INHIBITION OF AMP DEAMINASE OF BOVINE BRAIN AND LIVER BY FATTY ACYL-CoA DERIVATIVES

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1. Introduction

The importance of AMP deaminase in the purine nucleotide cycle [1,2], interconversion of adenine, inosine, and guanine nucleotides [3–6] and stabilization of adenylate energy charge [7] in various tissues makes this enzyme an interesting subject for enzymological and physico–chemical studies from the regulatory point of view. In the previous papers, we have reported some regulatory properties of AMP deaminase purified from chicken erythrocytes [8–11] and allosteric inhibition of the enzyme by phytic acid, the principal organic phosphate in the avian red cells [12]. We now report the potent inhibition of AMP deaminase by fatty acyl-CoA (FA-CoA), and discuss its physiological significance.

2. Materials and methods

2.1. *Materials*

Free fatty acids (FAs) and FA-CoA derivatives were obtained from Sigma. Nucleotides were products of Kyowa Hakko Co. All other chemicals were purchased from commercial sources.

2.2. *Preparation of AMP deaminase from bovine brain and liver*

Bovine brain and liver (100 g each) were homogenized in 5 vol. of 20 mM potassium phosphate buffer, pH 7.1, containing 0.1 M NaCl and 0.1% 2-mercaptoethanol (buffer A) with a polytron homogenizer. The extracts were centrifuged in a Hitachi 65P ultracentrifuge at 40 000 rev./min for 60 min to obtain supernatant. The supernatant was applied to a column (1.5 × 6 cm) of phosphocellulose equilibrated with buffer A. Elution of the enzyme was accomplished by first washing of the column with 100 ml of buffer A, and then using a 0.1–1.0 M NaCl linear gradient (300 ml) in the same buffer. In these conditions, the extracts yielded only one peak of activity on phosphocellulose column. The fractions with maximum activity were collected and dialyzed against buffer A. The dialyzed solution was applied to DEAE-cellulose column (2.5 × 25 cm) equilibrated with the above buffer. The enzyme was eluted with linear gradient from 0.1–1.0 M NaCl in 20 mM potassium phosphate buffer, pH 7.1 and 0.1% 2-mercaptoethanol. The fractions with maximum activity were pooled and used for kinetic experiments.

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2.3. *Assay of AMP deaminase activity*

Enzyme activity was measured colorometrically by estimating production of ammonia. For the kinetic experiments, the reaction mixture of 0.25 ml contained 60 mM potassium phosphate buffer, pH 7.1, 0.1 M NaCl, various concentrations of AMP and effectors, and the enzyme. The amount of ammonia was determined by the phenol–hypochlorite reagent [13]. The reaction was usually carried out at 37°C for 5 min.

3. Results

Table 1 shows the effects of various FA-CoA derivatives on the bovine brain AMP deaminase activity.

Table 1
Effect of fatty acids and their acyl-CoA derivatives on the bovine brain AMP deaminase activity

Additions	Concentrations (μM)	Relative velocity
None	–	1.00
CoA	12.5	1.08
Acetyl-CoA	12.5	1.03
Oleoyl-CoA	6.1	0.76
	12.5	0.44
Palmitoyl-CoA	6.1	0.28
	12.5	0.08
Stearoyl-CoA	6.1	0.05
	12.5	0.03
Oleic acid	100	0.99
	200	0.85
Palmitic acid	100	1.11
	200	1.39
Stearic acid	100	1.13
	200	1.39

The reaction mixture consisted of 50 mM potassium phosphate buffer, pH 7.1, 25 mM AMP, various concentrations of FA-CoA compounds or FAs, and the enzyme in a final volume of 0.25 ml. The assay conditions were described in Materials and methods.

As can be seen from the table, long-chain FA-CoA derivatives inhibited the enzyme to various extents, although CoA and acetyl-CoA were without effects, FAs at 100 μM have no inhibitory effect on the enzyme acitivity, while palmitate and stearate at 200 μM showed a tendency to activate the enzyme slightly.

The effect of increasing concentrations of oleoyl-, palmitoyl-, and stearoyl-CoA on the brain and liver enzyme activity was examined. As shown in fig.1, all FA-CoAs showed a powerful inhibition. The concentrations necessary for 50% inhibition of the brain enzyme activity, $I_{0.5}$ values for stearoyl-, palmitoyl-, and oleoyl-CoA were 2, 5, and 11 μM, respectively. $I_{0.5}$ values of the bovine liver enzyme for these acyl-CoA derivatives were 5, 12, and 25 μM, respectively, which are relatively higher in comparison with those of the brain enzyme.

We explored the mechanism of inhibition by FA-CoA and studied the affinity of the brain AMP deaminase for the substrate in the absence and presence of palmitoyl-CoA. Figure 2 indicates that

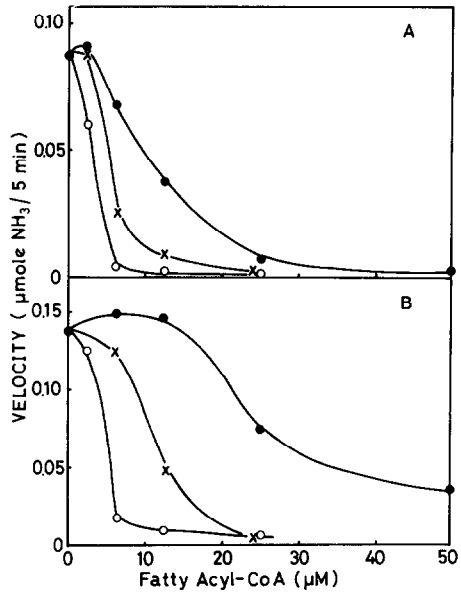

Fig.1. Effect of increasing concentrations of long-chain fatty acyl-CoA derivatives on the AMP deaminase activity. A. Bovine brain enzyme. B. Bovine liver enzyme. The reaction mixture consisted of 50 mM potassium phosphate buffer, pH 7.1, 25 mM AMP, various concentrations of FA-CoAs, and the enzyme in a final volume of 0.25 ml. The reaction was carried out at 37°C for 5 min. The amount of ammonia was determined by the phenol–hypochlorite reagent [13]. (•) Oleoyl-CoA. (X) Palmitoyl-CoA. (○) Stearoyl-CoA.

