Potassium Functionally Replaces the Second Lysine of the KMSKS Signature Sequence in Human Tyrosyl-tRNA Synthetase*

Joseph Austin‡ and Eric A. First§

From the Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center-Shreveport, Shreveport, Louisiana 71130

Unlike their bacterial homologues, a number of eukaryotic tyrosyl-tRNA synthetases require potassium to catalyze the aminoacylation reaction. In addition, the second lysine in the class I-specific KMSKS signature motif is absent from all known eukaryotic tyrosyl-tRNA synthetase sequences, except those of higher plants. This lysine, which is the most highly conserved residue in the class I aminoacyl-tRNA synthetase family, has been shown to interact with the pyrophosphate moiety of the ATP substrate in the Bacillus stearothermophilus tyrosyl-tRNA synthetase. Equilibrium dialysis and pre-steady-state kinetic analyses were used to determine the role that potassium plays in the tyrosine activation reaction in the human tyrosyl-tRNA synthetase and whether it can be replaced by any of the other alkali metals. Kinetic analyses indicate that potassium interacts with the pyrophosphate moiety of ATP, stabilizing the E{Tyr-ATP} complexes by 2.3 and 4.3 kcal/mol, respectively. Potassium also appears to stabilize the asymmetric conformation of the human tyrosyl-tRNA synthetase dimer by 0.7 kcal/mol. Rubidium is the only other alkali metal that can replace potassium in catalyzing tyrosine activation, although the forward rate constant is half of that observed when potassium is present. The above results are consistent with the hypothesis that potassium functionally replaces the second lysine in the KMSKS signature sequence. Possible implications of these results with respect to the design of antibiotics that target bacterial aminoacyl-tRNA synthetases are discussed.

Aminocacyl-tRNA synthetases have gained attention recently as potential targets for antibiotics (1–10). Identifying differences in the catalytic mechanisms of bacterial and human aminocacyl-tRNA synthetases will facilitate the development of antibiotics that selectively target the bacterial aminocacyl-tRNA synthetases. We are currently investigating the catalytic mechanisms of the human and Bacillus stearothermophilus tyrosyl-tRNA synthetases to elucidate the differences between these two enzymes.

Tyrosyl-tRNA synthetase catalyzes the attachment of tyrosine to tyrosine tRNA (tRNA^{Tyr}) by an ATP-dependent two-step reaction mechanism. In the first step, tyrosine is activated by MgATP to form an enzyme-bound tyrosyl-adenylate intermediate. The second step consists of the transfer of tyrosine to the 3′ end of tRNA^{Tyr}.

\[
\text{TyrRS} + \text{Tyr} + \text{MgATP} \rightleftharpoons \text{TyrRS} + \text{Tyr-AMP} + \text{PPi} \quad (\text{Eq. 1})
\]

\[
\text{TyrRS} + \text{Tyr-AMP} + \text{tRNA}^{Tyr} \rightleftharpoons \text{TyrRS} + \text{Tyr-tRNA}^{Tyr} + \text{AMP} \quad (\text{Eq. 2})
\]

Tyrosyl-tRNA synthetase is a homodimer that displays “half-of-the-sites” reactivity, with only one tyrosyl-adenylate molecule formed per dimer (11–13). Although most of the active site amino acids are conserved between the human and B. stearothermophilus tyrosyl-tRNA synthetases, several differences exist between the two enzymes, including the inability of the human and bacterial tyrosyl-tRNA synthetases to aminoacylate each other’s tRNA^{Tyr} (14, 15). In addition, sequence analyses indicate that the human tyrosyl-tRNA synthetase is <16% identical to the B. stearothermophilus enzyme and that four of the eighteen active site residues identified in the B. stearothermophilus enzyme, Cys^{35}, His^{48}, Thr^{51}, and Lys^{233}, are not conserved in the human tyrosyl-tRNA synthetase (14). Replacement of three of these four residues (Cys^{35}, His^{48}, and Lys^{233}) in the B. stearothermophilus tyrosyl-tRNA synthetase destabilizes the transition state complex for the tyrosine activation step by 1.2, 1.2, and 3.0 kcal/mol, respectively (16–22). Despite the absence of these amino acids, the stabilities of the transition states for tyrosine activation are virtually identical for the human and B. stearothermophilus enzymes (23). Lastly, potassium is required for catalysis of the tyrosine activation reaction in a number of eukaryotic tyrosyl-tRNA synthetases, but not their bacterial homologues (23–26).

