THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

Catalysis of tRNA Aminoacylation: Single Turnover to Steady-State Kinetics of tRNA Synthetases

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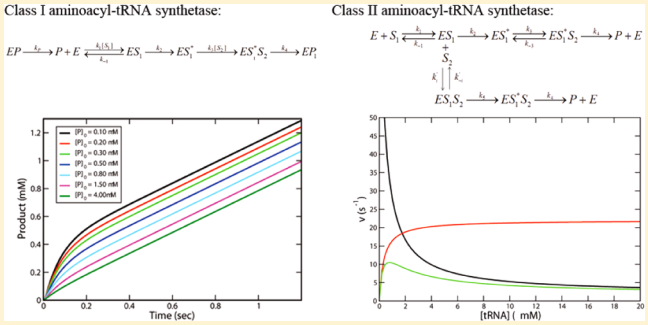
ABSTRACT: Aminoacyl-tRNA synthetases (aaRS) catalyze the bimolecular association reaction between amino acid and tRNA by specifically and unerringly choosing the cognate amino acid and tRNA. There are two classes of such synthetases that perform tRNA-aminoacylation reaction. Interestingly, these two classes of aminoacyl-tRNA synthetases differ not only in their structures but they also exhibit remarkably distinct kinetics under pre-steady-state condition. The class I synthetases show initial burst of product formation followed by a slower steady-state rate. This has been argued to represent the influence of slow product release. In contrast, there is no burst in the case of class II enzymes. The tight binding of product with enzyme for class I enzymes is correlated with the enhancement of rate in presence of elongation factor EF-TU. In spite of extensive experimental studies, there is no detailed theoretical analysis that can provide a quantitative understanding of this important problem. In this article, we present a theoretical investigation of enzyme kinetics for both classes of aminoacyl-tRNA synthetases. We present an augmented kinetic scheme and then employ the methods of time-dependent probability statistics to obtain expressions for the first passage time distribution that gives both the time-dependent and the steady-state rates. The present study quantitatively explains all the above experimental observations. We propose an alternative path way in the case of class II enzymes showing the tRNA-dependent amino acid activation and the discrepancy between the single-turnover and steady-state rate.



I. INTRODUCTION

The substrate specificity of aminoacyl-tRNA synthetase in aminoacylation of tRNA is important as the overall fidelity of protein synthesis partially relies on the charging of a tRNA with the cognate amino acid.[1] The overall aminoacylation reaction is composed of two steps.[2] In the first step, amino acid and ATP bind to the active site of the synthetase and the α-phosphate of ATP undergoes nucleophilic attack by the α-carboxylate of amino acid forming enzyme−aminoacyl−adenylate complex. In the second step of the reaction, the α-carbonyl of aminoacyl−adenylate is attacked by the 2′- or 3′- hydroxyl of the terminal adenosine of tRNA leading to the formation of aminoacyl-tRNA product.

$$\text{aaRS} + \text{aa} + \text{ATP} \rightleftarrows \text{aaRS.aa} - \text{AMP} + \text{PPi} \quad (1)$$

$$\text{aaRS.aa} - \text{AMP} + \text{tRNA} \rightleftarrows \text{aaRS} + \text{aa} - \text{tRNA} + \text{AMP} \quad (2)$$

Here "aa" is the amino acid and "aaRS" is the corresponding aminoacyl-tRNA synthetase.

There are 20 aminoacyl-tRNA synthetases for the corresponding 20 natural amino acids, and they can be divided into two classes based on their catalytic fold, regiochemistry, signature sequences, and binding with ATP and tRNA.[3−5] Class I synthetases have two signature motifs (HIGH and KMSKS) and their active site contains a Rossman nucleotide-binding fold which is absent in class II synthetases. On the other hand, class II synthetases contain three other signature motifs and their active site is composed of antiparallel β-sheets surrounded by α-helices.[6]

One of the consequences of the difference in active-site structure is that class I enzymes bind ATP in an extended conformation whereas class II enzymes bind it in a bent conformation.[4] The structural studies of synthetase:tRNA complex suggest that the class I enzymes approach the acceptor stem of tRNA from the minor groove side of tRNA with the variable groove side facing the solvent (except TyrRS which binds from major groove side of tRNA[7]) and the amino acid is attached to the 2′-OH of A76.[4] In the case of class II enzymes the approach of enzymes is in the reverse direction: enzymes approach from major groove site of the acceptor stem facing the variable groove of tRNA toward the synthetase (except AlaRS[8]) and the amino acid is attached to the 3′-OH[9] except PheRS (attaches to the 2′-OH[3,10]). Thus, both the structure of the active site of enzymes and resulting binding mode with tRNA and ATP distinctly classify all the 20 aminoacyl-tRNA synthetases into two classes.

The structural differences and binding modes are reflected on the overall enzymatic chemical reaction kinetics for the two classes of enzymes. Recent experimental studies of class I and

Received: May 24, 2012
Revised: August 20, 2012
Published: September 9, 2012

class II enzymes in pre-steady-state reveal that they follow different reaction mechanisms.[11,12] The enzymes of the same class share a common reaction mechanism and show similar time-dependent reaction rate. More recent experimental studies employing rapid chemical quench kinetics of aminoacylation reaction by class I *Escherichia coli* GlnRS show that the rate of reaction in single turnover is almost *1 order of magnitude higher than the steady-state rate, suggesting a* burst of product in the pre-steady-state condition.[13] From the observation of the burst kinetics, the authors concluded that the overall aminoacylation reaction is limited by the slow rate of product release. Class I ArgRS, CysRS, ValRS, and IleRS also show similar burst mechanism as a consequence of slow rate of product release[14−16] with the exception of GluRS which does not show any burst of product under the pre-steady-state condition.[17]

In contrast, recent work on class II enzyme, HisRS, did not show any pre-steady-state burst kinetics, suggesting that the product release step is fast. Several experiments of class II enzymes show that the overall rate of aminoacylation in the steady-state is determined by the amino acid activation step.[12] The similar results were also reported in case of the class II PheRS, SerRS,[18,19] and ThrRS.[20]

The important point in class II enzymes is that, although they do not exhibit burst, the single-turnover rate constant is 1 order of magnitude higher than the steady-state rate constant of class I enzymes. This leads to the conclusion that under steady-state condition the presence of tRNA decreases the rate of amino acid activation by 1 order of magnitude. Thus, the recent experimental studies indicate that the overall aminoacylation reaction is limited by amino acid activation for class II enzymes.

