THE EFFECT OF RIBOSOMAL PEPTIDYL-TRANSFERASE INHIBITORS IS ANTAGONIZED BY ELONGATION FACTOR G WITH GTP

A. S. SPIRIN and L. S. ASATRYAN

Institute of Protein Research, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

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1. Introduction

In a previous paper [1] it was shown that elongation factors with GTP increase the resistance of translating ribosomes to inhibitors. It was found that the elongation factor T_u (EF-T_u) with GTP counteracts such inhibitors of aminoacyl-tRNA binding with the ribosome as tetracycline and streptomycin and does not counteract erythromycin, spectinomycin and chloramphenicol; on the contrary, the elongation factor G (EF-G) with GTP proved to be an antagonist of erythromycin and spectinomycin which are known to be inhibitors of translocation, and did not affect the action of tetracyclin and streptomycin [1]. However, the fact that EF-G with GTP also counteracted chloramphenicol, which is known to be an inhibitor of ribosomal transpeptidation, seemed unusual.

In the present work the effect of the elongation factors with GTP on the action of some inhibitors of bacterial ribosome peptidyl-transferase, such as chloramphenicol, lincomycin, amicetin, gougerotin and griseoviridin, was studied. The result was unexpected: all the tested inhibitors of ribosomal peptidyl-transferase were antagonized by EF-G (but not EF-T_u) with GTP. The result is discussed in the light of the concept of reversibility of the transpeptidation reaction in the ribosome.

2. Materials and methods

Escherichia coli MRE-600 ribosomal 30 S and 50 S subparticles, purified elongation factors EF-T_u and EF-G and tRNA aminoacylated with [^{14}C]phenylalanine (513 Ci/mol, Amersham) were prepared as described earlier [2].

All the translation systems were prepared in buffer containing 10 mM Tris–HCl, 100 mM KCl, 6–13 mM $MgCl_2$, 1–2 mM dithiothreitol, final pH 7.1–7.4 at 25°C. The $MgCl_2$ concentration was 10 mM for the factor-free system, 6 mM for the EF-T_u-promoted system, 13 mM for the EF-G-promoted system and 10 mM for the (EF-T_u + EF-G)-promoted system, which corresponds to the Mg^{2+} optima of the respective systems [2].

In all the cases each 50 μl sample contained 7 μg of 30 S subparticles, 14 μg of 50 S subparticles, 10 μg of poly(U) and 60 μg of total tRNA aminoacylated with [^{14}C]phenylalanine. In addition, samples of the EF-T_u-promoted system contained 1 μg of EF-T_u and 3 μg of GTP; samples of the EF-G-promoted system contained 0.5 μg of EF-G and 3 μg of GTP; samples of the (EF-T_u + EF-G)-promoted system contained 1.75 μg of EF-T_u, 0.5 μg of EF-G and 3 μg of GTP.

Incubation of the factor-free system was done for 3 h at 25°C; incubation of the EF-T_u-promoted and the EF-G-promoted systems was carried out for 2 h at 25°C; the (EF-T_u + EF-G)-promoted system was incubated for 30 min at 25°C. In all cases the end of incubation corresponded to the linear region of the kinetic curve of polyphenylalanine synthesis in the respective system (see [2]).

Treatment of the samples with hot trichloroacetic acid and radioactivity counts were done as described earlier [2].

Poly(U), dithiothreitol and gougerotin were from Calbiochem. GTP was from Fluka, Switzerland. Chloramphenicol was provided by Dr Yu. O. Sazykin,

Institute of Antibiotics, Moscow; griseoviridin was a gift of Dr D. Vazquez, Instituto de Biologia Cellular, Madrid; lincomycin and amicetin were generously provided by Dr G. B. Whitfield of the Upjohn Company, Kalamazoo, Michigan.

