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NEGATIVE COOPERATIVITY IN ADENYLATE FORMATION CATALYSED BY BEEF PANCREAS TRYPTOPHANYL-tRNA SYNTHETASE

Influence of tRNA^{Trp}

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1. Introduction

Beef pancreas tryptophanyl-tRNA synthetase (EC 6.1.1.2) seems to be the most thoroughly studied aminoacyl-tRNA synthetase from multicellular organisms (see [1]). It consists of 2 probably identical subunits and contains 2 interacting active sites [2–5]. In the absence of inorganic pyrophosphatase, negative cooperativity is observed when the enzyme catalyses formation of the tryptophanyl–adenylate complex [6]. In the presence of pyrophosphatase 2 mol adenylate are formed without remarkable cooperativity [7].

Formation of the tryptophanyl–adenylate · enzyme complex has not been yet studied by the stopped-flow technique in comparison with the data of steady-state kinetics. This paper presents a short report of such a study.

The following major conclusions have been made. Formation of the first mole of adenylated proceeds with a rate constant $>200 \text{ s}^{-1}$ as opposed to the second mole of adenylate which is formed more slowly by 2 orders of magnitude. The latter value increases when inorganic pyrophosphatase is added. Moreover, in the presence of bovine or yeast tRNA^{Trp} (both native and lacking the terminal A) the rate constant of formation of the second mole of adenylate is higher than 150 s^{-1}, i.e., approaches that of the first mole. From these data and those available in the literature, it is assumed that the slow formation of the second adenylate is caused by the presence of pyrophosphate on the first active site, not by the anti-cooperative binding of tryptophan as suggested in [8]. Dissocia-tion from the enzyme · adenylate complex is induced or greatly facilitated by tRNA^{Trp} or, artificially, by yeast pyrophosphatase leading to the opening of the second active site for adenylate formation.

2. Materials and methods

Tryptophanyl-tRNA synthetase was prepared and its concentration determined as in [1]. Yeast inorganic pyrophosphatase was from Worthington. Yeast tRNA^{Trp} was a gift of Dr V. Scheinker, tRNA^{Trp} from beef liver was isolated as in [9]. Fluorescent titrations and kinetic parameters were measured with a Durrum D-110 spectrofluorometer. The excitation wavelength was 290 nm. The emission was measured with the cut-off filter (0-54 Corning C. S.) which transmitted wavelengths >300 nm. In stopped-flow experiments the solutions were mixed in the ratio 1:1 (v/v). The reaction was followed by changes in fluorescence intensities with time monitored on the recording oscillograph Textronix 5103 N. The recorded values in these experiments were close to those obtained in stationary fluorescent titration measurements.

All other experimental details are given in the legends to figures.

3. Results and discussion

In [7] the fluorescence of beef pancreas trypto-phanyl-tRNA synthetase was practically unaffected

by binding of ATP and tryptophan being, however, sensitive to tryptophanyl—adenylate formation. A 24% fluorescent quenching is observed when 2 mol adenylate are formed by a dimeric enzyme. This observation allows one to follow the kinetics of adenylate formation by measuring the changes of the fluorescence intensity of the enzyme [7].

Fig.1 shows the dependences of the fluorescence intensities on the concentration of various ligands added to the synthetase in the course of adenylate formation. Qualitatively, it follows directly from the

shape of the curves (fig.1 A,D), that tryptophan has a very high affinity for the enzyme whereas ATP binds more weakly. This result is to be expected since it is known (see [1]) that both K_d- and K_m-values for tryptophan are much smaller than those for ATP.

