Size-dependent Allosteric Effects of Monovalent Cations on Rabbit Liver Fructose-1,6-bisphosphatase*

(Received for publication, January 16, 1976)

KUNIO NAKASHIMA AND SYOZO TUBOI
From The Department of Biochemistry, Yamagata University School of Medicine, Yamagata, Japan 990-23

Effects of monovalent cations on the neutral rabbit liver fructose-1,6-bisphosphatase are multifunctional and dependent on their nonhydrated ionic size. (a) The maximal velocity is increased by addition of monovalent cations with the optimum stimulation occurring with a nonhydrated ionic radius of 1.2 Å in the presence of a chelating agent such as EDTA. (b) Activation curves are sigmoidal with n values varying from 1.5 to 2.3 as ionic radius of monovalent cation increases. The apparent $K_a$ values from 16.0 to 180 mM, obtained for various monovalent cations, show the inhibitory effect and the apparent $K_a$ for fructose 1,6-bisphosphate is increased as the concentration of monovalent cation is increased. A linear relationship is obtained between the slopes of increase in the $K_a$ and the reciprocals of ionic volume of monovalent cations. (d) The apparent $K_a$ for Mg$^{2+}$ is also increased as the concentration of monovalent cation is increased, and a linear relationship is obtained again between the increases in $K_a$ and the reciprocals of ionic volume of monovalent cations. The cooperative nature for Mg$^{2+}$ saturation is decreased as the $K_a$ increases. (e) The apparent $K_a$ for AMP is also linearly altered as the concentration of monovalent cation is varied. However, the alteration of the $K_a$ is unusual, that is, the smaller cations than K$^+$ increase the $K_a$ (Li$^+$ > Na$^+$ > NH$_4^+$), whereas the larger cations decrease the value (CH$_3$OH)CNH$_2^+$ > Ca$^+$ > Rb$^+$). The effect of K$^+$ is insignificant. Alterations in the $K_a$ are also linearly related to the reciprocals of ionic volume of monovalent cations. The cooperative nature for AMP inhibition is decreased or increased as the $K_a$ increased or decreased. (f) In the absence of the chelating agent, the curves for Mg$^{2+}$ saturation and AMP inhibition were hyperbolic without monovalent cations. By addition of monovalent cation the $K_a$ for Mg$^{2+}$ or $K_a$ for AMP is increased and cooperative nature for binding of both ligands are induced.

For nonspherical monovalent cations, the application of "functional ionic radius" is proposed. Functional ionic radii of NH$_4^+$, (CH$_3$OH)CNH$_2^+$, and (CH$_3$CH$_2$OH)N$^+$ are estimated to be 1.17, 2.55, and 2.87 Å, respectively.

The presence of two distinct sites for the actions of monovalent cations is suggested.
To clarify the mode of action of monovalent cations on neutral rabbit liver fructose-1,6-bisphosphatase, the kinetic parameters of the enzyme in the presence of cations are investigated and the sequence specificity of monovalent cations is analyzed. Evidence is presented for size-dependent actions of cations on two distinct sites of enzyme.

**EXPERIMENTAL PROCEDURE**

**Materials**—Glucose-6-P isomerase and glucose-6-P dehydrogenase were obtained from Boehringer Mannheim and mixed before use. For these enzyme preparations were dialyzed against a thousand volumes of 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4, for 18 hours at 4°. Fructose-1,6-P, NaNADP+, and NaAMP were purchased from Sigma. P-cellulose (P11) was obtained from Whatman and washed with 1 N NaOH and HCl, and then extensively with water. All other chemicals were reagent grade.

