COMPARISON OF THE MECHANISM OF ACTION OF CYCLIC 11,12-ERYTHROMYCIN A CARBONATE AND ERYTHROMYCIN A *

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Synthesis of polyphenylalanine and polylysine in the E. coli MRE 600 and Q13 cell-free systems was inhibited by erythromycin A and cyclic 11,12-erythromycin A carbonate to a similar or identical extent. Both compounds inhibited translation of phage f2 RNA in the E. coli Q13 cell-free system. Neither antibiotic affected binding of initiator tRNA or phage f2 RNA to E. coli ribosomes, and neither inhibited translation of BMV RNA in the wheat-germ cell-free system.

Erythromycin, an antibiotic produced by Streptomyces erythreus, is a macrolide antibiotic. It inhibits growth of gram-positive and some gram-negative bacteria by blocking polypeptide synthesis (Brock & Brock, 1959; Taubman et al., 1963; Wolfe & Hahn, 1964). The site of its action is the 50-S ribosomal subunit with which, in the presence of K⁺ or NH₄⁺, erythromycin forms a dissociable complex (Mao & Putterman, 1969; Oleinick & Corcoran, 1969; Taubman et al., 1966; Wilhelm & Corcoran, 1967). The protein L-4 responsible for binding of erythromycin was isolated from the 50-S subunit of E. coli Q13 ribosomes (Otaka et al., 1970; Tanaka et al., 1968; Wittmann et al., 1973). This protein, located in direct proximity to the active site "P" of the 50-S subunit, stimulates the action of another protein, L-27, indispensable for binding of peptidyl-tRNA to ribosomes (Pongs et al., 1974). Thus erythromycin A by affecting the transpeptidation-translocation reaction, renders impossible elongation of the polypeptide.

The aim of these studies was to compare the mechanism of action of erythromycin A and its synthetic derivative, cyclic 11,12-erythromycin A.

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carbonate (Bojarska-Dahlig & Slawiński, 1972; Slawiński et al., 1975). The synthetic product is about twice as active as the original antibiotic against *Bacillus pumilus* (NCTC 8241) test strain with reduced at the same time the LD$_{50}$ value (Korzybski et al., 1977). According to Pestka (1971), the activity of macrolide antibiotics increases with the size of their lactone ring. It was, therefore, possible that cyclic 11,12-erythromycin A carbonate, having an additional ring in the position 11,12 of its lactone moiety (Scheme 1), is more active than erythromycin A.

![Erythromycin A](image)

**Scheme 1**

Experiments were performed in the cell-free systems from *Escherichia coli* Q13, MRE 600 and wheat germ. Synthetic polynucleotides – polyuridylic and polyadenylic acids – and natural mRNA’s isolated from phage f2 and BMV (bromegrass mosaic virus) were used as templates.

**MATERIAL AND METHODS**

Erythromycin A (ERY-A) and cyclic 11,12-erythromycin A carbonate (ERY-AC) were from Tarchomińskie Zakłady Farmaceutyczne (Warszawa, Poland). Bromegrass mosaic virus RNA (BMV RNA) and transfer RNA from wheat germ were a kind gift of Dr P. KAESBERG (The University of Wisconsin, U.S.A.). Wheat germ was from General Mills (Minnesota, U.S.A.).

*Preparation of cell-free extracts.* Ribosomes from *E. coli* MRE 600 and Q13 were prepared according to Nirenberg (1963), and the 30 000 g (S-30) supernatant, by the method of Zagórski et al. (1972). The 23 000 g supernatant from wheat germ was obtained according to Zagórski (1978).
Phage f2 RNA. Growth of phage f2, labelling and isolation of f2 RNA were performed as described by Zagórski et al. (1972). [3H]N-Formylmethionyl-tRNA ([3H]Met-tRNA) was synthesized by the method of Lelong et al. (1970).

Translation of polyuridylic acid [poly(U)]. The incubation mixture contained in a total volume of 125 μl: 2 mM-adenosine triphosphate (ATP), 0.5 mM-guanosine triphosphate (GTP), 10 mM-phosphoenolpyruvate (PEP), 80 mM-Tris/HCl buffer, pH 7.8, 14 mM-magnesium acetate, 30 mM-KCl, 6 mM-2-mercaptoethanol, 2.5 μg PEP kinase, 50 μg E. coli tRNA, 20 μg poly(U), 25 nmol of [14C]phenylalanine (spec. act. 10 Ci/mol), unlabelled amino acids (25 nmol each), and 1 mg of E. coli S-30 protein. After 45 min at 37°C, the acid-insoluble material was washed using the filter-paper technique of Mans & Novelli (1961). Radioactivity was measured in Packard-TriCarb liquid scintillation counter.