The data plotted in fig.1 A were analysed by computer simulation taking into account the comparable concentrations of the enzyme and ATP and the existence of 2 ATP binding sites. The apparent dissociation constants for ATP at the first and the second active sites were found to be very similar. Thus, we were
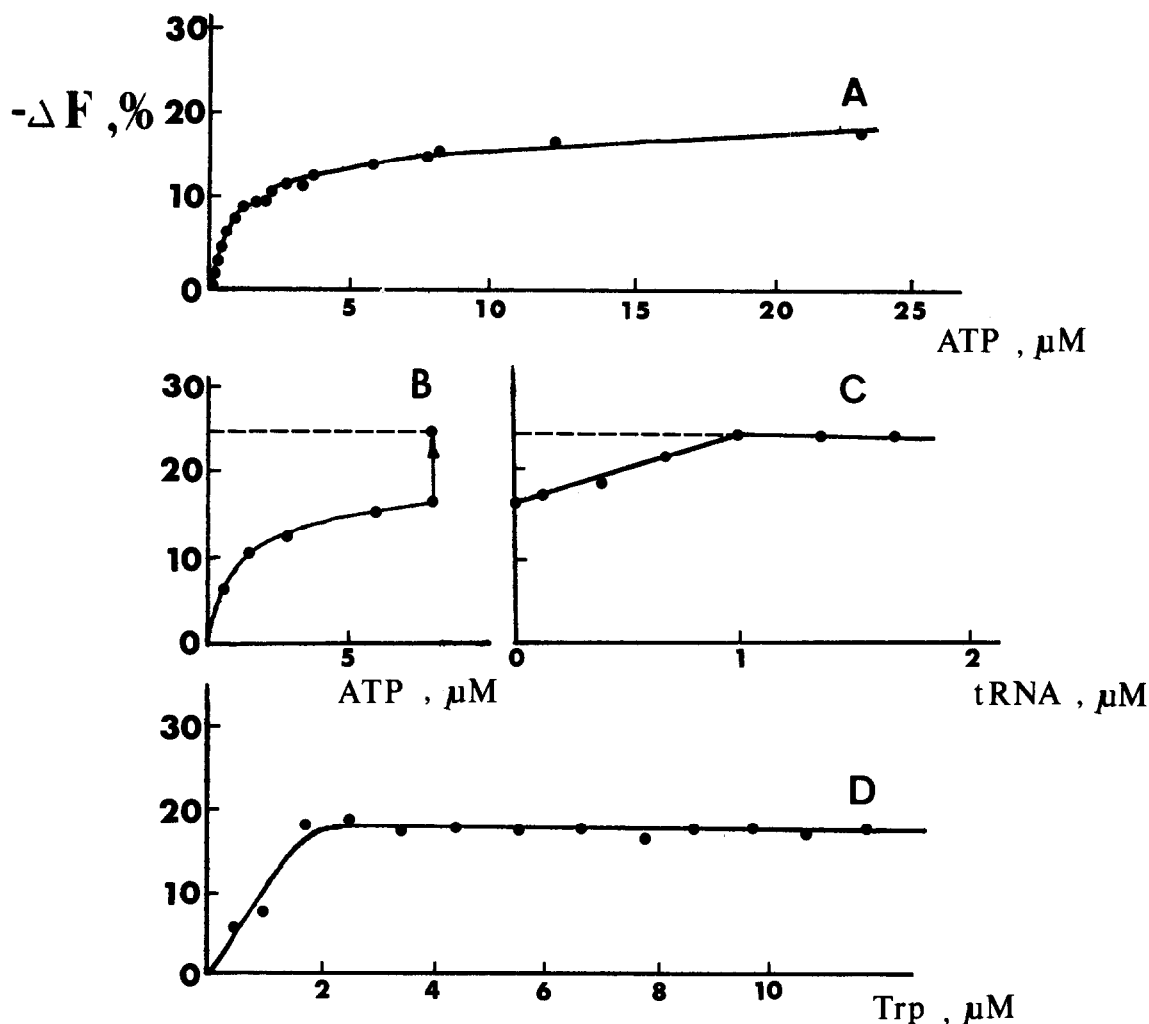

