically contained 50 mM imidazole-HCl (pH 6.5)/100 mM KCl/AMP at the indicated concentrations enzyme/water to a final volume of 0.3 ml. The reaction was carried out in a 3 ml cuvette containing a silica insert to reduce the light path to 0.1 cm. In the enzyme-RNA binding experiments, enzyme activity was measured colorimetrically by estimating production of ammonia. The reaction mixture of 0.25 ml contained 30 mM AMP/20 mM potassium phosphate, pH 7.0/0.15 M NaCl/0.1% 2-mercaptoethanol/30 μg bovine serum albumin. The amount of ammonia was determined by the phenol-hypochlorite reagents [17]. The reaction was usually carried out at 37°C for 10 min. 1 unit deaminase activity is defined as the amount of enzyme which consumes 1 μmol AMP/min.

18 S and 28 S RNAs were prepared from rat liver ribosomes, by phenol extraction followed by centrifugation through sucrose density gradient according to the method described by Girard [18]. tRNA was also prepared from the pH 5 fraction of rat liver.

Results

Effect of RNAs on the enzyme activity of three AMP deaminase isozymes

In Fig. 1 the effect of 28 S ribosomal RNA at a concentration of 0.027 A_{260} unit/ml on the enzyme activity of muscle, liver and heart AMP deaminases is shown. As each of the three parental isozymes has a different K_m value for AMP, the effect of RNA was tested in the presence of different concentrations of AMP. At any given level of AMP, the muscle enzyme was activated, whereas the liver and heart enzymes were inhibited.

The effect of various concentrations of 28 S RNA on the activity of three isozymes was then tested. As shown in Fig. 2, the muscle enzyme was activated at low concentration of RNA and the concentration required to give half-maximal activation was about 0.001 A_{260} unit/ml. In contrast to the muscle enzyme, the liver and heart enzymes were inhibited by 28 S RNA. The liver enzyme was inhibited at lower concentrations of RNA in comparison with those required to inhibit the heart enzyme. The concentration required to give half-maximal inhibition

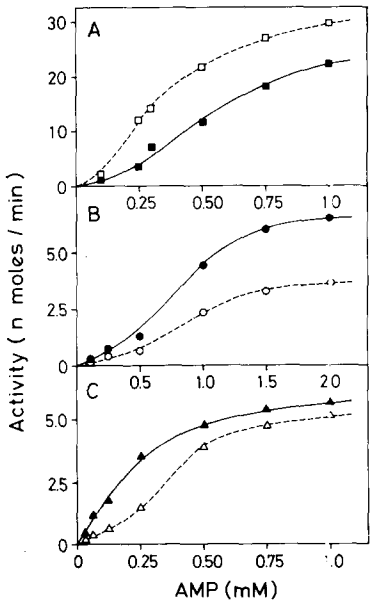

Fig. 1. Effect of 28 S ribosomal RNA on the velocity of the skeletal muscle, liver and heart enzymes. The reaction mixture contained 50 mM imidazole-HCl, pH 6.5/100 mM KCl/ various concentrations of AMP/enzyme in a final volume of 0.3 ml. Enzyme activity was measured by following the decrease in absorbance at 265 nm minus 300 nm at 25°C. The reaction was carried out in the presence (open symbols) or absence (closed symbols) of 0.027 A_{260} unit/ml 28 S RNA. A, isozyme A from skeletal muscle; B, isozyme B from liver; C, isozyme C from heart.

was less than 0.0005 A_{260} unit/ml for the liver enzyme, but greater than 0.01 A_{260} unit/ml for the heart enzyme. Thus, heart AMP deaminase required 20-times or more higher concentrations of RNA to achieve the inhibition comparable to those elicited by RNA with liver enzyme.

The effect of 18 S RNA and tRNA on the enzyme activity was also tested. They both activated the enzyme activity of muscle enzyme, and inhibited the activity of the liver and heart enzymes. On the basis of a concentration of A_{260} unit/ml, 28 S RNA and 18 S RNA were equally effective in activating the muscle enzyme and inhibiting the liver and heart enzymes, but tRNA was less active compared to 18 S or 28 S RNA.

To test whether the effect of RNA is specific to RNA, the effect of calf thymus DNA treated with pronase and RNAase was also examined under the experimental conditions described in the legend to

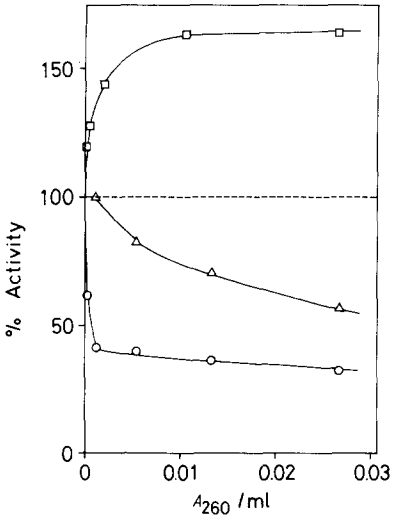

Fig. 2. Effect of various concentrations of 28 S RNA on enzyme activity. Enzyme activity was measured by following the decrease in absorbance at 265 nm minus 300 nm. The reaction mixtures contained 0.3, 1 and 0.1 mM AMP for muscle (□), liver (○) and heart (△) enzymes, respectively.