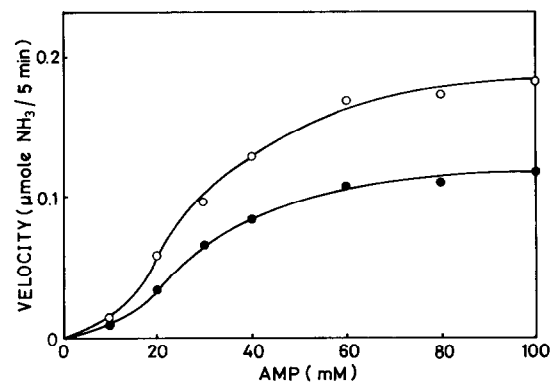

Fig.2. Effect of fatty acyl-CoA on the velocity with respect to AMP concentration. The reaction mixture was similar to that in fig.1 except that the concentration of AMP was varied, and the palmitoyl-CoA concentration was fixed at 6.25 μM. (○) No addtion. (•) 6.25 μM Palmitoyl-CoA added.

the inhibition of the enzyme by palmitoyl-CoA is exerted through a 'non-competitive' mechanism and it involves no change in the affinity of the enzyme for the substrate.

It has been reported that free FAs or their acyl-CoA derivatives dissociate and consequently inactivate the polymeric enzymes [14,15]. In order to examine this possibility, we investigated the effects of preincubation of the enzyme with FA-CoA on the activity and molecular size of the enzyme. As a result of the experiments, prolonged preincubation of the enzyme with FA-CoA compounds did not change the enzyme activity, and furthermore, sucrose density gradient centrifugation revealed that the *s* value of the enzyme is approximately 10 *s* in the absence and presence of FA-CoA. Therefore, the association or dissociation of the enzyme seems not to be involved in the inhibition by FA-CoA.

4. Discussion

Activation and inhibition of various enzyme activities by free FAs and FA-CoAs were demonstrated in connection with metabolic control [17–19]. However, as is well known, long-chain acyl-CoA derivatives are strong surfactants and consequently the regulatory significance of FA-CoAs and free FAs as negative effectors has been questioned largely because of the difficulties in distinguishing possible non-specific detergent effects from more specific regulatory interactions with enzymes including glucose 6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.3) and malate dehydrogenase (EC 1.1.1.37) [20]. FA-CoA-inhibition of several enzymes was shown to be due to the ligand-induced irreversible dissociation of polymeric enzymes [14,15], which was thought to be non-specific detergent effects. A doubt is thus thrown on the physiological significance of the irreversible inactivation by FA-CoA, since the reversibility is one of the requirements for an effector [16]. Recently, Hsu and Powell have demonstrated that (1, N^6) oleoyl-CoA, which is a more potent detergent than oleoyl-CoA, is much less inhibitory to citrate synthase (EC 4.1.3.4) and not appreciably bound [21]. Thus, the detergency and the inhibition were experimentally separable, demonstrating that the inhibition was a specific characteristic of FA-CoA and was independent of its detergent properties. A specific interaction between the adenine moiety of oleoyl-CoA and citrate synthase was suggested. A physiological role of FA-CoAs as negative effectors for enzymes such as citrate synthase seems reasonable.

It is evident that AMP deaminase was reversibly inhibited by low concentration of FA-CoA compounds, whereas free FAs have no remarkable effect at the same concentrations, suggesting that the polar protion of FA-CoA (including the adenine of CoA) is responsible for the inhibition by FA-CoAs. However, since both CoA and acetyl-CoA have no inhibitory effect on the enzyme, the non-polar portion of FA-CoA is necessary for a specific interaction between AMP deaminase and FA-CoA molecules.

The physiological function of AMP deaminase has remained obscure, although AMP deaminase is known to be physiologically important in the interconversion of adenine and guanine nucleotides [3–6], and as a key enzyme in the purine nucleotide cycle [1,2]. Recently, the importance of the enzyme in the stabilization of adenylate energy charge* was demonstrated in rat liver [7]. It is generally known that a relatively small drop in the energy charge value is accompanied by a decrease in total concentration of adenine nucleotides, when animal is subjected to metabolic stress. The AMP deaminase activity increased with decreasing energy charge is suggested to have a buffering effect on the adenylate energy charge, i.e., the decrease in charge will be opposed by AMP deamination (since removal of AMP must increase the mole fraction of ATP and ADP) [7]. AMP deaminase is thus assumed to act as a part of the system that protects against wide excursions of adenylate energy charge. Fatty acid respiration is well known to decrease the adenylate energy charge in cells: Williamson et al. [22] reported that the addition of oleic acid to perfused liver system decreased the energy charge with the small decrease in total adenine nucleotides pool. These results are accounted for as follows: the potent inhibition of AMP deaminase by FA-CoA metabolized from FA diminishes the removal of AMP and as a consequence causes the decrease in energy charge. The results presented

*Adenylate energy charge = ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]) see, ref. [7].

here are consistent with the hypothesis that AMP deaminase functions as a stabilizer of adenylate energy charge in animal cells.

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