Fig.1. Fluorescence changes of the tryptophanyl-tRNA synthetase at 37°C at various concentrations of the substrates. Concentrations of the components (in μM) for A and B: enzyme, 0.9; Trp, 10.0; titration with ATP. The arrow (B) indicates the fluorescence change after addition of inorganic pyrophosphatase (2 U/ml): (C) enzyme, 0.9; Trp, 10.0; ATP, 80; titration with tRNA$^{Trp}_{-A}$; (D) enzyme, 0.9; ATP, 20.0; titration with Trp. (B,C) The dotted lines correspond to the fluorescence change in the presence of the pyrophosphatase (B) and tRNA (C).

unable to reveal any significant differences between 2 active sites with respect to their affinity for ATP in the presence of tryptophan.

From fig.1B it is seen that addition of inorganic pyrophosphatase causes a sharp decrease in the fluorescence intensity up to 23—24% which corresponds (not shown) to the binding of ~2 mol adenylate/dimeric tryptophanyl-tRNA synthetase in accordance with the data in [7].

Since it is known for many aminoacyl-tRNA synthetases (see [12]) that cognate tRNAs affect the process of amino acid activation it was of interest to study the rate of tryptophanyl—adenylate formation in the presence of tRNA^{Trp}. To exclude aminoacylation of tRNA due to the transfer of the tryptophanyl residue from adenylate to the 3'-terminus of tRNA, we used tRNA^{Trp} without terminal 3'-adenosine. It is known that tRNA^{Trp} without terminal A is able to induce conformational changes of the tryptophanyl-tRNA synthetase [13,14]. Addition of bovine or yeast tRNA^{Trp} to the tryptophanyl—adenylate · enzyme complex formed in situ causes a fluorescence quenching corresponding to the 1:2 ratio between the enzyme and the adenylate. The tRNA^{Trp}-induced quenching is much faster as compared to the same effect produced by pyrophosphatase (see below). Thus, at small ATP concentrations the 1:1 complex is predominantly formed whereas either addition of ATP, tRNA^{Trp} or inorganic pyrophosphatase leads to an increase in the stoichiometry of formation of the enzyme · adenylate complex.

To gain further insight on the mechanism of adenylate formation we used rapid kinetic methods to determine the rate constants of the enzyme adenylate formation in various conditions. The rate constant of the first adenylate formation with the 1:1 stoichiometry was so high ($k > 200$ s^{-1}) in the given conditions that we were unable to measure it. At higher ATP concentrations, when at stationary conditions a ~2:1 stoichiometry is observed, 2 processes were recorded: the first one with $k_1 > 150$ s^{-1} (not shown) and the second, a slow fluorescence quenching with $k_2 = 2.3$ s^{-1} (fig.2). From a comparison of these and the steady-state kinetic data it seems reasonable to assume that k_1 is a rate constant for the formation of the first mole of adenylate, whereas the k_2 value corresponds to formation of the second mole of adenylate. These values differ considerably from those published in [8].

