The role of AMP deaminase (EC 3.5.4.6) reaction in the stimulation of the regulatory enzymes of glycolysis was investigated using permeabilized yeast cells. 1) The addition of polyamine activated AMP deaminase in situ, resulting in the subsequent increase in ammonium production, which can stimulate the activity of 6-phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40). 2) Zn²⁺ inhibited AMP deaminase activity, followed by a decrease in ammonium ion concentration which reduced the activity of phosphofructokinase. 3) Polyamine and Zn²⁺ did not activate or inhibit directly the activity of phosphofructokinase and pyruvate kinase. 4) A simple Michaelis-Menten relationship was observed between the various levels of ammonium ion and of fructose 1,6-biphosphate formed in situ, indicating that phosphofructokinase activity or glycolytic flux was dependent upon the level of ammonium ion produced through the action of AMP deaminase. 5) The increase in P, concentration resulted in the decreased magnitude of activation by NH₄⁺ and marked stimulation by P, itself of phosphofructokinase, and further reduced the production of NH₄⁺ through the inhibition of AMP deaminase, suggesting that phosphofructokinase activity may not be regulated by the level of NH₄⁺ but by P, concentration under conditions of increased P, levels.

The AMP deaminase-ammonium system shows a regulatory function in glycolysis of yeast cells in the presence of physiological P, levels, whereas glycolysis may be principally controlled by P, level under the conditions of elevated P, concentration. Polyamines may play a part in the stimulation of glycolysis through the elevated level of ammonium ion under the conditions of increased ATP utilization during cell proliferation, and can participate in the catabolic processes as well as anabolic processes through the stimulation of the AMP deaminase-ammonium system.

The glycolytic pathway is controlled by a large amount of metabolites, many of which reflect the energy status of the cells (1). 6-Phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) are nonequilibrium reactions under \textit{in vivo} conditions. These enzymes, which are considered the regulatory enzymes of glycolysis, are activated by ammonium and potassium ions, and phosphofructokinase activity is stimulated by phosphate (2-7). In keeping with their origin as the major effectors of the glycolytic pathway, the increase in the concentration of ammonium ion and P, under conditions of increased ATP utilization in certain tissues, is suggested to play a part in the stimulation of glycolysis through the effect on these glycolytic key enzymes (8-12). The role of AMP deaminase (EC 3.5.4.6) in the production of NH₄⁺ from AMP was discussed in relation to the control of phosphofructokinase by Sugden and Newsholme (8) and Winder et al. (13), although direct evidence was not presented. AMP deaminase may be important in the regulation of adenylate energy charge, adenylate pool size (14-18), and the control of the purine nucleotide cycle (19): the importance of the cycle has been discussed in relation to the control of glycolysis in some tissues. Recently, we presented a permeabilization method which allows the assay of intracellular enzymes within the boundaries of yeast cells (20), and the yeast AMP deaminase was demonstrated to be regulated \textit{in situ} by a variety of effectors. Furthermore, preliminary evidence was presented suggesting that ammonium ion, produced through the activation of AMP deaminase, effectively stimulates yeast phosphofructokinase in the presence of polyamine (21). In the present study, we demonstrate that the levels of NH₄⁺, produced through the action of AMP deaminase, qualitatively and quantitatively correlate with the activity of phosphofructokinase and pyruvate kinase \textit{in situ}, which can stimulate glycolysis. The specificity and physiological significance of polyamine effect is discussed in relation to the participation in the stimulation of glycolysis through the activation of AMP deaminase-ammonium system under the conditions of increased ATP utilization during cell proliferation.

**EXPERIMENTAL PROCEDURES**

Materials

Nucleotides were obtained from Yamasa Co. (Tokyo, Japan). Spermine and firefly lantern extract (FLE-50) were products of Sigma. Enzymes used for the determination of glycolytic intermediates were purchased from Boehringer. Commercial baker's yeast (Saccharomyces cerevisiae) was obtained locally.

