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Magnesium dependence of the measured equilibrium constants of aminoacyl-tRNA synthetases

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Abstract

The apparent equilibrium constants (K') for six reactions catalyzed by aminoacyl-tRNA synthetases from *Escherichia coli* were measured, the equations for the magnesium dependence of the equilibrium constants were derived, and best-fit analyses between the measured and calculated values were used. The K' values at 1 mM Mg^{2+} ranged from 0.49 to 1.13. The apparent equilibrium constants increased with increasing Mg^{2+} concentrations. The values were 2–3 times higher at 20 mM Mg^{2+} than at 1 mM Mg^{2+}, and the dependence was similar in the class I and class II synthetases. The main reason for the Mg^{2+} dependence is the existence of PP_i as two magnesium complexes, but only one of them is the real product. AMP exists either as free AMP or as MgAMP, and therefore also has some effect on the measured equilibrium constant. However, these dependences alone cannot explain the measured results. The measured dependence of the K' on the Mg^{2+} concentration is weaker than that caused by PP_i and AMP. Different bindings of the Mg^{2+} ions to the substrate tRNA and product aminoacyl-tRNA can explain this observation. The best-fit analysis suggests that tRNA reacts as a magnesium complex in the forward aminoacylation direction but this given Mg^{2+} ion is not bound to aminoacyl-tRNA at the start of the reverse reaction. Thus Mg^{2+} ions seem to have an active catalytic role, not only in the activation of the amino acid, but in the posttransfer steps of the aminoacyl-tRNA synthetase reaction, too.

Keywords: Synthetase; tRNA; Aminoacyl-tRNA synthetase; Equilibria; Magnesium

1. Introduction

An enzyme does not change the equilibrium of the catalyzed reaction. However, it is known that the measured, apparent, equilibrium constant can be dependent on metal ion or proton concentrations if total concentrations of the substrates or products are used in the calculations [1,2]. The enzyme is then specific for a given complex of the substrate or product. The real enzymic equilibrium is formed only between the real substrates and products, and is independent of the reactions in the reaction mixture before or after the enzyme reaction. In the aminoacyl-tRNA synthetase (aaRS) reactions two of the substrates (ATP and tRNA) and all three products (PP_i, AMP and aminoacyl-tRNA) can exist in different magnesium complexes and all substrates and products can exist in different ionic states. Therefore the measured, apparent, equilibrium constant must be dependent on measurement conditions, namely pH, magnesium concentration and ionic strength.

The equilibrium constant for threonyl-tRNA synthetase (ThrRS) has previously been measured as 0.37 and for valyl-tRNA synthetase (ValRS) as 0.32 [3,4]. I recently published a description of the magnesium dependence of the measured equilibrium constant for the arginyl-tRNA synthetase (ArgRS) reaction [5]. The K' increased from 1 to 2.2 when the concentration of Mg^{2+}_{free} was changed from 0.5 to 17 mM. The magnesium dependence of the K' value could not be explained only by the existence of both $MgPP_i$ and Mg_2PP_i but different Mg^{2+} complexes of tRNA and aatRNA are involved, too. The present work expands the ArgRS studies to six other aminoacyl-tRNA synthetases, two from class I and four from class II. In all cases the interpretation of the Mg^{2+} dependences of the equilibrium constants require different binding of the Mg^{2+} ion to tRNA and aminoacyl-tRNA.

2. Theory

Scheme 1 shows the known magnesium and proton equilibria of ATP, PP_i and AMP. The most important complexes at about pH 7 and about 1 mM Mg^{2+}_{free} are $MgATP^{2-}$ and $MgPP_i{}^{2-}$ but

