Regulation of D-Fructose 1-Phosphate Kinase by Potassium Ion*

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SUMMARY

Purified Aerobacter aerogenes D-fructose 1-phosphate kinase (ATP: D-fructose 1-phosphate 6-phosphotransferase), an enzyme which does not exhibit cooperative kinetics, was activated by \( K^+ \), \( Rb^+ \), and \( NH_4^+ \), inhibited by \( Li^+ \), and not significantly affected by \( Na^+ \) or \( Cs^+ \). The enzyme exhibited some activity when assayed in tetramethylammonium-glycylglycine buffer even in the absence of other monovalent cations, suggesting that the requirement for monovalent cations is not absolute. \( K^+ \) increased the reaction velocity about 4-fold in the presence of excess ATP and D-fructose-1-P, and about 10-fold when both substrates were present at concentrations near their \( K_m \) values (0.3 mM). \( K^+ \) also exerted a much greater activating effect under conditions of inhibiting ATP levels than when \( Mg^{++} \) was present at a sufficiently high level to prevent inhibition by ATP. The \( K_m \) for \( K^+ \) (about 3 mM) was not affected by the \( Mg^{++} \) or ATP concentration, but was increased by decreased levels of D-fructose-1-P. \( K^+ \) decreased the \( K_m \) values for both ATP and D-fructose-1-P. These kinetics are consistent with Michaelis-Menten theory and suggest a simple model in which the enzyme exists in two active forms, one form predominating in the presence of \( K^+ \) and the other predominating in its absence.

\( D\)-Fructose 1-phosphate kinase (\( D\)-fructose-1-P + ATP \( \rightarrow \) D-fructose-1,6-diP + ADP) is an inducible enzyme which is instrumental in the metabolism of \( D\)-fructose in Aerobacter aerogenes PRL R3 (1, 2). Unlike the constitutive \( D\)-fructose 6-phosphate kinase (\( D\)-fructose-6-P + ATP \( \rightarrow \) D-fructose-1,6-diP' + ADP'; EC 2.7.1.11) from this organism, it exhibits non-cooperative kinetics; ATP inhibits \( D\)-fructose-1-P kinase when the \( Mg^{++} \) to ATP ratio is less than 2:1, but the inhibition cannot be relieved by D-fructose-1-P or by various nucleoside mono- and diphosphates (3).

Because the intracellular concentration of \( K^+ \) in microbial cells is dependent upon their metabolic state (4–6), and because \( K^+ \) is required by many enzymes for maximal activity (7–9), the possibility exists that \( K^+ \) may serve a regulatory role in microbial metabolism. \( D\)-Fructose-6-P kinase from various sources is activated by both \( K^+ \) (10–13) and \( NH_4^+ \) (11–16), but \( D\)-fructose-1-P kinase from Bacteroides symbiosus apparently is not activated by monovalent cations (17). In this paper we describe the effect of \( K^+ \) on \( D\)-fructose-1-P kinase from \( A. \) aerogenes PRL R3. \( K^+ \) increased the \( V_{max} \) increased the affinity of the enzyme for ATP and \( D\)-fructose-1-P, and partially overcomes inhibition by excess ATP. A model is proposed for the activation of \( D\)-fructose-1-P kinase by \( K^+ \) which is consistent with the observed kinetics.

MATERIALS AND METHODS

Materials—The \( D\)-fructose-1-P kinase used in these studies was the 315-fold purified preparation (specific activity, 47.0) described previously (3). Bovine serum albumin was obtained from Sigma, and tetramethylammonium hydroxide (25% in water) from Mallinckrodt Chemical Works. Other chemicals were obtained as described previously (3).

Removal of Activating Monovalent Cations from Reaction Components—Prior to assay, the \( D\)-fructose-1-P kinase was diluted 160-fold with a solution of bovine serum albumin (2 mg per ml) in 0.1 M tetramethylammonium-glycylglycine buffer (pH 7.5). The residual (NH\(_4\))\(_2\)SO\(_4\) and sodium acetate in the diluted enzyme solution amounted to approximately 8 \( \mu \)M and 0.1 \( \mu \)M, respectively, in the final reaction mixture. Bovine serum albumin, \( \alpha \)-glycerophosphate dehydrogenase triose phosphate isomerase, and \( D\)-fructose-1,6-diP aldolase were freed from activating monovalent cations by passage through a column of Sephadex G-25 equilibrated with 0.1 M tetramethylammonium-glycylglycine buffer (pH 7.5). Fractions were collected by eluting with the same buffer. Sodium ions were removed from ATP and NADH by dialyzing the nucleotides (sodium salts) in 0.2 M tetramethylammonium-glycylglycine buffer (pH 7.5) and passing the solutions through a column of Sephadex G-15 equilibrated with the same buffer. The concentrations of the nucleotides in the eluted fractions were determined by absorbance at 265 nm. Barium \( D\)-fructose-1-phosphate was treated with Dowex 50 (H\(^+\)) to remove barium ions, and the resulting free acid was neutralized with (CH\(_3\))\(_2\)NOH. \( D\)-Fructose-1-phosphate concentrations were determined enzymically (3).

