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ROLE OF AMP DEAMINASE REACTION IN THE RESPONSE OF PHOSPHOFRUCTOKINASE TO THE ADENYLATE ENERGY CHARGE

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Summary The role of NH_4^+ ion and AMP deaminase reaction in the activation of phosphofructokinase with respect to its response to the adenylate energy charge was investigated using permeabilized yeast cells. (a) Phosphofructokinase and AMP deaminase were activated by the decrease in the adenylate energy charge. The addition of NH_4^+ further stimulated the phosphofructokinase activity in the presence of intracellular level of K^+, and the optimal energy charge value giving the maximal response of the enzyme was shifted from 0.3 to the value above 0.5. (b) The increase in NH_4^+ ion produced through the activation of AMP deaminase by spermine which shows no direct action on the phosphofructokinase activity can activate phosphofructokinase with shift of the optimal energy charge value of the enzyme to 0.5 in the presence of K^+, whereas the optimal energy charge value for AMP deaminase reaction was not affected by the addition of spermine. Phosphofructokinase can be activated most effectively by the physiological decrease in the energy charge under the condition of increased NH_4^+ in the presence of K^+. The possibility that the interaction of phosphofructokinase with AMP deaminase under hypoxic condition might be a contributing factor to the Pasteur effect is discussed.

Phosphofructokinase (EC 2.7.1.11) plays a principal role in the control of glycolysis (1-3). Ammonium ion was shown to act as an effective activator of phosphofructokinase, and the effect has been implicated in the mechanism of the Pasteur effect (1, 2, 4). The decrease in the adenylate energy charge and the elevated level of Pi as well as NH_4^+, which result from the increased hydrolysis and the decreased synthesis of ATP under the hypoxic and ischemic conditions (5-10), are suggested to play a part in the stimulation of phosphofructokinase and of the glycolytic flux (1-3, 11-14). Our previous papers have shown that the activation of phosphofructokinase can be essentially ascribed to the elevated NH_4^+ level under the conditions of increased ATP hydro-

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lysis (15-17) in permeabilized yeast (18). However, the effect of NH_4^+ on the modulation of phosphofructokinase by the energy charge has not been examined. Permeabilized yeast cells offer an excellent experimental system for the study on control of the adenylate energy charge, because of near equilibrium of adenylate kinase (EC 2.7.4.3) reaction, which can form AMP rapidly after increased hydrolysis of ATP (19). This paper describes the regulatory role of NH_4^+ in the response of phosphofructokinase to the variation in the energy charge: NH_4^+ activated the phosphofructokinase activity with the shift of the optimal energy charge value giving the maximal activity in the presence of physiological level of K^+: a key role of AMP deaminase reaction was demonstrated in the activation of glycolysis under the simulated *in vivo* conditions.

MATERIALS AND METHODS

Materials - All chemicals were obtained from commercial sources as mentioned in previous papers (15-19). Baker's yeast (*Saccharomyces cerevisiae*) was obtained locally.

Incubation Conditions and Determination of Metabolites - Baker's yeast was permeabilized as described previously (18). Reaction mixture of 0.5 or 0.25 ml contained 4 mM ATP plus AMP, 8 mM $MgCl_2$, 2.4 mM glucose 6-phosphate, 20 mM cacodylate buffer (pH 7.1), 40-60 mM KCl, and toluenized yeast cells (4 or 8 mg/ml) in the absence and presence of NH_4^+, spermine or linolenate. Aliquots of 0.2 ml were deproteinized after 5 min-incubation and then neutralized as described previously (16, 17). The supernatant was utilized for the determination of ammonia and fructose 1, 6-bisphosphate (Fru-1, 6-P_2) plus triose phosphates.

