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STABILIZATION OF THE ADENYLATE ENERGY CHARGE BY THE DEPLETION OF ADENYLATES WITHOUT GLYCOLYTIC STIMULATION

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A relationship between the AMP deaminase reaction and the recovery of the energy charge was examined using permeabilized yeast cells. Citrate inhibited the glycolytic flux and the recovery of the energy charge. The addition of spermine enhanced the recovery of the energy charge without the reversal of the inhibition of glycolysis in the presence of excess citrate.

The AMP deaminase reaction can participate in the stabilization of the energy charge only by the depletion of total adenylates, and not by the glycolytic stimulation under the conditions where citrate is highly increased during aerobic growth conditions.

Control of the adenylate energy charge is closely related to the action of AMP deaminase. The enzyme stabilizes the energy charge by the depletion of adenylates (1), and further by the stimulation of glycolysis, which causes the regeneration of ATP (2-5). The stimulation of glycolysis accompanied by the depletion of adenylate pool as a result of the increased ATP hydrolysis (6-9) can be accounted for by the increase in ammonium ion produced by the AMP deaminase reaction (2,5). However, it remained obscure whether the stabilization of the energy charge by the depletion of adenylates only, which was pointed out by Chapman and Atkinson, was operative or not under the physiological conditions. We here report that the energy

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charge can be stabilized by the depletion of adenylates without glycolytic stimulation when glycolysis is inhibited by excess citrate. This mechanism may contribute to maintaining the higher energy charge under the aerobic conditions (10).

MATERIALS AND METHODS

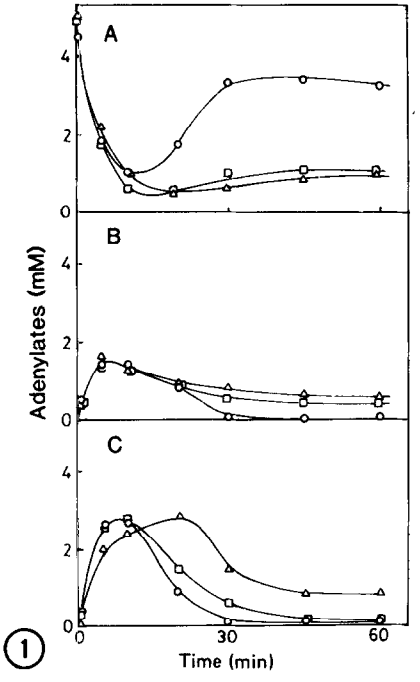
Materials - All chemicals were obtained from commercial sources as described previously (2-5). Baker's yeast (Saccharomyces cerevisiae) was purchased locally.

Incubation Conditions and the Determination of Metabolites- Baker's yeast was permeabilized as described previously (11). Reaction mixtures for the experiments of the energy charge control and the determination of metabolites were essentially similar to those described previously (2-5) except that various concentration of citrate was included.

RESULTS

Variation of adenine nucleotide concentration was examined in permeabilized yeast cells. After the addition of ATP and glucose, the decrease in ATP and the increase in ADP and AMP was observed (Fig.1) with the phosphorylation of glucose. Citrate markedly inhibited the regeneration of ATP (Fig.1A), possibly due to the inhibition of glycolysis as mentioned below. No difference in the regeneration of ATP between the group added with citrate and the one with citrate plus spermine (Fig.1A) suggests that spermine cannot enhance the ATP regeneration in the presence of citrate; however, the addition of spermine decreased the AMP level through the activation of AMP deaminase (Fig.1C).

Changes in the energy charge, total adenylates and ammonium ion were shown in Fig.2. The decrease in total adenylates had occurred before the energy charge began to rise, and the stoichiometric production of ammonium ion, corresponding to the decrease in the adenylates was observed (Fig.2B). The recovery of the energy charge was markedly inhibited by citrate (Fig.2A);



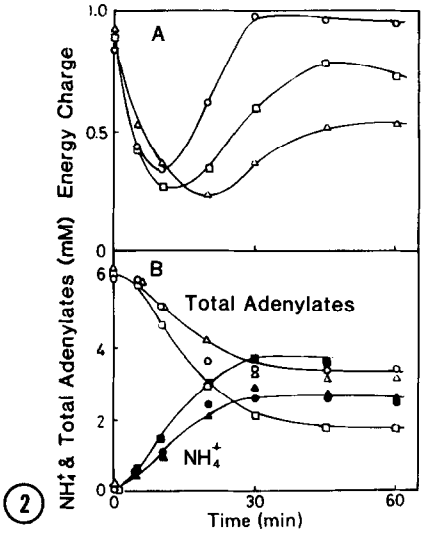


Fig. 1 Effects of citrate and spermine on the changes in the adenine nucleotides in permeabilized yeast cells. Reaction mixture of 1.5 ml contained 5 mM glucose, 6 mM ATP, 10 mM $MgCl_2$, 2 mM Pi, 1 mM NAD, 10 mM cacodylate buffer (pH 6.5), 40 mM K^+ and the permeabilized yeast cells (40 mg/ml) in the absence and presence of 10 mM citrate with or without 1.5 mM spermine. A. ATP; B. ADP; C. AMP. ○, No addition; △, 10 mM citrate added; □,10 mM citrate plus 1.5 mM spermine added.

