Aminoacyl-tRNA synthetases: inter-site interaction as a possible proofreading mechanism

Sergey Kh. Degtyarev

All-Union Research Institute of Molecular Biology, Koltsovo, Novosibirsk Region, 633159, USSR

Received 8 February 1983

Abstract not received

Enzyme specificity *Binding site interaction* *Aminoacyl-tRNA synthetase*
Inter-site interaction *Proofreading mechanism* *Tryptophanyl-tRNA synthetase*

1. INTRODUCTION

Aminoacyl-tRNA synthetases play an important role in the biosynthesis of proteins. The majority of these enzymes has two active centers, and apparently all enzymes are able to bind 2 mol amino acid [1-5]. The existence and interaction of multiple binding sites leads to coupling of reactions in the active centers. This interaction, presumably, enhances the catalytic efficiency of these enzymes [1,6]. However, the possible contribution of the dimeric functional structure to the specificity of these enzymes remains unclear. Here, I discuss this possible contribution. It is assumed that inter-site interaction may be involved in correction after aminoacyl-adenylate formation. The proofreading mechanism assumed is based on:

(i) Trigger character of enzyme action;
(ii) Existence of the active sites interaction;
(iii) Alteration of amino acid position in enzyme complex in the course of the adenylate formation.

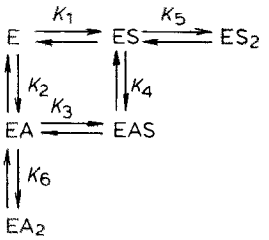
Model schemes for tryptophanyl-tRNA synthetase are given which explain experimental data.

2. INTERACTION OF BINDING SITES AND INCREASE OF THE ENZYME SPECIFICITY

The formation of enzyme-substrate complex is the first and probably the main step in discrimination between specific (correct) and non-specific (wrong) substrates. Discrimination only at this step will be considered supposing that the rate of product formation is the same in all enzyme-substrate complexes. In the case of two competing substrates (correct (S) and wrong (A)) discrimination at this step may be characterized by the coefficient α, which represents the ratio of the sum of the enzyme forms containing S to the sum of the enzyme forms containing A. If the enzyme has only one binding site:

$$\alpha = \frac{[ES]}{[EA]} = \frac{K_2 \cdot [S]}{K_1 \cdot [A]} \qquad (1)$$

where K_1 and K_2 are dissociation constants of ES and EA, and [S] and [A] concentrations of S and A, respectively. If the enzyme has two binding sites (scheme 1):



$$\alpha = \frac{[ES] + 2 \cdot [ES_2] + [EAS]}{[EA] + 2 \cdot [EA_2] + [EAS]} = \frac{K_2 \cdot [S]}{K_1 \cdot [A]} \cdot \left[1 + \frac{2 \cdot [S]}{K_5} + \frac{[A]}{K_4}\right] \Big/ \left[1 + \frac{2 \cdot [A]}{K_6} + \frac{S}{K_3}\right]$$

Published by Elsevier Science Publishers
00145793/83/0000-0000/$3.00

where K_i is the dissociation constant of the corresponding stage (i) in scheme 1.

As compared with the one-center case, α for scheme 1 contains additional terms, which may differ from 1. If $K_5 \ll K_3$ and $K_6 \ll K_4$, at high concentrations of S and A, α may be considerably greater than that in ratio (1). These conditions are quite reasonable and correspond to the existence of an inter-site interaction which impedes the formation of complexes EAS and EA_2, but does not affect the complex ES_2. One may imagine such an interaction if one admits closeness of the binding sites in the enzyme molecule and the presence of additional groups in the molecule A as compared with S. In this case, unstability of the enzyme complexes with A will be a mere consequence of direct steric contact of ligand molecules residing in the two binding sites of the enzyme.

Thus, the enzyme with two binding sites may distinguish the substrate from some of its analogues more effectively, due to interaction of the binding sites.

The two-center binding scheme has been analysed previously [1,7,8], where the authors concluded that no increase of the discrimination factor may be achieved compared with the one-center scheme. However, formal thermodynamic analysis applied to the two-center scheme did not take into account the possibility of interaction between S and A in the binding sites in the ternary complex and considered only interactions of the enzyme with substrate and its analogue [8].

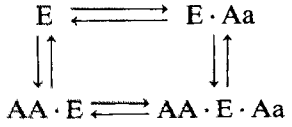
3. INTER-SITE INTERACTIONS IN AMINOACYL-tRNA SYNTHETASES

Possible participation of the interaction of binding sites in discrimination between substrates should be considered in the case of aminoacyl-tRNA synthetases. The majority of these enzymes contains two active centers or at least two amino acid binding sites.

The fidelity of tRNA charging by amino acid is very high and the error does not exceed 3×10^{-4} [9,10]. As follows from analysis [1] aminoacyl-tRNA synthetases should have a special mechanism to increase specificity. A number of such proofreading mechanisms has been proposed [1,11,12]. However, the possible contribution of the functional dimerity of these enzymes has not been considered.