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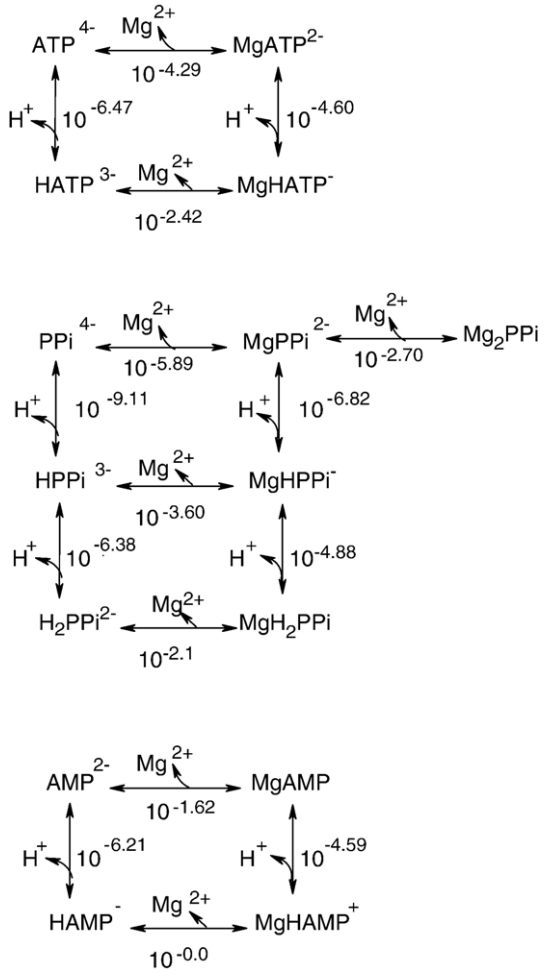


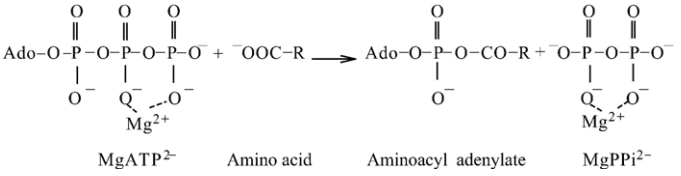
Scheme 1. The known proton and magnesium equilibria of ATP, PP_i and AMP which can affect the measured equilibrium constants of the aminoacyl-tRNA synthetases. The dissociation constant values (M) are from references [6] and [7].

also Mg_2PP_i and $MgHPP_i^-$ are present. AMP is mainly as AMP^{2-} but also $HAMP^-$ and MgAMP exist in low amounts.

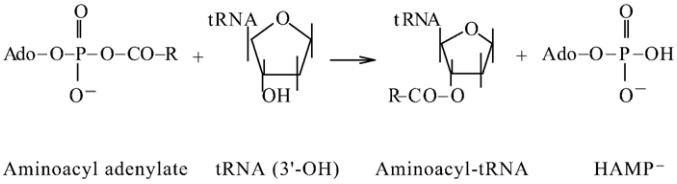
The reaction catalyzed by the aminoacyl-tRNA synthetases is given by Eq. (1). K' is the apparent equilibrium constant of this reaction. (aa is the amino acid and aatRNA aminoacyl-tRNA.)

$$\mathrm{ATP} + \mathrm{aa} + \mathrm{tRNA} = \mathrm{PPi} + \mathrm{AMP} + \mathrm{aatRNA} \quad (1)$$

Eqs. (2) and (3) show the ionic and Mg^{2+} balances in the two main steps of the (class I) aaRS reaction. $MgATP^{2-}$ is the real substrate and $MgPP_i^{2-}$ the real product [8]. tRNA and aatRNA also exist as Mg^{2+} complexes but they are not written in Eqs. (2) and (3). The form of the AMP product is $HAMP^-$ which is dissociated to AMP^{2-} after the enzymic reaction. This feature exists generally in the ATP hydrolysis reactions [9].



(2)



(3)

In the class I aminoacyl-tRNA synthetases PP_i is freed as $MgPP_i^{2-}$ (as shown in Eq. (2)). The class II aminoacyl-tRNA synthetases differ from class I in having three Mg^{2+} ions (some enzymes two) in the ATP·E complex. The differences in the magnesium dependences between the two classes have been confirmed both by crystal structure studies [10] and kinetic studies [11]. Therefore the corresponding product also seems to be $MgPP_i^{2-}$ in class I and Mg_2PP_i in class II. The kinetic studies support this difference [11].

