Tryptophanyl-tRNA synthetase: Evidence for an anhydrous bond involved in the tryptophanyl enzyme formation

ARTICLE *in* FEBS LETTERS · DECEMBER 1978

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(78)80056-8 · Source: PubMed

4 AUTHORS, INCLUDING:

Olga O Favorova
Pirogov Russian National Research M…

154 PUBLICATIONS **1,552** CITATIONS

SEE PROFILE

TRYPTOPHANYL-tRNA SYNTHETASE: EVIDENCE FOR AN ANHYDROUS BOND INVOLVED IN THE TRYPTOPHANYL ENZYME FORMATION

G. K. KOVALEVA, S. G. MOROZ, O. O. FAVOROVA and L. L. KISSELEV
Institute of Molecular Biology, USSR Academy of Sciences, Moscow V-334, USSR

Received 21 August 1978

1. Introduction

The reaction catalyzed by aminoacyl-tRNA synthetases proceeds via the formation of an intermediate aminoacyl adenylate–enzyme complex [1,2]. Some data are available which suggest the existence of another form of the substrate amino acid activation involving a covalent bond with the enzyme [3,4].

We have identified and isolated a covalent derivative formed between the tryptophan residue and tryptophanyl-tRNA synthetase from beef pancreas [5,6]. One mole or less of tryptophan residue is covalently bound per mole of the dimeric enzyme (EC 6.1.1.2). The tryptophan residue in this derivative is believed to be in the activated state since it:

(i) Exchanges with exogeneous tryptophan rather than with other amino acids;
(ii) Reacts with NH_2OH yielding a tryptophanyl hydroxamate;
(iii) Aminoacylates $tRNA^{Trp}$ in the absence of ATP (other tRNAs are not aminoacylated).

Here we show that the tryptophanyl enzyme is a mixed anhydride formed between the carboxylic groups of the tryptophan moiety and of the protein molecule. Blocking of this group in the protein inactivates the enzyme's ability to catalyze the aminoacylation reaction but has no effect on the amino acid activation reaction.

2. Materials and methods

2.1. *Materials*

$^{14}CH_3ONH_2$ (55 Ci/mol) was obtained from Isotope (USSR). The sources of other materials were as in [6].

Tryptophanyl-tRNA synthetase (a dimer of the α_2 type with mol. wt 120 000, homogeneous after electrophoresis) was obtained as in [7], and used as a tryptophanyl enzyme preparation [5,6].

2.2. *Methods*

The enzyme activity was assayed as in [6]. The [^{14}C]tryptophanyl enzyme was obtained by exchange between unlabeled tryptophanyl enzyme and [^{14}C]-tryptophan [6]. Free enzyme was obtained by acid precipitation of the tryptophanyl enzyme in 0.5 M Na-acetate buffer (pH 4.5); the precipitate was dissolved in 0.5 M Tris·HCl (pH 7.5) and dialyzed against 0.05 M Tris·HCl (pH 7.5) at 4°C.

The enzyme was incubated with $^{14}CH_3ONH_2$ at 37°C for 30 min in 10 mM K-phosphate buffer (pH 7.5), containing 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.05 mM EDTA and 12 mM KCl. The preparation was subjected to gel filtration on a Sephadex G-50 (fine) column (0.9 × 40 cm) equilibrated at 4°C with 0.02 M K-phosphate (pH 7.5), containing 25 mM KCl, 1 mM dithiothreitol and 0.1 mM EDTA. The elution rate was 20 ml/h, the fraction volume, 1 ml. The amount of the ^{14}C-label bound to the protein was measured and the low molecular weight radioactive fraction was analyzed by ascending chromatography on carboxymethylcellulose paper (Whatman CM82) in 10 mM K-phosphate buffer, (pH 7.0). For isolation of the labeled peptide(s), the protein treated with $^{14}CH_3ONH_2$ was dialysed against 0.05 M triethylammonium bicarbonate buffer (pH 7.5), and heated at 50°C for 15 min. The mix-

ture was cooled to 37°C and adjusted to pH 8.4 with crystalline ammonium bicarbonate; the preparation was digested with trypsin for 3 h at 37°C with an enzyme/substrate ratio of 1/50 (w/w), adding a fresh portion of trypsin solution after each hour of incubation.

3. Results and discussion

The energy-rich bond which makes it possible for the tryptophan in the tryptophanyl enzyme to be substituted by free tryptophan with a protonated amino group may be an anhydride [8].