The presence of multiple binding sites in the molecule of synthetases leads to coupling of the reactions in the active centers [1,6]. Trigger mechanism of enzyme action has been outlined in order to take into account this circumstance [6,13,14]. In accordance with this mechanism, active centers of the enzyme function in an alternative regime; i.e., if amino acid is present in one center, aminoacyl-adenylate occupies the other one. The more general case will be considered further: both the two-center enzymes and synthetases are able to form only 1 mol adenylate with subsequent binding of amino acid. There are 4 possible productive ternary complexes in which inter-site interactions may take place: Aa·E·S,Aa·E·A,Sa·E·A and Sa·E·S, where Sa and Aa are the correct and the wrong adenylate, respectively.

The properties of these complexes have been detailed in the case of tryptophanyl-tRNA synthetase from beef pancreas [15]. This enzyme forms aminoacyladenylate complexes with 4-,5-,6- and 7-fluorotryptophans. The stoichiometry of the complexes is 1:1. They are stable and survive gel-filtration. However, in the presence of tryptophan(S) or its fluoro-derivative(A), aminoacyl-adenylate complexes with 5-, 6- and 7-fluorotryptophan (E·Aa) become unstable and dissociate according to the scheme:



where AA is either A or S.

However, adenylate complex with tryptophan (E·Sa) is stable in the presence of both tryptophan and its fluoro-derivative.

A similar phenomenon was observed with isoleucyl-tRNA synthetase from *E.coli* [16]. This enzyme forms the wrong aminoacyl-adenylate complex with valine, which dissociates in the presence of isoleucine, while isoleucyladenylate complex is stable in the presence of isoleucine.

Hence, inter-site interactions within synthetases not only exist, but have a quite definite character: they lead to dissociation of wrong adenylate complexes.

To explain this phenomenon it is nesseasary to take into account that the formation of adenylate seems to be accompanied by a change of amino acid position in the enzyme complex. Analysis of the creation of inter-site interactions may be carried out using model schemes. The analysis will be done for tryptophanyl-tRNA synthetase assuming for simplicity that the active centers are very close to each other and that interaction of active sites is a consequence of direct interaction of ligands in the ternary complex.

Fig. 1 shows tryptophan, 5-fluorotryptophan and their aminoacyl-adenylates in graphic image. Fig. 2 demonstrates possible ternary complex in model schemes: (– – –) border of active centers. In these schemes, contact of a part of the graphic image of ligand with (– – –) means the existence of inter-site interaction leading to dissociation of the complex. It is seen that interactions are present only in the case of wrong adenylate complexes.

4. DISCUSSION

These model schemes are certainly speculative, but all the assumptions are based on experimental results. Closeness of the active centers of beef pancreas tryptophanyl-tRNA synthetase follows from much indirect evidence [17–20]. This enzyme exhibits negative cooperativity in binding of 2 molecules of tryptophan and in the formation of 2 mol adenylate [21,22]. Intrinsic fluorescence of enzyme is changed only after adenylate formation while interaction of the enzyme with $tRNA^{Trp}$ or tryptophanyl-tRNA does not alter the intensity of fluorescence [23,24]. This indicates that the enzyme undergoes essential conformational rearrangement in the course of adenylate formation.

Scheme of direct interaction of ligand gives a simple explanation to experimental data in the case of tryptophanyl-tRNA synthetase. The conformational rearrangement of the enzyme after adenylate formation may also create inter-site interactions. In the latter case interaction of active centers may be transferred through the enzyme molecule and there is no nesessity for the closeness of the active centers. Such a mechanism seems to be the case with isoleucyl-tRNA synthetase because valine is smaller than isoleucine, and direct interactions of ligands are excluded.

Analysis of the scheme shows that this mechanism may take place only for definite analogues of substrate. Furthermore, as follows from fig. 2, substitution in the first position of the indole ring (in the conditions of the model scheme) may lead to destabilization of the correct adenylate. However, it is noteworthy that in vivo there are only a few amino acid analogues which both have a free -COOH group and possess a high affinity to the enzyme. Hence, the mechanism does not seem to be unrealistic. This mechanism might

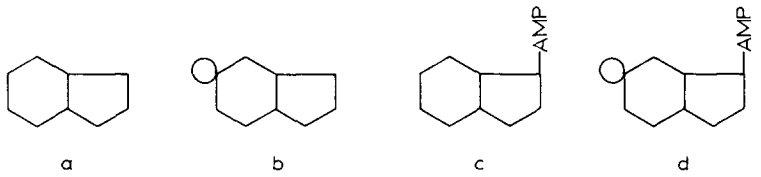


Fig. 1. Graphic images of: (a) tryptophan; (b) 5-fluorotryptophan; (c) tryptophanyladenylate; (d) 5-fluorotryptophanyladenylate.