Fig. 2. At concentration of 0.007 A_{260} unit/ml, the activation or inhibition was not observed, but at 10-times higher concentration of DNA the activities of muscle, liver and heart enzymes were all inhibited approx. 30%.

To test further whether the macromolecular structures of RNA are required for modulation of enzyme activity, 28 S RNA was digested by RNAase T_1 and the effect of products was reexamined. As shown in Table I, the effects of RNA disappeared after RNAase T_1 treatment. Essentially the same results were observed by RNAase T_2 treatment. These results indicated that the macromolecular structure of RNAs is essential for activation of inhibition of AMP deaminase.

Binding of AMP deaminase to RNAs

Sedimentation velocity experiments were then performed using AMP deaminase purified from rat skeletal muscle and RNAs. The results of mixing AMP deaminase and RNAs in 10 mM imidazole-HCl/0.1 M KCl, pH 6.5, at the excess molar ratios of tRNA, 18 S and 28 S RNAs to the enzyme are shown in Fig. 3. Fig. 3A shows the sedimentation velocity patterns for AMP deaminase. When tRNA was added, the faster sedimenting peak which

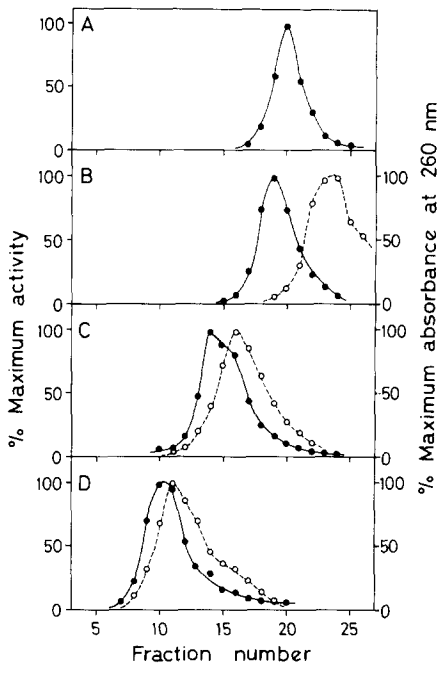

Fig. 3. Binding of muscle AMP deaminase to RNAs. Muscle AMP deaminase (4 units) was mixed with 1.8 A_{260} units tRNA, 18 S RNA and 28 S RNA in the presence of 20 mM imidazole-HCl, pH 7.0/0.1 M KCl. The mixture or the deaminase alone was then applied onto 4.8 ml 5–25% sucrose gradient containing 20 mM imidazole-HCl, pH 7.0/0.1 M KCl. Absorbance at 260 nm (○) and the enzyme activity (●) in the fractions were determined after centrifugation in a Hitachi RPS-50 rotor at 45 000 rev./min for 3 h at 4°C. A, muscle AMP deaminase alone; B, enzyme + tRNA; C, enzyme + 18 S RNA; D, enzyme + 28 S RNA.

represents a complex between the enzyme and tRNA appeared (Fig. 3B). The pattern in Fig. 3C shows the results of mixing AMP deaminase and 18 S ribosomal RNA. AMP deaminase activity sedimented much faster than the free enzyme and a little faster than 18 S RNA. Sucrose density gradient centrifugation experiments were also performed using AMP deaminase and 28 S ribosomal RNA and the data are shown in Fig. 3D. The enzyme activity peak which appears to represent a complex between the enzyme and 28 S RNA has a larger S value than RNA and sedimented much faster than the unbound enzyme.

The data suggest the formation of a complex composed of 1 mol RNA bound/mol enzyme under excess molar ratio of RNA to enzyme, since the complex composed of 2 mol or more RNA/mol enzyme

TABLE I

EFFECT OF RNAase T_1 TREATMENT OF 28 S RNA ON THE ENZYME ACTIVITY

RNAase treatment was carried out at 37°C for 30 min. A reaction mixture of 0.5 ml contained 50 mM Tris-HCl, pH 7.5/2 mM EDTA/250 units RNAase T_1 and 0.8 A_{260} unit 28 S RNA. 28 S RNA or RNAase T_1 alone was also incubated under the same conditions. 10 μl solution were added to the assay mixture (0.3 ml) for AMP deaminase and the effect was determined (% activity). AMP concentrations used were 0.3, 1 and 0.1 mM for muscle, liver and heart enzymes, respectively.