RESULTS

When the reaction was started by the addition of ATP and glucose 6-phosphate, formation of Fru-1, 6-P_2 and NH_4^+ was plotted against the energy charge value (Fig. 1). Phosphofructokinase responded to variation in the energy charge, and the slope of the energy charge response curve indicated an activation of the enzyme with the decrease in the energy charge. The addition of NH_4^+ effectively activated phosphofructokinase even in the presence of K^+, and the optimal value of the energy charge giving maximal activity was shifted from 0.3 to 0.5 (Fig. 1A). Production of NH_4^+ exhibited a typical characteristic of the ATP-regenerating enzyme: the AMP deaminase activity increased with the decrease in the energy charge (Fig. 1B), but the optimal

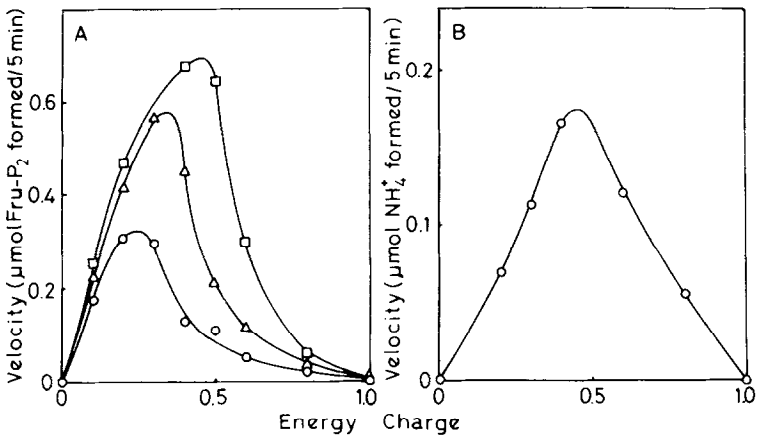

Fig. 1 Response of the activity of phosphofructokinase (A) and AMP deaminase (B) to variation in the adenylate energy charge in the absence and presence of NH_4^+ ion. Reaction mixture of 0.5 ml contained 4 mM ATP plus AMP, 8 mM $MgCl_2$, 2.4 mM glucose 6-phosphate, 20 mM cacodylate buffer (pH 7.1), 60 mM KCl, and toluenized yeast cells (4 mg/ml) in the absence and presence of NH_4^+. Aliquots of 0.2 ml were deproteinized after 5 min-incubation and then neutralized as described previously (16). The supernatant was utilized for the determination of Fru-1, 6-P_2 plus triose phosphates and ammonia (16). ○ , None; △ , 2 mM NH_4^+; □ , 5 mM NH_4^+ added.

energy charge value giving maximal response of the enzyme was not affected by the addition of NH_4^+ (data not shown).

The influence of the activation or inhibition of AMP deaminase reaction on the relationship between the phosphofructokinase activity and the energy

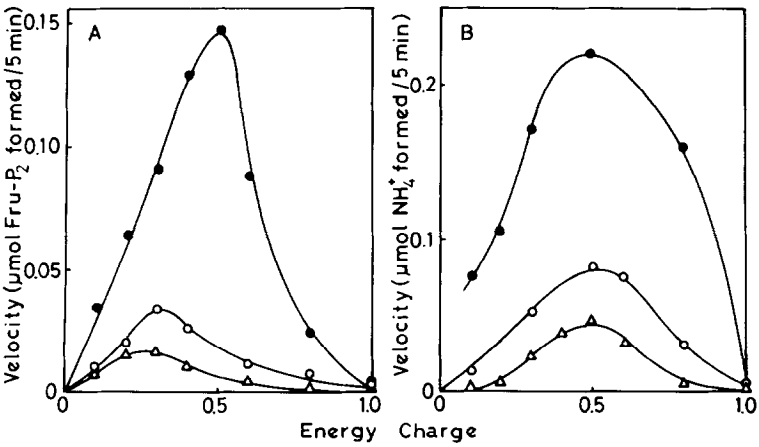

Fig. 2 Response of the activity of phosphofructokinase (A) and AMP deaminase (B) to variation in the adenylate energy charge in the absence and presence of 1 mM spermine or 2 mM linolenate. Reaction mixture of 0.25 ml was similar to that of Fig. 1 except that 8 mg/ml permeabilized yeast cells and 40 mM KCl were included. ○ , None; ● , 1 mM spermine added; △ , 2 mM linolenate added.