The affinity of class I enzymes toward the product (aa-tRNA) is observed to be higher than that toward substrates, as evident from the slow release of product.[11] The tight binding of class I enzyme and the product may require the assistance of the elongation factor EF-TU to release the aa-tRNA from the enzyme at higher rate. Thus, it is expected to obtain a higher rate of aminoacylation for class I enzymes in the presence of EF-TU. Though recent study on class I CysRS shows increased rate of aminoacylation in the presence of the elongation factor EF-TU,[11] earlier studies of class I IleRS do not exhibit any change in the rate in the presence of EF-TU.[21,22] The reason for this behavior is not clear.

In spite of so many experimental studies, there is no satisfactory theoretical analysis that can capture all the above experimental findings quantitatively. Here we present a theoretical analysis constructing a kinetic scheme based on methods of time-dependent probability statistics. We analyze both the pre-steady-state and steady-state dynamics for both class I and class II enzymes. The assumption of product release step as the rate-determining step for class I enzymes leads to an explanation of the burst kinetics in the time dependence of the reaction. We obtain the rate of overall reaction and the aminoacyl transfer step for class I enzyme. Both of them show a single rate constant and are the same as obtained in a recent experimental study of CysRS.[11] The time dependence of the overall reaction rate shows the burst kinetics in pre-steady-state. The magnitude of the burst is dependent on the amount of product initially present in the reaction mixture. As expected, the burst magnitude decreases with increasing concentration of initial product present in the system. Though the presence of EF-TU enhances the rate of aminoacylation for no-editing class I enzymes, it does not affect the rate for editing class I enzymes.

In the case of class II enzymes, we propose an alternative pathway (that is, the pathway in which enzyme binds tRNA before aminoacyl-adenylate formation) in the presence of tRNA that leads to the slow rate of amino acid activation. This alternative pathway can capture the tRNA concentration dependence of amino acid activation as well as the discrepancy in the rate of overall reaction in single-turnover and steady-state condition. The absence of burst kinetics of class II enzymes can also be understood from the present scheme.

II. CLASS I ENZYMES: KINETIC SCHEME AND THEORETICAL FORMULATION

On the basis of experimental observations, we construct a kinetic scheme of aminoacylation for class I enzyme as follows

$$EP \xrightarrow{k_p} P + E \underset{k_{-1}}{\overset{k_1[S_1]}{\rightleftharpoons}} ES_1 \xrightarrow{k_2} ES_1^* \xrightarrow{k_3[S_2]} ES_1^*S_2 \xrightarrow{k_4} EP_1$$

where EP is the enzyme−product complex, P and P_1 are products, E is the enzyme in the free state, S_1 is the amino acid (aa), S_1^* is aa-AMP, S_2 is the tRNA, and the k's are the rate constants of the corresponding elementary steps. The enzyme can start reacting from either of two states, free enzyme (E) and enzyme−product complex (EP), or from both the states depending on the initial population of these states. In single-turnover reaction in the absence of initial product in the reaction mixture, the reaction starts solely from free enzyme (E). After single turnover of a free enzyme, it goes to the enzyme-bound product state (EP). Here, we should mention that a few class I enzymes (GlnRS, GluRS, ArgRS, and LysRS I) do not activate amino acids in the absence of tRNA. In those cases, the above scheme needs to be modified so that the enzyme binds tRNA first and then it binds the amino acid, to be followed by the amino acid activation step. Since, the product release is the slowest among all the elementary steps, the binding sequence of the substrates (aa and tRNA) does not affect the results of our present study.

To obtain the rate of reaction starting from both E and EP states, we follow a time-dependent probability statistics of mean first passage time, often employed in the study of enzyme kinetics at single molecular level.[23] The rate of reaction in single molecular enzyme is identical to the inverse of the mean first passage time.[24] Therefore, our first goal is to obtain an expression of first passage time (waiting time) distribution. Once the waiting time distribution is calculated, the steady-state rate can be easily computed. The method of waiting time (first passage time) distribution computation is described in the Appendix.

Equations A.1−5 in the Appendix can be represented in matrix form as

$$s\hat{\mathbf{f}}(s) = \mathbf{Q}\hat{\mathbf{f}}(\mathbf{s}) + \mathbf{r} \tag{3}$$

where

$$\hat{\mathbf{f}}(s) = \begin{pmatrix} \hat{f}_{T_{EP}}(s) \\ \hat{f}_{T_E}(s) \\ \hat{f}_{T_{ES_1}}(s) \\ \hat{f}_{T_{ES_1^*}}(s) \\ \hat{f}_{T_{ES_1^*S_2}}(s) \end{pmatrix}, \quad \mathbf{r} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ k_4 \end{pmatrix}$$

$$\mathbf{Q} = \begin{pmatrix} -k_p & k_p & 0 & 0 & 0 \\ 0 & -k_1[S_1] & k_1[S_1] & 0 & 0 \\ 0 & k_2 & -(k_{-1}+k_2) & k_{-1} & 0 \\ 0 & 0 & 0 & -k_3[S_2] & k_3[S_2] \\ 0 & 0 & 0 & 0 & -k_4 \end{pmatrix}$$