In this study, we address the question of how potassium promotes catalysis of the tyrosine activation reaction in human tyrosyl-tRNA synthetase. Specifically, the hypothesis that potassium stabilizes the transition state complex for the tyrosine activation reaction through interactions with the pyrophosphate moiety of MgATP is tested. This hypothesis is based on the following reasoning. Stabilization of the transition state for tyrosine activation in B. stearothermophilus tyrosyl-tRNA synthetase is largely due to the formation of interactions between the pyrophosphate moiety of MgATP and active site amino acids in the enzyme (reviewed in Refs. 27 and 28). In particular, the second lysine in the KMSKS signature sequence, which is replaced by a serine in the human tyrosyl-tRNA synthetase, stabilizes the transition state for tyrosine activation by 3.0 kcal/mol in the B. stearothermophilus enzyme (14, 19, 21). The observation that the stabilities of the transition states for tyrosine activation are virtually identical for the human and B.
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*steatorrhophilus* enzymes suggests that the human tyrosyl-tRNA synthetase compensates for the absence of the second lysine in its KMSKS signature sequence. Because potassium is required for catalytic activity in the human tyrosyl-tRNA synthetase, but not in the *B. steatorrhophilus* enzyme, it is a candidate for functionally replacing the second lysine in the active site of the human enzyme.

The results presented in this study provide kinetic evidence that potassium stabilizes the transition state complex for tyrosine activation by interacting with the pyrophosphate moiety of ATP. These observations suggest that potassium functionally replaces the second lysine in the KMSKS signature motif, either directly through interactions with the pyrophosphate moiety or indirectly through its effects on active-site amino acids in the human tyrosyl-tRNA synthetase. We further show that of the alkali metals, only rubidium can substitute for potassium, indicating that the effects of potassium are ion-specific. Based on the observation that potassium appears to functionally replace the most highly conserved amino acid in the class I aminoacyl-tRNA synthetase family, namely, the second lysine in the KMSKS signature sequence, we propose that the replacement of this lysine by potassium provides a selective advantage that has resulted in its presence being maintained in eukaryotic tyrosyl-tRNA synthetases.

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents were purchased from the following sources: nickel-nitritotriacetic acid resin and Polypropylene columns were from Qiagen; l-[^14C]tyrosine was from Moravek Biochemicals; inorganic pyrophosphate, tetrasodium pyrophosphate, NaI, and RbCl were from Sigma; nitrocellulose filters were from Schleicher & Schuell; and EMD-103 membranes and NAP-25 columns were from Amersham Biosciences. All other reagents were purchased from Fisher Scientific.

**Equilibrium Dialysis**—Equilibrium dialysis was performed using [14C]tyrosine and recombinant human tyrosyl-tRNA synthetase from *Escherichia coli* have been described previously (14, 23).

**Experimental Determination of Potassium Dependence**—The human tyrosyl-tRNA synthetase was expressed and purified as described previously (23, 29). The data were analyzed by both nonlinear and linear curve fitting using the following equations:

\[
\alpha = \frac{n}{K_{TyrFree}}[E]_T + [TyrFree]^{-1} \frac{K_{TyrBase}}{[E]}_T
\]

where \( \alpha = \frac{[TyrBound]}{[TyrFree]} \), \( K_{TyrFree} \) is the dissociation constant for tyrosine, \( [TyrBound] \) is the concentration of tyrosine bound to the enzyme, \( [TyrFree] \) is the concentration of unbound tyrosine at equilibrium, \( n \) is the number of active sites, and \( [E]_T \) is the total enzyme concentration as determined by absorbance at 280 nm. The concentration of bound tyrosine was determined by subtracting the concentration of tyrosine in the dialysis chamber that contained no enzyme ([TyrFree]) from the concentration of tyrosine in the dialysis chamber containing the enzyme.

