The Effect of Monovalent Cations on Isoleucyl Transfer-RNA Synthetase Reaction from Baker's Yeast

T. LÖVGREN

Department of Biochemistry and Pharmacy, Åbo Akademi, SF-20500 Åbo, Finland

The effect of monovalent cations on isoleucyl-tRNA synthetase from baker's yeast has been analyzed. The aminoacylation of tRNA^{Ile} is strongly activated by potassium ions. Maximal rate of aminoacylation was obtained in the presence of approximately 140 mM potassium chloride. The rate of the reaction is proportional to $[K^+]^{2.35}$ over the entire range to 140 mM. No activation of the ATP–PPi exchange reaction can be demonstrated but the activity decreased continuously.

The kinetic constants for the aminoacylation reaction show that any activating effect of potassium ions (including those affecting the binding of substrates) must obviously be on the rate of the reaction. Ionic interactions play a major role in the process of tRNA-binding to the enzyme.

In contrast to the specific functions of certain divalent cations, the mode of action of monovalent cations for activating many enzymes has remained relatively obscure.¹ It is generally considered that the active conformation of enzymes activated by monovalent cations is adjusted or stabilized by the ion.

Monovalent cations, usually potassium, are included in the reaction mixtures of almost all work done on aminoacyl-tRNA synthetases. However, very few reports have been published on the action of the monovalent cations. Svensson^{2,3} has done some studies of the effect of various salts on the aminoacylation reaction of methionyl-tRNA synthetase. The effect of inhibiting concentrations of salt has been discussed by Loftfield.^{4,5} Yarus⁶ notes that the major effect of salts is on the association of tRNA and enzyme, not on the rate of aminoacylation. The best explanation seems to be a conformational state

of tRNA which is optimal for binding. Pingoud *et al.*⁷ estimated the equilibrium and rate constants for the interaction of seryl-tRNA synthetase with tRNA^{Ser} at various concentrations of salt.

The present paper reports the activating effect of monovalent cations on isoleucyl-tRNA synthetase (EC 6.1.1.5) from baker's yeast. No activating effect on the ATP–PPi exchange reaction has been found while concentrations above 0.2 M inhibit both the esterification and the ATP–PPi exchange reaction. The site of action of the activating ion is discussed.

MATERIALS AND METHODS

Isoleucyl-tRNA synthetase was prepared from baker's yeast according to a method used to obtain the enzyme from *E. coli*.⁸ The yeast cells were broken by sonication and 10 % glycerol was included to the buffer solutions. The specific activity of the enzyme was 53 units/mg protein. One unit of enzyme activity is defined as the aminoacylation of 1 nmol tRNA in 1 min at 30 °C.

Aminoacylation was performed according to Ref. 8. The assay mixture (150 μ l) contained, unless otherwise noted, 100 mM Tris–HCl buffer pH 7.5, 2 mM ATP, 4 mM Mg²⁺, 60 μ g tRNA, 60 μ M [¹⁴C]-isoleucine, 140 mM KCl, 2.5 mM GSH, 0.02 % bovine serum albumin and enzyme.

The pyrophosphate exchange assay was performed according to Ref. 9 except that the anion-exchange paper was soaked in 0.15 M Na₄P₂O₇ at pH 8.0 and eluted with a 0.1 M Na₄P₂O₇ of the same pH. The enzyme activity is given as the incorporation of 1 μ mol [³²P]-pyrophosphate into ATP per min at 30 °C. tRNA^{Ile} was prepared from unfractionated baker's yeast tRNA according to the method

Fig. 1. (a) Dependence of initial rate of isoleucyl-tRNA formation on activating KCl concentrations, (●) 140 mM, (○) 100 mM, (×) 80 mM, (■) 60 mM, (□) 40 mM, (▲) 20 mM and (△) in the absence of added KCl. The concentration of tRNA^{ile} was 3.6×10^{-7} M and the test was carried out as described in Materials and methods. (b) The data of Fig. 1a replotted as the specific activity of isoleucyl-tRNA synthetase versus KCl concentration. (c) The data of Fig. 1b replotted as the logarithm of the potassium ion activated rate of reaction versus the logarithm of the potassium ion concentration. $v_{K^+} = v - v_0$, v is the activated rate, and v_0 is the rate when no potassium ions were added.

of Gillam *et al.*¹⁰ The purified tRNA had an acceptor activity of 0.9 nmol/ A_{260} unit at pH 7.0.

RESULTS

In the presence of 140 mM potassium chloride a 20-fold increase in the rate of amino-

acylation is obtained compared to the rate observed in the presence of less than 0.1 mM potassium. A typical experiment for the determination of the effect of potassium chloride on the rate of aminoacylation is shown in Fig. 1a. A similar effect was obtained with potassium sulfate. Sodium ions are also almost as effective as potassium ions. When the rate

Fig. 2. (a) The dependence of the rate of isoleucyl-tRNA formation of inhibiting KCl concentrations. The concentration of tRNA^{ile} was 1.7×10^{-7} M and the test was carried out as described in Materials and methods. (b) The data of Fig. 2a replotted as the logarithm of rate of reaction versus $\sqrt{\mu}/(1 + \sqrt{\mu})$. The estimated ionic strength was 0.1 M in the absence of added KCl.