$\hat{\mathbf{f}}(s)$ is the vector composed of the first passage time components in Laplace space that are labeled by their respective subscripts. $\mathbf{Q}$ is the rate matrix and $\mathbf{r}$ is the vector as shown above. Equation 3 can be inverted to yield the following expression of $\hat{\mathbf{f}}(s)$:

$$\hat{\mathbf{f}}(s) = (s\mathbf{I} - \mathbf{Q})^{-1}\mathbf{r} \tag{4}$$

Once we obtain the Laplace transform, $\hat{\mathbf{f}}(s)$, the Laplace transform of waiting time distribution of EP, $f_{T_{EP}}(s)$, can be obtained as

$$f_{T_{EP}}(s) = (1\ \ 0\ \ 0\ \ 0\ \ 0)\hat{\mathbf{f}}(s) \tag{5}$$

Similarly, the Laplace transform of waiting time distribution of E, $f_{T_E}(s)$, can be obtained as

$$f_{T_E}(s) = (0\ \ 1\ \ 0\ \ 0\ \ 0)\hat{\mathbf{f}}(s) \tag{6}$$

The waiting time distributions, $f_{T_{EP}}(t)$ and $f_{T_E}(t)$, can be easily computed by inverse Laplace transformation of $\hat{f}_{T_{EP}}(s)$ and $\hat{f}_{T_E}(s)$, respectively. The mean first passage time (MFPT), $\tau_{EP} = \int_0^\infty dt\ t f_{EP}(t)$ is readily given by $\tau_{EP} = -[(d\hat{f}_{T_{EP}}(s)]/[ds)|_{s=0}]$. Using eqs 4 and 5 we have

$$\tau_{EP} = (1\ \ 0\ \ 0\ \ 0\ \ 0)\mathbf{Q}^{-2}\mathbf{r} \tag{7}$$

One can obtain $\hat{\mathbf{f}}(s)$ by analytically solving the above matrix equation, and the final expression of $\hat{f}_{T_E}(s)$ is given as

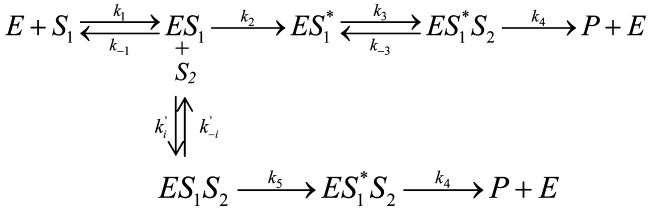
$$\hat{f}_{T_E}(s) = \frac{k_1k_3k_4k_{-1}[S_1][S_2]}{(k_3[S_2]+s)(k_4+s)[k_1[S_1](k_{-1}+s)+s(k_2+k_{-1}+s)]} \tag{8}$$

The mean first passage time of EP is

$$\tau_{EP} = \frac{1}{k_3[S_2]} + \frac{1}{k_4} + \frac{1}{k_{-1}} + \frac{k_2+k_{-1}}{k_1k_{-1}[S_1]} + \frac{1}{k_p} \tag{9}$$

III. CLASS II ENZYMES: KINETIC SCHEME AND THEORETICAL FORMULATION

Class II enzymes do not show any burst mechanism in the pre-steady-state condition, confirming that the product release step is not the rate-limiting step.[11,12,18,19] The interesting feature of class II enzymes is that though they do not show any crossover in pre-steady-state rate the rate of reaction in steady-state is much lower than the single-turnover rate. The reason for this discrepancy could be the fact that the tRNA, acting as an inhibitor, slows down the activation of amino acid in the steady state. As in single-turnover reaction very low amount of tRNA is used, there is weak effect of tRNA on amino acid activation. Though the experimental studies on SerRS and TyrRS indicate the lowering of amino acid activation rate in steady-state on increasing the tRNA concentration, the mechanism is still not clear.[25,26] Here we construct a kinetic scheme for class II enzyme with an alternative path of amino acid activation in presence of tRNA as follows:



As already described in the previous section, E is the enzyme, S_1 is the amino acid (aa), S_2 is tRNA, S_1^* is aa-AMP, and P is the product. The tRNA binds to the E.aa.ATP complex prior to the activation of amino acid and reduces its concentration. After tRNA binding, the enzyme undergoes structural rearrangements in the active sites.[27,28] We suppose this structural change in the active sites reduces the rate of amino acid activation. Thus, tRNA inhibits the activation of amino acid in an uncompetitive manner. There is another possibility: the tRNA can bind to the free enzyme prior to the binding of amino acid, which results in competitive inhibition of amino acid activation. Both types of inhibitions will show similar effect on overall amino acid activation rate. In general, tRNA inhibits in both ways (competitive and noncompetitive). The competitive inhibition affects the Michaelis–Menten constant whereas the uncompetitive inhibition affects the catalytic constant of the steady-state rate. In practice, most of the experiments are carried out at high amino acid and ATP concentration. Under such condition there will be very negligible effect of competitive inhibition of tRNA on the amino acid activation rate. Because of this and for the sake of simplicity, in the present scheme we have ignored the competitive inhibition. In the case of complete inhibition, the rate constant k_{cat} is expected to be 0. Experimental observations of nonzero k_{cat} suggest that tRNA acts as a partial inhibition.[11,12] The present scheme considers the partial inhibition by tRNA as shown in the above scheme.

The tRNA concentration ($[S_2]$) dependent steady-state rate of amino acid activation in presence of nonchargeable tRNA is

$$v_{act} = \frac{\left(k_2 + k_5\frac{[S_2]}{K_I'}\right)[S_1]}{K_m + \alpha'[S_1]} \tag{10}$$

where $\alpha' = 1 + ([S_2]/K_I')$. K_I' is the dissociation constant of the ES_1S_2 complex and K_m is the Michealis–Menten constant of aminoacyl-adenylate formation.

The steady-state rate of amino-acid transfer to the tRNA for preformed aminoacyl-adenylate complex, i.e., the second step of overall aminoacylation reaction, is

$$v_{tran} = \frac{k_4[S_2]}{K_m' + [S_2]} \tag{11}$$

The steady-state rate of overall aminoacylation reaction is

dx.doi.org/10.1021/jp305045w | J. Phys. Chem. B 2012, 116, 11809–11817

$$v = \frac{k_4\left(k_2 + k_5\frac{[S_2]}{K_I'}\right)}{k_4\left(\frac{K_m}{[S_1]} + \alpha'\right) + k_2\left(\frac{K_m'}{[S_2]} + 1\right) + k_5\left(\frac{K_d'}{K_I'} + \frac{[S_2]}{K_I'}\right)} \quad (12)$$

where K_m' is the Michealis−Menten constant of the second step, i.e., the amino acid transfer step, and K_d' is the dissociation constant of $ES_I^*S_2$ complex. Following the method described in Appendix, we can obtain the single-turnover rate of the overall aminoacylation reaction.