Fig.3A,B shows the influence of 2 concentrations of inorganic pyrophosphatase on the fluorescence
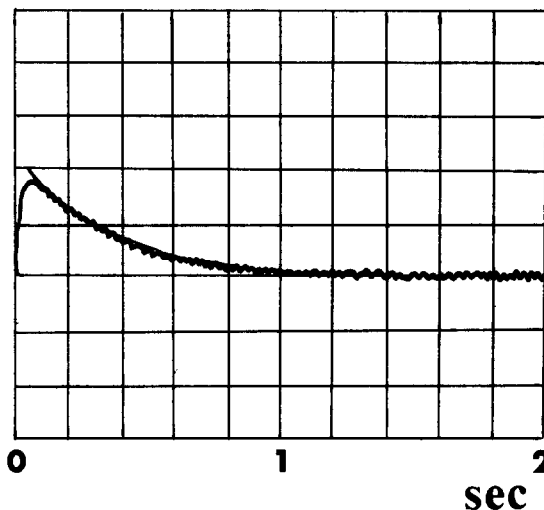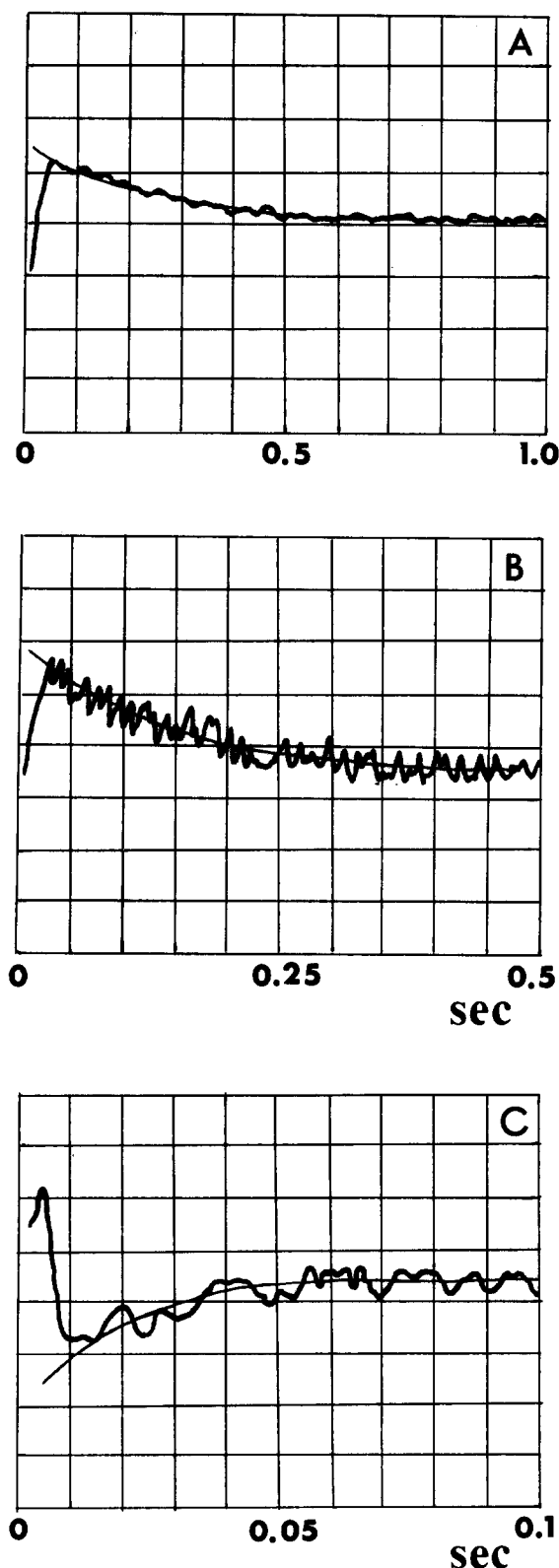

Fig.2. Kinetics of the fluorescence change of the tryptophanyl-tRNA synthetase after addition of ATP and Trp at 37°C. The incubation mixture contained (in μM): (E) 0.9; ATP, 25; Trp, 2.0; and Tris—HCl (pH 7.5), 50 mM; MgCl$_2$, 10 mM. Oscillograph data: 200 mV/division, the full signal 2 V.

quenching. It is seen that pyrophosphatase addition in quantities comparable with that of the synthetase causes an increase of the k_2 value proportionally to the concentration of the pyrophosphatase added. However, the effect is less significant as compared with the addition of tRNA^{Trp} (yeast, beef). In the latter case, the k_2 value dramatically increases and exceeds 150 s^{-1} being roughly equal to k_1. Similar results were obtained with the native tRNA^{Trp} (not shown).