It is known that NH_2OH reacts with mixed anhydrides of carboxylic acids to yield the hydroxamates of both. If the tryptophan residue forms an anhydride bond with the enzyme, treatment of the tryptophanyl enzyme with NH_2OH can yield a protein hydroxamate in addition to tryptophanyl hydroxamate. To reveal the possible coupled formation of the hydroxamates of tryptophan and of the enzyme, we treated the tryptophanyl enzyme containing 0.72 mol covalently bound tryptophan/mol enzyme with $^{14}CH_3ONH_2$ and isolated, by gel filtration on a Sephadex G-50 column, a labeled protein material containing 0.26 mol $^{14}CH_3ONH_2$ residues/mol enzyme. Carboxymethylcellulose chromatography of a low molecular weight material obtained after gel filtration revealed the presence of 0.52 mol tryptophanyl-*O*-[^{14}C]-methylhydroxamate/mol enzyme. However, in principle labeling of the protein with $^{14}CH_3ONH_2$ could be the result of hydroxylaminolysis of Asn-Gly [9] or amide [10] bonds in the protein. To rule out this possibility, the following experiment was done. $^{14}CH_3ONH_2$-treated protein was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis. No scission of the polypeptide chains was observed. In addition, ^{14}C-incorporation into the control sample (the enzyme devoid of covalently bound tryptophan) was considerably lower than into the tryptophanyl enzyme (0.06 compared to 0.26 mol/mol enzyme). Thus, we concluded that the reaction of the tryptophanyl enzyme with $^{14}CH_3ONH_2$ proceeds with the simultaneous formation of the *O*-methylhydroxamates of both enzyme and tryptophan, the latter being formed preferentially (0.56 and 0.26–0.06 mol/mol enzyme, respectively). These results indicate the presence of an anhydride bond between the tryptophan moiety and the protein carboxylic group in the tryptophanyl enzyme.

Fractionation of the tryptic digest of the $^{14}CH_3ONH_2$-labeled tryptophanyl enzyme revealed two radioactive peptides (fig.1). Spot 1 contained much more material than spot 2.

The pH dependence of the stability of the tryptophanyl enzyme is shown in fig.2; it was measured using two procedures:

(i) By retaining the ^{14}C-labeled tryptophanyl enzyme preincubated at various pH values on nitrocellulose filters;

(ii) By aminoacylation of $tRNA^{Trp}$ with the tryptophanyl enzyme preincubated with [^{14}C]tryptophan without ATP at various pH's (see legend to fig.2).

The pH dependence of the tryptophanyl enzyme's stability can be explained if one considers the chemical properties of the anhydride bond in the mixed

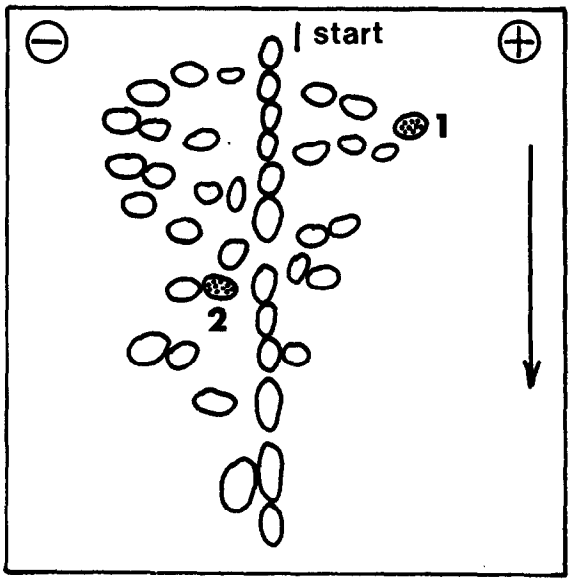

Fig.1. Separation of the peptides in a tryptic hydrolysate of the tryptophanyl enzyme treated with $^{14}CN_3ONH_2$. The hydrolysate was separated on Whatman 3 MM paper in two directions: (1) electrophoresis in pyridine–acetic acid–water (25:1:225 v/v/v), pH 6.5, 100 V/cm, 30 min; (2) chromatography in *n*-butanol–acetic acid–pyridine–water (15:3:10:12 by vol.). The chromatogram was developed with a 0.001% ninhydrin solution in acetone. Radioactive peptides visualized by radioautography are shaded.