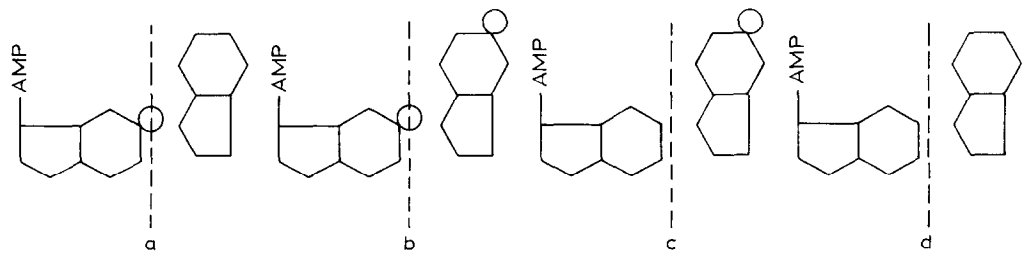


Fig. 2. Model schemes of different ternary complexes: (a) Aa·E·S; (b) Aa·E·A; (c) Sa·E·A; (d) Sa·E·S.

be used to discriminate between tryptophan and 5-hydroxytryptophan in the case of tryptophanyl-tRNA synthetase, and between isoleucine and valine in the case of isoleucyl-tRNA synthetase.

The mechanism differs from other proofreading mechanisms by the absence of wasteful hydrolysis of ATP. Indeed, once incorrect adenylate complex has formed, it will dissociate after binding of the amino acid, and this adenylate may be bound by specific enzyme. The possibility of such a transfer is shown in [16].

Hopfield had suggested the kinetic proofreading mechanism [11]. According to this mechanism, the amino acid undergoes two steps of discrimination: binding with the enzyme followed by preferential destruction of the wrong adenylate. However, it is well-known that both incorrect and correct adenylates are stable [25,26] and the significance of this mechanism remains unclear. Within the hypothesis outlined above it may be suggested that at the first step in the course of substrate binding the enzyme discriminates among correct and all wrong substrates and then, after adenylate formation, the mechanism of inter-site interaction is used to discriminate between correct and wrong adenylate(s).

ACKNOWLEDGEMENTS

I thank Professor D.G. Knorre and Dr E.G. Malygin for the useful discussions and Dr M.A. Grachev for the critical reading of manuscript.

REFERENCES

[1] Fersht, A. (1975) Biochemistry 14, 5-12.
[2] Jakes, R. and Fersht, A. (1975) Biochemistry 14, 3344-3350.
[3] Fersht, A., Mulvey, R. and Koch, G. (1975) Biochemistry 14, 13-18.
[4] Mulvey, R. and Fersht, A. (1976) Biochemistry 15, 243-249.
[5] Igloi, G.L. and Cramer, F. (1978) in: Transfer RNA, Altman, S. ed. pp. 294-349, MIT Press, Cambridge MA, London.
[6] Fasiolo, F., Ebel, J.P. and Lazdunski, M. (1977) Eur. J. Biochem. 73, 7-15.
[7] Bosshard, H.R. (1976) Experientia 32, 949-963.
[8] Fersht, A. (1977) Enzyme structure and mechanism, pp. 279-280, W.H. Freeman, Reading, San Francisco CA.
[9] Loftfield, R. (1963) Biochem. J. 89, 89-92.
[10] Loftfield, R. and Vanderjagt, D. (1972) Biochem. J. 128, 1353-1356.
[11] Hopfield, J. (1974) Proc. Natl. Acad. Sci. USA 71, 4135-4139.
[12] Fersht, A. (1977) Enzyme structure and mechanism, pp. 194-196, W.H. Freeman, Reading, San Francisco CA.
[13] Kochkina, L.L., Akhverdyan, V.Z. and Malygin, E.G. (1976) Molekul. Biol. 10, 1127-1131.
[14] Kisselev, L.L., Malygin, E.G., Akhverdyan, V.Z. (1978) Dokl. Acad. Nauk USSR 238, 1475-1479.
[15] Degtyarev, S.Kh., Malygin, E.G., Favorova, O.O., Kisselev, L.L. (1982) Molekul. Biol. 16, 170-176.
[16] Fersht, A. (1977) Biochemistry 16, 1025-1030.
[17] Kisselev, L.L., Favorova, O.O., Kovaleva, G.K. (1979) Methods Enzymol. 59, 234-257.
[18] Favorova, O.O., Madoyan, I.A., Drutsa, V.L. (1981) FEBS Lett. 123, 161-164.
[19] Madoyan, I.A., Favorova, O.O., Kovaleva, G.K., Sokolova, N.I., Shabarova, Z.A., Kisselev, L.L. (1981) FEBS Lett. 123, 156-160.
[20] Degtyarev, S.Kh., Kovaleva, G.K., Zinoviev, V.V., Malygin, E.G. (1981) Molekul. Biol. 15, 176-181.
[21] Graves, P., Masat, J., Juquelin, H., Labouesse, J., Labouesse, B. (1979) Eur. J. Biochem. 96, 509-518.
[22] Kovaleva, G.K., Degtyarev, S.Kh. and Favorova, O.O. (1979) Molekul. Biol. 13, 1237-1246.
[23] Graves, P., Bony, J., Masat, J. and Labouesse, B. (1980) Biochimie 62, 33-41.
[24] Degtyarev, S.Kh., Beresten, S.F., Denisov, A.Y., Lavrik, O.I. and Kisselev, L.L. (1982) FEBS Lett. 137, 95-99.
[26] Baldwin, A. and Berg, P. (1966) J. Biol. Chem. 241, 839-845.