**Methods**—Neutral fructose-1,6-bisphosphatase was purified from fresh male rabbit liver according to the methods of Tashima et al. (16) and Traniello et al. (17) with the following modifications. The animal was killed by a blow on the head, and the liver was perfused with cold 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4, before removal, and was chilled in the same solution. The liver was chopped into small pieces, homogenized with 5 volumes (w/v) of the perfusion medium at 4° in a Potter-Elvehjem type homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 12,000 x g for 20 min, and to each liter of the supernatant fraction 243 g of (NH4)2SO4 was added slowly. After the precipitate was removed by centrifugation at 12,000 x g for 20 min the supernatant (400 ml) was dialyzed several times against 5 liters of 0.1 M EDTA, pH 7.0, at 4°. The pH of the solution was adjusted to 6.5 by addition of 5 N acetic acid, and the P-cellulose paste corresponding to 2 g of dry powder was added, keeping the pH constant with 2 N NaOH. The suspension was filtered by filtration on a Buechner funnel, and the filtrate was adjusted to pH 6.1 with 5 N acetic acid. Additional P-cellulose paste corresponding to 2 g of dry powder was added to absorb fructose-1,6-bisphosphatase. During the adsorption the pH was kept constant with 2 N NaOH. The suspension was filtered, and the P-cellulose was packed in a column (2.5 x 20 cm) and washed with 10 liters of 0.23 M acetate buffer, pH 6.3, containing 0.1 M NaOH and HCl, and then extensively with water. All other chemicals were reagent grade.

**RESULTS**

**Activation by Monovalent Cation**—Monovalent cation activation was observed only in the presence of concentrations of fructose-1,6-P2 higher than 0.025 mM (Fig. 1). With respect to Li+, activation was not observed at any concentration. The activity in the absence of alkali metal ions or NH4+ corresponds to that in the presence of 40 mM triethanolamine alone. The enzyme showed a partial dependency on monovalent cation, and at lower concentrations of triethanolamine than 25 mM, enzyme activity became constant to approximately 40% of that observed in the presence of saturating NH4+. Moreover, activation by 40 mM triethanolamine, which was used for the buffer solution in the studies of the effects of monovalent cations, was almost negligible (less than 3%). Thus it was practicable to analyze the kinetic parameters in the presence of added monovalent cations in this buffer. Activation curves were found to be sigmoidal, and this cooperative nature increased as ionic size of the monovalent cation increased. The estimated n values for Na+, NH4+, K+, Rb+, Cs+, Tria, and triethanolamine were 1.5, 1.5, 1.6, 1.6, 1.7, 2.1, and 2.3, respectively.

Although activation was observed with potassium salt of any anion tested, the sulfate salt was most effective as was previously observed with kidney enzyme (3), whereas phosphate salt diminished the activating effect by about 40%. In the following experiments, sulfate salts of monovalent cations were used throughout.

These activating effects of monovalent cations were not evident in the absence of a chelating agent such as EDTA (Table I). Unless otherwise noted, 0.1 mM EDTA was added in the assay system throughout the experiments. $K_v$ and Ionic Radius—Apparent activation constants ($K_v$) for monovalent cations obtained from the concentrations for 50% activation in Fig. 1 were 16.0, 33.6, 48.3, 58.2, 75.6, 152, and 180 mM for Na+, NH4+, K+, Rb+, Cs+, (CH3OH)2CNH+, and (CH3CH2OH)2N+, respectively. A linear relationship was observed between the $K_v$ values for Na+, K+, Rb+, Cs+ and nonhydrated ionic radii of these cations (Fig. 2A).

When the reported nonhydrated ionic radius of 1.43 Å (21) or 1.48 Å (22) is employed for NI4+, the position in the figure deviates from the line, probably because of the nonspherical

![Fig. 1. Activation of rabbit liver fructose-1,6-bisphosphatase by monovalent cations. Enzyme activity was determined in the presence of variable concentrations of monovalent cations. Each point represents the mean value of duplicated determinations.](http://www.jbc.org/)

**FIG. 1.** Activation of rabbit liver fructose-1,6-bisphosphatase by monovalent cations. Enzyme activity was determined in the presence of variable concentrations of monovalent cations. Each point represents the mean value of duplicated determinations.
Size-dependent Effects of Monovalent Cations

TABLE I

Requirement for EDTA in monovalent cation activation

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Fructose-1,6-bisphosphatase activity (μmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CH₂CH₂OH)₂N⁺</td>
</tr>
<tr>
<td></td>
<td>-EDTA</td>
</tr>
<tr>
<td>15</td>
<td>0.14</td>
</tr>
<tr>
<td>40</td>
<td>0.14</td>
</tr>
<tr>
<td>150</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*a* Contained 40 mM triethanolamine.

