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IN VITRO AND IN SITU STUDIES ON THE INHIBITION OF YEAST AMP DEAMINASE BY FATTY ACIDS

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The effect of various fatty acids on the purified and in situ AMP deaminase (AMP aminohydrolase, EC 3 5 4 6) was investigated both the purified AMP deaminase and the permeabilized system of yeast cells were used as the enzyme sources (1) All the saturated fatty acids, longer than 10 in the hydrocarbon chain, were inhibitors of the purified enzyme in the absence of ATP, whereas no or little inhibition of the enzyme was observed in the presence of ATP Unsaturated fatty acids acted as more potent inhibitors of the purified enzyme, although the addition of ATP increased the $I_{0\,5}$ values for these fatty acids Fatty acids acted as non-competitive inhibitors without alteration of the affinity for the substrate in the absence and presence of ATP (2) Unsaturated fatty acids showed a powerful inhibition of the in situ AMP deaminase, and the presence of ATP could scarcely affect the inhibition of the in situ enzyme by these fatty acids On the other hand, no or little inhibition of the in situ enzyme by saturated fatty acids was observed in the absence and presence of ATP The difference in the kinetic properties between the in situ and the purified enzyme suggests that there is difference in protein interactions for AMP deaminase in situ and in vitro

Introduction

AMP deaminase (AMP aminohydrolase, EC 3 5 4 6), which is distributed in a variety of eukaryotes such as those from mammals, birds, amphibians [1], yeasts [2] and higher plants [3,4], may be important in the stabilization of adenylate energy charge, the control of adenylate pool size [5–7], and as a regulatory enzyme in the purine nucleotide cycle [8] in various mammalian tissues The AMP deaminase activity increasing with decreasing energy charge is considered to act as a part of the system that protects against wide excursions of adenylate energy charge at the expense of the concentration of the adenylate pool size [5] The activity of AMP deaminase is regulated by fatty acids [9] and their coenzyme A esters [6] the inhibition of the enzyme by fatty acyl-CoA and unsaturated fatty acids can explain the decrease in the adenylate energy charge during fatty acid respiration and under hypoxic conditions However, the physiological significance of the effects of fatty acids on the enzyme cannot be assessed directly from the kinetic study of the purified enzyme Recently, we presented a permeabilization method which allows the assay of intracellular enzymes within the boundaries of yeast cells, and the yeast AMP deaminase was demonstrated to be regulated in situ by a variety of effectors [10] In this study, we examined the effect of fatty acids on the AMP deaminase activity, both the purified yeast enzyme and the permeabilized system of yeast cells Fatty acids inhibited the enzyme in vitro and in situ unsaturated fatty acids acted as more potent inhibitors than saturated fatty acids did The inhibition of AMP deaminase by fatty acids suggests some differences in protein interactions for AMP deaminase in situ and in vitro

Materials and Methods

Materials Fatty acids were obtained from Sigma Chemical Co Nucleotides were obtained from

Yamasa Co (Tokyo, Japan) Commercial baker's yeast (*Saccharomyces cerevisiae*) was purchased locally

Methods Baker's yeast AMP deaminase was purified as a homogeneous preparation according to the method described previously [2] Preparation of permeabilized yeast cells was as reported previously, which included toluene treatment at 40–42°C [10] The enzyme activity was determined as described previously [2] the reaction mixture of 1 0 ml contained 10 mM cacodylate buffer, pH 7 1, 0 1 M KCl, various concentrations of AMP and effectors and 0 1 μg purified enzyme or 4 mg permeabilized yeast cells

For the preparation of fatty acid solution, each fatty acid was sonicated in 10 mM cacodylate buffer, pH 7 1, for 5 min with a Branson sonifier The reaction was carried out at 37°C for 5 min The progress curves of the reaction were linear with time in the presence or absence of fatty acids under these conditions The amount of ammonia was determined by the phenol-hypochlorite reagent [11]

