COLICIN E3 TREATMENT RENDERS RIBOSOMES MORE RESISTANT TO STREPTOMYCIN AND REDUCES MISCODING

Gernot SANDER

Laboratoire de Biochimie (Laboratoire associé no. 240 du CNRS), Ecole Polytechnique, 91128 Palaiseau Cédex, France

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1. Introduction

The aminoglycoside antibiotic streptomycin is known to inhibit moderately the poly(U)-dependent poly(phenylalanine) synthesis [1] and binding of aa-tRNA to ribosomes [2]. This is accompanied by a strong increase in the level of misreading of the genetic code both in vitro [1] and in vivo [3]. A certain similarity between the action of the antibiotic and that of the bacteriocin, colicin E3, has been noted [4]. Moreover, streptomycin inhibits the nucleolytic action of the bacteriocin [5]. Colicin E3 treatment does not however increase misreading with poly(U) as message [6]. In [7], I have shown that the cleavage in situ of the 16 S RNA by the colicin leads to a strong decrease in A-site-specific enzymatic binding of aa-tRNA to the ribosome, and to an equally pronounced increase of the concomitant EF-Tu-dependent GTPase activity. The A-site being the decoding site on the ribosome, it was of interest to check for possible functional relations between actions of streptomycin and colicin E3. The results with the poly(U)-dependent poly(phenylalanine) synthesis system, reported here, show that streptomycin leads to a partial phenotypic repair of the damage caused by colicin E3. Inversely, colicin E3 treatment markedly enhances polypeptide synthesis in the presence of the antibiotic and reduces concomitant miscoding.

2. Materials and methods

Ribosomes, 70 S (either standard 0.5 M NH_4Cl-washed [8] or 'tight couples' [9]) 20 pmol, were incubated for 30 min at 30°C in 15 μl 20 mM Tris–HCl (pH 7.8), 50 mM NH_4Cl and 10 mM $MgCl_2$, with or without 100 pmol colicin E3, an amount sufficient to cleave virtually all the 16 S RNA, as checked by polyacrylamide gel electrophoresis (data not shown, see also [10]). The preincubation mixtures were cooled to 0°C, and the other components were added to yield, in 75 μl final reaction mixtures, 80 mM Tris–HCl (pH 7.8), 33 μM or 200 mM KCl plus 10 mM NH_4Cl, 15 mM 2-mercaptoethanol, the $[MgCl_2]$ indicated in the figures and: 0.5 mM GTP, 0.5 mM ATP, 2.7 mM phosphoenolpyruvate (K^+ salts), 1 μg pyruvate kinase, 3 μg poly(U) (all from Boehringer), 5 μl postribosomal supernatant, 3 A_{260} units total tRNA (Schwarz), 150 pmol [^{3}H]phenylalanine (180 cpm/pmol) and 100 pmol [^{14}C]isoleucine (361 cpm/pmol; the latter two from the Centre d'Energie Atomique, Saclay). Streptomycin was 5×10^{-5} M when present. After incubation at 30°C, incorporation of radioactivity into hot trichloroacetic acid-insoluble material was measured on 20 μl aliquots as in [7]. For each $[Mg^{2+}]$ and $[K^+]$, the time course of incorporation was measured. The incubation time in the reported experiments was within the linear range of the incorporation kinetics, both for phenylalanine and isoleucine, in all the ionic conditions. The colicin E3 present in the reaction mixtures interfered neither with incorporation of amino acids, nor with the effect of streptomycin, since identical results were obtained with ribosomes isolated from colicin E3-treated cells and washed free of the bacteriocin (not shown; see also [7,11]).

3. Results

3.1. *Incorporation of phenylalanine*

Figure 1 shows the poly(U)-dependent incorporation of phenylalanine as a function of $[Mg^{2+}]$ at 33 mM K^+ and 10 mM NH_4^+. The control without colicin E3 treatment (panel A) shows that streptomycin caused not only an overall inhibition but also a shift of the Mg^{2+} optimum to a higher value [12]; accordingly, the inhibition ranges from 80% at 5 mM Mg^{2+} to 50% at 20 mM Mg^{2+}. This resembles the effect of colicin E3 (panel B, lower curve, and [7]). Treatment of the ribosomes with the bacteriocin reduced their sensitivity towards streptomycin (compare panels A and B, filled symbols); in fact, the antibiotic restored the synthetic activity of the treated particles to near-normal levels in the upper range of $[Mg^{2+}]$.