The standard equilibrium constants for the enzymic reaction can be written in class I as Eq. (4) and in class II as Eq. (5), where the real magnesium complexes and the real ionic forms of the substrates and products are taken into account.

$$K_I = \frac{\left[\mathrm{MgPP_i}^{2-}\right]\left[\mathrm{HAMP}^-\right]\left[\mathrm{Mg}_n(\mathrm{aatRNA})^{(q-1)}\right]\left[\mathrm{Mg}^{2+}\right]^{(m-n)}}{\left[\mathrm{MgATP}^{2-}\right]\left[\mathrm{aa}\right]\left[\mathrm{Mg}_m(\mathrm{tRNA})^{q-}\right]} \quad (4)$$

$$K_{II} = \frac{\left[\mathrm{Mg_2PP_i}^{2-}\right]\left[\mathrm{HAMP}^-\right]\left[\mathrm{Mg}_n(\mathrm{aatRNA})^{(q-1)-}\right]\left[\mathrm{Mg}^{2+}\right]^{(m-n)}}{\left[\mathrm{MgATP}^{2-}\right]\left[\mathrm{Mg}^{2+}\right]\left[\mathrm{aa}\right]\left[\mathrm{Mg}_m(\mathrm{tRNA})^{q-}\right]} \quad (5)$$

The amount of Mg^{2+} ions bound to aatRNA (n) or tRNA (m) can be different for different tRNA's, and as well the amount of negative charges (q) can be different. As a first assumption m=n, then the term $[Mg^{2+}]^{(m-n)}$ does not exist in the numerator since $m-n=0$.

Remarkable simplifications can be made in Eqs. (4) and (5). If the measurements are done at constant pH values, the role of the proton equilibria can be omitted but the dissociation constants for the magnesium complexes and the equilibrium constants of the total reaction have values specific for that pH. At pH 7.4 the K_d value for Mg_2PP_i is 2.6 mM and for MgAMP 14 mM [7,12].

Another simplification comes from the strong binding of the first Mg^{2+} ion to ATP and PP_i. The first dissociation constants of Mg^{2+}, K_d(MgATP)=60 μM [12] and K_d($MgPP_i$)=55 μM [7], are so low that the uncomplexed species do not practically exist at normal measurement or normal natural conditions of the aaRS reactions. Then $[ATP_{tot}] \approx [MgATP]$ and $[PP_{i,tot}] \approx ([MgPP_i] + [Mg_2PP_i])$. The second dissociation constant for PP_i, K_d (Mg_2PP_i)=2.6 mM at pH 7.4 [7], is high enough to allow the existence of both $MgPP_i$ and Mg_2PP_i when $[Mg^{2+}_{free}]$ is in the millimolar range. At higher magnesium concentrations AMP is also in a magnesium complex, K_d(MgAMP)=14 mM [12]. ATP, apparently, does not bind a second Mg^{2+} ion [12].

The third type of simplification concerns the Mg^{2+} binding to tRNA. If the Mg^{2+} ions are bound as strongly to the substrate

tRNA as to the product aatRNA, they do not have an effect to the equilibrium constant, and can be omitted from the equations. In practice the best-fit analyses show that one critical Mg^{2+} ion is required to be bound to tRNA but this Mg^{2+} ion does not remain bound, or is very weakly bound to aatRNA. In Eqs. (4) and (5) this means that $m=1$ and $n=0$ or 1. There are several mechanisms regarding the Mg^{2+} binding to aatRNA, fitting somehow to the results (discussed later). The simplifications mentioned above are taken into account in the following equations, and the reaction mechanism is chosen where the given Mg^{2+} ion does not remain bound to aatRNA.

The apparent, measured, equilibrium constant for the reaction in Eq. (1) is given by Eq. (6), where total substrate and product concentrations are used.

$$K' = \frac{[PP_{i,tot}][AMP_{tot}][aatRNA]}{[ATP_{tot}][aa][tRNA]} \quad (6)$$

The dissociation of the Mg^{2+} ion from Mg_2PP_i, MgAMP and MgtRNA is shown by Eqs. (7)–(10). K_{M2PP} is the dissociation constant for the dissociation of one Mg^{2+} ion from Mg_2PP_i ($Mg_2PP_i \rightarrow Mg^{2+} + MgPP_i$), K_{MAMP} the dissociation constant for $MgAMP \rightarrow Mg^{2+} + AMP$, and K_{MR} for the dissociation $Mg{\cdot}tRNA \rightarrow Mg^{2+} + tRNA$.