**Kinetic Analyses**—All kinetic analyses were performed at 25 °C in the standard buffer, which contained 150 mM Tris-Cl, pH 7.5, 10 mM MgCl2, and 20 mM β-mercaptoethanol. The different metal ions were added, in the form of LiCl, NaCl, KCl, RbCl, or CsCl solutions, to standard buffer at a final concentration of 150 mM. Stopped-flow fluorescence studies were used to monitor the pre-steady-state kinetics for the MgATP dependence of tyrosyl-adenylate formation and the pyrophosphate dependence of the reverse reaction (13). In these studies an SX 18.MV stopped-flow spectrophotometer (Applied Photophysics) was used to monitor the decrease in fluorescence of the human tyrosyl-tRNA synthetase that is associated with the formation of the TyrRS-Tyr-AMP complex (\( \gamma \), noncovalent bond; \( \delta \), covalent bond) and the increase in fluorescence that is associated with the reverse reaction (\( \lambda_{es} = 295 \) nm, \( \lambda_{em} > 320 \) nm) (23). In the MgATP dependence experiments, one of the syringes of the stopped-flow instrument contained the enzyme (0.3–0.5 μM), whereas the other syringe contained varying concentrations of MgATP. Both syringes contained standard buffer, pyrophosphatase, and 200 μM tyrosine. For the rubidium dependence assay, one syringe contained human tyrosyl-tRNA synthetase (0.3–0.5 μM), 10 mM MgATP, and pyrophosphatase in standard buffer, whereas the other syringe contained 400 μM tyrosine and varying concentrations of RbCl (5–200 mM). Upon mixing equal volumes from each syringe, the decrease in fluorescence of the protein was monitored over time. For analysis of the reverse reaction, the TyrRS-Tyr-AMP complex was pre-formed by incubating the enzyme in 150 mM Tris, pH 7.5, 10 mM MgCl2, 20 mM β-mercaptoethanol, and 2 units/ml inorganic pyrophosphatase with 50–100 μM tyrosine and 10 mM MgATP at 25 °C for 1 h (18). The TyrRS/Tyr-AMP complex was separated from free tyrosine and MgATP by gel filtration on NAP-25 columns (Amersham Biosciences) pre-equilibrated with the standard buffer (18). The reverse reaction was monitored by mixing varying concentrations of tetrasodium pyrophosphate (Na4PP4) with the TyrRS/Tyr-AMP complex (0.3 μM) in standard buffer with and without either 75 mM KCl or 100 mM RbCl at 25 °C and measuring the increase in the intrinsic fluorescence of the protein (13, 23). All kinetic data were fit to a single exponential floating end point equation using the Kaleidagraph and Applied Photophysics stopped-flow software packages to determine the observed rate constants. The Kaleidagraph software was used to plot the observed rate constants versus the substrate concentrations and to fit these plots to the following hyperbolic function:

\[
k_{obs} = \frac{k_3[S]}{K_{ATP} + [S]}
\]

where \( k_3 \) is the forward rate constant for the formation of tyrosyl-adenylate, \( [S]_T \) is the total substrate concentration, and \( K_{ATP} \) is the dissociation constant of the substrate of interest (30). In the absence of potassium, MgATP inhibits the reaction at concentrations above 40 mM. However, the relevant kinetic constants could be determined from the data by fitting the resulting \( k_{obs} \) versus [MgATP] plot to the following equation that describes uncompetitive inhibition:

\[
k_{obs} = \frac{k_1}{1 + [S]_T} + \frac{k_3[S]}{K_{ATP} + [S]}
\]