charge was investigated in permeabilized cells. The addition of spermine or linolenate effectively enhanced or inhibited the formation of Fru-1, 6-P_2, and the AMP deaminase activity (Fig. 2), respectively. Noteworthy is the finding that the optimal energy charge value giving maximal response of phosphofructokinase increased in the presence of spermine, but decreased a little in the presence of linolenate (Fig. 2A). On the other hand, the optimal energy charge value for the AMP deaminase activity was not altered with the activation or inhibition of the enzyme (Fig. 2B). These results suggest that NH_4^+ ion produced through the activation of AMP deaminase by spermine can participate in the stimulation of phosphofructokinase by enhancing the sensitivity of the response to the physiological decrease in the energy charge *in vivo*.

DISCUSSION

Phosphofructokinase, which plays a key role in glycolytic regulation (1, 2), is controlled by the multimodulation mechanism (20): the enzyme is inhibited by ATP and citrate, and is activated by AMP, Pi, NH_4^+ and K^+ under the *in vitro* and *in vivo* conditions (1-3, 20). Phosphofructokinase shows a typical characteristic of the ATP-regenerating enzyme with regard to the response to the energy charge; that is, the decrease in the energy charge resulting from the increased hydrolysis of ATP activates phosphofructokinase in the permeabilized cells (see, Fig. 1) as well as in the purified enzyme form (11). This mechanism has been explained by the reversal of the ATP-inhibition of the enzyme and further by the increase in the activator AMP produced through the action of adenylate kinase reaction (11). The increase in AMP level can enhance the activity of AMP deaminase, resulting in the depletion of total adenylates and in the increase in NH_4^+ level (19, 21). The adenylate energy charge can be highly stabilized by the depletion of total adenylates (19) and by the stimulation of glycolysis through the elevated NH_4^+ level (16, 17).

Phosphofructokinase belongs to the NH_4^+-activated type: K^+ can act as an activator of the enzyme, but its Ka value is extremely larger as compared to that for NH_4^+ (16). Binding sites of the enzyme for NH_4^+ are possibly distinct from the K^+-sites (4). The high specificity of the activation of phospho-

fructokinase by NH_4^+ was demonstrated in the purified enzyme from rabbit muscle (4), yeast (22) and Clostridium (23), and NH_4^+ is regarded as a physiological activating cation. Ammonium ion activated phosphofructokinase in the presence of K^+ at physiological level, and further modified the response to the variation in the energy charge as demonstrated in this paper: the optimal energy charge value giving the maximal response of the enzyme increased in the presence of NH_4^+. These results suggest that phosphofructokinase can be activated most effectively by the physiological decrease in the energy charge (0.6 -0.9) under the condition where NH_4^+ was produced.

Spermine activated the activity of AMP deaminase (18, 24), resulting in the increase in NH_4^+ level (15, 16). Action of spermine on the phosphofructokinase activity was demonstrated to be indirect and is ascribed to the elevated NH_4^+ level (16). Activation of AMP deaminase by spermine was closely correlated with the activation of phosphofructokinase with the shift of the optimal energy charge giving the maximal response of the enzyme. These data may present further evidence supporting a substantial role of NH_4^+ in the stimulation of glycolysis by spermine.

The decrease in the energy charge is closely correlated with the production of NH_4^+ under the condition where the adenylate kinase-AMP deaminase reaction cannot be inhibited (19). The increased hydrolysis or decreased formation of ATP under the hypoxic condition elevates the NH_4^+ concentration. Activating effect of NH_4^+ on phosphofructokinase was earlier discussed in relation to the Pasteur effect (4). Combined action of the decreased energy charge and increased NH_4^+ ion on phosphofructokinase may participate in a principal part of the glycolytic enhancement in hypoxic cells. The Pasteur effect might be attributed, at least in part, to the interaction of AMP deaminase with phosphofructokinase in vivo. The analysis presented here using permeabilized cells will serve as a guide to a metabolic regulation by the enzymes,which respond to variation in the energy charge, including ATP-regenerating and ATP-utilizing types.

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