A difference was noticed between the cognate tRNA$^{Trp}_{-A}$ from beef and yeast in its action on the adenylate formation: in the case of heterologous tRNA after formation of the second mole of adenylate we observed a decrease of fluorescence intensity with $k = 58$ s^{-1} which proceeded slower than the adenylate synthesis (fig.3C). We did not study this process in detail. However, the following explanation can be suggested: an additional conformational change of the enzyme · adenylate · tRNA$^{Trp}_{-A}$ complex may proceed with non-homologous substrates. Certain differences between tRNA^{Trp} from yeast and beef have been noticed earlier in experiments on the limited hydrolysis of tryptophanyl-tRNA synthetase · tRNA^{Trp} complexes [13,14].

Thus, tRNA$^{Trp}_{-A}$ and inorganic pyrophosphatase, being profoundly different chemically, cause a quali-

tatively similar (although quantitatively different) action on the rate of formation of the second molecule of tryptophanyl–adenylate by dimeric synthetase. We suppose that a common feature in their action consists in removal of the pyrophosphate molecule formed simultaneously with the synthesis of the first adenylate molecule. Pyrophosphate is probably released from the active site when $tRNA^{Trp}_{-A}$ is added whereas pyrophosphatase slowly decomposes the bound PP_i, since its affinity to the synthetase $\cdot PP_i$ complex is low. Thus, the presence of the PP_i molecule at the first active site hinders formation of the second adenylate molecule. In other words, the negative cooperativity manifests itself as a sharp kinetic non-equivalence of the 2 active sites in adenylate formation which is caused, at least, partly by the presence of bound pyrophosphate. A cognate tRNA is able to overcome this effect presumably by inducing a release of bound PP_i.

These conclusions are indirectly supported by some observations made earlier with the same enzyme:

(i) One molecule of $tRNA^{Trp}$ covalently fixed on the dimeric tryptophanyl-tRNA synthetase [2] alters the kinetic parameters of the tryptophanyl–adenylate formation at the second (non-modified) active site [3];

(ii) $tRNA^{Trp}$ being added to the enzyme $\cdot$ adenylate complex completely abolished the protective action of adenylate on the limited proteolysis of the tryptophanyl-tRNA synthetase [13,14];

(iii) Pyrophosphate formed at one active site is probably not released from the enzyme [4] which has the other active site selectively blocked by affinity modification [15]; pyrophosphatase treatment partly restores the enzymic activity of the non-modified site;

(iv) From the experiments in [4,8] a high affinity of pyrophosphate for the enzyme $\cdot$ adenylate complex is suggested.

Fig.3. Kinetics of the fluorescence change of the tryptophanyl-tRNA synthetase $\cdot$ adenylate complex after addition of inorganic pyrophosphatase (A,B) or yeast $tRNA^{Trp}_{-A}$ (C). Incubation mixtures contained (in μM): (E) 0.9; ATP, 3.0; Trp, 2.0; and Tris–HCl (pH 7.5), 50 mM; $MgCl_2$, 10 mM. Pyrophosphatase 1 U/ml (A) or 3 U/ml (B), $tRNA^{Trp}_{-A}$ (yeast, 1 μM (C). Oscillograph data: (A) 10 mV/div, full signal 600 mV; (B) 10 mV/div, full signal 600 mV; (C) 20 mV/div, full signal 1 V.

The negative cooperativity during adenylate formation has been clearly shown in [16,17]. The k_2 values for formation of the second adenylate molecule were much smaller than k_1 for both tyrosyl-tRNA synthetases from *E. coli* and *B. stearothermophilus* and for methionyl-tRNA synthetase from the latter source. We assume that in these cases the cause of diminution of the second rate constant for adenylate formation was due to the presence of the tightly bound pyrophosphate in the neighbouring active site after the first adenylate molecule being formed.