3. Results and discussion

The contribution of the elongation factors to the resistance of ribosomes against inhibitors was studied by comparing the inhibitor effect on the factor-dependent and factor-free translation systems [1]. Chloramphenicol, lincomycin, amicetin, gougerotin and griseoviridin, known to be specific inhibitors of the peptidyl-transferase center of bacterial ribosomes (see review [3]), were tested. Figure 1 shows the dependence of inhibition of the factor-free, the EF-T_u-promoted, the EF-G-promoted and the (EF-T_u + EF-G)-promoted poly(U)-directed translation systems on the inhibitor doses for the five indicated inhibitors of the peptidyl-transferase. It is seen that all the tested antibiotics suppress the factor-free translation very effectively. However, in the presence of the two elongation factors (EF-T_u + EF-G) and GTP, the poly(U)-directed translation proves to be much more resistant to all the inhibitors of the peptidyl-transferase. The most interesting fact is that a similar high resistance to the antibiotics is observed in the presence of only EF-G with GTP, while the presence of just EF-T_u with GTP in the system does not contribute to the resistance of the ribosome against inhibitors of the peptidyl-transferase.

Table 1 shows the amount of antibiotic molecules per ribosome required for a 50% inhibition of the corresponding translation systems. It is seen that the factor-free and the EF-T_u-promoted systems are similarly sensitive to the peptidyl-transferase inhibitors. At the same time, both the EF-G-promoted and the two-factor-promoted systems require essentially greater amounts of antibiotic for the half-inhibition; these amounts exceed those in the case of the factor-free and EF-T_u-promoted systems by at least an order of magnitude or more.

Hence, it is the presence of EF-G with GTP that proves to be necessary and sufficient to strongly increase the resistance of translating ribosomes against peptidyl-transferase inhibitors. This fact appears to be novel and requires special discussion.

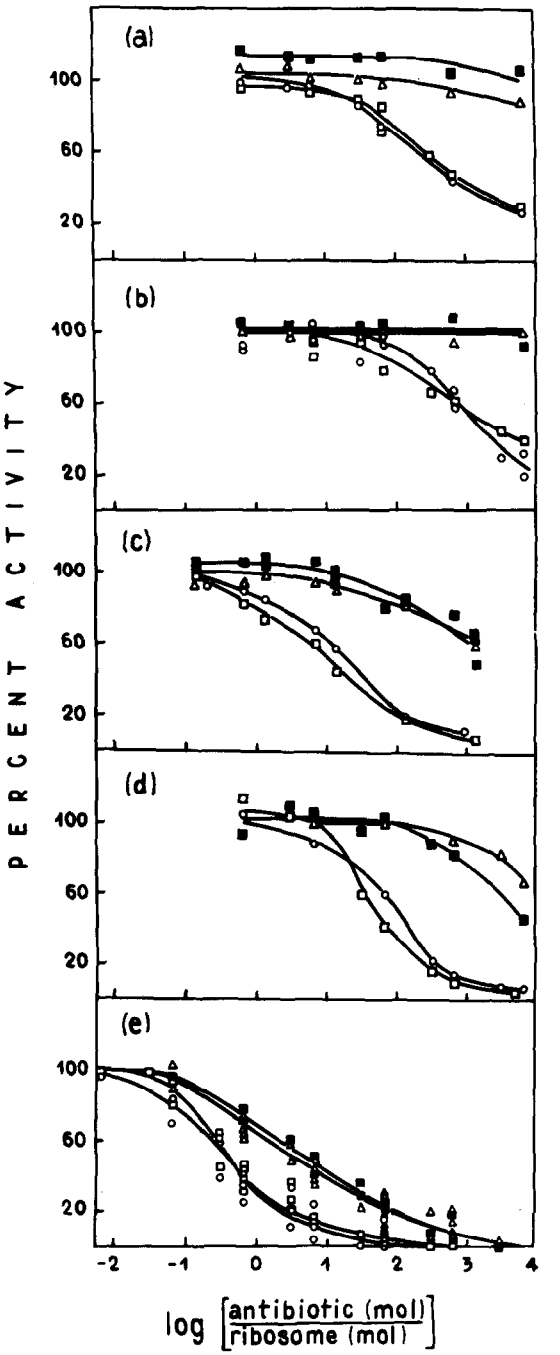

Fig.1. Dependence of inhibition of the poly(U)-directed factor-free (○), the EF-T_u-promoted (□), the EF-G-promoted (△), and the (EF-T_u + EF-G)-promoted (■), translation systems on the amount of inhibitor per ribosome. Ribosome concentration 1.5 × 10^{-7} M. (a) chloramphenicol; (b) lincomycin; (c) amicetin; (d) gougerotin; (e) griseoviridin.