Results

Effect of fatty acids on the purified AMP deaminase

Fig 1 shows the effect of various fatty acids on the activity of purified AMP deaminase It is clear that all the saturated fatty acids, longer than 10 in the hydrocarbon chain, inhibit the enzyme in the absence of ATP the $I_{0.5}$ values, the concentrations necessary for 50% inhibition of the enzyme, were shown to decrease with the increasing chain length of the saturated fatty acids (Fig 1) A notable exception appears to be lauric acid which acted as the most powerful inhibitor These results suggest that the hydrophobic interactions are involved in the inhibition of the enzyme by fatty acids When 1 mM MgATP was included, saturated fatty acids gave no or little inhibition of the purified enzyme

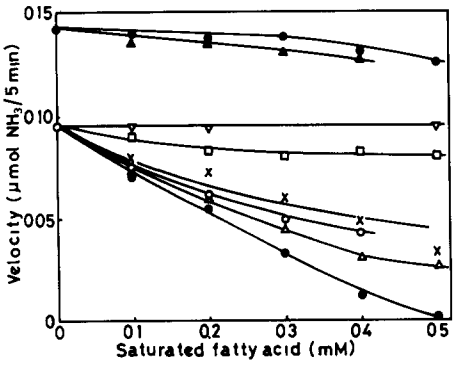

Fig 1 Inhibition of purified AMP deaminase by saturated fatty acids in the absence and presence of ATP The reaction mixture of 1 ml consisted of 10 mM cacodylate buffer, pH 7 1, 5 mM AMP, 100 mM KCl, various concentrations of fatty acids, and the enzyme in the absence and presence of 1 mM ATP and 1 mM $MgCl_2$ ▽——▽, caprylate, □——□, caprate, ⊕——⊕, laurate, X——X, myristate, ○, ●, palmitate, △, ▲, stearate Open and closed symbols show the values in the absence and presence of ATP and $MgCl_2$, respectively

Unsaturated fatty acids acted as more potent inhibitors of the enzyme in the absence of ATP the $I_{0.5}$ values for arachidonate, oleate, linoleate and linolenate were 0 03–0 06 mM (Fig 2), which are remarkably low in comparison with those for the saturated fatty acids The addition of ATP also decreased the degree of the inhibition by unsaturated fatty acids the $I_{0.5}$ values for these fatty acids increased to 0 09–0 15 mM in the presence of MgATP

In order to examine the mechanism of the inhibition by fatty acids, we studied the affinity of the purified AMP deaminase for the substrate in the absence and presence of fatty acids The effect of fatty acids was largely on the maximal velocity of the enzyme the increase in the concentration of fatty acids decreased the maximal velocity when the

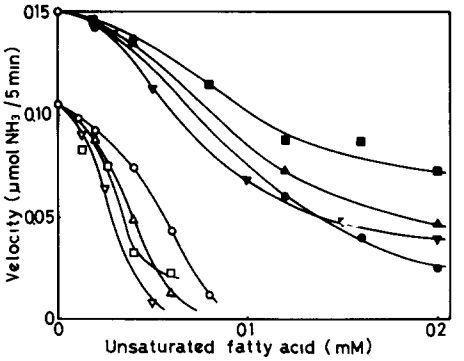

Fig 2 Inhibition of purified AMP deaminase by unsaturated fatty acids in the absence and presence of ATP The reaction mixture was similar to that described in Fig 1 □, ■, oleate, △, ▲, linoleate, ○, ●, linolenate, ▽, ▼, arachidonate Open and closed symbols show the values in the absence and presence of ATP and $MgCl_2$, respectively