*b* At the concentration of 0.1 mM.

![Figure 2](image_url)

**Fig. 2.** Ionic radius dependency of monovalent cation activation. A, apparent activation constants (Kᵦ) estimated statistically from the data of Fig. 1 as described under "Experimental Procedure" were plotted against nonhydrated ionic radii (21) of monovalent cations. B, the maximal velocities in the presence of monovalent cations were obtained from double reciprocal plots of monovalent cation concentration versus (v - v₀) at high cation concentrations, where v₀ is the velocity without added monovalent cation, and was expressed as percent of v₀. Ionic radii of NH₄⁺, Tris, and triethanolamine (TEA) were obtained from A.

form of the ion. To satisfy the linear relationship in this biological system, "functional radius" was postulated for NH₄⁺, being 1.17 Å from the line in Fig. 2A. This value was found to be appropriate in cases of alteration in the Kᵦ for fructose-1,6-P₃, Kᵦ for Mg²⁺, and Kᵦ for AMP (see below). Functional nonhydrated ionic radii of Tris and triethanolamine were also estimated to be 2.55 and 2.87 Å, respectively, from their Kᵦ values.

**Maximal Activation and Ionic Radius**—When the maximal velocities in the presence of various monovalent cations were plotted against ionic radii of cations, optimal ionic radius for the maximal activation was observed at about 1.2 Å (Fig. 2B).

**Fructose-1,6-P₃ Saturation and Monovalent Cation**—The saturation kinetics of the enzyme for fructose-1,6-P₃ was affected by the presence of a monovalent cation, and at lower concentrations of fructose-1,6-P₃ the monovalent cation showed an inhibitory effect (Fig. 3). The Michaelis constant for fructose-1,6-P₃, which was estimated from the data at concentrations lower than 0.02 mM, was linearly increased as the concentration of monovalent cation increased (Fig. 4). The Kᵦ in the absence of alkali metal ions or NH₄⁺ corresponds to the value of 6.1 x 10⁻⁴ M for 40 mM triethanolamine alone which is present as the buffer. This value is similar to the values for neutral kidney enzyme of 5 to 10 x 10⁻⁴ M in the presence of 40 mM triethanolamine and 40 mM diethanolamine (10). From Fig. 4, the Kᵦ of neutral rabbit liver enzyme for fructose-1,6-P₃, which would be obtained in the absence of monovalent cations, was estimated to be 4.0 x 10⁻⁴ M.

**Kᵦ and Ionic Volume**—Increase in the Kᵦ was linearly correlated with the reciprocal of ionic volume of monovalent cation (Fig. 4, inset). Ionic volumes obtained for NH₄⁺ and (CH₂CH₂OH)₂N⁺ from the data of Fig. 2A are also in good correlation to the increases in the Kᵦ value.

These effects of monovalent cations on the Kᵦ were also observed in the absence of a chelating agent.

**Mg²⁺ Saturation and Monovalent Cation**—Monovalent cations altered the affinity of neutral rabbit liver fructose-1,6-bisphosphatase for the cofactor divalent cation, Mg²⁺, increasing its apparent Kᵦ value obtained as the concentration for 50% activation. Fig. 5 shows the effect of Na⁺ on Mg²⁺ saturation curves in the presence of 0.1 mM EDTA. The curves were sigmoidal and the increased Kᵦ values were obtained by addition of Na⁺ as previously reported for 150 mM K⁺ (3).

Similar effects were observed with other monovalent cations, and the Kᵦ for Mg²⁺ linearly increased as the concentration of each monovalent cation increased (Fig. 6). The Kᵦ in 40 mM triethanolamine buffer alone was 0.20 mM, and the basic Kᵦ for Mg²⁺, which would be obtained in the absence of any monovalent cation, was estimated to be 0.16 mM.