where \( k_1 \) is the forward rate constant for tyrosine activation, \( K_{ATP} \) is the dissociation constant, and \( K_{ATP} \) is the binding constant for ATP at the site of inhibition. A rapid equilibrium assumption is used for the binding of tyrosine and MgATP (25). Due to the low solubility of the MgPP4 complex, it was not possible to reach saturating pyrophosphate concentrations in the pyrophosphate dependence reaction. As a result, \( k_{obs} \) and \( K_{PP} \) could not be independently determined for the reverse reaction. Instead, the reverse reaction was monitored under conditions where \( K_{PP} \gg [PP]_T \), and \( k_{obs} \) was determined by fitting the data to the resulting linear approximation of Eq. 5. In the absence of either KCl or RbCl, the reaction was sufficiently slow to allow the pre-steady-state kinetics to be monitored using a filter binding assay. In this assay, the enzyme-bound tyrosyl-adenylate complex was prepared as described above using [14C]tyrosine. The TyrRS-[14C]Tyr-AMP complex was preincubated in standard buffer at 25 °C, and the reverse reaction was initiated by the addition of tetrasodium pyrophosphate (0.4–1.8 mM). The reaction was monitored by removing and filtering 25-μl aliquots through nitrocellulose filters over a 5-min time period. The filters were washed with two 5-ml aliquots of ice-cold 150 mM Tris, pH 7.5, dried, and counted by scintillation counting in 5 ml of Cytoscint (Fisher Scientific).

**Calculation of Relative Free Energies**—The relative free energies for each state along the reaction pathway were calculated from the rate and dissociation constants using the following equations:

\[
\Delta G_{TyrRS/Tyr-AMP} = RT\ln K_{Tyr-AMP}
\]

\[
\Delta G_{TyrRS/Tyr-AMP} = RT\ln (K_{Tyr-AMP})
\]

\[
\Delta G_{TyrRS/Tyr-AMP} = RT\ln (K_{Tyr-AMP})
\]

\[
\Delta G_{TyrRS/Tyr-AMP} = RT\ln (K_{Tyr-AMP})
\]

where \( \Delta G \) is the Gibbs free energy relative to that of the unliganded
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enzyme \( (G_{\text{TyRS}} = 0) \), \( R \) is the gas constant, \( T \) is the absolute temperature, \( k_b \) is the Boltzmann constant, \( h \) is Planck's constant, and \( [\text{TyrRS-Tyr-ATP}] \) is the transition state complex (18). The Gibbs activation energy for the formation of tyrosyl-adenylate was calculated by taking the difference in free energies between the transition state and the TyrRS-Tyr-AMP complex that immediately precedes the transition state. All free energies are calculated relative to the unliganded enzyme, assuming a standard state of 1 M ATP, 1 M tyrosine, and 1 M pyrophosphate.

**Monitoring the Stability of the Enzyme-bound Tyrosyl-adenylate Intermediate**—The stability of the enzyme-bound tyrosyl-adenylate intermediate was determined by measuring the rate for the dissociation of \([\text{14C}\text{Tyr-AMP}] \) from the TyrRS-[\text{14C}]Tyr-AMP complex at 25 °C. The TyrRS-[\text{14C}]Tyr-AMP complex was prepared from l-[\text{14C}]tyrosine, MgATP, and tyrosyl-tRNA synthetase using the procedure described above. The decay of the complex was measured by adding 200 µl of the TyrRS-[\text{14C}]Tyr-AMP complex to standard buffer with and without 150 mM KCl and removing 20-µl aliquots periodically. Each aliquot was added to a 2.5-cm nitrocellulose filter overlaid with 3 ml of ice-cold 150 mM Tris-Cl, pH 7.5. The filters were washed with 5 ml of ice-cold 150 mM Tris-Cl, pH 7.5, and dried. The residual TyrRS-[\text{14C}]Tyr-AMP bound to the filters was assayed by scintillation counting in 5 ml of Cytoscient scintillation mixture. The rate constant for the dissociation of \([\text{14C}]\text{Tyr-AMP} \) from the TyrRS-[\text{14C}]Tyr-AMP complex \( (k_{\text{off}}) \) was determined by fitting a plot of the residual radioactivity (cpm) versus time (in minutes) to the first order rate equation. Based on the assumption that potassium has no effect on the binding of tyrosyl-adenylate to the enzyme \( (k_{\text{on}}) \), the free energy difference for the dissociation of Tyr-AMP in the absence and presence of potassium was calculated from the following equation:

\[
\Delta G = RT\ln \left( \frac{k_{\text{on}}}{k_{\text{off}}} \right) \quad \text{(Eq. 12)}
\]

where \( k_{\text{on}} \) and \( k_{\text{off}} \) are the rate constants for the dissociation of Tyr-AMP from the TyrRS-Tyr-AMP complex in the presence and absence of potassium, respectively.

**RESULTS**

**Catalysis of Tyrosine Activation in the Absence of Potassium**—To determine whether potassium increases the affinity of human tyrosyl-tRNA synthetase for its substrates, the dissociation constants of tyrosine and MgATP were determined in the absence of potassium and compared with the values of the dissociation constants previously determined in the presence of 150 mM KCl (23). The dissociation constant for tyrosine \( (K_{\text{Tyr}}) \) was determined by equilibrium dialysis. In the absence of potassium, \( K_{\text{Tyr}} \) is 95 ± 10 μM (Fig. 1A). This is ~5-fold higher than the \( K_{\text{Tyr}} \) value determined in the presence of potassium (Table I). Pre-steady-state kinetic methods were used to determine the dissociation constant of MgATP from the TyrRS-Tyr complex \( (K'_{\text{ATP}}) \) and the forward rate constant for tyrosine activation \( (k_b) \). In the absence of potassium, MgATP inhibits the reaction at concentrations above 40 mM (Fig. 1B). This inhibition is not observed when potassium is present (Fig. 1B, inset). By fitting the resulting \( k_{\text{obs}} \) versus [MgATP] plot to Eq. 6, it was possible to determine \( k_b \) and \( K'_{\text{ATP}} \), as well as the binding constant for ATP to the site of inhibition, \( K_{\text{ATP}} \). The values of \( k_b \), \( R_{\text{ATP}} \), and \( K_{\text{ATP}} \) determined in the absence of potassium are 1.7 ± 0.1 s⁻¹, 71 ± 15 mM, and 43 ± 1 mM, respectively.

The reverse rate constant \( (k_{\text{off}}) \) and the dissociation constant of pyrophosphate \( (K_{\text{PP}}) \) cannot be determined independently of each other because the low solubility of the MgPP complex prevents the use of concentrations sufficient to saturate the enzyme (23). However, the \( k_{\text{off}}/K_{\text{PP}} \) ratio can be determined by monitoring the reaction under conditions where \( K_{\text{PP}} > [\text{PP}] \) and fitting the data to the resulting linear approximation of Eq. 5 (Fig. 1C). The \( k_{\text{off}}/K_{\text{PP}} \) ratio was found to decrease by ~1600-fold in the absence of potassium. A summary of the rate and dissociation constants determined in the absence and presence of potassium is presented in Table I.

**Free Energy Calculations for Each Step Along the Reaction Pathway**—To quantify the effect of potassium at each step in the reaction pathway, Gibbs free energy values were calculated for each bound state relative to the free energy of the unbound enzyme (Fig. 2). Due to the inability to determine \( k_{\text{on}} \) and \( K_{\text{PP}} \) independently of each other, the free energy of the TyrRS-Tyr-AMP-PP complex could not be calculated. As a result, the relative stability of the TyrRS-Tyr-AMP-PP complex has been omitted from the free energy diagram (Fig. 2). Although potassium has a small effect on the stability of the TyrRS-Tyr-AMP complex (0.6 kcal/mol), the primary effect of potassium is on those
complexes in which ATP is present. Specifically, potassium stabilizes the TyrRS-Tyr-AMP and TyrRS-[Tyr-AMP-PP] complexes by 2.3 and 4.3 kcal/mol, respectively. This interaction appears to involve the pyrophosphate moiety of ATP because potassium has no effect on the stability of the TyrRS-Tyr-AMP complex.