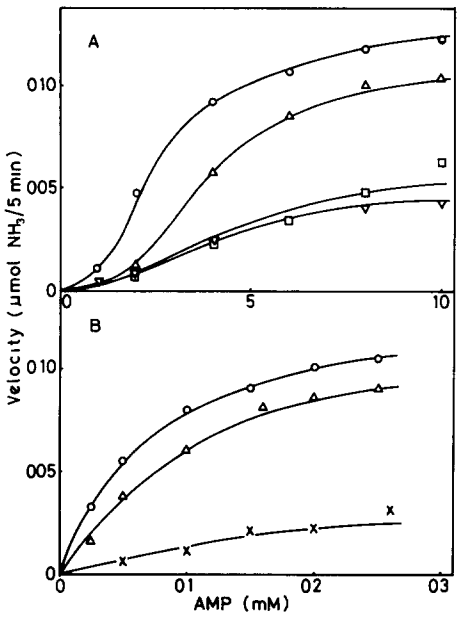

Fig 3 Effect of fatty acids on the velocity of purified AMP deaminase with respect to AMP concentration in the absence (A) and presence of ATP (B) The reaction mixture was similar to that described in Fig 1 except that the concentration of AMP was varied and the fatty acid concentration was fixed ○———○, no addition, △———△, 0 02 mM linolenate added, □———□, 0 03 mM linolenate added, X———X, 0 04 mM linolenate added, ▽———▽, 0 5 mM palmitate added

kinetics with respect to AMP were sigmoid (Fig 3A), or normalized (Fig 3B) in the absence or presence of ATP, respectively

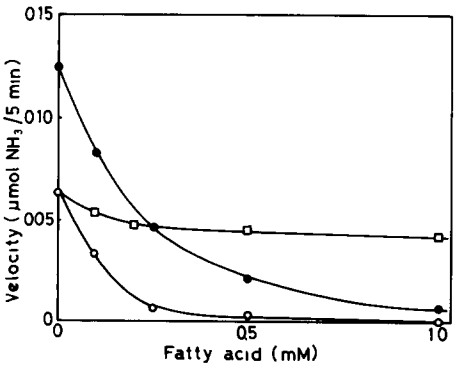

Fig 4 Inhibition of AMP deaminase in situ by fatty acids in the absence and presence of ATP The reaction mixture was similar to that described in Fig 1 except that the permeabilized yeast cells were used as the enzyme ○, ●, linolenate, □, oleate Open and closed symbols show the values in the absence and presence of ATP and $MgCl_2$, respectively

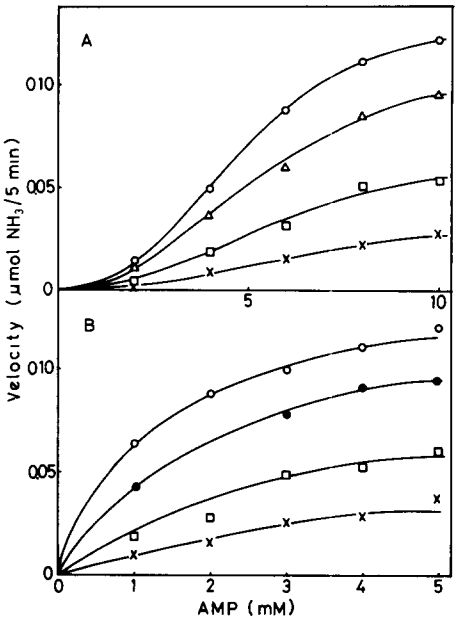

Fig 5 Effect of linolenate on the velocity of AMP deaminase in situ with respect to AMP concentration in the absence (A) and presence of ATP (B) The reaction mixture was similar to that described in Fig 1 except that the concentration of AMP was varied and the permeabilized yeast cells were used as the enzyme ○———○, no addition, ●———●, 0 1 mM linolenate added, △———△, 0 2 mM linolenate added, □———□, 0 3 mM linolenate added, X———X, 0 5 mM linolenate added

Effect of fatty acids on AMP deaminase in situ

We further examined the inhibitory properties of fatty acids on the AMP deaminase in situ using the system of permeable yeast cells Unsaturated fatty acids acted as an inhibitor of the in situ enzyme, whereas no or little inhibition of the enzyme by saturated fatty acids was observed linolenate showed a powerful inhibition in the absence of ATP, and the addition of ATP could scarcely affect the inhibition of the enzyme by linolenate (Fig 4)

The affinity of the in situ AMP deaminase for the substrate was examined in the absence and presence of fatty acids Fig 5 shows that the effect of linolenate was largely on the maximal velocity of the enzyme in the absence (Fig. 5A) and presence of ATP (Fig 5B), as demonstrated for the purified enzyme