The sigmoidal nature of Mg²⁺ saturation curves for other fructose-1,6-bisphosphatases had been reported previously (3). Neutral rabbit liver enzyme also showed the cooperative nature in Mg²⁺ saturation with the n value of 1.95 in 40 mM triethanolamine, and this value was decreased in the presence of Li⁺ or Na⁺. With 30 mM Li⁺ and 300 mM Na⁺, the n values of 0.33 and 1.12 were obtained, respectively. On addition of larger monovalent cations, decrease in the value was very slight.

**Kᵦ for Mg²⁺ and Ionic Volume**—The increase in the Kᵦ for Mg²⁺ was also found to be related to ionic volume of monovalent cation. A linear relationship was observed between the rates of increase in the Kᵦ and the reciprocals of ionic volume of monovalent cations (Fig. 7).

**Effect of EDTA**—The Kᵦ and n values for Mg²⁺ saturation were influenced by removal of the chelator EDTA. In 40 mM triethanolamine buffer containing no EDTA, the Mg²⁺ saturation curve was found to be almost hyperbolic showing the n value and Kᵦ of 1.02 and 0.42 mM, respectively (Fig. 8). High concentrations of the monovalent cation, however, induced the sigmoidal nature of the Mg²⁺ saturation curve in the absence of EDTA, and with 150 mM K⁺ the n and Kᵦ values were increased to 1.25 and 2.11 mM, respectively. Thus in the absence of EDTA, increase in the Kᵦ for Mg²⁺ by addition of monovalent cation was more marked, whereas increase in the maximal velocity was very slight.

**AMP Inhibition and Monovalent Cation**—Effect of monovalent cation on the affinity of rabbit liver fructose-1,6-bisphosphatase for AMP was investigated (Fig. 9). In the absence of Mg²⁺, the n value and Kᵦ for AMP decreased as the concentration of Li⁺ increased, but it increased in the presence of Na⁺. In the presence of Mg²⁺, the effect of Na⁺ was similar to that of Mg²⁺, whereas the effect of Li⁺ was moderate. In the absence of Mg²⁺, the effect of Na⁺ and Mg²⁺ was similar, but it was different in the presence of Li⁺.
Size-dependent Effects of Monovalent Cations

**FIG. 3** (left). Initial velocity pattern for neutral rabbit liver fructose-1,6-bisphosphatase. Reciprocal specific activities are plotted as a function of fructose-1,6-P* (FDP) concentration at the fixed concentrations of K* indicated.

**FIG. 4** (right). Effect of monovalent cations on the Michaelis constant for fructose-1,6-P*. The apparent $K_m$ values were estimated using a least squares method as described under “Experimental Procedure,” and were plotted as a function of monovalent cation concentration. The slopes and intercepts of the figure were also obtained with a least squares method, writing the $K_m$ values and the cation concentration + 0.1 for $y$ and $x$, respectively, in Equation 2. The slopes were replotted against the reciprocals of ionic volume of monovalent cations in the inset. Ionic radius of NH$_4^+$ and (CH$_3$CH$_2$OH)$_3$N$^+$ were obtained from their $K_m$ values as described in the text.

**FIG. 5.** Mg$^{2+}$ saturation profile in the presence of Na$.^+$ Enzyme activity was determined at the varied concentrations of Mg$^{2+}$ in 40 mM triethanolamine in the absence or presence of the fixed concentrations of Na$^+$ indicated. Each point represents the mean value of duplicated determinations. The inset shows Hill plots of the same data. The maximal velocity was obtained from double reciprocal plots of Mg$^{2+}$ concentration versus $v$ at higher concentrations of Mg$^{2+}$. The lines were drawn with the slopes (n) and Mg$^{2+}$ concentrations for 50% activation ($K_a$) obtained by a least squares method as described under “Experimental Procedure.”

The apparent $K_i$ value for AMP was interesting. Larger cations than K$^+$ increased the affinity, whereas smaller cations decreased it (Fig. 9). The n for AMP inhibition of 1.76 was also altered slightly in the presence of high concentrations of monovalent cations. Larger cations than K$^+$ increased the value and smaller ones decreased it.

