TEMPLATE-FREE RIBOSOMAL SYNTHESIS OF POLYLYSINE FROM LYSYL-tRNA

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1. Introduction

Escherichia coli ribosomes can bind lysyl-tRNA in the absence of poly(A) as a template polynucleotide [1]. The ribosome can also retain polylysyl-tRNA without codon–anticodon interaction [2]. It was unclear, however, whether the ribosome without messenger can synthesize (elongate) polylysine peptide chain using lysyl-tRNA.

Here we show that *E. coli* ribosomes are capable of synthesizing oligolysines from lysyl-tRNAs without poly(A). EF-Tu-induced binding of lysyl-tRNA, ribosomal peptidyl transferase and EF-G-promoted translocation are strictly required for this template-free elongation.

2. Materials and methods

Escherichia coli MRE-600 ribosomes washed 4 times with 1 M NH_4Cl with 10 mM $MgCl_2$ [3,4] were used. The purified ribosomes were stored in the frozen state at −70°C in the 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 [5,6].

Preparations of commercial *E. coli* tRNA (Boehringer-Mannheim) and tRNA isolated in this laboratory from 105 000 × *g* postribosomal supernatant of *E. coli* by phenol deproteinization were aminoacylated enzymatically with [^{14}C]lysine (486 mCi/mmol, Amersham) as in [7]; the final preparations contained 1480 and 880 pmol [^{14}C]lysyl-tRNA/mg total tRNA, respectively.

For kinetic measurements the reaction mixtures were prepared in the buffer consisting of 20 mM Tris–HCl, 100 mM NH_4Cl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT ($pH_{37°C}$ 7.6). Each 50 μl aliquot contained 20 pmol ribosomes, 170 pmol EF-Tu, 37 pmol EF-G, 100 μg of total tRNA (148 or 88 pmol [^{14}C]lysyl-tRNA), 8–16 nmol GTP (Fluka), 100 nmol phosphoenolpyruvate (Fluka), 1 μg phosphoenolpyruvate kinase (Boehringer-Mannheim). Incubation was at 37°C. The reaction was stopped by 5% trichloroacetic acid with 0.25% Na_2WO_4 [8] and the suspension formed was hydrolyzed at 90°C for 15 min. The hot acid-insoluble precipitates were collected on GFF glass filters (Whatman) and their radioactivities were measured in the standard toluene–PPO–POPOP mixture using Beckman LS-100 scintillation spectrometer.

Dependence of the activities of the poly(A)-directed and the template-free syntheses of polylysine on [Mg^{2+}] was recorded under the same conditions as given above except that $MgCl_2$ was varied from 5–20 mM and incubation time was 30 min. 20 μg poly(A) (Calbiochem) were present in each 50 μl aliquot of the poly(A)-directed system.

For comparative measurements of inhibition of the poly(A)-directed and the template-free syntheses of polylysine the reaction mixtures were done in the same buffer as above except that $MgCl_2$ was 14 mM. Aliquots of 100 μl containing 20 pmol ribosomes and variable concentrations of antibiotics were added to 50 μl portions of the mixture of [^{14}C]lysyl-tRNA, EF-TU, EF-G, GTP, phosphoenolpyruvate and pyruvate kinase in the same amounts as indicated above; in the case of the poly(A)-directed system the mixture contained also 20 μg poly(A). After incubation for 30 min at 37°C the samples were treated as above.

For the separation and identification of [^{14}C]-olygolysines CM-cellulose 52 (Whatman) columns preequilibrated with 3 mM pyridine–acetate buffer (pH 5.2) were used [9]. Aliquots of 0.6 ml reaction

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mixture with 240 pmol ribosomes and corresponding amounts of the other components were taken for each column. After incubation for 30 min at 37°C the aliquots were extracted with phenol. RNA fraction of each aliquot was precipitated with ethanol and precipitates were dissolved in 50 μl 0.2 N NaOH. The solutions were incubated for 15 min at 37°C (under these conditions lysine and oligolysine residues were split off from tRNA), diluted to 5 ml with distilled water adjusted to pH 5.0 by acetic acid and applied to the column (0.25 cm × 33 cm). Lysine peptides from dilysine up to heptalysine were eluted by the exponential gradient from 3–950 mM pyridine–acetate buffer (pH 5.2). Fractions of 1 ml were collected and their radioactivity was counted in the mixture of toluene–PPO–POPOP and Triton X-100 (Serva) in 2:1 ratio.