$$[PP_{i,\,tot}] = [MgPP_i]\left(1 + \frac{[Mg^{2+}]}{K_{M2PP}}\right) \quad (7)$$

$$[PP_{i,tot}] = [Mg_2PP_i]\left(1 + \frac{K_{M2PP}}{[Mg^{2+}]}\right) \quad (8)$$

$$[AMP_{tot}] = [AMP]\left(1 + \frac{[Mg^{2+}]}{K_{MAMP}}\right) \quad (9)$$

$$[tRNA_{tot}] = [MgtRNA]\left(1 + \frac{K_{MR}}{[Mg^{2+}]}\right) \quad (10)$$

In the class I synthetases the standard equilibrium constant (at a constant pH) is given by Eq. (11), and the relationship between the standard (K_I) and apparent, measured (K'_I) equilibrium constants is in Eq. (12) (where Eqs. (7)–(10) have been taken into account).

$$K_I = \frac{[MgPP_i][AMP][Mg^{2+}][aatRNA]}{[MgATP][aa][MgtRNA]} \quad (11)$$

$$K'_I = K_I\left(1 + \frac{[Mg^{2+}]}{K_{M2PP}}\right)\left(1 + \frac{[Mg^{2+}]}{K_{MAMP}}\right)\Big/\left([Mg^{2+}] + K_{MR}\right) \quad (12)$$

Similarly in the class II aminoacyl-tRNA synthetases, K_{II} is the standard and K'_{II} the apparent equilibrium constant:

$$K_{II} = \frac{[Mg_2PP_i][AMP][aatRNA]}{[MgATP][aa][MgtRNA]} \quad (13)$$

$$K'_{II} = K_{II}\left(1 + \frac{K_{M2PP}}{[Mg^{2+}]}\right)\left(1 + \frac{[Mg^{2+}]}{K_{MAMP}}\right)\Big/\left(1 + \frac{K_{MR}}{[Mg^{2+}]}\right) \quad (14)$$

Eqs. (12) and (14) finally show the forms used in the best-fit analyses (of the best mechanism). The value of K_{M2PP} at pH 7.4 is 2.6 mM [7] and K_{MAMP} is 14 mM [12]. If the effects of MgAMP and MgtRNA are not taken into account, the plot K'_I *vs.* $[Mg^{2+}]$ from Eq. (12) is a straight line with intersection points of $-K_{M2PP}$ (=−2.6 mM) at the abscissa axis and K_I at the ordinate axis. When the MgAMP effect is also included, the plot is a curve which intersects the ordinate axis at K_I but rises then faster (curved downwards) than the straight line. (These plots are shown as the dotted lines in Fig. 1.)

Eq. (14) for class II also gives a straight line if only the Mg_2PP_i effect is taken into account. It intersects the Mg^{2+} axis at $-K_{M2PP}$, but K_{II} is given by the slope. When the MgAMP effect is also included, a similar plot curved downwards, as for the class I synthetases, is obtained.

3. Materials and methods

3.1. Materials

The enzyme preparations and tRNA preparations were as described previously [11]. The absence of pyrophosphatase in the enzyme preparations was checked as in [13]. SerRS was purified from *E. coli* MRE600 by a procedure which included precipitation of nucleic acids with polyethylenimine, chromatography on DEAE-Sepharose CL-6B at pH 6 using a gradient of 0–300 mM NaCl, solubilization of the ammonium sulphate-precipitated protein in a column of Sepharose CL-2B by a decreasing gradient of ammonium sulphate, gel filtration on Sephacryl S-300, chromatography on Poros Q ion exchanger at pH 6 using a gradient of 0–200 mM NaCl, and chromatography on hydroxylapatite (HA Calbiochem) using a gradient from 10 to 100 mM potassium phosphate pH 7. The specific activity of the preparation was 0.96 μmol min^{-1} mg^{-1}.