Methods

**Procedures for Incubation**—Permeabilized yeast cells were prepared according to the method described previously (20). (A) Glycolytic activity was examined at 37 °C in the reaction mixture containing 4.5 mM glucose, 4.5 mM ATP, 10 mM MgCl₂, 2 or 10 mM P, 0.1 mM NAD, 10 mM cacodylate buffer (pH 6.5), and toluenized yeast cells (40 mg/ml) in the absence and presence of 1 mM spermine or 0.2-0.5 mM Zn²⁺ in a total volume of 4 ml. (B) When only the activity of phosphofructokinase \textit{in situ} was quantitatively examined, P, and NAD were excluded in the reaction mixture so that Fru-P₃ and triose

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**AMP Deaminase Reaction as a Control System of Glycolysis in Yeast**

**ACTIVATION OF PHOSPHOFRUCTOKINASE AND PYRUVATE KINASE BY THE AMP DEAMINASE-AMMONIA SYSTEM**

Masataka Yosho and Keiko Murakami

From the 4 Department of Biochemistry, Yokohama City University School of Medicine, Yokohama 232, Japan and the 5 Department of Laboratory Medicine, St. Marianna University School of Medicine, Kawasaki 213, Japan
phosphates cannot be further metabolized. Aliquots of 0.2 ml were deproteinized by 0.4 ml of 7.5% perchloric acid at appropriate intervals. After standing for 15 min at 0 °C, 0.6 ml of potassium carbonate was added and the potassium perchlorate formed was removed by centrifugation. The supernatant was utilized for the determination of adenylates, ammonia, and glycolytic intermediates.

**Determination of Metabolites**—ATP was analyzed by luciferin-luciferase reaction, and ADP and AMP were measured as the difference after enzymatic conversion to ATP (22). Ammonia was estimated by the method of Chaney and Marbach (23). Glucose was estimated by the method of Bergmeyer et al. (24), glucose 6-phosphate (Glc-6-P) and fructose 6-phosphate (Fru-6-P) by the method of Lang and Michal (25), Fru-P₂ and combined triose phosphates by the method of Michal and Beutler (26), pyruvate, P-enolpyruvate, and glycerate 2-phosphate by the method of Czok and Lamprecht (27), and ethanol by the method of Bernt and Gutmann (28).

**RESULTS**

**Stimulation of Glycolysis**—Fig. 1 shows the variation of the concentrations of hexose 6-phosphates, Fru-P₂, and pyruvate in permeabilized yeast cells. After the addition of ATP and glucose, hexose 6-phosphates rapidly increased and then gradually decreased (Fig. 1A), followed by an increase in Fru-P₂ (Fig. 1B) and further by a formation of pyruvate (Fig. 1C). Glucose recovery as glycolytic intermediates including Glc-6-P, Fru-6-P, Fru-P₂, triose phosphates, and pyruvate is above 95 and 85% 10 and 20 min after initiation of the reaction, respectively (Fig. 1C); thus, we can evaluate the effects of these ligands on the glycolytic activity under these conditions. As can be seen in Fig. 1, the addition of spermine and Zn²⁺ stimulated and inhibited the formation of Fru-P₂ and pyruvate, respectively, although glucose was decreased with the identical rate under the experimental conditions.

Adenine nucleotide concentrations were also determined under the same conditions (Fig. 2). After the addition of ATP and glucose, ATP decreased rapidly, and AMP and ADP³ increased (Fig. 2A), resulting in a drastic drop in the adenylate energy charge (Fig. 2B). The decrease in total adenylates, which had occurred before the energy charge began to rise, was essentially ascribed to the degradation of AMP through the action of AMP deaminase. This assumption was also verified by the stoichiometric production of ammonium ion corresponding to the decrease in total adenylates (Fig. 2C).

The 5'-nucleotidase (EC 3.1.3.5) and adenosine deaminase (EC 3.5.4.4) cannot be responsible for the degradation of adenylates and for the production of NH₄⁺ because of the lower activity of adenosine deaminase as discussed previously (17, 18). Recovery of the energy charge and the depletion of total adenylates were significantly enhanced by the addition of spermine, the activator of AMP deaminase (29), whereas the energy charge was more slowly recovered with a slower decrease in total adenylates in the presence of Zn²⁺, the powerful inhibitor of AMP deaminase (29, 30) (Fig. 2, B and C).