IV. CLASS I ENZYMES: RESULTS AND DISCUSSION

The single-turnover rate is obtained from inverse Laplace transform of eq 8. Since the expression of $\hat{f}_{T_E}(s)$ is complicated, it is very difficult to get an analytical expression of the single-turnover rate $f_{T_E}(t)$. It is convenient to obtain the time-dependent single-turnover rate for a set of rate constants and substrate concentrations. Recent experimental study reports the rate constants of overall two-step aminoacylation reaction and the transfer of amino acid to tRNA for CysRS.[11] They also obtained the dissociation constant of enzyme-Cys and enzyme-$tRNA^{Cys}$ complexes. Here we use most of their rate constants values in order to obtain the rate of aminoacylation both in single-turnover and steady conditions. The rate constants are follows:

$$k_1 = 5\mu M^{-1}\,s^{-1},\ k_{-1} = 350\ s^{-1},\ k_2 = 200\ s^{-1},\ k_3 = 50\ \mu M^{-1}\,s^{-1},\ k_4 = 13.7\ s^{-1},\ k_p = 1.685\ s^{-1}$$

The substrate concentrations are $[S_1] = 500\ \mu M$, $[S_2] = 5\ \mu M$.

For the above set of parameters the rate of single turnover obtained from eq 8 is

$$f_{T_E}(t) = 57.44e^{-320.5t} - 72.50e^{-250t} + 15.22e^{-13.7t} \quad (13)$$

The corresponding time-dependent product concentration can be obtained as

$$P(t) = [E]_{tot}\int_0^t dt_1 f_{T_E}(t_1) \quad (14)$$

The time-dependent rate of single-turnover reaction (eq 13) shows three exponential decays with slowest rate constant coming from the transfer of amino acid to tRNA ($k = 13.7\ s^{-1}$). The time-dependent product formation is shown in Figure 1A. The product formation for the single aminoacyl transfer step is also shown in the same plot. Both of them show effectively single-exponential kinetics with same rate constant value, suggesting the amino acid transfer step as the rate-limiting step. This is in complete agreement with the experimental result.[11] The little difference in time dependence of the two rates is due to the contribution from the pretransfer steps.

In pre-steady-state experiments, the enzyme initially stays in the free state (E). After single-turnover reaction the enzyme−product complex (EP) is formed. Since the rate of product release for class I enzymes is slow, the concentration of EP increases during the single-turnover process. After completion of single turnover, all the enzymes start the reaction from EP state maintaining a steady-state rate. Thus, during single turnover the concentration of free enzyme (E) decreases and all the free enzymes go to the EP state at the end of the single turnover. The rate of aminoacylation reaction can be considered as the sum of the contributions coming from E and EP states. Thus, the overall rate can be written as

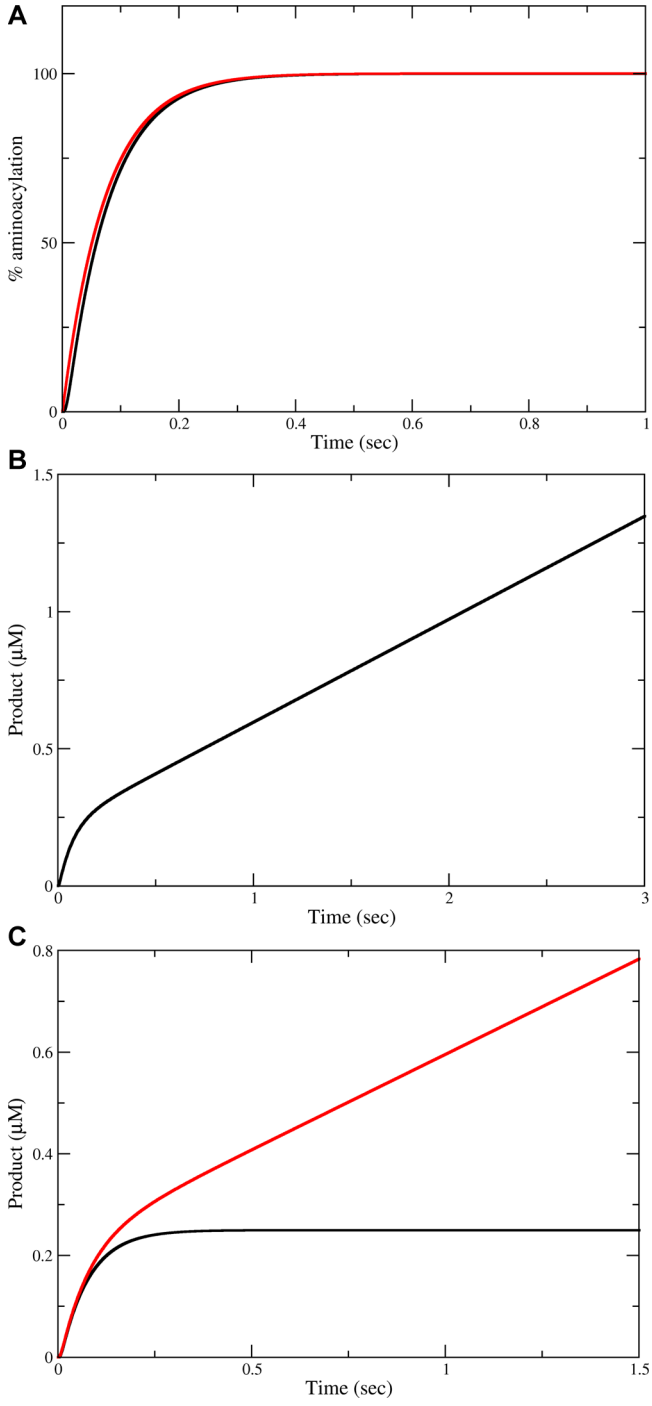


Figure 1. (A) Time course of aminoacylation in single-turnover reaction of class I enzyme. The black line represents the two-step aminoacylation reaction and the red line represents the second step, i.e., the aminoacyl transfer step of the overall aminoacylation reaction. (B) Time dependence of aminoacylation of class I enzyme in pre-steady-state. The presence of burst shows initial high rate in single turnover followed by steady-state rate. The concentration of enzyme is 0.25 μM. (C) Replot of (A) and (B) to show that the magnitude of burst is identical to the maximum product formed in single-turnover reaction which is equal to the initial concentration of free enzyme. The concentration of enzyme is 0.25 μM.

