PEPTIDYL PUROMYCIN SYNTHESIS; EFFECT OF SEVERAL ANTIBIOTICS WHICH ACT ON 50 S RIBOSOMAL SUBUNITS

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Received 5 December 1970

The effects of several antibiotics which are known to bind with 50 S ribosomal subunits, on the formation of several di- and tri-peptidyl puromycins have been examined. Tylosin and spiramycin inhibited the formation of phenylalanyl-^{14}C-phenylalanyl-puromycin, glycyl-^{14}C-phenylalanyl-puromycin, leucyl-^{14}C-phenylalanyl-puromycin, N^{ϵ}-carbobenzoxylysyl-^{14}C-phenylalanyl-puromycin, and valyl-glycyl-^{14}C-phenylalanyl-puromycin as well as *N*-acetyl-^{14}C-phenylalanyl-puromycin. Of these compounds, erythromycin and oleandomycin selectively inhibited the formation of phenylalanyl-^{14}C-phenylalanyl-puromycin. Although chloramphenicol and lincomycin inhibited the formation of most of these peptidyl puromycins, the formation of phenylalanyl-^{14}C-phenylalanyl-puromycin and leucyl-^{14}C-phenylalanyl-puromycin was found to be resistant to these antibiotics. So far, no significant effect of siomycin has been observed on peptidyl puromycin formation in the absence of G factor.

1. Introduction

It has been reported that puromycin reacts on ribosomes with polylysyl-tRNA [1, 2], polyphenylalanyl-tRNA [3], formylmethionyl-tRNA [4, 5], and *N*-acetyl-phenylalanyl-tRNA [6, 7] in the absence of additional supernatant factors. These puromycin reactions have been studied as useful analogues of peptide bond formation. Recently a number of di- and tri-peptidyl tRNAs were chemically synthesized and their reaction with puromycin was studied [8].

This paper reports an investigation of the effect of several antibiotics, which have been shown to bind with 50 S ribosomal subunits, on the formation of peptidyl puromycin by *Escherichia coli* ribosomes.

2. Materials and methods

Ribosomes were prepared from *E. coli* Q13 and washed with an NH_4Cl solution according to Pestka [9]. *N*-Acetyl-^{14}C-phenylalanyl-tRNA was prepared by the method of Lapidot et al. [10]. Peptidyl-tRNAs were prepared by condensation of *N*-hydroxy-succinimide esters of *o*-nitrophenylsulfenyl amino acids or peptide with ^{14}C-phenylalanyl-tRNA followed removal of the *N*-blocking group from the resulting *o*-nitrophenylsulfenyl-peptidyl tRNA according to Lapidot et al. [11]. Peptidyl puromycin formation was assayed by measuring the puromycin dependent release of peptide from peptidyl tRNA [8, 12]. The spontaneous release of peptides from each peptidyl tRNA was corrected for, using the value obtained in the absence of puromycin. Siomycin used in this study was a water soluble derivative (monothiomalic acid–siomycin A) [13] which was kindly supplied by Dr. Ebata and his collaborators in this laboratory.

3. Results and discussion

As shown in table 1, at quite low concentrations tylosin and spiramycin strongly inhibited all of the peptidyl puromycin reactions examined. Erythromycin and oleandomycin, which are also classified as macrolide antibiotics, as are tylosin and spiramycin, selectively inhibited phenylalanyl-^{14}C-phenylalanyl-puromycin synthesis, the other peptidyl puromycin syntheses being resistant to these two antibiotics. Since chloramphenicol and lincomycin have been considered to be inhibitors of the peptidyl transfer reaction [14, 15], it is interesting to note that compared

Table 1
Effect of antibiotics on peptidyl puromycin synthesis.

Peptidyl-PM	Antibiotics (μM)						
	Ery (80)	Ole (80)	Tyl (5)	Spi (5)	CAP (500)	Lin (800)	Sio (50)
N-Ac-^{14}C-Phe-PM	106%	112%	16%	15%	0%	9%	113%
Gly-^{14}C-Phe-PM	102	123	37	43	2	24	126
Phe-^{14}C-Phe-PM	22	23	19	34	77	86	99
Leu-^{14}C-Phe-PM	103	99	33	38	79	92	109
N^{ϵ}-Cbz-Lys-^{14}C-Phe-PM	92	93	13	41	39	67	130
Val-Gly-^{14}C-Phe-PM	117	111	28	25	22	65	102

The reaction mixture contained the following components in a final volume of 125 μl: 50 mM tris-HCl (pH 7.8), 16 mM magnesium acetate, 3.8 A_{260} units of ribosomes, approximately one A_{260} unit of peptidyl-tRNA (ca. 12,000 cpm), 50 μg of polyuridylic acid, 0.4 mM puromycin and 80 mM KCl. Incubation was carried out at 37° for 15 min. After incubation, 50 μl aliquots were applied to filter paper discs and cold trichloroacetic acid precipitable unreacted peptidyl tRNA was assayed [12].

The values obtained in the absence of antibiotics were taken as 100%. Ery: erythromycin, Ole: oleandomycin, Tyl: tylosin, Spi: spiramycin, CAP: chloramphenicol, Lin: lincomycin, Sio: siomycin, PM: puromycin, *N*-Ac-^{14}C-Phe-PM: *N*-acetyl-^{14}C-Phe-PM.

Table 2
Effect of oleandomycin and siomycin on *N*-acetyl-^{14}C-phenylalanyl-puromycin synthesis.

