STEPWISE tRNA RECOGNITION MECHANISM AND ITS KINETIC CONSEQUENCES

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1. Introduction

The Michaelis equation is widely used for the treatment of enzyme kinetic data, maximum velocity V_m and Michaelis constant K_M being the final values obtained. The first is usually assumed to be the measure of the velocity of the catalytic step of the process, the latter is regarded as a measure of the affinity of substrate to enzyme. With tRNA's as substrates of ARSases* (E.C. 6.1.1) or aa-tRNA's as ribosomal substrates, several points of the enzyme–substrate interaction should be considered. It is reasonable to suggest the enzyme–substrate complex formation to be a multi-step process. This possibility has been discussed [1].

In the present paper the two-step recognition mechanism is analyzed as the most simple model of the multi-step recognition. It is demonstrated that in this case, the above mentioned simple interpretation of the K_M and V_m values may be wrong, namely significant changes in V_m may occur without any changes in the catalytic rate constant of the process, due to changes in the recognition step. Some consequences for the problem of the specificity of tRNA amino acylation and of translation are discussed.

2. Description of the model

The enzymatic reaction with two-step mechanism of the catalytically active complex formation may be represented as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} ES^* \xrightarrow{k_3} E + P$$

(E – enzyme saturated with low-molecular-weight substrates, S – high-molecular-weight substrate, P – reaction product, ES, ES* – first (inactive, low specificity) and second (active, high specificity) enzyme–substrate complexes, k_i – rate constants).

The first step may be considered to result from interactions of substrate with enzyme of relatively low specificity. It may be interactions common to some set of tRNA's capable of being aminoacylated with one ARSase [2,3] or all elongator aa-tRNA's interacting with a ribosome-mRNA complex with one codon exposed to translation [4,5]. The existence of these common interactions seems to be reasonable because of the suggested common tertiary structure of tRNA's [6]. The existence of a common structural basis of the interactions between tRNA's and ARSases was recently proposed by Rich and Schimmel [7]. The second step may be regarded as a final adjustment of the high molecular weight substrate on the enzyme due to highly specific interactions. This step results in an appropriate orientation of the reacting group (say, 2′-OH group of the 3′-terminal adenosine residue of tRNA) towards the catalytic center of the enzyme.

In the steady-state approximation the kinetic equation may be written in the usual Michaelis–Menten form using

$$V_m = \frac{k_3 e}{1 + \dfrac{k_{-2} + k_3}{k_2}}$$

$$K_M = \frac{k_{-1}}{k_1} \cdot \frac{\dfrac{k_3}{k_{-1}} + \dfrac{k_{-2} + k_3}{k_2}}{1 + \dfrac{k_{-2} + k_3}{k_2}}$$

* *Abbreviations:*
aa-tRNA, aminoacyl-tRNA; ARSase, aminoacyl-tRNA synthetase.

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It is seen, that V_m is equal to k_3e, velocity of the catalytic step, only when $(k_{-2} + k_3)/k_2 \ll 1$. If we consider a set of substrates with equal k_3 and $(k_{-2} + k_3)/k_2$ changing from 0 to ∞ V_m will change from k_3e to zero. In the same set K_M will change from k_3/k_1 to k_{-1}/k_1, and in the case when k_3 and k_{-1} do not differ significantly from each other, only slight changes in K_M values should be observed.

Therefore, the changes in V_m values in the series of substrates in the case of two-step recognition does not necessarily reflect changes in the catalytic step but may result from changes in the second recognition step.

It should be emphasized that the apparent dissociation constant is always equal to or less than K_1:

$$K_{app} = \frac{[E]\,s}{[ES] + [ES^*]} = \frac{K_1}{1 + \frac{k_2}{k_{-2}}}$$

(s – substrate concentration, $K_1 = k_{-1}/k_1$). If $k_2/k_{-2} < 1$ or ~ 1, the stability of the enzyme–substrate complex is determined mainly by the first recognition step of low specificity.