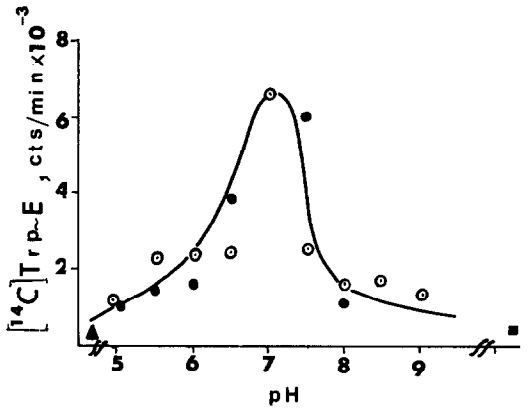

Fig.2. pH dependence of the stability of the tryptophanyl enzyme. Method 1. Labelled tryptophanyl enzyme was formed by incubating 6 μM unlabelled tryptophanyl enzyme with 60 μM [^{14}C]tryptophan in 0.2 M phosphate buffer, (pH 7.5), for 10 min. Then phosphate buffers of different pH values up to 1 M (●), trichloroacetic acid up to 5% (▲), or KOH up to 0.25 M (■) were added and the mixtures kept for 10 min at 25°C. Samples were then applied to nitrocellulose filters (Synpor N 6, Chemapol) which were rinsed with 20 ml cold 0.02 M phosphate buffers of the same pH values (●), 3% trichloroacetic acid (▲), or water (■). Method 2. [^{14}C]Tryptophanyl-tRNA was formed by incubating 10 μM tryptophanyl enzyme with 0.1 mM [^{14}C]tryptophan and 10 mM $MgCl_2$ in 4 ml 0.07 M phosphate buffers of different pH values for 1 h at 25°C. 50 μl of these mixtures were added to 125 μl of a solution containing 6 μM $tRNA^{Trp}$, 10 mM $MgCl_2$ in 0.02 M phosphate buffer (pH 7.5), and incubated for 3 min at 25°C. The amount of [^{14}C]tryptophanyl-tRNA formed was measured as in [6] (◎).

anhydrides of *N*-acylamino acids and peptides [8]. The relatively high stability of the bond formed between the tryptophanyl residue and the enzyme at neutral pH (the half-life at 4°C is 30 h [6]) may be attributed to significant hydrophobicity of the tryptophan binding site, by analogy with increased stability of the phosphoanhydride bond in aminoacyl adenylate-enzyme complexes [11,12]. Near the isoelectric point of aminoacyl-tRNA synthetases (pH ~5) the anhydride bond becomes accessible to water.

In accord with the energy-rich character of anhydride bonds the tryptophanyl residue in the tryptophanyl enzyme is able to be transferred during denaturation with 8 M urea from the carboxylic to the SH-group of the enzyme (to be published).

Recently Mulvey and Fersht [13] have found for valyl-tRNA synthetase from *E. coli* that formation of 1 mole of bound adenylate is followed by binding of an additional mole of valine [see also 3 and 6]. On the basis of the trichloroacetic acid lability of the complex between valine and the protein, these authors concluded that the complex is a non-covalent one. However, taking into account the acid lability observed for the tryptophanyl enzyme, it is likely that the valyl residue is bound to valyl-tRNA synthetase via the anhydride bond.

The activity of the enzyme treated with $^{14}CH_3ONH_2$ was compared to that of the tryptophanyl enzyme, in the reactions of ATP–$^{32}PP_i$ exchange and acylation of tRNA. Blocking of the carboxylic group of the protein had no effect on the enzyme activity in the isotope exchange reaction but decreased its activity in the aminoacylation reaction (fig.3). The degree of inactivation (~28%) fitted in well with the molar ratio of $^{14}CH_3ONH_2$ incorporation into the enzyme. Although this correlation was observed at relatively low levels of the protein modification, it was fully reproduced in several experiments, and supported the idea that the protein carboxylic group involved in tryptophanyl enzyme formation was essential for the tRNA acylation reaction. This conclusion is in agreement with the earlier observation that an activated tryptophan residue is directly transferred from the tryptophanyl enzyme to a specific $tRNA^{Trp}$ molecule in the absence of ATP [6].

The results obtained make it possible to consider this essential carboxylic group as a nucleophilic one which participates in the transfer of an activated amino acid moiety from aminoacyl adenylate to a specific tRNA as has been postulated [14,15]. The concept of the catalytic role of this group is also consistent with the phenomenon of the half-of-the-sites reactivity observed both for tRNA binding [16,17] and tryptophanyl enzyme formation [6]. Moreover, tryptophanylation of one subunit of the dimeric enzyme prevents aminoacyl adenylate formation or binding to the same subunit [6].

The existence of a covalent bond between aminoacyl-tRNA synthetase and a substrate amino acid as well as its chemical nature have been proved, to the best of our knowledge, for the first time; however, further studies are necessary in order to elucidate the functional role of the tryptophanyl enzyme.