3. Results

Figures 1–3 present the kinetics of [^{14}C]lysyl incorporation into hot trichloroacetic acid–Na_2WO_4-insoluble product in the course of incubation of the ribosomal cell-free system without poly(A). The results suggest that a significant synthesis of polylysine from lysyl-tRNA occurs in the system. From fig.1 it is seen that the presence of both the ribosomes and the two elongation factors are strictly required for the synthesis.

It is interesting that EF-Tu-promoted (enzymatic) binding of lysyl-tRNA is found to be very important for the poly(A)-independent synthesis of polylysine (fig.1).

As shown in fig.2, the polylysine synthesis in the absence of poly(A) has an absolute requirement for EF-G-promoted translocation: either the omission of EF-G from the system or the addition of an inhibitor of the EF-G function (such as guanyl-5′-yl methylenediphosphonate or fusidic acid) practically blocks the polylysine elongation.

The system does not work in the cold (fig.2).

Fig.3 demonstrates that non-cognate template polynucleotides, such as poly(U) and poly(C), significantly inhibit the ribosomal polylysine synthesis in the absence of poly(A).

Length distribution of the polypeptide product synthesized is shown in fig.4. It is seen that the complete system without poly(A) synthesizes oligolysines up to 7 lysine residues in length (fig.4A). At the same

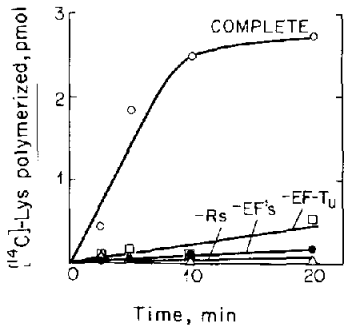

Fig.1. Kinetics of [^{14}C]lysyl incorporation into hot trichloroacetic acid–Na_2WO_4-insoluble product in the poly(A)-independent cell-free system of *E. coli*: (○) complete template-free system (ribosomes, [^{14}C]lysyl-tRNA, EF-Tu, EF-G, GTP); (△) the system without ribosomes; (●) the system without EF-Tu and EF-G; (□) the system without EF-Tu.

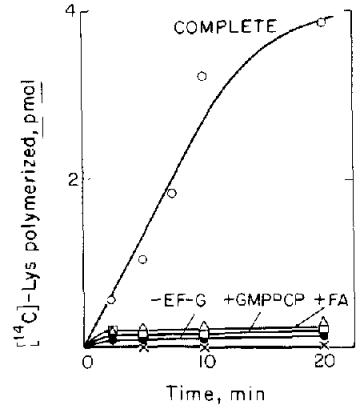

Fig.2. The same as in fig.1: (○) complete template-free system; (●) the system without EF-G; (□) 0.4 mM guanyl-5′-yl methylenediphosphonate is present instead of GTP; (△) 0.4 mM fusidic acid is added; (x) complete template-free system at +4°C.

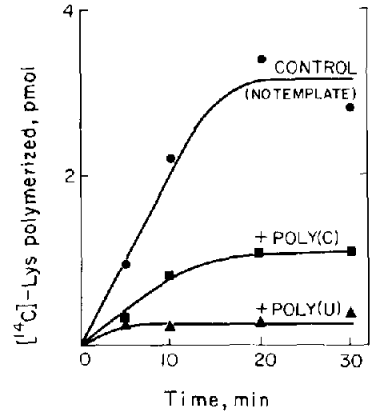

Fig.3. The same as in fig.1: (●) complete template-free system; (▲) 4 μg poly(U) is added; (■) 4 μg poly(C) is added.

