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Template-free ribosomal synthesis of polypeptides from aminoacyl-tRNA

Polyphenylalanine synthesis from phenylalanyl-tRNA^{Lys}

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Misacylated phenylalanyl-tRNA^{Lys}, just as lysyl-tRNA^{Lys}, but not phenylalanyl-tRNA^{Phe}, have been shown to serve as substrates for ribosomal synthesis of polypeptides (polyphenylalanine and polylysine, respectively) in the absence of a template polynucleotide (poly(A)). The conclusion was made that it is the structure of tRNA that determines the ability of the aminoacyl-tRNA^{Lys} to participate in peptide elongation on ribosomes without codon-anticodon interactions.

Ribosomal polypeptide synthesis *Template-free peptide elongation* *tRNA conformation* *Translocation* *Aminoacyl-tRNA binding*

1. INTRODUCTION

It has been demonstrated previously that *Escherichia coli* ribosomes can use lysyl-tRNA and certain other aminoacyl-tRNAs as substrates for polypeptide synthesis in the absence of a polynucleotide template [1–3]. Among 16 aminoacyl-tRNAs tested lysyl-, seryl-, threonyl- and aspartyl-tRNAs proved to be the best substrates for the template-free ribosomal synthesis of homopeptides, whereas phenylalanyl-, asparaginyl-, methionyl-, isoleucyl- and some other tRNAs could not be substrates for elongation in the absence of a messenger [2,3]. However, it was not clear what determines the capability of an aminoacyl-tRNA to serve as a substrate for template-free polypeptide synthesis, viz. the peculiarities of the tRNA structure or the nature of the amino acid residue. The use of misacylated tRNAs seems to be adequate to answer this question.

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It is shown here that phenylalanyl-tRNA^{Lys}, just as lysyl-tRNA^{Lys}, is capable of serving as a substrate for homopolypeptide synthesis on the ribosome in the absence of poly(A) and any other template polynucleotide. It was concluded that it is the structure of tRNA that determines the capability of aminoacyl-tRNA^{Lys} to participate in peptide elongation without codon-anticodon interaction.

2. MATERIALS AND METHODS

E. coli MRE-600 ribosomes were washed 4 times with 1 M NH_4Cl [4,5]. The purified ribosomes were stored at −70°C in buffer containing 20 mM Tris-HCl, 100 mM NH_4Cl, 10 mM $MgCl_2$, 0.1 mM EDTA and 10% glycerol ($pH_{37}°_C$ 7.6). Purified elongation factors EF-Tu and EF-G were prepared from *E. coli* MRE-600 mainly by the procedures in [6,7].

The total *E. coli* tRNA (Boehringer-Mannheim) was enzymatically aminoacylated with [^{14}C]lysine (Amersham, 348 mCi/mmol) [8]; the tRNA ac-

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dividual [^{14}C]lysyl-tRNA^{Lys} was prepared by affinity chromatography of the total [^{14}C]lysyl-tRNA on Sepharose-immobilized EF-Tu from *Thermus thermophilus* [9]. The [^{14}C]lysyl-tRNA^{Lys} after chromatography had 1000–1100 pmol [^{14}C]lysine per A_{260} unit. (One A_{260} unit corresponds to 1500 pmol tRNA.)

To produce a misacylated tRNA^{Lys}, the [^{14}C]lysyl-tRNA^{Lys} was deacylated in 100 mM Tris-HCl buffer, pH 8.9 for 1 h at 37°C. Misacylation of the tRNA^{Lys} by [^{3}H]phenylalanine (Amersham, 50 Ci/mol) was done using phenylalanyl-tRNA synthetase from yeast as in [10]. The specific activity of the yeast phenylalanyl-tRNA synthetase sample given to us by Dr P. Remy, Institute of Molecular and Cellular Biology, Strasbourg, was 3000–3500 units/mg protein. The resultant [^{3}H]phenylalanyl-tRNA^{Lys} had 750 pmol phenylalanine per A_{260} unit. Contamination of tRNA^{Phe} in the preparations of tRNA^{Lys} was estimated using aminoacylation of tRNA^{Lys} by [^{3}H]phenylalanine with the enzyme from *E. coli* under standard conditions; tRNA^{Phe} contamination in tRNA^{Lys} was no more than 20–25 pmol per A_{260} unit, i.e. not exceeding 2–2.5%.