3.2. Enzyme assays

The equilibria were assayed as the normal aminoacylation of the tRNA [11], but ten times higher enzyme activities (about 300 nM/min) and ten times longer reaction times (2 h) were used, and Cl^- ions were avoided [14]. A typical reaction mixture (100 μl) contained 50 mM Hepes/25 mM KOH (pH 7.4 at 30 °C), 0.02% chicken egg albumin, tRNA of 0.46–1.9 μM amino acid-binding capacity, 2 mM ATP, about 50,000 cpm of [^{14}C]amino acid, 2–5 μM non-radioactive amino acid, 0.25–0.5 mM PP_i, 0.25–1 mM AMP, Mg-(acetate)$_2$ as indicated, 0 or 1 mM spermidine, 50 mM K-acetate, and 1 mM dithiothreitol. Reaction temperature was 30 °C.

The constancy of the equilibrium constant values was checked by varying the amino acid, PP_i, and AMP concentrations. The formation of the equilibrium in the reverse reaction was checked by running the reaction to the end without PP_i,

Table 1
Measured equilibrium constant values (K') at different magnesium concentrations for two aminoacyl-tRNA synthetases from class I (isoleucyl- and tyrosyl-tRNA synthetases) and four from class II (seryl-, histidyl-, lysyl- and phenylalanyl-tRNA synthetases)

Enzyme	Magnesium (mM)		Measured K'	
	Total	Free	No spd	1 mM spd
IleRS				
	3	0.6	0.45	0.54
	4	1.5	0.56	0.61
	5	2.3	0.63	0.65
	6	3.2	0.69	0.72
	8	5.1	0.83	0.80
	10	7.0	0.94	0.92
	12	9.0	1.04	1.02
	16	13.0	1.22	1.17
	20	16.9	1.29	1.28
TyrRS				
	2	0.32	0.91	0.95
	2.5	0.62	0.96	0.98
	3	0.99	0.96	1.09
	4	1.8	1.07	1.09
	5	2.8	1.21	1.21
	6	3.7	1.31	1.35
	8	5.6	1.56	1.80
	12	9.4	1.99	2.16
	16	13.3	2.69	2.46
	20	17.3	3.03	2.91
SerRS				
	3	1.0	0.54	0.52
	4	1.9	0.60	0.57
	5	2.9	0.73	0.70
	8	5.7	1.00	0.91
	12	9.6	1.34	1.35
	16	13.5	1.69	1.62
	20	17.4	2.13	1.66
HisRS				
	3	0.6	1.23	0.93
	4	1.5	1.24	1.03
	5	2.3	1.33	1.13
	6	3.2	1.43	1.35
	8	5.1	1.65	1.65
	10	7.0	1.82	1.84
	12	9.0	2.00	1.96
	16	13.0	2.16	2.39
	20	16.9	2.36	2.75
LysRS				
	3	0.6	0.85	1.16
	4	1.5	1.17	1.22
	5	2.3	1.31	1.33
	6	3.2	1.43	1.46
	8	5.1	1.68	1.67
	10	7.0	1.92	1.90
	12	9.0	2.05	1.98
	16	13.0	2.34	2.40
	20	16.9	2.63	2.72
PheRS				
	3	1.0	0.87	0.96
	4	1.9	1.01	1.04
	5	2.9	1.17	1.17
	8	5.7	1.41	1.38
	12	9.6	1.74	1.75
	16	13.5	2.25	2.04
	20	17.4	2.41	2.49

The total magnesium concentrations include free Mg^{2+} and magnesium complexed in ATP, PP_i and AMP. The K' values were measured both without spermidine (spd) and with 1 mM spermidine.
The start concentrations of the reaction components were:
IleRS: 5.9 μM Ile, 2 mM ATP, 1.3 μM $tRNA^{Ile}$, 0.5 mM PP_i and 0.25 mM AMP.
TyrRS: 3.2 μM Tyr, 2 mM ATP, 1.11 μM $tRNA^{Tyr}$, 0.1 mM PP_i and 1 mM AMP.
SerRS: 3.8 μM Ser, 2 mM ATP, 0.76 μM $tRNA^{Ser}$, 0.025 mM PP_i and 1 mM AMP.
HisRS: 5.65 μM His, 2 mM ATP, 1.9 μM $tRNA^{His}$, 0.5 mM PP_i and 0.25 mM AMP.
LysRS: 5.8 μM Lys, 2 mM ATP, 1.4 μM $tRNA^{Lys}$, 0.5 mM PP_i and 0.25 mM AMP.
PheRS: 2.6 μM Phe, 2 mM ATP, 0.46 μM $tRNA^{Phe}$, 0.025 mM PP_i and 1 mM AMP.

then adding PP_i (0.1–1 mM) to cause equilibration in the reverse direction. The same equilibrium states were attained as in the corresponding forward reactions.