Fig. 2 Effects of citrate and spermine on the changes in the adenylate energy charge, total adenylates and ammonium ion concentration. Incubation conditions were similar to those described in Fig. 1. Total adenylate concentrations were taken from Fig. 1. A. Energy charge. B. Total adenylates (open symbols) and ammonium ion concentrations (closed symbols). ●,○, No additon; ▲,△, 10 mM citrate added; ■,□, 10 mM citrate plus 1.5 mM spermine added.

however, the depletion of adenylates and the production of ammonium ion were not at all affected by citrate (Fig.2B). It is noteworthy that the addition of spermine enhanced the recovery of the energy charge through the depletion of adenylates without the regeneration of ATP in the presence of excess citrate (Fig.2).

Glycolytic flux was also determined under the same conditions. Citrate inhibited the glycolytic flux or the formation of pyruvate as demonstrated previously (3), and the

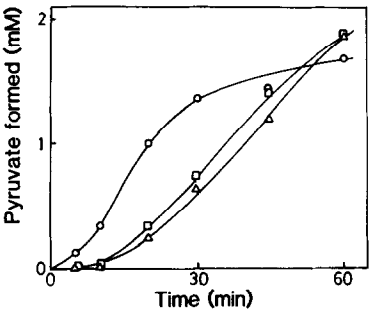


Fig. 3 Effects of citrate and spermine on the formation of pyruvate in permeabilized yeast cells. Incubation conditions were similar to those in Fig. 1. ○, No addition; △, 10 mM citrate added; □, 10 mM citrate plus 1.5 mM spermine added.

addition of spermine with citrate could not enhance the glycolytic flux (Fig.3) as well as the ATP regeneration in the presence of citrate (Fig.1A). However, spermine enhanced the recovery of the energy charge without the regeneration of ATP or the glycolytic stimulation in the presence of excess citrate. These results suggest that the energy charge can be recovered by the depletion of adenylates, but not by the stimulation of glycolysis under the experimental conditions.

DISCUSSION

AMP deaminase is responsible for the stabilization of the adenylate energy charge (1), and acts as a control system of glycolysis (2-5) and as a regulatory enzyme in the interconversion of purine nucleotides (12). Chapman and Atkinson pointed out that AMP deaminase participates in the stabilization of the energy charge by the depletion of adenylates, because the removal of AMP must increase the mole fraction of ATP and ADP (1). Recently, we have analyzed the relationship between the degradation of adenylates and glycolytic enhancement in yeast cells: the ammonium ion produced by the AMP deaminase reaction as a key enzyme in the adenylate degradation can play a principal role in the stimulation of phosphofructokinase (EC

2.7.1.11) and pyruvate kinase (EC 2.7.1.40)(2-5). Activation of AMP deaminase thus causes the recovery of the lowered energy charge through the glycolytic stimulation in yeast (2-5), although the removal of AMP may contribute more or less to the rise in the energy charge. Stimulation of glycolysis coupled to the decrease in the adenylate pool as a result of the decrease in ATP or the energy charge under the hypoxic or ischemic conditions (6-9), can be accounted for by the interaction of AMP deaminase with phosphofructokinase activity through the increased level of ammonium ion in various cells and tissues.

As shown in this paper, the principal factor in maintaining the energy charge is the depletion of adenylates, and not the glycolytic stimulation in the presence of excess citrate, which inhibits glycolytic flux. Yeast cells contain 5 or 10 mM of citrate under the anaerobic and aerobic growth conditions, respectively (13). Citrate, which increases in the aerobically grown cells, acts as an effective inhibitor of phosphofructokinase, and is considered to play a key role in the metabolic shift of glycolysis to respiration (13). The depletion of adenylates without glycolytic enhancement may be the principal factor responsible for the stabilization of the energy charge in the aerobically grown cells, whose glycolysis is inhibited by the increased citrate. Recently, Ashby et al. reported that the elimination of AMP deaminase activity had no effect on the glycolytic rate in platelets and they concluded that AMP deaminase play a role in maintaining the energy charge at 0.9 only by the depletion of adenylate pool (10). The role of AMP deaminase in the control of the energy charge in platelets may be accounted for by the same mechanism as described here.

The adenylate energy charge can be stabilized by the glycolytic stimulation coupled to the degradation of adenine nucleotides or by the depletion of the adenylate pool alone. The level of citrate may act as a principal factor which affects the interconversion between the two control systems of the energy charge.

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