The commercial preparation of tRNA^{Phe} (Boehringer-Mannheim, 1259 pmol per A_{260} unit) was enzymatically aminoacylated by [^{3}H]phenylalanine (Amersham, 50 Ci/mmol) [8]. It had 1250 pmol phenylalanine per A_{260} unit.

All samples of aminoacylated tRNA were stored in 10 mM CH_3COONa (pH 4.5 at −70°C).

The kinetics of ribosomal synthesis of polypeptides from aminoacyl-tRNA was examined in 20 mM Tris-HCl buffer (pH 7.6) containing 12 mM $MgCl_2$, 100 mM NH_4Cl, 1 mM DTT and 0.1 mM EDTA. The Mg^{2+} dependence of peptide synthesis was studied in the same buffer at Mg^{2+} concentrations varying from 5 to 20 mM. 4 pmol ribosomes, 50 pmol [^{14}C]lysyl-tRNA or [^{3}H]phenylalanyl-tRNA, 75 pmol EF-Tu, 1.5 pmol EF-G, 15 pmol GTP, 100 nmol phospho*enol*pyruvate and 1 μg phospho*enol*pyruvate kinase were present per 50 μl aliquot. Incubation was done at 37°C.

In the case of polyphenylalanine synthesis the reaction was stopped by addition of 3 ml of 5% trichloroacetic acid. The suspension was hydrolyzed at 90°C for 20 min, and the precipitates collected on GF/F glass filters (Whatman), washed with cold 5% trichloroacetic acid and their radioactivities measured in the standard toluene-PPO-POPOP mixture using a Beckman LS-9800 scintillation spectrometer. The counting efficiency was 94% for ^{14}C and 28% for ^{3}H.

In experiments with polylysine synthesis the reaction was stopped by addition of 50 μl of 1 N NaOH, hydrolysis was conducted for 10 min at 37°C, the hydrolysate was cooled and neutralized with 50 μl of 1 M CH_3COOH, then 2 ml of 5% trichloroacetic acid with 0.25% Na_2WO_4 (pH 2.0) was added, and the mixture kept at 4°C for 10 min [11]. The precipitate was collected on a GF/F glass filter, washed with trichloroacetic acid-Na_2WO_4 mixture, dried, and the radioactivity measured as described above.

3. RESULTS

The control kinetic curves of polypeptide synthesis on template-programmed ribosomes using [^{14}C]lysyl-tRNA^{Lys}, [^{3}H]phenylalanyl-tRNA^{Lys} and [^{3}H]phenylalanyl-tRNA^{Phe} as substrates are presented in fig.1. It is seen that the rates of poly(A)-directed syntheses of polyphenylalanine from [^{3}H]phenylalanyl-tRNA^{Lys} and polylysine from [^{14}C]lysyl-tRNA^{Lys} are similar. Polyphenylalanine synthesis on the poly(U)-programmed ribosomes using [^{3}H]phenylalanyl-tRNA^{Phe} as a substrate is shown to be much faster.

Fig.2 demonstrates the kinetics of [^{14}C]lysine incorporation into the trichloroacetic acid-Na_2WO_4-insoluble product from [^{14}C]lysyl-tRNA^{Lys} during incubation of ribosomes in a cell-free system in the absence of poly(A). It is seen that the *E. coli* ribosomes use the individual lysyl-tRNA as a substrate and polymerize lysyl residues without template polynucleotide. This process strictly depends on the presence of EF-G in the system.

Fig.3 shows that *E. coli* ribosomes without template polynucleotide are capable of polymerizing phenylalanine residues as well using [^{3}H]phenylalanyl-tRNA^{Lys} as a substrate. In this case polypeptide elongation also strictly requires the presence of EF-G in the system (not shown). As follows from comparison of figs 3 and 2, the rates of polyphenylalanine and polylysine syntheses on ribosomes without template polynucleotide from phenylalanyl-tRNA^{Lys} and lysyl-tRNA^{Lys}, respec-