\( \phi H \) measurements during preparation of the buffer and neutralization of \( D\)-fructose 1-phosphate were made with a Sargent miniature combination electrode (manufactured by Jena Glass Works, Mainz, Germany), which has an extremely low electrolyte leak rate. Possible contamination of the reagents with KCL from the electrode was determined with a Coleman flame.
TABLE I

Effect of monovalent cations on d-fructose-1-P kinase activity
The standard assay was used with the monovalent cations added as the chloride salts.

| Monovalent cation | Activity at a cation concentration of: |
|-------------------|---------------------------------------|
|                   | 6.7 mM | 40.0 mM |
| Potassium         | 260    | 340     |
| Rubidium          | 140    | 207     |
| Ammonium          | 100    | 133     |
| Sodium            | 100    | 107     |
| Cesium            | 100    | 107     |
| Lithium           | 73     | 27      |

* Expressed as percentage of rate with no additions.

Results

Effect of Monovalent Cations on d-Fructose-1-P Kinase Activity—Table I shows the effect of various monovalent cations on the reaction catalyzed by d-fructose-1-P kinase. K+, Rb+, and NH4+ activated to different degrees, with K+ being the most effective. Li+ inhibited, and Na+ and Cs+ had little or no effect on the activity. The activating effects of KCl, RbCl, and NH4Cl could not have been due to Cl−, since NaCl, CsCl (Table I), and increased levels of MgCl2 (3) did not stimulate the reaction. The enzyme exhibited the same level of activity when NaOH was used instead of (CH3)4NOH to neutralize the reagents used for the assay.

Effect of K+ on the Km for ATP and D-Fructose-1-P—Figs. 1 and 2 show the effect of KCl concentration on the Km for d-fructose-1-P and ATP. In the absence of KCl, the Km values for d-fructose-1-P and ATP were 0.8 mM and 0.7 mM, respectively. Both Km values were decreased to about 0.3 mM in the presence of 40 mM KCl.

Effect of Monovalent Cations on d-Fructose-1-P Kinase Activity—Table II shows the effect of various monovalent cations on the reaction catalyzed by d-fructose-1-P kinase. K+, Rb+, and NH4+ activated to different degrees, with K+ being the most effective. Li+ inhibited, and Na+ and Cs+ had little or no effect on the activity. The activating effects of KCl, RbCl, and NH4Cl could not have been due to Cl−, since NaCl, CsCl (Table I), and increased levels of MgCl2 (3) did not stimulate the reaction. The enzyme exhibited the same level of activity when NaOH was used instead of (CH3)4NOH to neutralize the reagents used for the assay.

Effect of K+ on the Km for ATP and d-Fructose-1-P—Figs. 1 and 2 show the effect of KCl concentration on the Km for d-fructose-1-P and ATP. In the absence of KCl, the Km values for d-fructose-1-P and ATP were 0.8 mM and 0.7 mM, respectively. Both Km values were decreased to about 0.3 mM in the presence of 40 mM KCl.

Activation by K+ at Different Concentrations of ATP and D-Fructose-1-P—The percentage activation of d-fructose-1-P kinase by 40 mM KCl was least when both ATP and d-fructose-1-P were
Fig. 3. Activation of d-fructose-1-P kinase by 40 mM KCl under varying ATP and d-fructose-1-P concentrations. The two substrates were varied simultaneously and the enzyme concentration was constant. Experimental points are given as ○ and ●. The solid lines are curves generated by the Michaelis equation (18) as given under "Discussion."

Fig. 4. Lineweaver-Burk plot showing the effect of d-fructose-1-P concentration on the apparent $K_A$ for KCl. Velocity (nanomoles of d-fructose-1,6-di-P formed per min) is represented as the rate in the presence of KCl minus the rate in the absence of KCl, with the enzyme concentration constant.