A graphical representation of the changes in the concentration of glycolytic intermediates and adenylates is given in Fig. 3. As noted above, glucose added was almost completely recovered as glycolytic intermediates including Glc-6-P, Fru-6-P, Fru-P₂, triose phosphates, and pyruvate after the first 10 min of the reaction (Fig. 1C); the level of phosphoglycerate was negligible. The values after 10 min of the reaction are given in % change of the control values. Using the theory of the crossover theorem (31), these results indicate that the addition of spermine or Zn²⁺ can affect the activity of phosphofructokinase. Variation of the levels of adenylates also indicates that AMP deaminase reaction was influenced by the presence of spermine or Zn²⁺. Equilibrium of adenylate kinase (EC 2.7.4.3) reaction was scarcely affected by these ligands.

**Activation of Phosphofructokinase Activity**—The mechanism of the effects of spermine and Zn²⁺ on the phosphofructokinase activity and adenylate metabolism was studied. The experiment was designed to examine the activity of phosphofructokinase in situ only; thus, P₃ and NAD were excluded in the reaction mixture so that Fru-P₂, the reaction product of the enzyme and the triose phosphates formed from it, cannot be further metabolized. When ATP was used as phosphoryl donor, the production of Fru-P₂ increased with the increase in the concentration of spermine; however, NH₄⁺ formed through the adenylate kinase-AMP deaminase system as a result of ATP hydrolysis was also increased under these conditions (Fig. 4A). It was still an open question whether the activity of phosphofructokinase was stimulated by NH₄⁺ or by spermine directly. When ITP was used as phosphoryl donor, the system was not able to produce NH₄⁺; the increase in the concentration of spermine did not stimulate the formation of Fru-P₂ under these conditions, although the phosphofructokinase activity was increased.
Activation of Glycolysis by AMP Deaminase-Ammonia System

Fig. 2. Effects of spermine and Zn\textsuperscript{2+} on the changes in adenine nucleotides, NH\textsubscript{4}\textsuperscript{+}, and the adenylate energy charge in permeabilized yeast cells. Toluennized yeast cells (40 mg/ml) were incubated at 37 °C as described in the legend to Fig. 1. Adenine nucleotides and NH\textsubscript{4}\textsuperscript{+} were determined according to the luciferin-luciferase method (22) and phenol-hypochlorite reagent (23), respectively. A, ATP (open symbols) and AMP (closed symbols). B, adenylate energy charge. The values were calculated as follows: energy charge = [ATP] + 1/2[ADP]/[ATP] + [ADP] + [AMP]. C, NH\textsubscript{4}\textsuperscript{+} (open symbols) and total adenylates (closed symbols). ○, no addition; △, 1 mM spermine added; □, 0.2 mM Zn\textsuperscript{2+} added; ▼, 0.5 mM Zn\textsuperscript{2+} added.

Activity remained 25–30% of the activity obtained for ATP. The addition of NH\textsubscript{4}\textsuperscript{+} to the reaction mixture at various concentrations, the levels of ammonium and Fru-P\textsubscript{2} varied from 0 to 2 mM, and 0.1 to 1.2 mM, respectively. A relationship between the levels of NH\textsubscript{4}\textsuperscript{+} and Fru-P\textsubscript{2} represents a simple Michaelis-Menten type saturation curve (Fig. 5). Furthermore, NH\textsubscript{4}\textsuperscript{+} added was able to activate phosphofructokinase under the conditions where AMP deaminase was completely inhibited by the addition of Zn\textsuperscript{2+}: the same response was obtained in Fig. 5 with NH\textsubscript{4}\textsuperscript{+} added as with the NH\textsubscript{4}\textsuperscript{+} produced through the AMP deaminase reaction in situ. The apparent K\textsubscript{s} value of phosphofructokinase for NH\textsubscript{4}\textsuperscript{+} was calculated to be 1.43

Fig. 3. Percentage changes in glycolytic intermediates and adenine nucleotides. The data were taken from Figs. 1 and 2. The 0% line represents the control (no addition) values. Only trace amount of 2- and 3-phosphoglycerates, and 1, 3-biphosphoglycerate were observed. G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1, 6-biphosphate; Pyr, pyruvate.