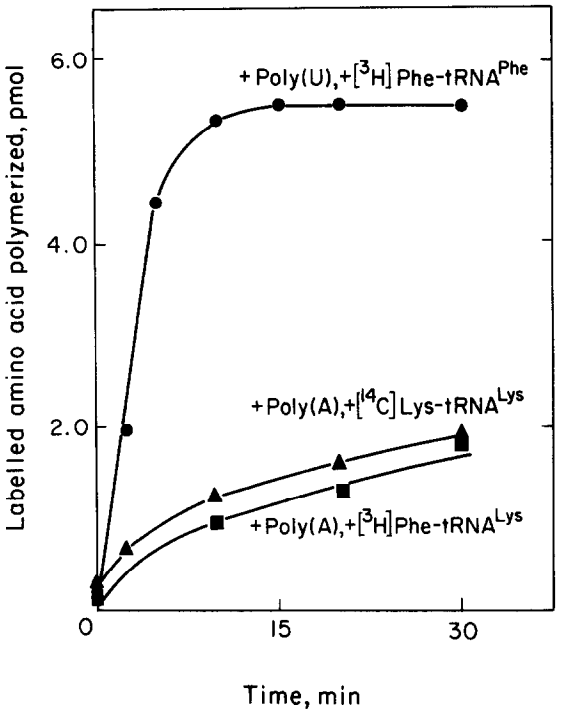

Fig.1. Kinetics of the syntheses of polypeptides on ribosomes in the presence of template polynucleotides from individual aminoacyl-tRNAs. 37°C, 12 mM $MgCl_2$.

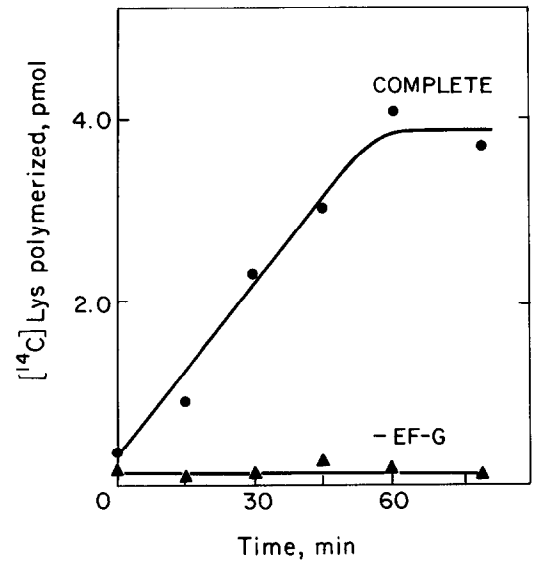

Fig.2. Kinetics of the synthesis of polylysine on ribosomes in the absence of a template polynucleotide from [^{14}C]lysyl-tRNA^{Lys}. (●) Complete template-free system including EF-Tu, EF-G and GTP. (▲) The same but without EF-G. 37°C, 12 mM $MgCl_2$.

tively, are similar (in some experiments the difference in the synthesis rates reached 30%; e.g. see below, fig.4). This indicates the decisive role of the tRNA residue, but not of the amino acid residue, in the efficacy of utilization of aminoacyl-tRNAs by ribosomes for peptide elongation.

Fig.2 also shows that the ribosomes not programmed with a template polynucleotide are unable to use phenylalanyl-tRNA^{Phe} as a substrate for polymerization of phenylalanyl residues. The interaction of tRNA^{Phe} with the codon seems to be an obligatory condition for ribosomal polyphenylalanine synthesis.

The dependence of the rates of poly(A)-independent polypeptide syntheses using [^{14}C]lysyl-tRNA^{Lys} and [^{3}H]phenylalanyl-tRNA^{Lys} on Mg^{2+} concentration are presented in fig.4A,B. In both cases the template-free polypeptide syntheses have the same Mg^{2+} optimum, at 11 mM $MgCl_2$. Fig.4A,B also shows that EF-G omission prevents template-free polypeptide synthesis on the ribosomes throughout the range of Mg^{2+} concentrations.