Fig. 5. Lineweaver-Burk plot showing the lack of modification of the apparent $K_A$ for KCl by varying concentrations of MgCl$_2$ and ATP. ○, 3.3 mM ATP and 6.7 mM MgCl$_2$; □, 5.0 mM ATP and 20.7 mM MgCl$_2$; ●, 0.7 mM ATP and 1.4 mM MgCl$_2$. The d-fructose 1-P concentration was constant at 6.7 mM. The velocity (nanomoles of d-fructose-1,6-di-P formed per min) is represented as the rate in the presence of KCl minus the rate in the absence of KCl, with the enzyme concentration constant.

Fig. 6. ATP inhibition of d-fructose-1-P kinase in the presence and absence of KCl. The concentrations of MgCl$_2$, ATP, and KCl are indicated on the graph. The enzyme concentration was constant.

Two substrates in which neither substrate affects the $K_n$ for the other (18). The experimental points coincided well with the theoretical values. Activation by K$^+$ in the presence of 3 mM ATP and d-fructose-1-P was about 4-fold, became greater as the concentration of both substrates was lowered, and was about 10-fold when both ATP and d-fructose-1-P were present at concentrations near their $K_n$ values.

$K_A$ for K$^+$—The apparent $K_A$ for K$^+$ was dependent on the concentration of d-fructose-1-P (Fig. 4), being about 7 mM at 0.63 mM d-fructose-1-P and decreasing to about 3 mM when the

Table II.

present at high concentrations, became greater when the concentration of either substrate was lowered, and was maximal when both substrates were present in small amounts (Table II).

Fig. 3 shows graphically the effect of 40 mM KCl on the d-fructose-1-P kinase reaction in the presence of various concentrations of ATP and d-fructose-1-P. The theoretical curves were generated from the Michaelis equation for enzymic reactions involving
n-fructose-1-P concentration was increased to 12.6 mM. On the other hand, the apparent $K_e$ for $K^+$ was independent of the concentration of ATP and $Mg^{2+}$ (Fig. 5).

Effect of $K^+$ on the Inhibition of n-Fructose-1-P Kinase Activity by ATP—ATP inhibited n-fructose-1-P kinase activity when $Mg^{2+}$ to ATP ratios were less than 2:1 (3). Although 40 mM KC1 did not totally overcome ATP inhibition, a marked activation by $K^+$ was observed when $Mg^{2+}$ to ATP ratios were less than 2:1 (Fig. 6). At 6.6 mM ATP and 3.3 mM $Mg^{2+}$, 40 mM KC1 activated the enzyme over 100-fold, whereas at 6.6 mM ATP and 13.2 mM $Mg^{2+}$, the activity by the same concentration of KC1 was only about 4-fold.

**Discussion**

In many cases of $K^+$ activation, an absolute requirement for a monovalent cation has been demonstrated (7–9, 13, 19–21). In contrast, *A. aerogenes* n-fructose-1-P kinase, although activated, does not seem to require $K^+$ absolutely for activity; removal of possibly activating monovalent cations from the reaction components prior to assay did not result in a total loss of activity, and the tetraethylammonium ion used in the assay has been reported to be nonactivating for enzymes affected by monovalent cations (8, 21, 22). $Na^+$ had no effect since n-fructose-1-P kinase activity was identical when either (CH$_3$)$_2$NOH or NaOH was used to neutralize the assay reagents, and addition of NaCl to the assay containing (CH$_3$)$_2$NOH had no significant effect on the reaction velocity. Like many other enzymes activated by $K^+$ (7), n-fructose-1-P kinase was partially activated by Rub+ and $NH_4^+$ and was inhibited by Li+.

$K^+$ affected not only the maximal velocity of the n-fructose-1-P kinase reaction, but also the $K_m$ for both ATP and n-fructose-1-P. The greater percentage activation observed at lower substrate concentrations is to be expected because of the ability of $K^+$ to increase the apparent affinity of the enzyme for both substrates. In contrast to the finding with acetyl CoA synthetase (8), $K^+$ activation of n-fructose-1-P kinase was not modified by excess $Mg^{2+}$ (Fig. 5). Inhibition of activity by ATP could not be relieved by n-fructose-1-P, ADP, AMP, or various other nucleo-side mono- and diposphates (3). However, $K^+$ was effective in partially overcoming ATP inhibition when the $Mg^{2+}$ concentration was less than twice that of ATP (Fig. 6), although the requirement of the reaction for $Mg^{2+}$ could not be replaced by $K^+$. $NH_4^+$ has been reported to relieve ATP inhibition of rat liver n-fructose-6-P kinase (15), but not that from rabbit muscle (11, 16).