Fig. 4. Effect of concentration of spermine and Zn\textsuperscript{2+} on the activity of phosphofructokinase in situ. The reaction mixture contained 10 mM cacodylate buffer (pH 6.5), 10 or 20 mM glucose, 5 mM ATP or 10 mM ITP, 10 mM MgCl\textsubscript{2}, the indicated concentration of spermine or Zn\textsuperscript{2+}, and the toluenized yeast cells (10 mg/ml) in a final volume of 0.2 ml. Fru-P\textsubscript{2} and NH\textsubscript{4}\textsuperscript{+} were determined after the 10-min incubation. ○, □, Fru-P\textsubscript{2}; △, NH\textsubscript{4}\textsuperscript{+}. Open and closed symbols show the Fru-P\textsubscript{2} formation determined in the presence of 10 mM NH\textsubscript{4}\textsubscript{Cl} with ATP as the substrates, respectively. Square symbols show the Fru-P\textsubscript{2} formation determined in the presence of 10 mM NH\textsubscript{4}\textsubscript{Cl} with ATP as the substrates. A, effect of spermine. B, effect of Zn\textsuperscript{2+}. 

activity remained 25–30% of the activity obtained for ATP. The addition of NH\textsubscript{4}\textsuperscript{+} to the reaction mixture gave a full activity of phosphofructokinase without stimulation by spermine (data not shown). The effect of Zn\textsuperscript{2+} on the in situ phosphofructokinase activity was also examined. The inhibition of Fru-P\textsubscript{2} formation by Zn\textsuperscript{2+} paralleled inhibition of NH\textsubscript{4}\textsuperscript{+} production under the conditions where ATP was used as phosphoryl donor, and no inhibitory effect of Zn\textsuperscript{2+} on the phosphofructokinase was observed when excess NH\textsubscript{4}\textsuperscript{+} was included (Fig. 4B). From these results, Zn\textsuperscript{2+} action on phosphofructokinase is suggested to be indirect, as in the case of spermine action. Furthermore, Zn\textsuperscript{2+} showed no inhibitory effect on the phosphofructokinase activity under the conditions where ITP was used as phosphoryl donor: the formation of Fru-P\textsubscript{2} remained 25–30% of the activity obtained for ATP, but the full activity of phosphofructokinase without inhibition by Zn\textsuperscript{2+} was obtained when excess NH\textsubscript{4}\textsuperscript{+} was included (Fig. 4B).
tion conditions were similar to those of Fig. 4 except that the concentrations of spermine and Zn\(^{2+}\) were varied from 0 to 1 mM, and from 0 to 0.5 mM, respectively, and that 5 mM ATP and 10 mM glucose were used as substrates. Fr-u-P\(_2\) and NH\(_4^+\) were determined after the reaction was terminated at 10 min. The values of Fr-u-P\(_2\) concentrations were plotted against those of NH\(_4^+\) (circle). Triangle symbols show the values of Fr-u-P\(_2\) produced by NH\(_4^+\) added under the conditions where AMP deaminase was completely inhibited by the addition of 0.2 mM Zn\(^{2+}\). The theoretical line is drawn from the following equation and values \(v = \frac{V - [A]}{K_v + [A]} + v_0\), where \([A]\) is the concentration of NH\(_4^+\), \(K_v\) the concentration required for 50% activation. \(K_v\) was determined to be 1.43 mM from the double reciprocal plot (inset). \(v\) is represented as the concentration of Fr-u-P\(_2\) formed/10 min. \(v_0\), the Fr-u-P\(_2\) formed under the conditions where AMP deaminase is completely inhibited by the addition of 0.2 mM Zn\(^{2+}\), is 0.1 mM (per 10 min).

**Activation of Pyruvate Kinase Activity**—The effect of AMP deaminase-ammonium system on the activity of pyruvate kinase, one of the regulatory enzymes of glycolysis, was investigated. The reaction system consisted of P-enolpyruvate, MgATP, and deoxyglucose: P-enolpyruvate and ADP, produced through the hexokinase reaction, can form ATP and pyruvate. ATP rapidly decreased and then slowly recovered, while ADP and AMP inversely increased; increased AMP was recovered, in particular, in the presence of spermine (Fig. 6). The decrease in total adenylates, which is ascribed to the action of AMP deaminase, and the stoichiometric production of NH\(_4^+\) were also observed (Fig. 7A). Depletion of total adenylates, and production of NH\(_4^+\) (Fig. 7A) as well as recovery of the energy charge (Fig. 6B) were remarkably enhanced by the presence of spermine. Formation of pyruvate, which was effectively stimulated by the addition of spermine (Fig. 7B), can be correlated with the activation of AMP deaminase since spermine itself did not affect the activity of pyruvate kinase.

