ELSEVIER

Biophysical Chemistry 131 (2007) 29–35

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

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

R. Kalervo Airas

Department of Biochemistry, University of Turku, FIN-20014 Turku, Finland

Received 10 July 2007; received in revised form 28 August 2007; accepted 28 August 2007
Available online 4 September 2007

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.

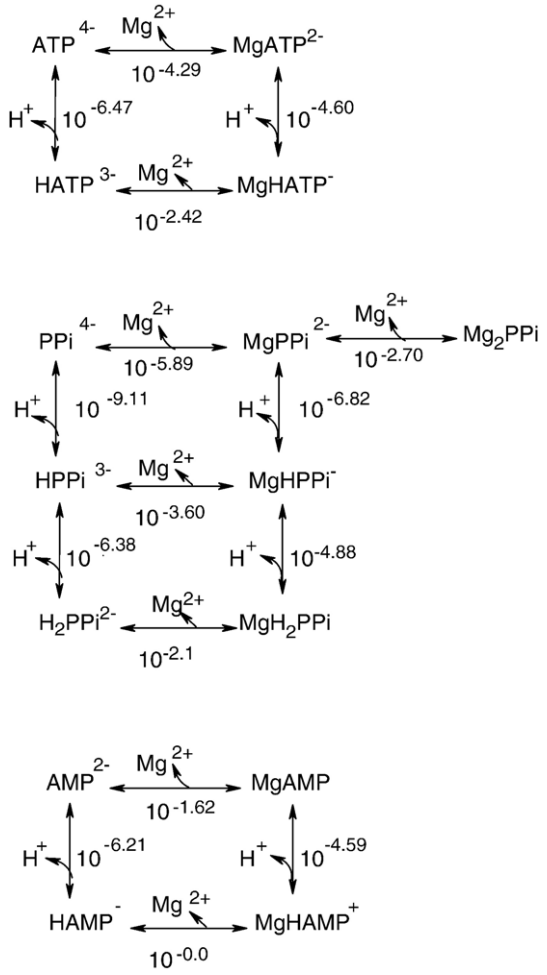
The equilibrium constant for threonyl-tRNA synthetase (ThrRS) has previously been measured as 0.37 and for valyl-tRNA synthetase (ValRS) as 0.32 [3,4]. I recently published a description of the magnesium dependence of the measured equilibrium constant for the arginyl-tRNA synthetase (ArgRS) reaction [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

E-mail address: kalervo.airas@utu.fi.

0301-4622/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2007.08.006



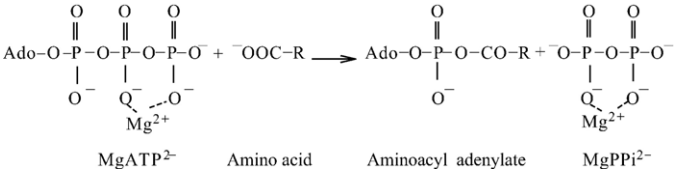
Scheme 1. The known proton and magnesium equilibria of ATP, PP_i and AMP which can affect the measured equilibrium constants of the aminoacyl-tRNA synthetases. The dissociation constant values (M) are from references [6] and [7].

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

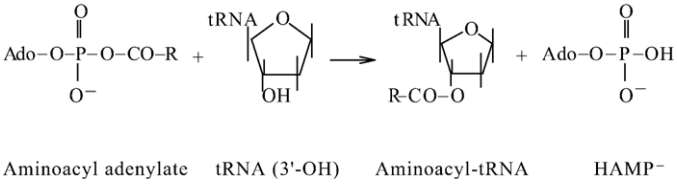
The reaction catalyzed by the aminoacyl-tRNA synthetases is given by Eq. (1). K' is the apparent equilibrium constant of this reaction. (aa is the amino acid and aatRNA aminoacyl-tRNA.)

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

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