Dissociation of Tyrosyl-adenylate from the Enzyme-bound Tyrosyl-adenylate Intermediate Complex.—To determine whether potassium affects the rate at which tyrosyl-adenylate dissociates from the TyrRS-Tyr-AMP complex, the rate constant for the decay of the pre-formed TyrRS-[14C]Tyr-AMP complex ($k_{-3}$) in the absence and presence of potassium was determined. [14C]Tyr-AMP dissociates from the TyrRS-[14C]Tyr-AMP complex with rate constants of 2.3 ± 0.3 × 10^{-5} s^{-1} and 7.7 ± 1.0 × 10^{-5} s^{-1} in the absence and presence of potassium, respectively (Fig. 3). This effect is specific to potassium because the rate of dissociation in the presence of 150 mM NaCl is the same as that observed in the absence of potassium (data not shown). In the B. stearothermophilus enzyme, dissociation of the TyrRS-[14C]Tyr-AMP complex occurs with a rate constant of 5.7 × 10^{-5} s^{-1} (29). Control experiments showed that there was no loss of enzyme activity after an 8-h incubation at 25 °C, indicating that the decay observed was not due to denaturation of the enzyme (data not shown). The free energy difference for the dissociation of Tyr-AMP in the absence and presence of potassium calculated from Eq. 12 is 0.65 kcal/mol.

Rubidium Can Substitute for Potassium in the Human Tyrosyl-tRNA Synthetase—To determine whether other alkali metal ions can substitute for potassium in the catalytic mechanism of the human tyrosyl-tRNA synthetase, pre-steady-state kinetic methods were used to calculate the observed rate constants ($k_{o,b}$) for the tyrosine activation reaction using 200 μM tyrosine, 10 mM MgATP, and 150 mM lithium, sodium, rubidium, or cesium (Table I). The values for $k_{o,b}$ obtained in the presence of lithium, sodium, or cesium are similar to those of $k_{o,b}$ when potassium is not present, indicating that they have no significant effect on the catalytic activity of the enzyme. In contrast, rubidium increases $k_{o,b}$ by 88-fold when compared with $k_{o,b}$ determined in the absence of any ion. Under similar conditions, potassium increases $k_{o,b}$ 220-fold. Due to the significant increase in $k_{o,b}$ in the presence of rubidium, the catalysis of tyrosine activation by the human tyrosyl-tRNA synthetase was further characterized in the presence of rubidium. Rubidium binds to the human tyrosyl-tRNA synthetase with an affinity similar to that of potassium ($K_{Rb+} = 40 ± 2$ mM versus $K_{K+} = 32 ± 2$ mM for the dissociation constants of rubidium and potassium, respectively) (Fig. 4). In the presence of 200 mM rubidium, the dissociation constants for tyrosine and MgATP and the forward rate constant are 29 ± 8 μM, 3.2 ± 0.4 mM, and 26 ± 2 s^{-1}, respectively. These values are similar to the values of the constants determined in the presence of potassium (34 ± 8 μM, 4.0 ± 0.1 mM, and 45 ± 4 s^{-1} for $K_{Ty}$, $K_{ATP}$, and $k_3$, respectively). Analysis of the reverse reaction in the presence of rubidium yields results similar to those obtained in the presence of potassium (Table I).

**DISCUSSION**

Potassium Functionally Replaces the Second Lysine in the KMSKS Signature Sequence in Human Tyrosyl-tRNA Synthetase—In human tyrosyl-tRNA synthetase, the second lysine in the KMSKS signature sequence is replaced by a serine (Ser^{1225}) (14). This lysine is the most highly conserved amino acid in the class I aminoacyl-tRNA synthetase family and is strictly conserved in bacterial tyrosyl-tRNA synthetases. Previous investigations indicate that despite the absence of the second lysine in the KMSKS signature sequence, the catalytic efficiencies of the human and B. stearothermophilus enzymes are nearly identical (23). In tyrosyl-tRNA synthetase, the KMSKS signature sequence is located on a mobile loop that moves ~10 Å to interact with the pyrophosphate moiety of ATP (19). In B.