**Effect of P\(_1\) on the Activity of Phosphofructokinase**—Conditions of increased ATP utilization and of decreased ATP formation, that is, hypoxia and ischemia, have been demonstrated to increase the levels of P\(_1\) and NH\(_4^+\) in several organisms and tissues (33-38). P\(_1\) can act as an effective activator and inhibitor of phosphofructokinase (2, 4, 6, 7) and AMP deaminase (39), respectively. We thus examined the effect of increased P\(_1\) on the activation by NH\(_4^+\) of phosphofructokinase and glycolysis. Ammonium ion exhibited a powerful activating effect on the activity of phosphofructokinase in the absence of P\(_1\); however, addition of up to 5 mM NH\(_4^+\) produced only little enhancement of the activation of the enzyme produced by higher P\(_1\) concentrations (Fig. 8). The data in Fig. 8 were replotted as the relationship between the formation of Fr-u-P\(_2\) and P\(_1\) concentration in the presence of different levels of NH\(_4^+\) (Fig. 8, inset). P\(_1\) can act as an effective activator of phosphofructokinase in the presence of lower concentration of NH\(_4^+\), but this ligand showed little or no stimulating effect when the enzyme was activated by excess NH\(_4^+\).

The effect of spermine on the adenylate regulation and the production of NH\(_4^+\) was examined under the conditions where 10 mM P\(_1\) was included (Fig. 9A): spermine showed little effects on the synthesis of ATP, the recovery of the energy charge (Fig. 9, B and C), or on the glycolytic activity (data not shown). The depletion of total adenylates and production of NH\(_4^+\) were significantly lower in comparison with those observed for the conditions of lower P\(_1\) (Fig. 9C). These results suggest that P\(_1\) appears to affect glycolysis and adenylate regulation system in two fashions: 1) NH\(_4^+\) ion shows much less magnitude of activation of phosphofructokinase in the presence of higher P\(_1\) levels, which can activate phosphofructokinase, and 2) P\(_1\) at higher concentration reduces the production of NH\(_4^+\) through the inhibition of AMP deaminase.
FIG. 8. Effect of the concentration of NH₄⁺ on the activity of phosphofructokinase in situ in the presence of different levels of Pi. The reaction mixture contained 5 mM glucose, 5 mM ATP, 10 mM MgCl₂, 15 mM KCl, 10 mM cacodylate buffer (pH 7.1), the indicated concentration of Pi, and of ammonium ion as chloride salt, 0.2 mM Zn²⁺ and the toluenized yeast cells (10 mg/ml). Fru-P₂ was determined after incubation for 10 min. ○, no addition; △, 2 mM Pi added; □, 5 mM Pi added; ▽, 10 mM Pi added. Inset, effect of concentration of Pi on the activity of phosphofructokinase in the presence of different levels of NH₄⁺. ○, 0.5 mM NH₄⁺ added; △, 5 mM NH₄⁺ added. Closed symbols show the values of Fru-P₂ and NH₄⁺ which were produced from the ATP-glucose system as shown in Fig. 1 in the absence (○) and presence of 1 mM spermine (△).

Thus, the AMP deaminase-NH₄⁺ system does not stimulate glycolysis when glycolysis is fully activated in the presence of higher Pi levels.

FIG. 9. Effect of spermine on the changes in adenine nucleotides and NH₄⁺ in the presence of higher concentration of Pi. The reaction mixture of 4 ml contained 4.5 mM glucose, 4.5 mM ATP, 10 mM MgCl₂, 0.1 mM NAD, 10 mM cacodylate buffer (pH 6.5), 2 or 10 mM Pi, and toluenized yeast cells (16 mg/ml) in the absence and presence of 1 mM spermine. A, ATP (open symbols) and AMP (closed symbols). B, adenylate energy charge. C, total adenine nucleotides (closed symbols) and NH₄⁺ (open symbols). ○, no addition; △, 1 mM spermine added with 2 mM Pi; □, no addition with 10 mM Pi; ▽, 1 mM spermine added with 10 mM Pi.  