	N-Acetyl-^{14}C-phenylalanyl-puromycin formed (as cpm) in the presence of KCl (mM)				NH_4Cl (mM)
	10	20	40	80	160
Exp. 1					
Control	0	563	1085		1898
Oleandomycin (160 μM)	480	1093	1819		2071
Exp. 2					
Control	0	321	1165	1806	1970
Siomycin (50 μM)	5	394	1253	1996	2105

Reaction conditions were the same as described in the legend to table 1 except that the concentration of KCl or NH_4Cl was changed as cited.

with the other peptidyl puromycins, the formations of phenylalanyl-^{14}C-phenylalanyl-puromycin and leucyl-^{14}C-phenylalanyl-puromycin were fairly resistant to both these antibiotics. Siomycin, which acts on 50 S ribosomal subunits [16], showed no remarkable effect on peptidyl puromycin formation in the absence of G factor.

We have previously found that erythromycin does not inhibit *N*-acetyl-^{14}C-phenylalanyl-puromycin formation, and in fact stimulates it at low concentration of K^+ or NH_4^+ where the puromycin reaction is normally hardly observed [17]. A similar effect was observed with oleandomycin but not with siomycin, although it also does not inhibit *N*-acetyl-^{14}C-phenylalanyl-puromycin formation at sufficient concentration of K^+ or NH_4^+ (table 2).

In this study, the compositions of the puromycin reaction mixtures were all the same, except for the different peptidyl tRNAs employed. Further, all the peptidyl tRNAs were derived from ^{14}C-phenylalanyl-tRNA, thus the differences in the sensitivity of the puromycin reaction to the antibiotics among these peptidyl tRNAs should be mainly due to differences in the interaction between the 50 S ribosomal subunit site and the peptidyl end of the peptidyl tRNAs. As suggested by de Groot et al. [8], the affinity of peptidyl tRNA to ribosomes may differ with the amino acid composition and chain length of the peptide attached to the tRNA. It has also to be noted that antibiotics examined in this study may be classified into following four groups according to their effect on peptidyl puromycin formation: 1) erythromycin and oleandomycin; 2) tylosin and spiramycin, 3) chloramphenicol and lincomycin, 4) siomycin. This classification is also supported by the previous findings that erythromycin, oleandomycin, chloramphenicol and

lincomycin inhibit polyadenylic acid dependent lysine incorporation to a much higher degree than polyuridylic acid dependent polyphenylalanine synthesis [18] and that the binding of ^{14}C-erythromycin to ribosomes is inhibited by the addition of an excess of oleandomycin, tylosin or spiramycin [19] but not by that of chloramphenicol [20], lincomycin [21], or siomycin (unpublished data), and that spiramycin, tylosin [22] and siomycin [23] strongly inhibit polyphenylalanine synthesis.

Considering these findings together, we may safely conclude that the binding of the respective antibiotics to ribosomes induces characteristic alterations in the conformation of the ribosome and consequent specific changes in the biological activity of the ribosome.

Acknowledgements

The authors wish to thank Dr. K.Inouye and Mr. M.Shin of this laboratory for their kind help in preparing the peptidyl tRNAs.

References

[1] M.E.Gottesman, J. Biol. Chem. 242 (1967) 5564.
[2] I.Rychlik, Biochim. Biophys. Acta 114 (1966) 425.
[3] B.E.H.Maden, R.R.Traut and R.E.Monro, J. Mol. Biol. 35 (1968) 333.
[4] M.S.Bretscher and K.A.Marcker, Nature 211 (1966) 380.
[5] A.Zamir, P.Leder and D.Elson, Proc. Natl. Acad. Sci. U.S. 56 (1966) 1794.
[6] J.Lucas-Lenard and F.Lipmann, Proc. Natl. Acad. Sci. U.S. 57 (1967) 1050.
[7] H.Weissbach, B.Redfield and N.Brot, Arch. Biochem. Biophys. 127 (1968) 705.
[8] N.de Groot, A.Panet and Y.Lapidot, European J. Biochem. 15 (1970) 215.
[9] S.Pestka, J. Biol. Chem. 243 (1968) 2810.
[10] Y.Lapidot, N.de Groot and I.Fry-Shafrir, Biochim. Biophys. Acta 145 (1967) 292.
[11] Y.Lapidot, D.Elat, S.Rappoport and N.de Groot, Biochem. Biophys. Res. Commun. 38 (1970) 559.
[12] S.Nishimura and G.D.Novelli, Biochim. Biophys. Acta, 80 (1964) 574.
[13] M.Ebata, K.Miyazaki and H.Otsuka, J. Antibiotics 22 (1969) 451.
[14] R.E.Monro and D.Vazquez, J. Mol. Biol. 28 (1967) 161.
[15] K.Igarashi, H.Ishitsuka and A.Kaji, Biochem. Biophys. Res. Commun. 37 (1969) 499.
[16] K.Tanaka, S.Watanabe, H.Teraoka and M.Tamaki, Biochem. Biophys. Res. Commun. 39 (1970) 1189.
[17] H.Teraoka and K.Tanaka, submitted for publication.
[18] D.Vazquez, Biochim. Biophys. Acta 114 (1966) 289.
[19] K.Tanaka, H.Teraoka and M.Tamaki, submitted for publication.
[20] K.Tanaka, H.Teraoka, T.Nagira and M.Tamaki, Biochim. Biophys. Acta 123 (1966) 435.
[21] H.Teraoka, K.Tanaka and M.Tamaki, Biochim. Biophys. Acta 174 (1969) 776.
[22] J.C.-H.Mao and R.G.Wiegand, Biochim. Biophys. Acta 157 (1968) 404.
[23] K.Tanaka, S.Watanabe and M.Tamaki, J. Antibiotics 23 (1970) 13.