The apparent $K_i$ value for AMP was also linearly altered as the concentration of monovalent cations increased (Fig. 10). K$^+$ exhibited almost no effect on the $K_i$ value at any concentration. This may be the reason why Hubert et al. (3) could not observe any significant effect of the monovalent cation on the $K_i$ of rabbit liver enzyme testing K$^+$. The $K_i$ in the absence of alkali metal ions of 1.16 x 10$^{-5}$ M corresponds to the value with 40 mM triethanolamine alone. The $K_i$ value in the absence of monovalent cations was estimated to be 1.32 x 10$^{-5}$ M.

These effects of monovalent cations on AMP inhibition were observed in the absence of the chelator EDTA, too. AMP inhibition curve in the absence of EDTA was almost hyperbolic, and by the addition of 150 mM Na$^+$ the $K_i$ and n values were altered from 1.66 x 10$^{-5}$ M and 1.1 to 4.37 x 10$^{-6}$ M and 1.4, respectively. That is, the monovalent cation in the absence of a chelator induces the cooperativity for AMP inhibition, as well as for Mg$^{2+}$ saturation.

$K_i$ and Ionic Volume—When the slopes of alteration in the $K_i$ were plotted against the reciprocals of ionic volume of monovalent cations, a linear relationship was obtained again as shown in Fig. 11. Critical ionic volume, which would cause no alteration in the $K_i$, was estimated to be 9.80 Å$^3$ corresponding to ionic radius of 1.33 Å.

**DISCUSSION**

The kinetic constants for all ligands, namely, the $K_m$ for fructose-1,6-P*, $K_a$ for Mg$^{2+}$, and $K_i$ for AMP, were increased or altered as the concentration of monovalent cations increased. As has been discussed previously by McGregor et al. (23), a general conformational change in enzyme protein may result in the alteration of the constants for all substances. One of the effects of a monovalent cation on rabbit liver fructose-1,6-bisphosphatase may be a general conformational change in enzyme protein. Linear relations are observed between the rates of alteration in these constants and the reciprocals of ionic volumes of monovalent cations (Figs. 4, 7, and 11). A possible explanation of the mechanism for these phenomena would be that the extent of conformational change is dependent on the amount of ions which could get into a specific “pocket,” distinct from the catalytic site or AMP binding site. The alteration in the $K_i$ for AMP is, however, different from that for the $K_m$ for fructose-1,6-P* or the $K_a$ for Mg$^{2+}$, that is, K$^+$ shows the neutral effect and smaller cations than K$^+$ increase the constant whereas larger ones decrease the value. Probably a general conformational change may give different effects on different loci of enzyme protein.
The presence of a chelator such as EDTA (Table I) whereas the inactivation is dependent on ionic radius of the monovalent cation with the optimum radius of 1.2 Å. It is of paramount interest that this activation by a monovalent cation requires the presence of a chelating agent EDTA whereas the inactivation is not due to the removal of trace heavy metal contaminants (26–31). The activation mechanisms suggested so far are: (a) the direct involvement in catalysis probably in such a chelate form as Mg²⁺EDTA (28–32) which would be acting on a different form of enzyme from that for free Mg²⁺ (29–31), (b) prevention or removal of ATP inactivation (33, 34), and (c) of GSSG inactivation (35). We now present evidence for additional actions of the chelating agent, that is, (d) participation in monovalent cation activation and (e) induction of cooperativities in Mg²⁺ saturation and AMP inhibition in the absence of monovalent cations.

In the activation reaction of rabbit liver fructose-1,6-bisphosphatase, saturation curves for monovalent cations are sigmoidal showing that the binding of a monovalent cation to the catalytic site is also cooperative (Fig. 1), while hyperbolic saturations are generally observed in other enzymes (6, 23, 36). The extent of cooperativity and the apparent activation concentration possess the tight relationships to ionic radius of the monovalent cation. When the previously reported ionic radius of NH₄⁺ deviates from the linear relationship. Non-metal cations such as NH₄⁺, Tris, or (CH₂OH)₂CNH₃⁺ are not completely spherical, so that their effective ionic radii or ionic volumes in the biological system may differ from the physically reported values. Using fructose-1,6-bisphosphatase activating system, it may be possible to obtain the “functional” radii of various monovalent cations.