dx.doi.org/10.1021/jp305045w | J. Phys. Chem. B 2012, 116, 11809−11817

$$f(t) = P_E(0) f_{T_E}(t) + P_{EP}(t) v_{EP} \quad (15)$$

where $f_{T_E}(t)$ is the time-dependent rate of product formation starting from free enzyme (E), v_{EP} is the steady-state rate of product formation starting from the enzyme-bound product complex (EP), $P_E(0)$ is the fraction of enzyme in the free state (E) initially present in the system, and $P_{EP}(t)$ is the fraction of EP at time t. The steady-state rate v_{EP}, obtained from eq 9, is 1.5 s^{-1}. $P_{EP}(t)$ can be obtained from $f_{T_E}(t)$ as

$$P_{EP}(t) = P_{EP}(0) + \int_0^t dt_1 f_{T_E}(t_1) \quad (16)$$

The amount of product formed at time t is given by

$$P(t) = [E]_{tot} \int_0^t dt_1 f(t_1) \quad (17)$$

where $[E]_{tot}$ is the total concentration of enzyme.

The time dependence of product formation ($P(t)$) shown in Figure 1B depicts the initial burst of product formation with the single-turnover rate followed by steady-state rate which is the characteristic feature of class I enzymes.[11,13,14] The burst kinetics clearly signifies that the aminoacylation reaction in steady-state is controlled by the product release step whereas the transfer of amino acid to the tRNA is the rate-limiting step in the course of single turnover.

The presence of product in the reaction mixture is found to decrease the magnitude of the burst. In experiment, to investigate the effect of product on rate of aminoacylation, the enzyme and product are mixed prior to the addition of substrates.[11] So, it is convenient to assume that initially E and EP are in equilibrium. From the dissociation constant of EP it is easy to obtain the fraction of E and EP initially present in reaction mixture. The fraction of EP at equilibrium is

$$P_{EP}(0) = \frac{([E]_{tot} + [P]_0 + K_D) - \sqrt{([E]_{tot} + [P]_0 + K_D)^2 - 4[E]_{tot}[P]_0}}{2[E]_{tot}} \quad (18)$$

where $[E]_{tot}$ is the total concentration of enzyme initially used, $[P]_0$ is the initial concentration of product, and K_D is the dissociation constant of EP complex. $P_E(0)$ is easily obtained from initial condition as

$$P_E(0) = 1 - P_{EP}(0) \quad (19)$$

In the presence of product in the initial reaction mixture, the overall rate of aminoacylation is obtained from eq 15. The product concentration-dependent aminoacylation is shown in Figure 2A. It clearly shows the decrease of burst magnitude on increasing concentration of initial product as previously observed in experiment.[11] This is to note that, though the magnitude of burst decreases, the rate of product formation at steady-state is independent of product concentration indicated by the same slope at long time for different product concentrations. This is because the initial presence of product affects only the population of free enzyme that shows burst kinetics, it does not change the rate of the product release step. The magnitude of burst is nothing but the initial concentration of enzyme present in the free state (E). Thus, the magnitude of burst can be written as

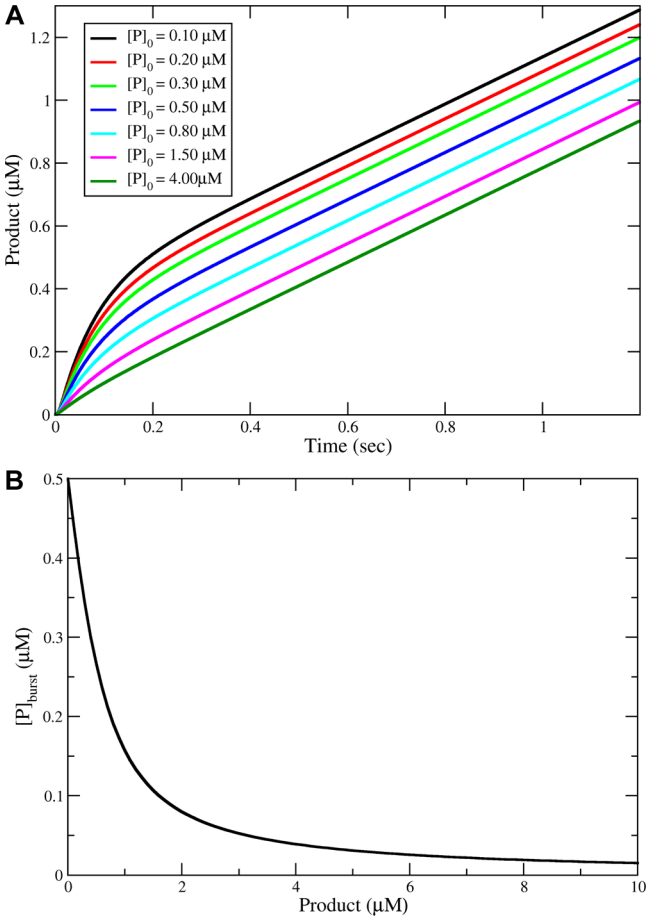


Figure 2. (A) Time course of aminoacyl-tRNA formation of class I enzyme in the presence of varying concentration of product initially present in the reaction mixture. On increasing the concentration of the initial product, the magnitude of burst decreases but the steady-state rate remains same. The concentration of enzyme is 0.5 μM. (B) Product concentration dependence of the burst magnitude. Concentration of enzyme is 0.5 μM.

$$[P]_{burst} = [E]_{tot} - \frac{([E]_{tot} + [P]_0 + K_D) - \sqrt{([E]_{tot} + [P]_0 + K_D)^2 - 4[E]_{tot}[P]_0}}{2} \quad (20)$$

The burst magnitude (Figure 2B) shows quadratic dependence on initial concentration of product. This result is in complete agreement with the experimental finding of Zhang et al. Thus, the present kinetic mechanism can explain most of the experimental findings quantitatively. To verify that the burst magnitude is same as the initial amount of free enzyme (E) present in the reaction mixture, we replot the Figure 1, A and B (see Figure 1C). The maximum amount of product formed in single turnover (i.e., the saturated value of $f(t)$) is identical to the amplitude of the burst.