The identification and characterization of the

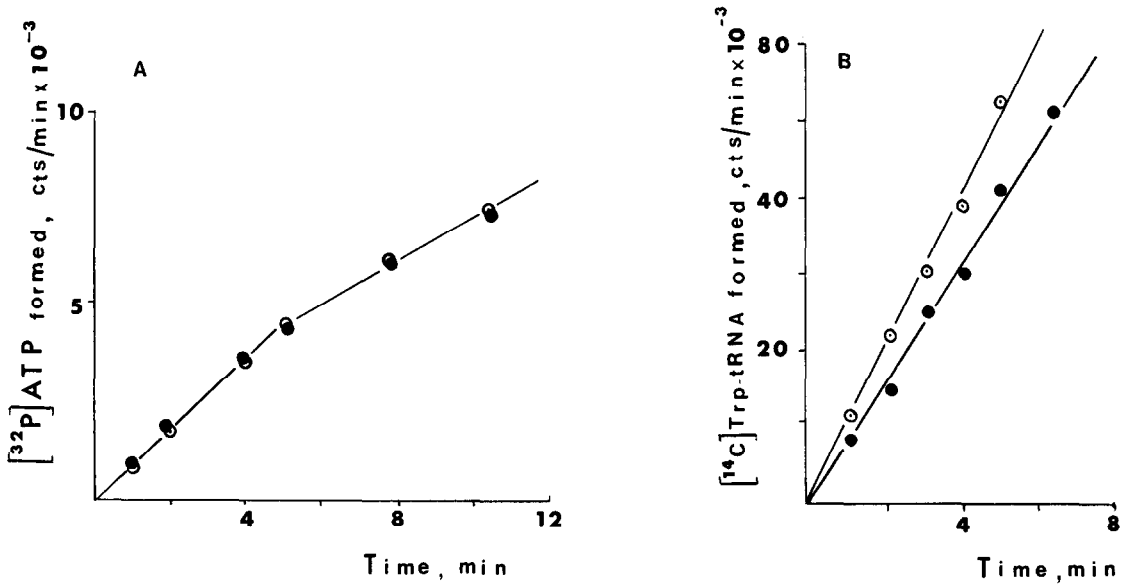

Fig.3. Comparison of the ATP–$^{32}PP_i$ exchange (A) and aminoacylation (B) activities of the $^{14}CH_3ONH_2$-treated (•) and initial (○) tryptophanyl enzyme. ^{14}C-Labeled enzyme contained 0.26 mol $^{14}CH_3ONH_2$ residues/mol protein

tryptophanyl enzyme were communicated at the USSR–FRG Symposium on the Chemistry of Proteins and Peptides (Dushanbe, April 1976), at the Symposium on the Structure and Function of Enzyme Active Sites (Puschino-on-Oka, August 1976) and at the IV Symposium on the Chemistry of Peptides and Proteins (Minsk, September 1977).

Acknowledgement

The authors are gratefully indebted to Dr A. A. Krayevsky for fruitful discussions.

References

[1] Kim, J.-J. P., Chakraburtty, K. and Mehler, A. H. (1977) J. Biol. Chem. 252, 2698–2702.
[2] Fersht, A. R., Bruton, C. J., Jakes, P., Koch, G. E. and Hartley, B. S. (1975) Biochemistry 14, 1–4.
[3] Thiebe, R. (1975) FEBS Lett. 60, 342–345.
[4] Remy, P. and Ebel, J.-P. (1976) FEBS Lett. 61, 28–31.
[5] Kovaleva, G. K., Favorova, O. O., Moroz, S. G., Krauspe, R. and Kisselev, L. L. (1976) Dokl. Akad. Nauk SSSR 229, 492–495.
[6] Favorova, O. O., Kovaleva, G. K., Moroz, S. G. and Kisselev, L. L. (1978) Molekul. Biol. 12, 588–601.
[7] Favorova, O., Kochkina, L., Shajgo, M., Parin, A., Khilko, S., Prasolov, V. and Kisselev, L. (1974) Molecul. Biol. (English edn) 8, 580–590.
[8] Bender, M. L. (1960) Mechanisms of Catalysis of Nucleophilic Reactions of Carboxylic Acid Derivatives. Chemical Reviews 60.
[9] Bornstein, P. and Balian, G. (1970) J. Biol. Chem. 245, 4854–4856.
[10] Ramachandran, L. K. and Narita, K. (1958) Biochim. Biophys. Acta 30, 616–620.
[11] Moldave, K., Castelfranco, P. and Meister, A. (1958) J. Biol. Chem. 234, 841–848.
[12] Parin, A. V., Savelyev, E. P. and Kisselev, L. L. (1970) FEBS Lett. 9, 163–167.
[13] Mulvey, R. S. and Fersht, A. R. (1977) Biochemistry 16, 4005–4013.
[14] Krayevsky, A. A., Kisselev, L. L. and Gottikh, B. P. (1973) Molekul. Biol. 7, 769–775.
[15] Lacey, J. C., jr and White, W. E., jr (1972) Biochem. Biophys. Res. Commun. 47, 565–573.
[16] Akhverdyan, V. Z., Kisselev, L. L., Knorre, D. G., Lavrik, O. I. and Nevinsky, G. A. (1977) J. Mol. Biol. 113, 475–501.
[17] Malygin, E. G., Zinoviev, V. V., Fasiolo, F., Kisselev, L. L., Kochkina, L. L. and Akhverdyan, V. Z. (1976) Mol. Biol. Rep. 2, 445–454.