Mixture contained	Isozymes from		
	Muscle	Liver	Heart
None	100	100	100
28 S RNA	182	40	44
RNAase T_1	100	113	89
28 S RNA + RNAase T_1	106	93	106

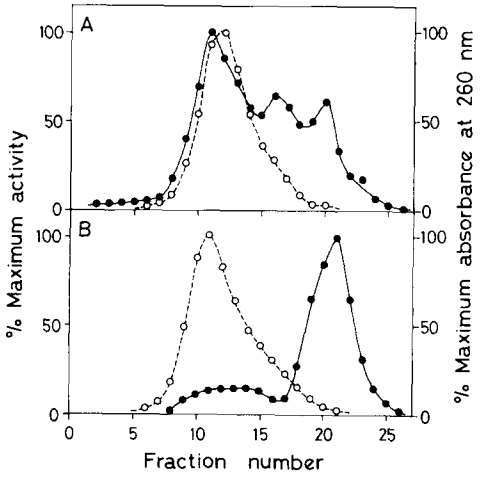

Fig. 4. Effect of KCl concentration on the release of the enzyme from RNA-enzyme complex. Muscle AMP deaminase (4 units) was mixed with 1.8 A_{260} units of 28 S RNA in the presence of 20 mM imidazole-HCl, pH 7.0/0.15 M KCl (A) or 0.2 M KCl (B). The mixture was then applied onto a 5–25% sucrose gradient containing 20 mM imidazole-HCl, pH 7.0/0.15 M KCl or 0.2 M KCl. Centrifugation was carried out under the same conditions described in the legend to Fig. 3. Absorbance at 260 nm (○); enzyme activity (●).

should have a much larger S value than those observed.

Effect of KCl and pH on the release of AMP deaminase from RNA-enzyme complex

In the experiments represented in Fig. 4, the mixture of AMP deaminase and 28 S RNA was centrifuged in a sucrose gradient of increasing concentrations of KCl. KCl had no effect on AMP deaminase release below 0.1 M, but there was a progressive increase in enzyme release between 0.1 and 0.2 M KCl. In 0.15 M KCl, the complex was partially dissociated and in 0.2 M KCl most of the enzyme activity was dissociated from the complex.

The effect of pH on the enzyme release from RNA-enzyme complex was also examined. In the presence of 0.1 M KCl, there was little enzyme release within the pH range 5.1–7.0, but 100% of bound enzyme was released when the pH was increased from 7.0 to 7.5.

Effect of nucleotides on the dissociation of the complex

A number of purine and pyrimidine nucleotides known as the effectors of AMP deaminase were tested for their ability to dissociate the enzyme from RNA. The results showed that ATP, GTP, ADP, CTP, UTP and pyrophosphate at 1 mM effectively dissociated the enzyme-RNA complex. Inorganic phosphate was relatively ineffective in promoting dissociation and no release of enzyme was observed at 1 mM concentration; but at 10 mM enzyme was released.

Saturability of AMP deaminase binding to RNA

In this experiment decreasing concentrations of 28 S RNA were mixed with a constant amount of AMP deaminase and sedimentation velocity experiments were then performed. As shown in Fig. 5, centrifugation of the solution in sucrose density gradient revealed many species with distinct sedimentation indicating the binding of different amounts of enzyme to RNA. In the presence of excess RNA, a single activity peak was observed (Fig. 5a). By increasing the ratio of enzyme to RNA, the original enzyme peak was reduced and faster moving peaks appeared (Fig. 5b, 5c), which represent a complex composed of more than 2 mol AMP deaminase bound/mol RNA. By further increasing the ratio of

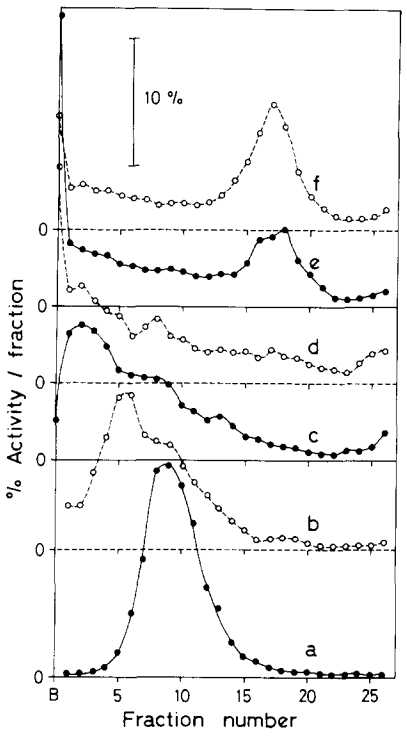

Fig. 5. Effect of RNA concentrations on the binding of the enzyme to RNA. 4 units muscle AMP deaminase were mixed with different amounts of 28 S RNA and sucrose density gradient centrifugation was carried out in a RPS-50 rotor at 20000 rev./min for 17 h at 8°C. The amounts of 28 S RNA used were: 0.58 A_{260} unit (a); 0.58/6 unit (b); 0.58/12 (c); 0.58/48 (d); 0.58/96 (e); 0.58/192 (f).