The presence of elongation factor EF-TU should increase the rate of aminoacylation as it binds to the product strongly, leading to the faster product release. Experiment on CysRS shows that EF-TU indeed increases the rate. The product release step can be faster if the EF-TU factor binds to the enzyme-bound product complex (EP) and stabilizes the corresponding transition state. In the case of CysRS the enzyme−product complex (EP) can bind to EF-TU, forming a ternary complex without any overlap between the binding sites

dx.doi.org/10.1021/jp305045w | J. Phys. Chem. B 2012, 116, 11809−11817

EF-TU and enzyme.[11] The common topologies of class I enzymes suggest that EF-TU should increase the rate of aminoacylation for all the enzymes belonging to this group. However, the modeling of IleRS indicates a steric overlap between the position of the editing domain with the binding site of EF-TU, suggesting that the ternary complex with EF-TU may not form for the class I enzymes that possess editing domain.[29−31] Thus, the editing class I enzymes should not show any increase of rate in the presence of EF-TU. Early studies show that in the case of IleRS there is no effect of EF-TU on the rate of aminoacylation,[22] though the ratio of aminoacyl product and AMP formation increases from 0.65 to 0.92 in the presence of EF-TU, indicating the ability of EF-TU to protect the product from hydrolysis.[32]

V. CLASS II ENZYMES: RESULTS AND DISCUSSION

To obtain numerical values of rate of amino acid activation and aminoacylation, we need to employ a set of rate constants that are within experimental range. The following set of parameters has been used in the present study of class II enzymes that are comparable to experimentally obtained rate constants.[11,12]

$k_1 = 20\ \mu M^{-1}\ s^{-1}$, $k_{-1} = 470\ s^{-1}$, $K_m = 30\ \mu M$, $k_2 = 130\ s^{-1}$, $k_3 = 353\ \mu M^{-1}\ s^{-1}$, $k_{-3} = 100\ s^{-1}$, $K'_m = 0.34\ \mu M$, $k'_i = 376\ s^{-1}$, $k'_{-i} = 100\ s^{-1}$, $K'_I = 0.266\ \mu M$, $K'_d = 0.28\ \mu M$, $k_4 = 22\ s^{-1}$, $k_5 = 2\ s^{-1}$, $[S_1] = 200\ \mu M$.

The steady-state rates obtained from eqs 10, 11, and 12 for the above set of parameters are

$$v_{act} = \frac{113.043 + 6.53[S_2]}{1 + 3.27[S_2]} \quad (21)$$

$$v_{tran} = \frac{22[S_2]}{0.34 + [S_2]} \quad (22)$$

$$v = \frac{(130 + 7.52[S_2])[S_2]}{2 + 7.15[S_2] + 4.10[S_2]^2} \quad (23)$$

The time-dependent single-turnover rate of the aminoacyl transfer at 2 μM tRNA concentration is

$$f_{tr}(t) = 19.67e^{-19.20t} - 19.67e^{-808.79t} \quad (24)$$

The single-turnover rate of overall aminoacylation reaction under identical condition is

$$f(t) = 51.22e^{-854.31t} - 53.12e^{-808.79t} - 61.15e^{19.20t} + 63.18e^{-15.08t} \quad (25)$$

The steady-state rate of amino acid activation (eq 21) shows that it monotonically decreases with increase of tRNA concentration and then it is saturated to the value 2 s^{-1} at high tRNA concentration (Figure 3A, black line). On the other hand, tRNA increases the aminoacyl transfer rate, i.e., the second step of overall aminoacylation reaction (Figure 3A, red line). As a result, there are two competitive effects of tRNA on two steps involved in the overall aminoacylation reaction. At low concentration of tRNA the overall reaction rate is determined by the rate of aminoacyl transfer step whereas at high concentration it is determined by the amino acid activation step. Thus, the overall reaction rate initially increases at low tRNA concentration and then decreases as the tRNA concentration is further increased (Figure 3A, green line). In general, in living cells tRNA concentration remains at high level. Thus, in practice, the aminoacylation reaction of class II