Fig. 3. The dependence of rate of exchange of $[^{32}\text{P}]$ -pyrophosphate on KCl concentration. The conditions were as described in Materials and methods.

of aminoacylation is plotted against the concentration of potassium chloride a sigmoidal curve is obtained (Fig. 1b). The rate of aminoacylation is very sensitive to salt concentrations above 140 mM (Fig. 2a). At 0.3 M potassium chloride only about 30 % of the optimal activity is left.

The rate of the ATP – PPi exchange reaction is not sensitive to potassium ions to the same extent as aminoacylation (Fig. 3). No activa-

tion whatsoever can be observed. The activity decreases continuously with an increasing potassium chloride concentration. At 0.3 M potassium chloride about 55 % of the original activity is still left.

The effect of potassium ions on the K_m of isoleucine, ATP and tRNA^{Ile} was determined by aminoacylation. Fig. 4 shows the effect of potassium ion activation on the estimation of K_m for tRNA. The Eadie plots show the variation of the rate of isoleucyl-tRNA formation with concentrations of tRNA. The data for all three substrates are summarized in Table 1. The K_m 's for isoleucine and ATP are substantially the same despite the difference in rate and the concentration of potassium ions. The K_m for tRNA is altered almost exactly as predicted from primary effect of salt on K_m .^{4,5}

Table 1. The kinetic constants for isoleucyl-tRNA synthetase determined by aminoacylation in the presence of different amounts of KCl. K_m are in units of mol/l and V_{max} are in units of nmol ile-tRNA/min mg protein.

[KCl]	0	140 mM	240 mM
K_m^{tRNA}	0.58×10^{-7}	3.0×10^{-7}	7.1×10^{-7}
K_m^{Ile}	4.2×10^{-8}	9.0×10^{-8}	8.7×10^{-8}
K_m^{ATP}	2.1×10^{-4}	2.4×10^{-4}	1.8×10^{-4}
V_{max}	3	95	65

Fig. 4. Eadie plots showing the variation of rate of isoleucyl-tRNA formation with concentrations of tRNA^{Ile} in the presence of (a) no added KCl, (b) 140 mM KCl, and (c) 240 mM KCl. Conditions were as described in Materials and methods.

DISCUSSION

According to Suelter¹ the enzymic reactions activated by monovalent cations can be divided into two main classes; phosphoryl transfer and elimination reactions. The usual representation of the aminoacylation of tRNA is through the intermediate formation of an enzyme bound aminoacyladenylate which represents a phosphoryl transfer reaction. Thus the ATP-Pi exchanges would have been expected to be sensitive to potassium ion activation but are not. The transfer of isoleucine from AMP to tRNA should not be sensitive to potassium ions but it is. As the rate of formation of isoleucyl-tRNA increases 20-fold in the presence of 140 mM potassium chloride the rate limiting step of aminoacylation has to be affected in one way or the other. Hence the potassium ions either affect a step of the reaction that they should not activate or then the concerted mechanism,⁵ which does not require the formation of enzyme bound aminoacyl-adenylate, has to be considered as an alternative that is sensitive to activating potassium ion concentrations. Based on the fact that the effect on the ATP-Pi exchange reaction is relatively small and inhibiting it seems likely that the enzyme is not substantially altered by potassium ions. On the other hand, in the presence of increasing amounts of potassium ions the K_m is constantly increasing even though the rate of the reaction is first increasing and then decreasing (Table 1 and Fig. 4). Any activating effect (including those affecting the binding of substrates) must obviously be on the rate of the reaction. The rate of the reaction is proportional to $[K^+]^{1.25}$ over the entire range to 140 mM (Fig. 1c). Potassium ions bind apparently to more than one site or one enzymatic species in the mechanism.

Loftfield^{4,5} feels that the major force in associating tRNA with ligase is interionic attraction. When the logarithm of the rate of aminoacylation of tRNA is plotted against the Debye-Hückel ionic strength function, $\log v = 0.5z^+z^- \sqrt{\mu}/(\sqrt{\mu} + 1)$, usually a linear curve has been obtained with a negative slope.^{4,5} If the data for inhibition is plotted according to the Debye-Hückel¹¹ approximation a negative slope of -15 is obtained (Fig. 2b). This observation is consistent with the idea that

ionic interactions play a major role in the process of tRNA-binding to the enzyme. The effect of salt on K_m for tRNA¹² is as predicted from other studies,⁴ but it is more interesting because the K_m is constantly increasing even though the rate of reaction is first increasing then decreasing.

More experimental data are obviously required for identification of the step in the catalytic process that is activated by potassium ions. It has recently been concluded that it is unlikely that enzyme-product dissociation is the rate-limiting step in the synthesis of aminoacyl-tRNA.¹³

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