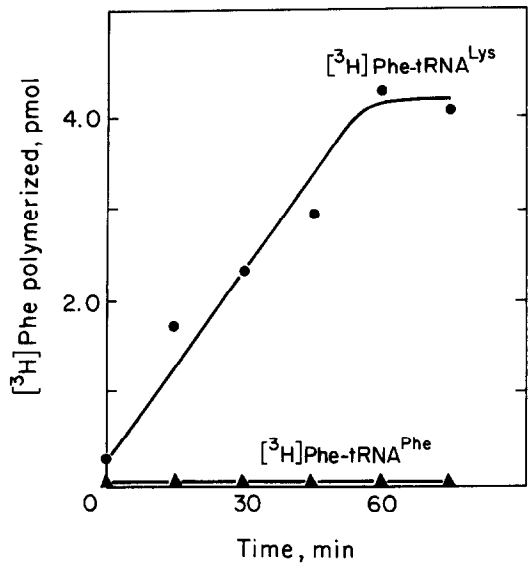

Fig.3. Kinetics of the synthesis of polyphenylalanine on ribosomes in the absence of template polynucleotides. 37°C, 12 mM $MgCl_2$.

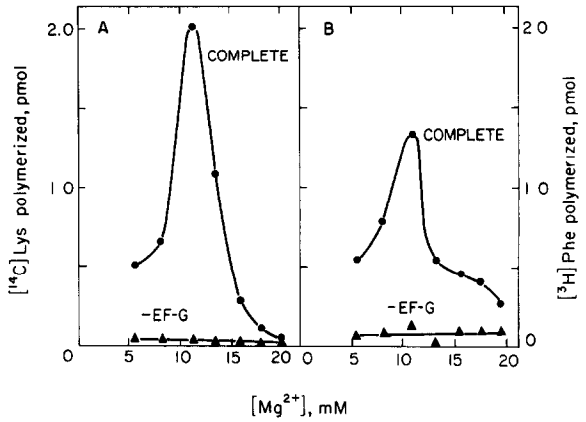

Fig.4. Dependences of template-free polypeptide syntheses on Mg^{2+} concentration in either complete template-free system including EF-Tu, EF-G and GTP or without EF-G. (A) Synthesis of polylysine from [^{14}C]lysyl-tRNA^{Lys}. (B) Synthesis of polyphenylalanine from [^{3}H]phenylalanyl-tRNA^{Lys}.

4. DISCUSSION

The results of using misacylated phenylalanyl-tRNA^{Lys} in template-free elongation experiments indicate that the structure of tRNA, and not the nature of the amino acid residue, determines the capacity of an aminoacyl-tRNA to serve as a substrate for peptide elongation on the ribosome in the absence of a template polynucleotide. It is likely that the structure of tRNA^{Lys} provides correct EF-Tu-dependent binding of an aminoacyl-tRNA^{Lys} to the A-site of the ribosome in the absence of the codon-anticodon interaction, resulting in ribosome-catalyzed transpeptidation between the P-site-bound and A-site-bound substrates. The presence of EF-G and GTP is found to be absolutely required for peptide elongation, thus suggesting the involvement of EF-G-catalyzed translocation of peptidyl-tRNA^{Lys} in the absence of a template polynucleotide. Thus, the interaction of tRNA with the codon seems to be obligatory neither for correct aminoacyl-tRNA binding to the ribosome nor for translocation. The latter is evidence that the primary (driving) force for translocation is provided by the shift of tRNA rather than that of the template.

Polypeptide synthesis is not observed when phenylalanyl-tRNA^{Phe} is used without poly(U). This demonstrates that the structure of tRNA^{Phe} cannot provide for a proper accommodation of the aminoacyl-tRNA as a substrate for peptide elongation on the ribosome, unless it is interacting with the codon. At present, it is not clear which step of the elongation cycle is not compatible with the structure of tRNA^{Phe} without codon; this could be either the binding and fitting of phenylalanyl-tRNA^{Phe} to the A-site, or the accommodation of tRNA^{Phe} in the P-site, or transpeptidation, or the EF-G-induced translocation of the peptidyl-tRNA^{Phe}. It seems to us that the inability of tRNA^{Phe} to be properly settled in the codon-free A-site is the most likely alternative. Stereochemical considerations suggest that in order to provide transpeptidation the A-site-bound tRNA must have a 5 Å longer distance between the anticodon and the acceptor end than the P-site-bound tRNA [12,13]. We hypothesize that some tRNAs, such as tRNA^{Phe}, require codon-anticodon interaction in order to be transformed into the A-site-suitable 'open' conformation, whereas other tRNAs, including tRNA^{Lys}, are more flexible or even pre-exist in the open form.

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