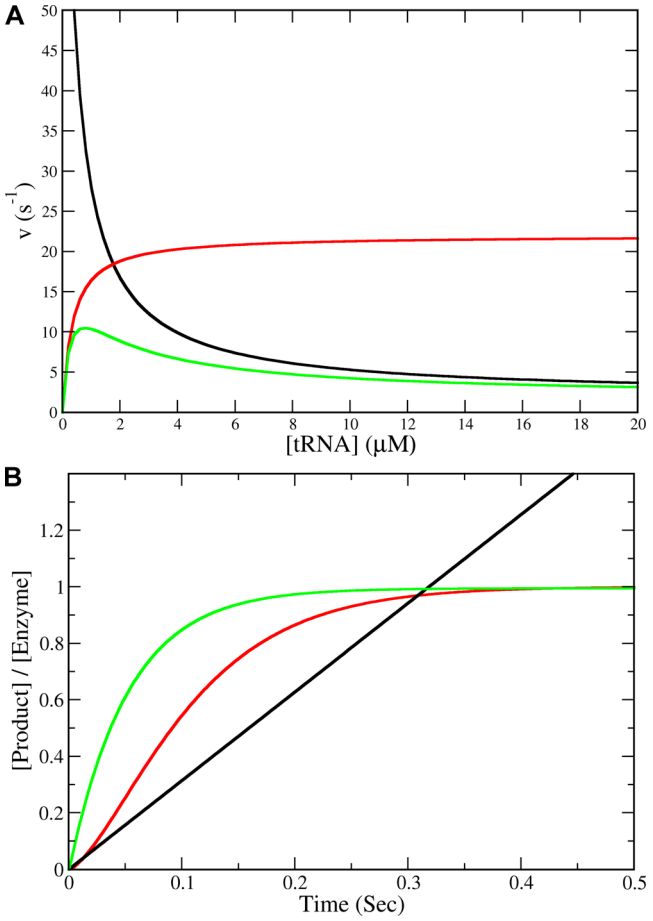


Figure 3. (A) tRNA-dependent steady rates of class II enzyme. The black line represents the rate of amino acid activation, red line represents the rate of aminoacyl transfer, and the green line represents the overall aminoacylation reaction. (B) Time dependence of aminoacylation of class II enzymes in steady-state (black line) and single turnover (red line). The green line represents the product formation in single-turnover reaction starting from the aminoacyl−adenylate complex, i.e., the second step of the overall aminoacylation reaction.

enzymes is controlled by the activation of amino acid. The early study reveals that HisRS activity is sensitive to the cellular energy charge.[33] Since the cellular energy charge determines the ATP level, this observation supports that the amino acid activation is the rate-limiting step in living cells.

The experimental studies of class II enzymes repeatedly find that the rate of single-turnover reaction is higher than the k_{cat} of steady-state reaction by 1 order of magnitude.[11,12,34] Zhang et al. reported the single-turnover and steady-state rate constants of class II enzymes (AlaRS and ProRS). Both of them show that the single-turnover rate is higher by 1 order of magnitude than the steady-state rate constant (k_{cat}). Another class II enzyme, HisRS, shows similar behavior. In case of HisRS, the single-turnover reaction which is carried out at 2 μM tRNA concentration shows that the rate of aminoacylation for preformed aminoacyl-adenylate complex (i.e., the second step of overall aminoacylation reaction) is 18.8 s^{-1} whereas the steady-state rate which is carried out at 20 μM tRNA concentration is found to be 3.14 s^{-1} and the steady-state rate constant k_{cat} is 2 s^{-1}.[12] The rate of amino acid activation in the absence of tRNA is 130 s^{-1}. The discrepancy between the observed rate of aminoacylation in single turnover and steady

dx.doi.org/10.1021/jp305045w | J. Phys. Chem. B 2012, 116, 11809−11817

state can be understood in terms of tRNA-dependent amino acid activation. Since the single-turnover reaction has been carried out on preformed aminoacyl−adenylate complex, the rate of reaction is determined by the rate of amino acid transfer which is 18.8 s^{-1} at 2 μM tRNA. The steady-state rate has been carried out in the presence of 20 μM tRNA. Under this condition, the rate of amino acid activation comes down from 130 to ∼3 s^{-1}, leading to the overall reaction rate 3.14 s^{-1} as shown in Figure 3A. Thus, the difference in experimental conditions for single-turnover and steady-state reaction makes the difference in overall aminoacylation rate. If we study single-turnover and steady-state reaction under identical condition, i.e., the same concentration of amino acid, ATP and tRNA in both cases the rates are expected to be same. In fact, the single-turnover rate at 20 μM tRNA concentration has been found to be 3.14 s^{-1} which is same as the steady-state rate for the same tRNA concentration.[2,12,35] If it is possible to carry out the steady-state reaction at 2 μM tRNA concentration then we can obtain the overall reaction rate same as single-turnover rate.

The time-dependent product formation in single-turnover reaction at 2 μM tRNA concentration is shown in Figure 3B with the corresponding product concentration under steady-state condition at 20 μM tRNA concentration. Product formation due to aminoacyl transfer in single turnover is also shown in the same plot. The figure clearly shows that both the overall aminoacylation and aminoacyl transfer rates in single turnover are higher than the steady-state rate.

VI. CONCLUSION

The present theoretical study systematically analyses the single-turnover and steady-state reaction mechanism/pathway for both classes of aminoacyl-tRNA synthetases. Each class of enzymes shares similar mechanism as expected from their structural similarities and substrate binding modes. These are reflected in their corresponding pre-steady-state rates of reaction. Though in single-turnover reaction the transfer of amino acid to the tRNA is the rate-limiting step for both classes of enzymes, under steady-state condition the product release step is the slowest step in the case of class I enzymes, showing the burst of product formation at short time.

The present study captures in a satisfactory fashion the gradual decrease of the burst magnitude on increasing product concentration in the initial reaction mixture. Presence of the elongation factor EF-TU enhances the overall rate of aminoacylation by facilitating the product release for class I enzymes. The enzymes that have editing domain such as IleRS do not show any effect of EF-TU on overall reaction, as EF-TU is unable to bind to the product due to steric hindrance of the editing domain.

In contrast to the class I enzymes, the amino acid activation is the rate-limiting step for class II enzymes, exhibiting no burst in product formation. Significantly, low steady-state rate compared to the single-turnover rate for class II enzymes has been shown to arise from inhibition effect of tRNA on amino acid activation step. The rate of reaction for class II enzymes is expected to be independent of EF-TU unless it affects the aminoacyl-adenylate formation.

Thus, in the case of class I enzymes the reaction starts from two different states, free enzyme (E) and enzyme−product complex (EP), with distinct rate constants whose contribution clearly depends on their relative population in the reaction mixture. The presence of the former in the mixture is responsible for the pre-steady-state burst. The magnitude of the burst is reduced by the presence of initial product. However, it does not affect the steady-state rate.

Though class II enzymes do not exhibit burst because of the fast product release, they exhibit different rates of reaction under single-turnover and steady-state conditions. Further study of single-turnover reaction of class II enzyme in the presence of excess tRNA and limited amino acid reports that the overall reaction rate is comparable to the steady-state rate[12] which supports that the tRNA binds to the enzyme before activation of amino acid.