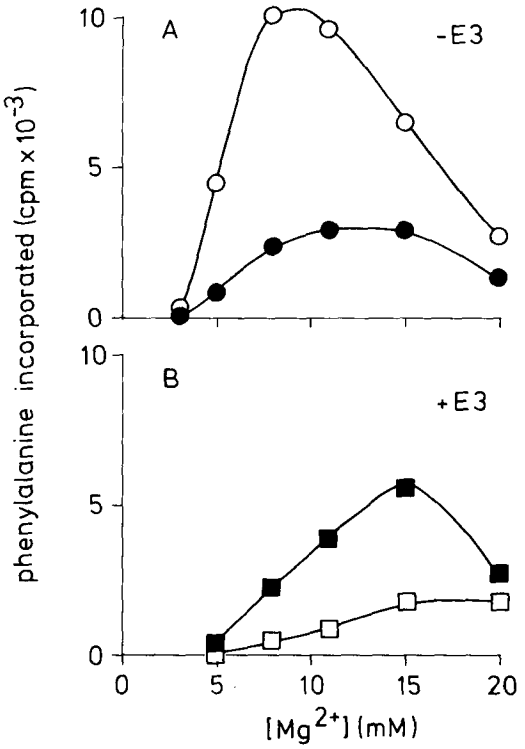

Fig.1. Poly(U)-dependent incorporation of phenylalanine into polypeptides: effect of streptomycin on control and colicin E3-treated ribosomes at 33 mM K^+. Incubation was for 25 min at 30°C. Other conditions as detailed in section 2. Open symbols: without streptomycin; filled symbols: with 50 μM streptomycin.

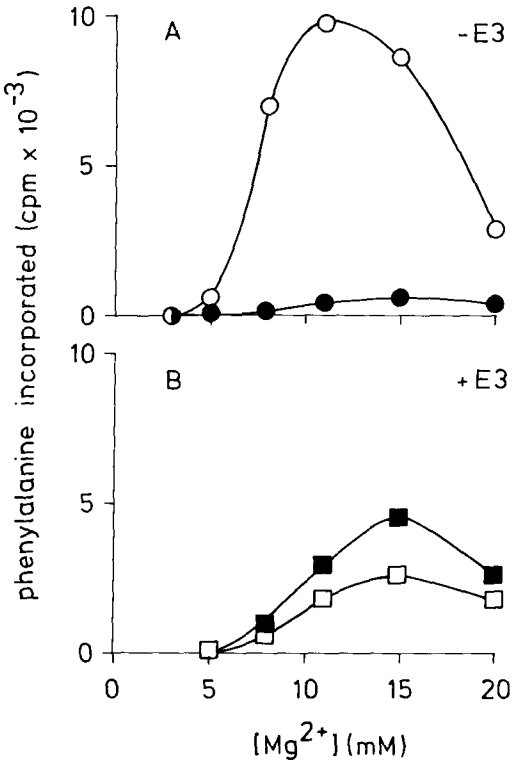

Fig.2. Poly(U)-dependent incorporation of phenylalanine into polypeptides: effect of streptomycin on control and colicin E3-treated ribosomes at 200 mM K^+. Other conditions were the same as in fig.1. Symbols, see fig.1.

Increasing K^+ to 200 mM (fig.2) caused a much stronger inhibition by streptomycin at all $[Mg^{2+}]$ concentrations tested, as well as a higher resistance of the colicin-treated ribosomes towards the antibiotic. As in fig.1, the combined action of colicin E3 and streptomycin increased poly(phenylalanine) synthesis well above the level found with either the bacteriocin or the antibiotic. The results shown were obtained with 'tight' 70 S ribosomes [9], but standard NH_4Cl-washed ribosomes [8] showed a very similar response, except for a 20–30% lower synthetic activity.