**TABLE I**

Comparison of binding and rate constants for the human tyrosyl-tRNA synthetase in the absence and presence of potassium

| Enzyme | $K_{Tyr}$ | $K_{ATP}$ | $k_3$ | $k_{o,b}$ | $K_{Rb+}$ |
|--------|----------|-----------|------|----------|-----------|
| Potassium | 95 (±10) | 71 (±15) | 1.7 (±0.1) | 13 (±1) | 36 (±3) |
| + Potassium | 34 (±8) | 4.0 (±0.1) | 45 (±4) | 115000 (±16000) | 58000 (±7000) |
| + Rubidium | 29 (±8) | 3.2 (±0.4) | 26 (±2) | 8300 (±500) | 30700 (±700) |

* The standard deviations for three to five repetitions of each experiment are indicated in parentheses.

+ $K_{Tyr}$, and the rates $k_{o,b}$ and $K_{Rb+}$ were all determined by pre-steady-state kinetics using stopped-flow fluorescence to monitor changes in the intrinsic fluorescence of the protein at 25 °C.

+ All values for the human tyrosyl-tRNA synthetase in the presence of potassium are taken from Ref. 23.

**FIG. 3.** The decay of the TyrRS-[Tyr-AMP] complex in the absence and presence of potassium. The TyrRS-[Tyr-AMP] complex was formed using [14C]tyrosine and isolated on a NAP-25 column. The decay of the complex was monitored by incubating the complex in 150 mM Tris, pH 7.5, 10 mM MgCl₂, and 20 mM β-mercaptoethanol at 25 °C and then removing and filtering aliquots over a period of three half-lives in the absence and presence of potassium. The residual radioactivity was measured in cpm in a Beckman scintillation counter.
stearothermophilus tyrosyl-tRNA synthetase, the second lysine in this sequence corresponds to lysine 233. Like potassium in the human enzyme, lysine 233 exerts its maximum effect in the transition state of the reaction and has no effect on the stability of the human enzyme, lysine 233 exerts its maximum effect in the B. stearothermophilus tyrosyl-tRNA synthetase, the second lysine in the human tyrosyl-tRNA synthetase is similar to that of the free energy difference determined for the dissociation of Tyr complex, even though it does not interact directly with the tyrosine substrate. This is indeed the case, with potassium stabilizing the symmetrical Tyr-RS-Tyr-AMP complex by 0.6 kcal/mol. These results are consistent with the hypothesis that potassium stabilizes the symmetrical Tyr-RS-Tyr-AMP complex relative to the unliganded asymmetric dimer, whereas the pre-steady-state kinetic assays are measuring the stability of the Tyr-RS-Tyr-AMP complex relative to the unliganded asymmetric dimer. In other words, the assay that measures the dissociation of tyrosyl-adenylate from the Tyr-RS-Tyr-AMP complex is measuring the stability of the Tyr-RS-Tyr-AMP complex relative to the unliganded asymmetric dimer, whereas the pre-steady-state kinetic assays are measuring the stability of the Tyr-RS-Tyr-AMP complex relative to the unliganded symmetric dimer.

The observation that tyrosine induces “half-of-the-sites” reactivity in tyrosyl-tRNA synthetase indicates that, like tyrosyl-adenylate, the tyrosine substrate must bind more tightly to the asymmetric dimer than it does to the symmetric dimer. If potassium stabilizes the asymmetric dimer, then it must also stabilize the Tyr-RS-Tyr complex, even though it does not interact directly with the tyrosine substrate. This is indeed the case, with potassium stabilizing the Tyr-RS-Tyr complex by 0.6 kcal/mol in the human tyrosyl-tRNA synthetase. This value is similar to the free energy difference determined for the dissociation of tyrosyl-adenylate in the absence and presence of potassium ($\Delta G = 0.65$ kcal/mol). These results are consistent with the hypothesis that potassium stabilizes the asymmetric dimer. Under normal physiological conditions, the concentration of potassium in the cell is $\sim 150$ mM (33). This concentration is sufficient to saturate the potassium-binding site on