The $Mg^{2+}{}_{free}$ concentrations were calculated as in [11]. by taking into account the formation of MgATP, $MgPP_i$, Mg_2PP_i, MgAMP, spermidine·ATP, and spermidine·PP_i.

4. Results and discussion

4.1. Measured equilibrium constants

The magnesium dependences of the equilibrium constants of six aminoacyl-tRNA synthetases were measured both without spermidine and in the presence of 1 mM spermidine (Table 1). The measured K' values were subjected to best-fit analyses using Eqs. (12) and (14) to obtain the plots in Fig. 1. All equilibrium constants are clearly dependent on the Mg^{2+} concentration. However, the slopes of the plots in Fig. 1 are much lower than they should be if the Mg^{2+} binding to PP_i only, or to PP_i and AMP, would be responsible for the Mg^{2+} dependence (shown by the dotted lines in Fig. 1). Therefore the Mg^{2+} binding to tRNA must have a role, too. The binding of some Mg^{2+} ions to aatRNA must be weaker than to the free tRNA. Spermidine has almost no effect on the K' values.

Table 2 gives the least-squares-optimized values of the standard equilibrium constants K_I and K_{II}, and the dissociation constant of Mg^{2+} from tRNA (K_{MR}). The average errors ($s_{y.x}$) in the optimization were below 3% for all enzymes, showing excellent fit. There are some differences in the equilibrium constants and K_{MR} values between individual synthetases but any systematic difference between class I and class II of the aminoacyl-tRNA synthetases cannot be observed. Table 2 also gives the K' values at 1 mM [Mg^{2+}]. These values may best represent the equilibrium constants at the conditions of the cell (pH 7.4, about 1 mM [Mg^{2+}], presence of spermidine). The corresponding values of constants for ArgRS were published earlier [5]. Its K_I is 2.55 mM, K_{MR}=2.4 mM and K' at 1 mM Mg^{2+} is 1.09.

If expressed otherwise, the results in Fig. 1 mean that the $\Delta_r G'^0$ values (calculated from the K' values) are lowered by higher Mg^{2+}