3.2. *Miscoding for isoleucine*

Poly(U)-dependent isoleucine incorporation induced by streptomycin was measured simultaneously with that of phenylalanine. Figure 3 shows the results at 33 mM K^+ (panel A) and 200 mM K^+ (panel B).

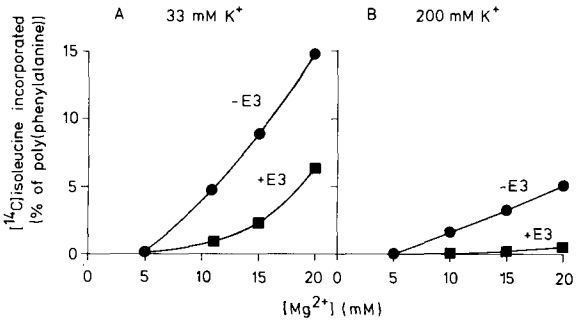

Fig.3. Streptomycin-induced misreading of isoleucine for phenylalanine at 33 mM and 200 mM K^+: effect of colicin E3. (A) 33 mM K^+; (B) 200 mM K^+. Values are expressed as % phenylalanine incorporation.

Misreading increases with rising $[Mg^{2+}]$ (see [1]) and decreases with higher $[K^+]$. In reaction mixtures containing 33 mM K^+, pretreatment of the ribosomes with colicin E3 reduced the level of misreading by 60–80%, depending on the $[Mg^{2+}]$. At 200 mM K^+ this effect became much stronger, streptomycin being virtually unable to induce miscoding at any of the $[Mg^{2+}]$ tested (fig.3).

4. Discussion

Streptomycin apparently binds to a site on the 30 S ribosomal subunit dependent on the protein S12 and influenced by proteins S4 and S5 [13,14]. Colicin E3 also very specifically affects the small ribosomal subunit, by cleaving the 16 S ribosomal RNA at about 50 nucleotides from the 3′-OH end but leaving the resulting fragment in its place (reviewed in [15]). I have previously proposed that a resulting 'wobble' of the codon–anticodon interaction may be responsible for both the decreased enzymatic binding of aa-tRNA to the ribosome and the concomitant increase of the associated GTP hydrolysis [7], an effect that is counteracted by high $[Mg^{2+}]$. Streptomycin also increases the Mg^{2+} optimum. Both Mg^{2+} and streptomycin might conceivably stabilize the colicin E3-treated ribosomes in a conformation favoring a productive alignment of the ternary complex EF-Tu·GTP·aa-tRNA on the damaged particles. Alternatively, streptomycin or Mg^{2+} may protect the ribosomes from the partial damage that occurs during each round of polypeptide synthesis following treatment with the bacteriocin [16], a process that eventually leads to total inactivation [16].

This work shows that the precision of the reading of the genetic message increases after treatment of the ribosomes with colicin E3. This holds even in the absence of streptomycin (data not shown). With streptomycin, this phenomenon is accompanied by an increase of polypeptide synthesis, indicating that the reduced miscoding is not related to irreversible inactivation of the treated ribosomes during chain elongation [16].

Recently, it has been found that, in conditions where the translocation reaction is slowed down, the accuracy of the mRNA–aa-tRNA interaction is enhanced, a possible explanation being that the mRNA·ribosome complex then has more time to select the proper aa-tRNA [17]. The present results show that colicin E3 treatment plus streptomycin can cause near-normal poly(phenylalanine) synthesis at high $[Mg^{2+}]$, accompanied by reduced miscoding, so that a different mechanism should be responsible in this case.

It should be noted that at 200 mM K^+ and 10 mM NH_4^+, i.e., in conditions close to the intracellular levels [15], the inhibitory action of streptomycin was much increased. In fact, some of the discrepancies in the literature concerning streptomycin action on in vitro polypeptide synthesis (see, e.g., [1,12,17]) may be due to the use of different ionic conditions. Another effect of the higher $[K^+]$ was its very considerable reduction of both the spontaneous misreading of isoleucine for phenylalanine (below the level of detection; not shown) and of the streptomycin-induced miscoding (see fig.3, upper curves of panels A and B). The intracellular ionic conditions may therefore be essential to maintain the observed low level of misreading in vivo.

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