DISCUSSION

AMP deaminase, which is widely distributed in various eukaryotes such as animals (39), yeast (17), and higher plants (40), may be important in stabilization of adenylate energy charge (14–18) and the interconversion of adenosine, imosine, and guanine nucleotides (19). AMP deaminase as an allosteric enzyme can be regulated by a variety of effectors (19); kinetic properties suggest that the enzyme may be related to the regulation of glycolysis (19) and fatty acid metabolism (15, 41). The purine nucleotide cycle, which consists of AMP deaminase, adenylosuccinate synthetase (EC 6.3.4.4), and adenylosuccinate lyase (EC 4.3.2.2), is closely correlated with the control of glycolysis (19). Ammonium ion and Pi, which increase under conditions of increased ATP utilization, are considered to be responsible for the stimulation of glycolysis (8–12). The increase in the concentration of NH₄⁺, Pi, and AMP, and decrease in ATP, citrate, and the energy charge values may play a role in the activation of phosphofructokinase, a regulatory enzyme of glycolysis (2, 4, 6, 7). Munz was the first to show that NH₄⁺ ion activated phosphofructokinase in yeast extract (10) and rat brain homogenates (11, 12). The participation of AMP deaminase in the production of NH₄⁺ from AMP was discussed in relation to the activation of phosphofructokinase by Sugden and Newsholme (8), although direct evidence had not been presented. The permeabilized system of yeast cells, which was presented by us (20), can offer an excellent experimental system for the study on the control of glycolysis. It is of particular interest to examine the effects of various regulatory ligands on the glycolytic activity under the conditions where intracellular levels of ATP and glucose were included, although some previous in situ studies on glycolysis were carried out in a high concentration of glucose and catalytic amount of ATP (42). The results presented in this paper show that NH₄⁺ produced through the action of AMP deaminase correlates with the activation of phosphofructokinase and pyruvate kinase in situ, which may stimulate glycolysis. Much less Fru-P₂ was formed under the conditions where AMP deaminase activity was inhibited by the addition of Zn²⁺, although the phosphofructokinase activity could be stimulated by the marked decrease in the energy charge and the increase in AMP concentration (see Figs. 1 and 2). Furthermore, pyruvate kinase activity was also stimulated by increased NH₄⁺ level rather than by the decrease in the energy charge and the increase in AMP or ADP (see Figs. 6 and 7). Thus, the AMP deaminase-NH₄⁺ system or the level of NH₄⁺ produced can play a principal part in the activation of phosphofructokinase and pyruvate kinase.

The average concentrations of NH₄⁺ in some tissues and organisms were found to be 0.24–0.34 mM (43–45). The concentration of NH₄⁺ is increased under conditions that cause an increase in glycolytic flux. In the rat muscle and mammalian brain the concentration of NH₄⁺ is increased from 0.2–0.3 mM to 1 mM and 5 mM by a tetanic stimulation (45) and prolonged ischemia (46), respectively. The increase in NH₄⁺ as well as Pi levels under conditions where glycolytic flux is stimulated may be a general phenomenon. The apparent K₅₀ of phosphofructokinase for NH₄⁺ calculated in this paper was 1.43 mM, a value which agreed well with that observed for the purified enzyme (32), and which was within the range of physiological concentration of this ion. The results of the present work, concerning the stimulation of phosphofructokinase and pyruvate kinase by the AMP deaminase-NH₄⁺.
system, can account for the experimental results on some tissues or cells in vivo (10-12, 45, 48).

It should be noticed, however, that the AMP deaminase-NH₄⁺ system is not necessarily responsible for the stimulation of glycolysis in the presence of higher concentration of P₄, which results in a decreased magnitude of activation by NH₄⁺, a remarkable stimulation by P₄ itself of phosphofructokinase, and a reduced production of NH₄⁺ through the inhibition of AMP deaminase. Glycolysis may not be regulated by the level of NH₄⁺ under the conditions where P₄ is significantly elevated. Little or no effect of spermine on the recovery of the energy charge was noticeable under these conditions (Fig. 9).

Inorganic ions such as K⁺, Mg²⁺, and P₄ were demonstrated to accumulate mainly in vacuoles of yeast cells; their concentrations are 60, 5, and 1 mM in the cytoplasm and 470, 73, and 110 mM in vacuoles, respectively (47). Efflux of these ions from vacuolar pool in the presence of glucose or fructose (48) may contribute to the stimulation of glycolysis or ATP synthesis.