enzyme to RNA, most of the enzyme activity was observed in the bottom fractions (Fig. 5d). Finally the binding was saturated and the enzyme peak corresponding to the free enzyme appeared (Fig. 5e, 5f). The maximum number of AMP deaminase molecules bound/mol RNA seems to be approx. 30, since the ratio of the enzyme to RNA was 22 : 1 in Fig. 5d, where no unbound form of enzyme was observed, and 44 : 1 in Fig. 5e, where the unbound form was detected. We assumed the molecular weight and specific activity of muscle AMP deaminase were 290000 and 1800 [10], respectively, and the molecular weight and A_{260} of a 0.1% solution of 28 S RNA as $1.75 \cdot 10^6$ and 22, respectively.

Isozyme specificity for formation of RNA-enzyme complex

To examine whether the binding to RNA was specific for muscle AMP deaminase, the isozymes from rat liver and heart were also tested for binding to 28 S RNA. Under the same experimental conditions as those used with the muscle enzyme (Fig. 3D), the enzyme activity peak was found in Fraction 10 with both liver and heart AMP deaminases, indicating the formation of a RNA-enzyme complex with both isozymes. The interaction of liver and heart enzymes as well as muscle enzyme with RNA was also confirmed by binding of enzyme to tRNA-Sepharose prepared according to Wagner et al. [19] by covalent coupling of rat liver tRNA to Sepharose 4-B. In the presence of 20 mM imidazole-HCl, pH 7.0/0.1 M KCl, the AMP deaminase activity of all three isozymes was retained on the RNA-Sepharose column. The enzyme activity adsorbed on the column was completely eluted with the buffer containing 0.1 M KCl/5 mM pyrophosphate. These findings indicate that although RNA inhibited the liver and heart enzymes in contrast to the muscle enzyme, which is activated by RNA, there was no isozyme specificity for the formation of RNA-enzyme complex.

Discussion

AMP deaminase has been found to interact with the erythrocyte membrane and myosin. Rao et al. [12] found that erythrocyte AMP deaminase associated with the erythrocyte membranes prepared by hypotonic lysis. Recently, Pipoly et al. [13] demonstrated the binding of purified human erythrocyte AMP deaminase to human erythrocyte membranes and its preferential and specific binding to the cytoplasmic surface of the membrane. In muscle, AMP deaminase has been observed to be a persistent minor contaminant of myosin preparation [14]. Ashby and Frieden [15] showed that rabbit skeletal muscle AMP deaminase binds to rabbit muscle myosin, heavy meromyosin and sub-fragment 2. But in rat, the enzyme from muscle interacted mainly with the light meromyosin portion of the myosin fraction [16].

It seems clear from the present studies that AMP deaminase interacts with tRNA, 18 S and 28 S RNA to form a complex. RNAs interacted with three parental AMP deaminase isozymes, all of which have been found to differ from one another in chromatographic and immunochemical properties [9].

Several analogies exist between the binding of AMP deaminase to the erythrocyte membrane, myosin and RNA. Like the erythrocyte membrane and myosin, the binding of AMP deaminase to RNAs was responsive to alterations of ionic strength, pH and concentrations of various ligands. Dissociation of the RNA-enzyme complex is found at physiological ionic strength and pH. The RNA-enzyme dissociation curve was steep between 0.1 and 0.2 M KCl, and also the association and dissociation occurred between pH 7.0 and 7.5. These results indicate that the minor fluctuations of ionic strength and pH within the physiological range could have major effects on enzyme binding. However, the evidence that physiogical levels of nucleotides such as ATP promote dissociation of the RNA-enzyme complex, makes their physiological meanings complicated.

A stoichiometry of about 2 mol AMP deaminase bound/mol myosin was observed in interaction between the muscle enzyme and myosin in rabbit [15]. But 0.38 mol AMP deaminase was bound to 1 mol myosin in rat [16]. Although we could not determine the precise number of AMP deaminase molecules bound/mol 28 S RNA, as shown in Fig. 5 a large number of enzyme molecules were observed to bind to RNA.

It has been reported that the binding of muscle AMP deaminase to myosin enhanced the activity [20], whereas the binding of erythrocyte enzyme to the erythrocyte membrane reduced the enzyme activity [13]. It is of interest to note that the interaction of enzyme with RNAs enhances the enzyme activity of the muscle enzyme but reduces the activity of the liver and heart enzymes, although all three enzymes associate with RNAs to form a complex.

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