At μM levels, PP_i inhibits the aminoacylation capacity of many aminoacyl-tRNA synthetases [18]. Probably, a high affinity of the synthetases for pyrophosphate is a general phenomenon. It is tempting to speculate that the absence of an isotopic PP_i–ATP exchange for some synthetases observed without tRNAs can be, at least partly, connected with the bound pyrophosphate released under the influence of cognate tRNA.

It has been suggested [8] that the biphasic process of adenylate formation is likely to be related to the anti-cooperative binding of tryptophan to the enzyme [11]. These authors have not observed, however, the biphasic curve for dependence of adenylate formation on the tryptophan concentration (fig.2 in [8]). We obtained similar data (fig.1D). As in [5] the anti-cooperativity in the tryptophan binding is not expressed when adenylate is formed. Therefore, we believe that the negative cooperativity in adenylate formation is connected with the presence of the tightly bound pyrophosphate rather than with the tryptophan binding.

In line with some other studies on catalysis by aminoacyl-tRNA synthetases one may conclude that cognate tRNA is much more than just a substrate, and pyrophosphate is more than simply a product in the aminoacyl-tRNA synthetase catalysed process of tRNA aminoacylation.

References

[1] Kisselev, L. L., Favorova, O. O. and Kovaleva, G. K. (1979) Methods Enzymol. 59/G, 234–257.

[2] Akhverdyan, V. Z., Kisselev, L. L., Knorre, D. G., Lavrik, O. I. and Nevinsky, G. A. (1977) J. Mol. Biol. 113, 475–501.

[3] Zinoviev, V. V., Rubtsova, N. G., Lavrik, O. I., Malygin, E. G., Akhverdyan, V. Z., Favorova, O. O. and Kisselev, L. L. (1977) FEBS Lett. 82, 130–133.

[4] Favorova, O. O., Madoyan, I. A. and Drutsa, V. L. (1981) FEBS Lett. 123, 161–164.

[5] Degtyarev, S. Kh., Kovaleva, G. K., Zinoviev, V. V. and Malygin, E. G. (1981) Molekul. Biol. 15, 176–181.

[6] Kovaleva, G. K., Degtyarev, S. Kh. and Favorova, O. O. (1979) Molekul. Biol. 13, 1237–1246.

[7] Graves, P., Bony, J., Masat, J. and Labouesse, B. (1980) Biochimie 62, 33–41.

[8] Merault, G., Labouesse, J., Graves, P.-V. and Labouesse, B. (1981) FEBS Lett. 123, 165–168.

[9] Gillam, I., Millward, S., Blew, D., Tigerstrom, M., Wimmer, E. and Tener, G. M. (1967) Biochemistry 6, 3043–3054.

[10] Pearson, B. J., Weiss, J. F. and Kelmers, A. D. (1971) Biochim. Biophys. Acta 228, 770–774.

[11] Graves, P. V., Mazat, J. P., Juguelin, H., Labouesse, J. and Labouesse, B. (1979) Eur. J. Biochem. 96, 509–518.

[12] Kern, D. and Lapoinet, J. (1980) Eur. J. Biochem. 106, 137–150.

[13] Scheinker, V. Sh., Beresten, S. F., Degtyarev, S. Kh. and Kisselev, L. L. (1979) Nucleic Acids Res. 7, 625–637.

[14] Beresten, S. F., Scheinker, V. Sh., Bolotina, I. A., Nurbekov, M. K., Mashkova, T. D., Avdonina, T. A. and Kisselev, L. L. (1981) Molekul. Biol. 15, 805–815.

[15] Madoyan, I. A., Favorova, O. O., Kovaleva, G. K., Sokolova, N. I., Shabarova, Z. A. and Kisselev, L. L. (1981) FEBS Lett. 123, 156–160.

[16] Mulvey, R. and Fersht, A. (1977) Biochemistry 15, 243–249.

[17] Mulvey, R. and Fersht, A. (1977) Biochemistry 16, 4005–4013.

[18] Dignam, J. and Deutscher, M. (1979) Biochemistry 18, 3165–3170.