**TABLE II**

**Rate of tyrosine activation in the presence of different monovalent metal ions**

| Ion$^a$ | $k_{obs}$ $^b$ | Ionic radii$^b$ |
|--------|---------------|----------------|
| No ion | $0.17 \pm 0.04$ | 1.69 |
| Li$^+$ | $0.10 \pm 0.01$ | 1.33 |
| Na$^+$ | $0.14 \pm 0.01$ | 1.48 |
| K$^+$  | $0.38 \pm 3$   | 1.33 |
| Rb$^+$ | $15 \pm 1$     | 1.48 |
| Cs$^+$ | $0.36 \pm 0.03$ | 1.69 |

$^a$ The ions were present as LiCl, NaCl, KCl, RbCl, or CsCl. $^b$ The reaction was measured by monitoring the decrease in the intrinsic fluorescence of the human tyrosyl-tRNA synthetase (0.5 $\mu M$) associated with the formation of Tyr-AMP in the presence of 200 $\mu M$ tyrosine and 10 mM MgATP at 25°C. The observed rate constants were determined from single exponential fits of the resulting reaction traces. $^c$ The values of the ionic radii are taken from Ref. 34.
human tyrosyl-tRNA synthetase and stabilize the high affinity asymmetric state of the enzyme ($K_d = 32 \text{ nm}$).

**Relevance of This Work to the Design of Tyrosyl-tRNA Synthetase Inhibitors**—One of the goals of this research is to identify differences between the catalytic mechanisms of human and bacterial tyrosyl-tRNA synthetases to design novel antibiotics that selectively inhibit bacterial tyrosyl-tRNA synthetases but not the human homologue. Amino acid sequence analysis indicates that there are four catalytically important active site residues in *B. stearothermophilus* tyrosyl-tRNA synthetase that are not present in the human enzyme (14). These four amino acids, Cys$^{15}$, His$^{48}$, Thr$^{71}$, and Lys$^{233}$, all interact with ATP in the transition state of the reaction (16–22). In addition, sequence analysis indicates that the tyrosine-binding site is highly conserved among bacteria (eubacteria), archaea, and eukaryotes (eukarya) (14). The only significant difference observed within the tyrosine-binding site is the replacement of a threonine in bacterial tyrosyl-tRNA synthetases (Thr$^{73}$ in the *B. stearothermophilus* enzyme) by a histidine in the archaeal and eukaryotic homologues. These observations suggest that the binding of tyrosine is similar among all tyrosyl-tRNA synthetases but that the interactions between the enzyme and ATP differ between bacteria and eukaryotes. The results presented in this study support this hypothesis and suggest that in order to design compounds that selectively inhibit bacterial tyrosyl-tRNA synthetases, one should take advantage of differences in the ATP-binding sites of the human and bacterial enzymes. Further support for this hypothesis comes from the observation that a species of *Micromonaspora* produces a highly selective inhibitor of bacterial tyrosyl-tRNA synthetases, binding to *Staphylococcus aureus* tyrosyl-tRNA synthetases with a 40,000-fold higher affinity than it binds to the *Saccharomyces cerevisiae* homologue (9). The structure of this inhibitor consists of a tyrosyl moiety covalently attached to a bicyclic sugar through a peptide bond (5). The bicyclic sugar binds to *S. aureus* tyrosyl-tRNA synthetase in the general region of the ribose-binding site and is presumably responsible for the specificity of the inhibitor.

In this study, we have presented evidence that potassium functionally replaces the second lysine in the KMSKS signature sequence in human tyrosyl-tRNA synthetase. These results suggest that selective inhibitors of tyrosyl-tRNA synthetase are more likely to target differences in the ATP-binding site than in the amino acid-binding site of the enzyme. Given the selective pressure to ensure that the cognate tRNA is aminocacylated by the correct amino acid, this principle is also likely to apply to the design of selective inhibitors for the other aminoacyl-tRNA synthetases.

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