Thus the presence of two distinct sites for the actions of the monovalent cation is suggested. One is the catalytic site in which the monovalent cation is promoting the enzymatic catalysis. Another is the allosteric site which is involved in the alteration of kinetic constants for the substrate and effectors indicating a general conformational change. The action of the monovalent cation on the former site can be distinguished from that on the latter by (a) the requirement for a chelating agent, (b) the cooperative nature for its binding, and (c) the correlation to ionic radius of the monovalent cation whereas ionic volume is an important factor for the latter.

Similarly, two distinct effects of monovalent cation are recently observed in the studies on rat liver pyruvate carboxylase. A monovalent cation is required for its activity (10), while pyridoxal 5’ phosphate, cysteine, β mercaptoethanol, or oleate on fructose-1,6-bisphosphatase have been extensively investigated and discussed.

The cooperativities which are observed for Mg²⁺ activation and AMP inhibition in the presence of a chelating agent EDTA are decreased or increased as the Kᵦ and Kᵦ increased or decreased, respectively, by addition of monovalent cations. In the absence of the chelator, however, Mg²⁺ activation and AMP inhibition are noncooperative and the monovalent cation induces the cooperativity for both cases. All these observations indicate that the monovalent cation acts as a potent allosteric effector on rabbit liver fructose-1,6-bisphosphatase.

Another role of the monovalent cation in the regulation of fructose-1,6-bisphosphatase activity may be the direct participation in enzymatic catalysis at the catalytic site. The increase in the maximal velocity is dependent on ionic radius of the monovalent cation with the optimum radius of 1.2 Å. It is of interest that this activation by a monovalent cation requires the presence of a chelator such as EDTA (Table I) whereas the conformational effect of the cation mentioned above can occur even in the absence of the chelator. These results suggest that in the presence of a chelating agent the monovalent cation is also participating in the direct catalysis as a bridge between ligands, or between enzyme and a ligand as has been suggested in other enzyme reactions (8–10, 23–25). The activating effects of chelating agents such as EDTA, histidine, citrate, malonate,
high concentrations of the cation affect the enzyme conformation and cause the dissociation of the native tetrameric enzyme into enzymatically active dimers or protomers, and finally into inactive protomers (37). With rabbit liver fructose-1,6-bisphosphatase, however, no evidence for the dissociation of enzyme protein in the presence of a monovalent cation has been obtained.

Initial velocity pattern for saturation of fructose-1,6-P₂ in the presence of monovalent cation (Fig. 3) is complex and shows the characteristic of double competitive substrate inhibition which has been first observed in yeast β-ketothiolase (38) and is characterized by Cleland (39). However, to clarify whether monovalent cation is acting as a co-substrate in the reaction of fructose-1,6-bisphosphatase, further examinations are needed.

Considering that K⁺ is the principal constituent of intracellular monovalent cations with the concentration reaching up to 150 meq per liter (40), it may be physiologically significant that K⁺ is one of the best activating monovalent cations for fructose-1,6-bisphosphatase with the optimum concentration of 150 mM. The increased Kₜ for Mg²⁺ in the presence of high concentrations of monovalent cations is also consistent with the very high intracellular concentration of Mg²⁺ of around 30 meq per liter (40). Conversely, it is of interest that K⁺ does not interfere in the regulatory action of the allosteric inhibitor AMP (20, 41) on liver fructose-1,6-bisphosphatase.

REFERENCES
1. Gomori, G. (1943) J. Biol. Chem. 148, 139-149
21. Handbook of Chemistry and Physics, 47th Ed (1966), The Chemical Rubber Co., Cleveland, Ohio
Size-dependent Effects of Monovalent Cations

Size-dependent allosteric effects of monovalent cations on rabbit liver fructose-1,6-bisphosphatase.
K Nakashima and S Tuboi


Access the most updated version of this article at http://www.jbc.org/content/251/14/4315

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/251/14/4315.full.html#ref-list-1