Table 1
Effect of ribosomal peptidyl-transferase inhibitors on the poly(U)-directed factor-free and factor-promoted translation systems

Antibiotics	Number of antibiotic molecules per ribosome resulting in 50% inhibition of the system:			
	Factor-free	EF-T_u-promoted	EF-G-promoted	(EF-T_u + EF-G)-promoted
Chloramphenicol	≈ 300	≈ 300	>> 10 000	>> 10 000
Lincomycin	≈ 1000	≈ 1000	>> 10 000	>> 10 000
Amicetin	10–20	10–20	> 1000	> 1000
Gougerotin	100	50	10 000	5000
Griseoviridin	0.5	0.5	5	5

The explanation could be a standard one: EF-G interacts with the ribosome, and in some way interferes with the binding sites of peptidyl-transferase inhibitors. Such an explanation, however, seems unlikely for the following reasons. (1) EF-G in the process of elongation must each time leave the ribosome before or during aminoacyl-tRNA binding [4–10], i.e. in any case prior to the transpeptidation reaction which is affected by the inhibitors. (2) EF-T_u, which interacts with the ribosome site common or overlapping with the interaction site of EF-G (see the literature cited above and also review [11]), in no cases counteracts the peptidyl-transferase inhibitors (fig.1 and table 1). (3) Ribosomal proteins involved in the binding and functioning of the elongation factors are different from those participating in the peptidyl-transferase center (see review [11]). (4) The tested antibiotics differ by their sites of binding with the peptidyl-transferase center of the ribosome [3], but nonetheless they all are antagonized by EF-G with GTP.

According to modern concepts, EF-G with GTP in the process of elongation comes into operation after the transpeptidation reaction (see, for example, review [12]). After formation of the oncoming peptide bond, the ribosome becomes competent for the binding of EF-G with GTP; the bound EF-G with GTP promotes the translocation of newly-formed peptidyl-tRNA conjugated with the shifting of mRNA and the release of deacylated tRNA. In what way can the promotion of translocation affect a preceding event such as transpeptidation?

An explanation can be the following. The reaction of transpeptidation in the ribosome is essentially reversible. This means that peptidyl-tRNA in the pre-translocation state is not yet the final product, but must be considered rather as a high-energy intermediate (e.g., owing to the conformationally-tense state of the pre-translocation ribosome complex):

$$\text{Peptidyl(n)-tRNA}'_{post} + \text{Aminoacyl-tRNA}''_{bound}$$

$$\xrightarrow[\text{transpeptidation}]{} \text{Peptidyl(n+1)-tRNA}''_{pre} + \text{tRNA}'_{bound}$$

$$\xrightarrow[\text{translocation}]{} \text{Peptidyl(n+1)-tRNA}''_{post} + \text{tRNA}'_{released}.$$

(where $_{post}$ is the post-translocation state and $_{pre}$ is the pre-translocation state of peptidyl-tRNA in the ribosome). The reversibility of the transpeptidation reaction in the ribosome has been already suggested earlier to explain the stimulating effect of EF-G with GTP on miscoding [2]. If transpeptidation, until translocation takes place, is reversible, then the antibiotics which are competitive inhibitors of substrate binding with the peptidyl-transferase center must interfere with the direct reaction, but the promotion of translocation by EF-G with GTP will draw the products of the reaction away from the equilibrium and thus shift the reaction to the right, i.e. will counteract the antibiotics.

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