Discussion

A large number of enzymes have been reported to be activated or inhibited by fatty acids and their

coenzyme A esters, and the physiological significance of these phenomena was discussed in connection with metabolic control the inhibition of glycolytic enzymes [12–14] and the activation of gluconeogenic enzyme [15] by fatty acids seem to have a physiological meaning in metabolic regulation Recently, we demonstrated the activation or inhibition of bovine brain and liver AMP deaminase by fatty acids [9] and their coenzyme A esters [6] Kinetic analysis of the effects of fatty acids on the enzyme have revealed that these effects can be qualitatively and quantitatively accounted for by a partial noncompetitive mechanism with respect to the substrate AMP Furthermore, a close correlation was observed between the fatty acid conformation and the kinetic parameters [9]

Kinetic studies on yeast AMP deaminase in vitro and in situ have revealed that the enzyme can be controlled by free fatty acids, as demonstrated for the mammalian enzyme [9] A striking difference in the effects of fatty acids on these AMP deaminases is the action of saturated fatty acids, which activate the bovine brain enzyme but inhibit the yeast AMP deaminase However, saturated fatty acids showed no or little inhibitory effect on the yeast enzyme in the presence of ATP (see Fig 1), whereas unsaturated fatty acids exhibited a powerful inhibition of the enzyme in vitro and in situ under the conditions where ATP was included or excluded (see, Figs 2 and 4) Fatty acid inhibition of several enzymes was shown to be due to the ligand-induced irreversible inactivation of enzymes [12–14], which was thought to be the effect of nonspecific detergent A doubt is thus thrown on the physiological significance of the irreversible inactivation by fatty acids, since the reversibility is one of the requirements for an effector [16] However, the inactivation of yeast AMP deaminase does not seem to be involved in the inhibition by fatty acids under these experimental conditions, because the progress curves of the reaction were linear with time in the presence of fatty acids These results suggest that yeast AMP deaminase is reversibly inhibited by fatty acids Furthermore, the reversibility of the fatty acid effect was more directly observed by dilution and then by adding bovine serum albumin (data not shown) Thus, unsaturated fatty acids can regulate the activity of AMP deaminase in yeast cells

Some kinetic parameters of the in situ enzyme such as K_m for AMP [10], $A_{0.5}$ for polyamines [10], $I_{0.5}$ for Zn^{2+} [10] and $I_{0.5}$ values for fatty acids are relatively higher in comparison with those of the purified enzyme [2,17,18] The decrease in the effective concentrations of these ligands within the cells through the interactions of these effectors with the lipid and/or protein of the membrane should be considered for the difference in the kinetic properties between the in situ and the purified enzyme However, the $I_{0.5}$ values of the purified AMP deaminase for unsaturated fatty acids were 0 03–0 06 mM, and markedly increased to approx 0 1 mM with the addition of ATP, whereas those values of the in situ enzyme remained as 0 10–0 15 mM with or without MgATP Thus, the difference in the kinetic properties between the in situ and the purified enzyme cannot be simply accounted for by the decreased concentrations of effectors through the membrane-ligand interactions, and thus suggests differences in protein interactions for AMP deaminase in situ and in vitro

Free fatty acids, in particular unsaturated fatty acids have a variety of metabolic functions The depletion of unsaturated fatty acids uncouples oxidative phosphorylation in yeast [19,20] unsaturated fatty acids are necessary for mitochondrial function as the membrane component Furthermore, as demonstrated in this paper, free unsaturated fatty acids can act as a physiological regulator of yeast AMP deaminase, which is responsible for the activation of phosphofructokinase [21], that is, glycolytic activity If the levels of free fatty acids are associated with the fatty acid content in membrane, the depletion of unsaturated fatty acids may result in the lowered activity of oxidative phosphorylation and the activation of AMP deaminase, which can stimulate glycolysis Free fatty acids have been considered to participate in the stimulation of gluconeogenesis and inhibition of glycolysis through the activation of gluconeogenesis enzyme and the inhibition or inactivation of glycolytic key enzymes [12–15] The levels of unsaturated fatty acids might further correlate with the metabolic interconversion between respiration and glycolysis in yeast

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