Monovalent cations participate in biological processes in a variety of ways. One of the important functions of these cations is as activators or inhibitors of enzymes (49, 50). As noted from tables of monovalent cation-activated enzymes compiled by Evans and Sager (51) and Suelter (49), most of these enzymes show relatively strict specificity toward activating cations. 1) Those enzymes activated by K⁺ are also usually activated by NH₄⁺ and Rb⁺, but are activated little by Na⁺ and Li⁺. 2) A few enzymes activated by Na⁺ and also activated by Li⁺ and Cs⁺ can be activated much less by K⁺, NH₄⁺, and Rb⁺. However, 3) some enzymes are activated equally efficiently by K⁺ and Na⁺ (52). AMP deaminase (53-55) and AMP nucleosidase (EC 3.2.2.4) (56, 57), which are typical enzymes of this group, show broad specificity toward activating cations: monovalent cations of both K⁺-NH₄⁺ and Na⁺-Li⁺ groups are equally efficient, and alkaline earth metals and transition metals also affect the enzyme activity. Polyamine is the most effective activator of these enzymes (52, 55, 58, 59).

The first group of monovalent cation-activated enzymes, K⁺-NH₄⁺ type, has been classified as a group of the enzymes which can be activated by both K⁺ and NH₄⁺. However, the enzymes of this group should be divided into the following subgroups: a) K⁺ type: enzymes which are activated equally efficiently by K⁺ and NH₄⁺ with A₀.₅ values of 50-100 mM; b) NH₄⁺ type: enzymes which are activated by NH₄⁺ with A₀.₅ values of 0.5-2.5 mM, and by K⁺ with A₀.₅ values of 50-100 mM. Ammonium ion at physiological level can be responsible for the control of the enzyme activities of the NH₄⁺ type, and K⁺ ion may act as a physiological activator of the enzymes of K⁺ type. Of particular interest is the fact that phosphofructokinase and pyruvate kinase, the regulatory enzymes of glycolysis belong to NH₄⁺ type; glycolysis may be one of the best targets for AMP deaminase-NH₄⁺ system. Enzymes belonging to this group are listed in Table I (3, 9, 60-71).

It should be noticed that polyamines can stimulate glycolytic activity through the enhancement of AMP deaminase-NH₄⁺ system. Polyamines, which accumulate accompanying cellular proliferation, are responsible for the increase in the rate of RNA and protein synthesis (72-74), and participate in the control of the activation of many enzymes (52). Recently, a new role of polyamines related to control of glucose metabolism was demonstrated; spermine and spermidine stimulate conversion of glucose to carbon dioxide and inhibit epinephrine-stimulated lipolysis isolated adipocytes (75, 76). Polyamines bind to fat cell membrane at a site distinct from the insulin receptor sites, resulting in the insulin-like effects of facilitating glucose transport and inhibiting lipolysis by suppressing cyclic AMP levels (77). The function of increased polyamines on glucose catabolism in cells has remained unknown, although polyamines may be responsible for the direct activation of several glucose-catabolizing enzymes (78-81).

The results presented here will serve as a guide to the analysis of the role of polyamines in vivo: polyamines can stimulate glycolysis by the activation of phosphofructokinase and pyruvate kinase through the increased NH₄⁺ produced from AMP, as well as facilitating glucose transport or direct activation of several enzymes in some tissues.

AMP deaminase, as an NH₄⁺-producing system, can participate in the regulation of glycolysis as well as the supply of some amino acids through the stimulation of threonine dehydratase activity in yeast (82). Polyamines and NH₄⁺ may play a key role in anabolic processes: polyamine can be responsible for the increase in the rate of nucleic acid synthesis and protein synthesis (69-71), and ammonium ion is utilized for the synthesis of amino acids (83). The present results as well as insulin-like effects of polyamines suggest that polyamines can participate in other processes, that is, glucose degradation. Polyamines and NH₄⁺ can participate in catalytic processes as well as anabolic processes through the stimulation of the AMP deaminase-ammonium system. Thus, these ligands may be termed as "amphibolic ligands."

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AMP deaminase reaction as a control system of glycolysis in yeast. Activation of phosphofructokinase and pyruvate kinase by the AMP deaminase-ammonia system.

M Yoshino and K Murakami


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