The work presented here consisted of the formulation of a suitable enzyme kinetic scheme which was then solved by the method of mean first passage time. Quantitative analysis could be carried out for the cases where required kinetic parameters are available. Within the experimentally studied systems, the present study provided explanation of many of the results.

APPENDIX: COMPUTATION OF WAITING TIME DISTRIBUTION

We assume the total time to form the enzyme−product complex (EP_1) starting from EP (shown in the kinetic scheme of section II) is a random variable T_{EP} that is governed by the first passage time distribution $f_{EP}(t)$. To obtain $f_{EP}(t)$, the quantity of prime interest, the waiting time distributions of all other intermediates are to be calculated. The times all the intermediates need to wait before formation of the enzyme−product complex (EP_1) are also considered as random variables. These times are denoted as T_E, T_{ES_1}, $T_{ES_1^*}$, and $T_{ES_1^*S_2}$. The subscripts indicate corresponding intermediates. The corresponding waiting time distributions are $f_{T_E}(t), f_{T_{ES1}}(t), f_{T_{ES1^*}}(t)$, and $f_{T_{ES1^*S2}}$.

Since each elementary step is random, we can consider the waiting time distribution of each step as a Poisson distribution:

$$f_{T_P}(t) = k_P \exp(-k_P t)$$

$$f_{T_1}(t) = k_1[S_1] \exp(-k_1[S_1]t)$$

$$f_{T_{-1}}(t) = k_{-1} \exp(-k_{-1}t)$$

$$f_{T_2}(t) = k_2 \exp(-k_2 t)$$

$$f_{T_3}(t) = k_3[S_2] \exp(-k_3[S_2]t)$$

$$f_{T_4}(t) = k_4 \exp(-k_4 t)$$

Let us imagine the system is initially in the state EP, so that the total time to complete the reaction and form EP_1 is the random variable T_{EP}. The probability that T_{EP} is realized within some time interval t, which we denote by $P(T_{EP}<t)$, can be written as

$$P(T_{EP} < t) = P(T_E + T_P < t)$$

In general, for any random variable X, $f_X(x) = dP(X<x)/dx$. The above equation is differentiated with respect to t to obtain corresponding waiting time distribution

$$f_{T_{EP}}(t) = f_{T_E+T_P}(t)$$

Since the distribution of the sum of two random variables is the convolution of the distributions of two individual random variables

dx.doi.org/10.1021/jp305045w | J. Phys. Chem. B 2012, 116, 11809−11817

$$f_{T_{EP}}(t) = \int_0^t dt_1 f_{T_E}(t - t_1) f_{T_P}(t_1)$$
$$= \int_0^t dt_1 f_{T_E}(t - t_1) k_P e^{-k_P t_1}$$

By taking Laplace transform, we obtain

$$\hat{f}_{T_{EP}}(s) = \frac{k_P \hat{f}_{T_E}(s)}{s + k_P} \quad (A.1)$$

where $\hat{f}_{T_{EP}}(s)$ is the Laplace transform of $f_{T_{EP}}(t)$. Similarly, we can derive the corresponding expressions of $f_{T_E}(t)$. The final expression is

$$\hat{f}_{T_E}(s) = \frac{k_1[S_1]\hat{f}_{T_{ES_1}}(s)}{s + k_1[S_1]} \quad (A.2)$$

In the case of ES_1 there are two ways. The first is the transition back to the E state, and the other choice is the forward step to ES_1^*. The probability that T_{ES_1} is realized within time t is

$$P(T_{ES_1} < t) = P(T_{-1} < T_2)P(T_E + T_{-1} < t)$$
$$+ P(T_2 < T_{-1})P(T_{ES_1^*} + T_2 < t)$$

In this expression, the first term indicates that ES_1 first goes back to the E state and from there it goes to the product EP_1. The second term corresponds to the forward path starting from ES_1 to the product EP_1. The first passage time of ES_1 is

$$f_{T_{ES_1}}(t) = P(T_{-1} < T_2) f_{T_E + T_{-1}}(t) + P(T_2 < T_{-1}) f_{T_{ES_1^*} + T_2}(t)$$

Expressing $f_{T_E + T_{-1}}$ and $f_{T_{ES1^*+T2}}(t)$ as convolution of two terms

$$f_{T_{ES_1}}(t) = \int_0^t dt_1 P(T_2 < t_1) f_{T_E}(t - t_1) f_{T_{-1}}(t_1)$$
$$+ \int_0^t dt_1 P(T_{-1} < t_1) f_{T_{ES_1^*}}(t - t_1) f_{T_2}(t_1)$$

We put the expressions of the probabilities and waiting time distribution of elementary steps and find the final expression

$$f_{T_{ES_1}}(t) = \int_0^t dt_1 f_{T_E}(t - t_1) k_{-1} e^{-(k_{-1} + k_2)t_1}$$
$$+ \int_0^t dt_1 f_{T_{ES_1^*}}(t - t_1) k_2 e^{-(k_{-1} + k_2)t_1}$$

Performing the Laplace transformation, we obtain

$$\hat{f}_{T_{ES_1}}(s) = \frac{k_{-1}\hat{f}_{T_E}(s)}{s + k_{-1} + k_2} + \frac{k_2 \hat{f}_{T_{ES_1^*}}(s)}{s + k_{-1} + k_2} \quad (A.3)$$

Following similar formalism, we obtain the first passage time distribution for all other intermediates in Laplace space

$$\hat{f}_{T_{ES_1^*}}(s) = \frac{k_3[S_2]\hat{f}_{T_{ES_1^*S_2}}(s)}{s + k_3[S_2]} \quad (A.4)$$

$$\hat{f}_{T_{ES_1^*S_2}}(s) = \frac{k_4}{s + k_4} \quad (A.5)$$

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Prof. Umesh Varshney for discussions and for pointing out valuable references. We thank Prof. Alan Fersht for helpful suggestions. M.S. thanks Mr. Rakesh Sharan Singh for discussions. This work was supported in part by grants from DST and CSIR (India). B.B. thanks DST for a JC Bose Fellowship.

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