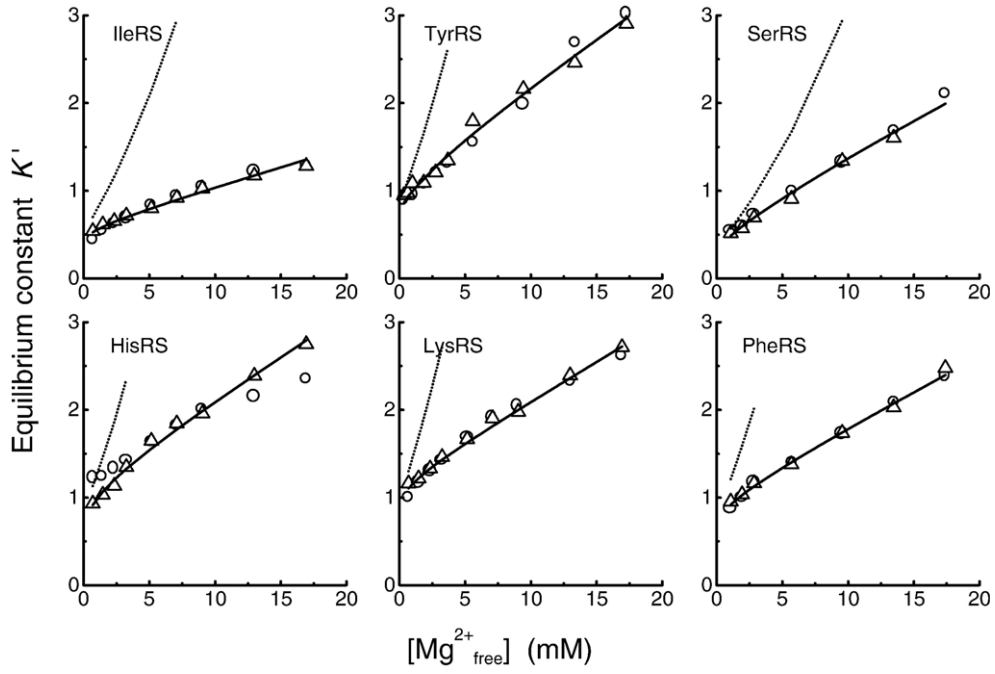


Fig. 1. Best-fit plots of the magnesium dependence of the measured equilibrium constants of aminoacyl-tRNA synthetases. The K' values from Table 1 were used. No spermidine (circles) or 1 mM spermidine (triangles) was present in the measurements. The solid lines are the best-fit plots which were calculated according to Eqs. (12) and (14) using the values measured in the presence of spermidine. The dotted lines show the theoretical magnesium dependence of the equilibrium constants if the Mg^{2+} binding to tRNA does not have any effect on K'.

concentrations. An increase in the Mg^{2+} concentration from 0.5 to 1.5 mM causes decreases of $\Delta G'^{0}$ by 255–505 J/mol in the different aminoacyl-tRNA synthetases. The corresponding decrease caused by PP_i and AMP would be 872 J/mol. The role of the Mg^{2+} binding to tRNA would thus be 280–620 J/mol in the different synthetases, and at higher Mg^{2+} concentrations this role would be still higher.

The K' value measured previously [3] for ThrRS, 0.37 at pH 7.0, is quite close to the value for SerRS in Table 2. The previous K' value for ValRS, 0.32, is, unfortunately, measured in the presence of 50 mM KF [4]. Fluoride forms complexes (and precipitates) both with magnesium pyrophosphate and magnesium ATP, and thereby affects the equilibrium.

4.2. Other possible mechanisms

If in some class I synthetases the product $MgPP_i$ receives another Mg^{2+} ion before dissociation from the enzyme, the case is represented by Eq. (14). Correspondingly, if in a class II synthetase the real product is $MgPP_i$ instead of Mg_2PP_i, the case is represented by Eq. (12). Both equations give similar dependences K' *vs.* $[Mg^{2+}]$, and therefore the different magnesium complexes of PP_i cannot be the reason for the difference between the calculated (dotted lines) and measured K' values.

The best-fit analysis suggests for all the studied synthetases that there is a binding of a magnesium ion to tRNA, with the K_{MR} value in the millimolar range, but that this magnesium ion is very weakly or not at all bound to aatRNA. The role of the binding to aatRNA allows some closely related mechanisms. Eqs. (4), (5), (12) and (14) and the above best-fit analyses have been done assuming that the given Mg^{2+} ion does not remain bound to aatRNA when this is dissociated from the enzyme. The Mg^{2+} ion would thus be dissociated after the transfer reaction and before the dissociation of the product aatRNA. Many aminoacyl-tRNA synthetases have at this step an editing reaction where the correctness of the aminoacyl group is tested on another reaction site after a major conformational reorientation of the CCA end of the aatRNA [15–17].

An almost identical case from the point of view of the equilibrium is met if both aatRNA and Mg·aatRNA can dissociate from the enzyme. Since the binding of the Mg^{2+} ion to aatRNA must be weak, the main route would anyway be the dissociation of Mg^{2+} from Mg·aatRNA·E first, and thereafter the dissociation of aatRNA from the enzyme. The same state of equilibrium would be attained on both routes, due to the microscopic reversibility.

In the model where it is obligatory for Mg·aatRNA to be the product, the best-fit analysis leads to very high K_d (Mg·aatRNA) values and low standard equilibrium constant K values so that K^*K_d remains constant. The fit is better the higher K_d. Therefore this model does not seem to be the correct choice.

There is still another mechanism which can explain the Mg^{2+} dependences of the apparent equilibrium constants in Fig. 1. If the Mg^{2+} ion is moved from the Mg·tRNA to AMP in the transfer reaction, MgAMP reacts then in the reverse reaction. This mechanism gives identical accuracies in the best-fit analysis compared with the above mechanism, and the optimized K_{MR} values are the same. However, this kind of involvement of a Mg^{2+} ion in the detailed mechanism of the transfer reaction has not been described in structural studies in neither class I [18] nor class II [19].