It is easy to demonstrate that in the presence of two substrates, S and $\bar{S}$, the second substrate acts as a competitive inhibitor of the transformation of the first substrate, and K_M of the second substrate appears as an inhibition constant. In accordance with the above considerations, a poor substrate with a low $\bar{V}_m$ may thus be a strong inhibitor due to the similarity of the K_1 and $\bar{K}_1$ values, that is due to similarity of the interactions at the first step of recognition. For example, it was demonstrated that in spite of the high specificity of protein biosynthesis [8] non-poly (U)-coded aa-tRNA's compete strongly with phenylalanyl-tRNA in polyphenylalanine synthesis in a cell free system from human tonsils [4] the K_i/K_M ratio being 25.

3. Discussion

The most detailed investigation of the kinetic reasons for the specificity of tRNA recognition in enzymatic aminoacylation was done by Ebel's group [2,3]. It was demonstrated that for a variety of ARSases $V_m/\bar{V}_m$ ratios for cognate and noncognate tRNA's exceeds significantly the $\bar{K}_M/K_M$ ratios for the same tRNA's. The authors conclude that 'more or less accurate recognition of a tRNA by aminoacyl-tRNA synthetase does not necessarily implicate the aminoacylation of the tRNA by this enzyme'. According to these authors, the recognition problem may be correctly dealt with only by 'distinguishing the recognition step from the catalytic one'. The preferential change of V_m value as compared with that of K_M was found for some tRNA's, differing in methylation level [9–11].

In the present paper it is demonstrated that in the case of two-step recognition the $V_m/\bar{V}_m$ ratio is not necessarily related to the ratio of the velocities of the catalytic steps. Moreover, the great difference between these velocities seems to be rather unreasonable. It was demonstrated that the fourth from the 3′-terminus nucleotide residues are identical for a set of tRNA's amenable to aminoacylation by the same enzyme and therefore are essential for aminoacylation [12]. This means that already this residue participates in the specific interaction with ARSase. The difference in the catalytic rate constant must reflect the difference in the fidelity of the arrangement of the reacting group within the catalytic center. It is rather difficult to imagine the way in which 2′-OH groups of the terminal adenosine of the identical flexible tetranucleotide sequences should behave differently in the common catalytic center. It seems more likely that due to differences in the structure of the second step recognition points either the time of the final arrangement of the CCA-end (that is k_2 values) or the life time of the specific complex (that is k_{-2} values) or both differ significantly for cognate and noncognate tRNA's.

The model presented is a first very rough approximation to the real multi-step recognition process. The analysis of more complicated schemes may become useful as the transient kinetic methods will supply some experimental data concerning rate constants of the tRNA-ARSase or aa-tRNA-ribosome interactions. But already this model demonstrates that the multi-step character of recognition must be taken into account when discussing the problem of the specificity of tRNA recognition.

References

[1] Yarus, M. and Berg, P. (1969) J. Mol. Biol. 42, 171–189.
[2] Ebel, J. -P., Giegé, R., Bonnet, J., Kern, D., Befort, N., Bollack, C., Fasiolo, F., Gangloff, J. and Dirheimer, G. (1973) Biochimie 55, 547–557.
[3] Giegé, R., Kern, D., Ebel, J. -P., Grosjean, H., De Henav, S. and Chantrenne, H. (1974) Eur. J. Biochem. 45, 351–362.
[4] Kreuzer, T., Bermeck, E. and Matthaei, H. (1972) Biochim. Biophys. Acta 281, 113–123.
[5] Scheulen, M., Schmidt, B. and Matthaei, H. (1973) Biochim. Biophys. Acta 299, 468–471.
[6] Kim, S. H., Sussman, J. L., Suddath, F. L., Quigley, G. J., McPherson, A., Wang, A. H. J., Seeman, N. C. and Rich, A. (1974) Proc. Natl. Acad. Sci. US 71, 4970–4974.
[7] Rich, A. and Schimmel, P. R. (1975) Nature (London), in press.
[8] Loftfield, R. B. and Eigner, E. A. (1966) Biochim. Biophys. Acta 130, 426–448.
[9] Roe, B., Sirover, M. and Dudock, B. (1973) Biochemistry 12, 4146–4154.
[10] Stulberg, M. P. and Shugart, L. R. (1971) Cancer Res. 31, 671–674.
[11] Roe, B., Sirover, M. and Dudock, B. (1973) Nature (London), New Biol. 246, 135–138.
[12] Roe, B. and Dudock, B. (1972) Biochem. Biophys. Res. Commun. 49, 399–406.