It is apparent that monovalent cations affect different enzymes in a variety of ways. For example, yeast pyruvate kinase, which has an absolute requirement for monovalent cations, displays a sigmoidal rate-monovalent cation relationship (21), and $K^+$ serves as an allosteric activator for muscle AMP deaminase, but has no effect on the maximal velocity of the reaction (22).

The simplest model which would account for our data may be represented as:

$$E + K^+ \rightleftharpoons E$-K^+$

where $E$ represents n-fructose-1-P kinase and $E$-K$^+$ represents an enzyme-$K^+$ complex. $E$, which would predominate in the absence of $K^+$, has $K_m$ values of about 0.7 to 0.8 mM for both ATP and n-fructose-1-P, whereas $E$-K$^+$, which would predominate in the presence of excess $K^+$, has $K_m$ values of about 0.5 mM for both substrates. $E$-K$^+$ exhibits about 3 to 10 times the activity of $E$, depending on the concentrations of n-fructose-1-P and ATP. The binding of either ATP or n-fructose-1-P to the enzyme is not affected by the concentration of the other substrate (3). Therefore, if this model is correct, the following version of the Michaelis equation, as derived for a two-substrate reaction (18), should apply:

$$v = \frac{k_e}{1 + \frac{K_{S1}}{[S_1]} + \frac{K_{S2}}{[S_2]}}$$

where $v$ is the velocity; $[S_1]$ and $[S_2]$ are the concentrations of n-fructose-1-P and ATP, respectively; $K_{S1}$ and $K_{S2}$ are the apparent $K_m$ values for n-fructose-1-P and ATP, respectively; and $k$ and $e$ are constants which reflect the activity and amount of the enzyme. Using experimentally determined values of $K_{S1}$, $K_{S2}$, and $k$ for both the $E$ and $E$-K$^+$ forms of the enzyme, the calculated velocities did, in fact, coincide well with the observed velocities (Fig. 9). The variance of the $K_4$ for $K^+$ with the n-fructose-1-P concentration (Fig. 4) may be explained by assuming the following reactions:

$$K^+ + E \rightleftharpoons k_1 E-K^+$$

$$K^+ + S_1E \rightleftharpoons k_3 S_1-E-K^+$$

where $S_1-E$ and $S_1-E-K^+$ represent complexes of n-fructose-1-P with $E$ and $E$-K$^+$, respectively. The $K_4$ for $K^+$ would tend to equal $k_3/k_1$ in the presence of limiting n-fructose-1-P, and $k_3/k_4$ in the presence of excess n-fructose-1-P. The data in Fig. 6 may be interpreted to mean that $E$-K$^+$ is less susceptible to inhibition by excess ATP than is $E$. It should be emphasized that this model is consistent with $K^+$ functioning either by interacting with substrate at the catalytic site or by inducing conformational changes through binding at an allosteric site. Although cooperative kinetics was not observed (3), it should be noted that allosteric modification of enzymes which exhibit hyperbolic rate-substrate relationships have been previously described (24–26), and such cases have been treated theoretically by Koshland (27). Since the proposed model does not necessarily require the binding of $K^+$ at the catalytic site, the mechanism of $K^+$ activation of n-fructose-1-P kinase could be an exception to the generalized mechanism of monovalent cation activation of phosphoryl-transfer enzymes suggested by Suelter (28).

n-Fructose-6-P, n-fructose-1,6-di-P, and citrate inhibit n-fructose-1-P kinase competitively with n-fructose-1-P (3). Such inhibition suggests possible control of activity in vivo. In view of the ability of $K^+$ to overcome ATP inhibition partially, it would be of interest to determine the effect of this cation on inhibition by the above compounds. It is not known whether the n-fructose-1-P kinase-catalyzed reaction is a rate-controlling step in n-fructose metabolism in *A. aerogenes*. If it is, then it is likely that inhibition of n-fructose-1-P kinase by ATP, n-fructose-6-P, n-fructose-1,6-di-P, and citrate, and activation by $K^+$, play significant roles in the regulation of n-fructose metabolism.
any event, the specific influx of $K^+$ that is known to accompany
the uptake and metabolism of sugars by microbial cells (4-6)
would tend to keep d-fructose-1-P kinase in a maximally active
state.

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