Table 2
The least-squares-optimized values for the standard, magnesium-independent equilibrium constants (K_I and K_{II} as in Eqs. (11) and (13)) at pH 7.4, and the dissociation constant of Mg^{2+} from the Mg·tRNA (K_{MR}), from the experiments in Fig. 1 with spermidine

Enzyme	K_I or K_{II}	K_{MR} (M)	K' at 1 mM Mg^{2+}_{free}
IleRS	0.00161±0.00008 M	0.0030±0.0004	0.59
TyrRS	0.00370±0.00022 M	0.0043±0.0006	1.04
SerRS	1.09±0.04	0.0076±0.0007	0.49
HisRS	1.37±0.06	0.0042±0.0005	1.01
LysRS	1.32±0.04	0.0035±0.0003	1.13
PheRS	1.14±0.04	0.0037±0.0004	0.94

The standard deviations were estimated by the grid-search method [28]. According to Eqs. (11) and (13) K_{II} is dimensionless in class II synthetases but K_I in class I has a concentration dimension.

4.3. Effect of spermidine

Spermidine activates aminoacyl-tRNA synthetases at low Mg^{2+} concentrations [11]. It replaces some Mg^{2+} ions which are bound to the reactive form of tRNA at the transfer reaction. Therefore its effects on the K' values were also measured. In Fig. 1 spermidine had no effect on the magnesium dependences of the apparent equilibrium constants of most enzymes, only in HisRS there can be some effect. Spermidine thus normally cannot replace the critical Mg^{2+} ion which is responsible for the change in the apparent equilibrium constant.

4.4. Some remarks

Previous kinetic analyses suggest the requirement of 1–3 tRNA-bound Mg^{2+} ions in the transfer reaction [11]. Dissociation of one or two of them after the transfer reaction increases the dissociation rate of the aatRNA from the enzyme. Although the roles of the tRNA-bound Mg^{2+} ions in the enzyme reaction and in the formation of the equilibria have some similarities, there are differences, too. The effects of spermidine, mentioned above, is one, and others come from the dissociation constants. The dissociation constants for the Mg·tRNA complexes in Table 2 are 2.4–7.3 mM. The enzyme reaction rate measurements with ArgRS, IleRS, LysRS, PheRS and SerRS show K_d values for Mg·tRNA at the same range of about 3–9 mM. The corresponding values for HisRS and TyrRS are lower. The dissociation constant value K_{MR} for TyrRS in Table 2 is 4.3 mM, but the dissociation constant values affecting the rate of the TyrRS reaction have been measured as 0.11 mM [14], or as 0.12 and 0.9 mM for two Mg^{2+} ions [11].

Several Mg^{2+} ions are known to be bound to the tRNA [20–23]. For instance, the yeast $tRNA^{Phe}$ has been reported to bind 11 Mg^{2+} ions [24]. Most of them have K_d values in the submillimolar Mg^{2+} concentration range, and they have a role in keeping the correct folding of the tRNA molecule. About 0.1 mM Mg^{2+} is sufficient to stabilize the native conformation. However, some Mg^{2+} ions in $tRNA^{Phe}$ have K_d values in the millimolar range, and their binding has been shown to bring the stems in a more stacked helical state [25,26]. In some mitochondrial tRNA molecules the binding of Mg^{2+} ions at about 2.5 mM causes unfolding of a part of the molecule [27]. The examples show that there are Mg^{2+} bindings to tRNA molecules in the millimolar range of Mg^{2+} concentrations, and that these can cause detectable conformational changes which can be important in the function of the tRNA molecules.

It cannot be yet said which Mg^{2+} ion bindings could be responsible for the change in the equilibrium constants. The critical binding to the free tRNA molecules occur at the millimolar range of Mg^{2+} concentrations, but in aatRNA this binding is much weakened. In the above examples the conformational changes have been detected at the D-stem and T-stem regions of the tRNA [26,27], but the single stranded CCA end can also be one candidate for the binding site of the critical Mg^{2+} ion.

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