PREVENTION OF ACYLATION OF AMINOACYL-tRNA BOUND IN A COMPLEX WITH EF-TU ELONGATION FACTOR

Juraj SEDLÁČEK, Jiří JONÁK and Ivan RYCHLÍK
Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia

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1. Introduction

Formation of the peptide bond in the elongation cycle is accompanied by cleavage of GTP in a ternary complex aminoacyl-tRNA–GTP–EF-Tu and by the liberation of EF-Tu–GDP + P_i from the ribosome. If the cleavage is prevented by replacing GTP with its non-cleavable analogue GMPPCP*, no peptide bond is formed in spite of the fact that the ternary complex which remains bound to the ribosome by a coded link, contains aminoacyl-tRNA [1–3]. The presence of EF-Tu on the ribosome is considered to be inhibitory [3,4]. Evidence is presented here that no peptide bond can be formed because the α-amino group of aminoacyl-tRNA is protected from acylation as long as it is bound in the complex aminoacyl-tRNA–GTP–EF-Tu. Specific chemical acylation of free and of EF-Tu–complexed aminoacyl-tRNA served as a model system.

2. Materials and methods

The *N*-hydroxysuccinimide ester of acetic acid was prepared according to [5]. [^{3}H]Phenylalanyl-tRNA containing 140 pmol phenylalanine per mg was prepared by charging total transfer RNA of *Escherichia coli* with [^{3}H]phenylalanine of specific activity 16.5 Ci/mmol (Amersham, England). The EF-Tu elongation factor was prepared in the crystalline form as EF-Tu–GDP according to [6]. GTP, phosphoenolpyruvate, phosphoenolpyruvate kinase were from Calbiochem (Switzerland), GMPPCP from P-L Biochemicals (USA), all the other chemicals were from Lachema (Czechoslovakia).

Time course of acylation: The reaction mixtures of 0.75 ml volume had the following final composition: 100 mM Tris-acetate (pH 7.6), 100 mM ammonium chloride, 10 mM magnesium acetate, 15 mM mercaptoethanol, 63 mM *N*-hydroxysuccinimide ester of acetic acid and 100 μg [^{3}H] phenylalanyl-tRNA. The complexes were formed by preincubations in mixtures of the same ionic composition as that of the reaction mixture. The complex of Phe-tRNA–GTP–EF-Tu was formed in a volume of 0.2 ml by preincubation of 40 μg EF-Tu–GDP with 1 μmol GTP, 75 μmol phosphoenolpyruvate and 30 μg pyruvate kinase for 10 min at 35°C and by subsequent preincubation at 0°C for 1 min with 100 μg Phe-tRNA. The complex of Phe-tRNA–GMPPCP–EF-Tu was formed in a volume of 0.2 ml by preincubation of 40 μg EF-Tu–GDP, 10 μg EF-Tu–EF-Ts and 10 μmol GMPPCP for 10 min at 35°C, and by subsequent preincubation at 0°C for 1 min with 100 μg Phe-tRNA. The acylation reaction was started by adding a solution of *N*-hydroxysuccinimide ester of acetic acid and was carried out at 0°C. Samples (0.1 ml) were removed from the reaction mixture at the times shown and combined with 0.1 ml solution of 100 mM glycine and 100 mM NaCl containing 100 μg carrier RNA and 1 ml cold 5% trichloracetic acid. The precipitates were centrifuged and the sediment was hydrolysed with 0.6 ml 100 mM NaOH for 30 min at 40°C. The samples were acidified with 0.1 ml 10 M hydrochloric acid and extracted with 1 ml

*GMPPCP: 5′-guanylyl methylene diphosphonate.

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ethyl acetate. The radioactivity of the dried organic phase representing the amount of acylated phenylalanyl-tRNA [see 7] was counted in a gas-flow counter.

3. Results

The rate of acylation of aminoacyl-tRNA both free and bound in a complex with the elongation factor was studied. The α-amino group of phenylalanyl-tRNA was chemically acetylated by the *N*-hydroxysuccinimide ester of acetic acid. The time course of the acetylation reaction in the presence of a large excess of the reagent and at neutral pH is shown in fig.1. Acetylation follows first-order kinetics. With free phenylalanyl-tRNA the half time of the acetylation reaction is about 30 sec. Acetylation of phenylalanyl-tRNA bound in complex of Phe-tRNA–GTP–EF-Tu proceeds at a 70 times slower rate, acetylation of phenylalanyl-tRNA in the complex with the non-cleavable analogue GMPPCP is about 40 times slower.

The individual components of the acetylation mixture have no substantial effect on the decrease of the reaction rate under conditions when complexes of the factor with aminoacyl-tRNA are not formed – no appreciable decrease is caused by EF-Tu–GDP or by GMPPCP or by phosphoenolpyruvate and pyruvate kinase.

The fact that the rate of acylation, though very low, is still detectable may be accounted for by the high but finite stability constant of the complexes. The relative difference in the rate of acylation between the complexes with GTP and with GMPPCP may reflect the difference in these stability constants.

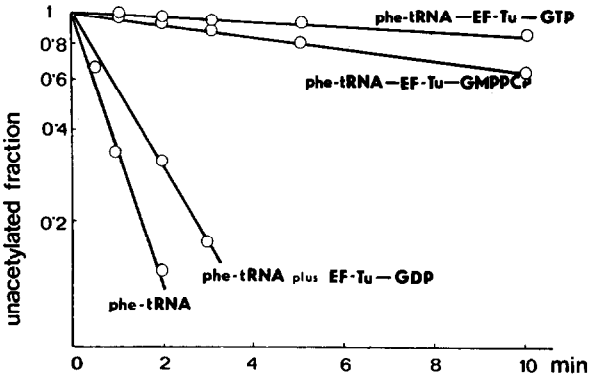

Fig.1. Acetylation of free and EF-Tu-complexed phenylalanine-tRNA.

4. Discussion

During the biosynthesis of proteins a new peptide bond is formed in analogy to the process studied in the model of acetylation of aminoacyl-tRNA with *N*-hydroxyccuccimnimide ester. The acylating agent is a peptide linked through an activated ester bond in peptidyl-tRNA. According to our experiments the ternary complex protects aminoacyl-tRNA from acylation so that it is not accesible even for the sterically non-demanding acetyl group. The ternary complex is rather stable so that one may assume that its components remain bound in the same way even on the ribosome provided the GTP was not yet split or the analogue used could not be split. The assumption is justified that the EF-Tu factor protects aminoacyl-tRNA in the ribosome from acylation by the growing peptide for the period during which the recognition is not yet concluded and the correct aminoacyl-tRNA has not yet been selected by the codon–anticodon checking process. As soon as the correct aminoacyl-tRNA is selected, GTP is split, the factor EF-Tu is liberated from the complex and the α-amino group is exposed for subsequent acylation with a peptide.

Apparently the EF-Tu factor plays a key role in the error-free decoding process during peptide elongation. Already at the stage of complex formation the ternary complex is formed only with aminoacyl-tRNA, discriminating thus against the incorrect derivatives to tRNA [see e.g. 8] that might stop the translation process on the ribosome. It enhances the interaction of correct aminoacyl-tRNA with the ribosome under rearrangement of the tertiary structure of aminoacyl-tRNA which permits the participation of another binding site on the 30 S subunit [9]. The present evidence that the α-amino group of aminoacyl-tRNA is protected from acylation in a complex of aminoacyl-tRNA–GTP–EF-Tu makes it possible to envisage another function of the EF-Tu factor, namely to prevent the formation of a peptide bond, unless the selection of the correct aminoacyl-tRNA has been properly completed.

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