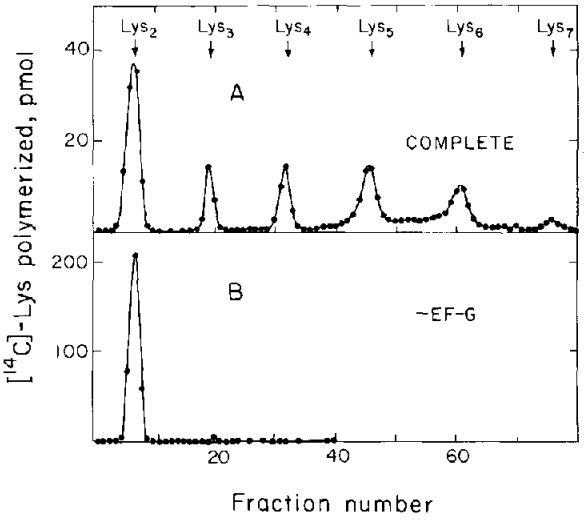

Fig.4. Elution profiles of [^{14}C]oligolysines from the CM-cellulose column: (A) synthesis in the complete template-free system; (B) synthesis in the system with omission of EF-G.

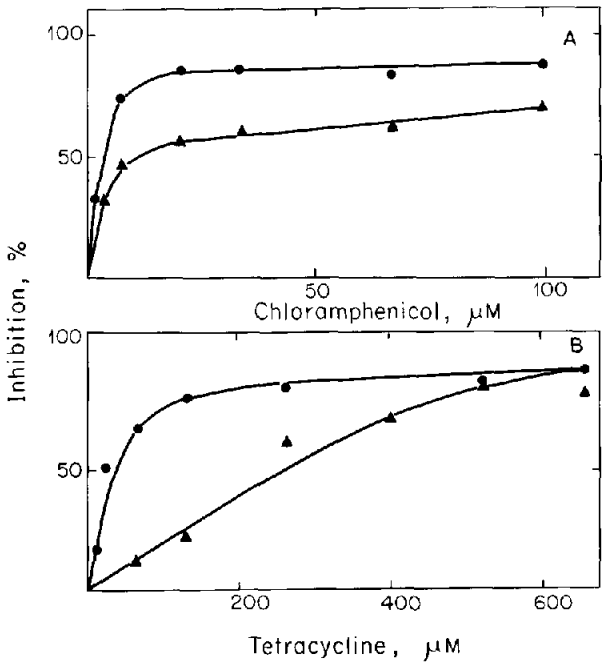

Fig.5. Inhibition of [^{14}C]polylysine synthesis depending on the concentration of antibiotics: (A) chloramphenicol; (B) tetracycline; (▲) poly(A)-directed system; (●) poly(A)-independent (template-free) system. Incubation time was 30 min at 37°C.

time, when EF-G is omitted, only dilysines are formed (fig.4B). The latter again suggests that normal ribosomal translocation is an obligatory pre-requisite for the template-free elongation.

Sensitivity of the ribosomal polylysine synthesis to typical ribosomal inhibitors such as tetracycline and chloramphenicol is demonstrated in fig.5. Here the difference between the template-free and the poly(A)-directed polylysine syntheses is noteworthy: the template-free elongation is found to be more sensitive.

One more difference between the template-free and the poly(A)-directed polylysine elongation can be revealed in the dependence of the activities of the two systems on Mg^{2+} concentration. As shown in fig.6, optimum [Mg^{2+}] for the template-free polylysine synthesis (~11 mM) is significantly lower than that for the poly(A)-directed elongation (~15 mM). (It is likely that the low Mg^{2+} shoulder in the poly(A)-directed system reflects the template-free synthesis in the reaction mixture.)

Thus, the template-free polylysine elongation on ribosomes can be distinguished from the classical poly(A)-directed polylysine synthesis at least by two criteria: more sensitivity to inhibitors; and lower Mg^{2+} concentrations required.

We have failed to detect polypeptide synthesis in the template-free ribosomal systems from Phe-tRNA. On the other hand, Arg-tRNA and Ser-tRNA have been also shown to serve as substrates for polypeptide elongation on template-free ribosomes.

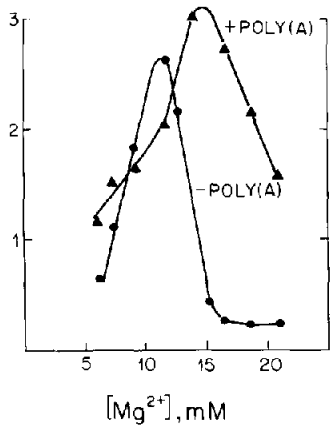

Fig.6. Dependence of the [^{14}C]polylysine synthesis on [Mg^{2+}]: (▲) poly(A)-directed system; (●) poly(A)-independent (template-free) system. Incubation time was 30 min at 37°C.

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