Translation of polyadenylic acid [poly(A)]. The incubation mixture contained in a total volume of 125 μl: 3 mM-ATP, 0.3 mM-GTP, 10 mM-PEP, 50 mM-Tris/HCl buffer, pH 7.8, 10 mM-magnesium acetate, 2.5 μg PEP kinase, 25 μg E. coli tRNA, 25 μg poly(A), 25 nmol of [14C]lysine (spec. act. 20 Ci/mol), unlabelled amino acids (25 nmol each), 250 μg of E. coli Q13 70-S ribosomes, and 300-500 μg of E. coli Q13 S-150 protein. After 45 min at 37°C, the mixture was pipetted onto filter-paper discs to stop the reaction. The discs were washed with 5% trichloroacetic acid in 0.25% sodium tungstate, and radioactivity was measured in the liquid scintillation counter.

Translation of phage f2 RNA. The incubation mixture contained in a total volume of 125 μl: 5 mM-ATP, 0.2 mM-GTP, 10 mM-PEP, 60 mM-Tris/HCl buffer, pH 7.8, 12.6 mM-magnesium acetate, 30 mM-KCl, 40 mM-NH4Cl, 4 mM-2-mercaptoethanol, 1.25 μg PEP kinase, 75 μg phage f2 RNA, 3 nmol of [14C]leucine (spec. act. 59 Ci/mol), unlabelled amino acids (12.5 nmol each), and 375 μg of E. coli Q13 S-30 protein. The reaction was carried out for 45 min at 37°C. The protein was washed according to Mans & Novelli (1961), and the radioactivity was measured in the liquid scintillation counter.

Translation of BMV RNA. The incubation mixture contained in a total volume of 50 μl: 1 mM-ATP, 0.375 mM-GTP, 12 mM-phosphocreatine kinase, 5 mM-Tris/acetate buffer, pH 7.6, 20 mM-4-(2hydroxyethyl)-1-piperazine ethanesulphonic acid-KOH buffer (HEPES), pH 7.6, 1 mM-magnesium acetate, 35 mM-potassium acetate, 1 mM-dithiothreitol (DTT), 25 μg wheat germ tRNA, 7.5 μg BMV RNA, 10 nmol of [14C]leucine (spec. act. 12 Ci/mol), unlabelled amino acids (10 nmol each), and 60 μg of the S-23 supernatant protein from wheat germ. After 2 h at 37°C, the protein was washed according to Mans & Novelli (1961) and the radioactivity was measured in the liquid scintillation counter.

Phage f2 RNA directed binding of [3H]Met-tRNA to E. coli 70-S ribosomes. The incubation mixture contained in a total volume of 50 μl:
50 mM-Tris/HCl buffer, pH 7.2, 100 mM-NH₄Cl, 5 mM-magnesium acetate, 1 mM-DTT, 1 mM-GTP, 40 pmol of 70-S ribosomes washed previously with 1 mM-NH₄Cl, 40 pmol of phage f2 RNA, 720 pmol [³H]Met-tRNA, and 30 µg protein of crude initiation factors from E. coli. The mixture was incubated for 20 min at 37 °C, chilled, diluted with cold 50 mM-Tris/HCl buffer, pH 7.5, supplemented with 160 mM-NH₄Cl and 12 mM-magnesium acetate, and passed through Millipore filter. After three successive washings with the same buffer, the filters were dried and the radioactivity was measured in the liquid scintillation counter.

Binding of phage f2 [¹⁴C]RNA to E. coli 70-S ribosomes. For this experiment, in the above-described incubation mixture, [³H]Met-tRNA and phage f2 RNA were replaced by 35 pmol of phage f2 [¹⁴C]RNA. After 20 min at 25 °C, the sample was chilled and diluted with cold 50 mM-Tris/HCl buffer, pH 7.5, supplemented with 160 mM-NH₄Cl and 12 mM-magnesium acetate.

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**Fig. 1.** Effect of erythromycin A and cyclic 11,12-erythromycin A carbonate on A, poly(U)-dependent [¹⁴C]phenylalanine incorporation in the E. coli Q13 S-30 system, and B, poly (A)-dependent [¹⁴C]lysine incorporation in the E. coli Q13 fractionated system. For details see text. ●, ERY-A; ○, ERY-AC.
acetate. For washing of samples and measurement of their radioactivity the same methods were used as in the experiment on binding of $[^{3}H]$Met-tRNA to ribosomes.

RESULTS AND DISCUSSION

According to Wolfe & Hahn (1964), erythromycin inhibits by about 50% the poly(U)-dependent synthesis of polypheynylalanine in the S-30 system from E. coli, and almost completely inhibits the poly(A)-directed synthesis of polylysine in the fractionated systems derived from sensitive strains (Tanaka & Teraoka, 1968). Analogous comparative experiments were performed with erythromycin A and its carbonate derivative. As it can be seen from Fig. 1A, both antibiotics inhibited by 40 - 50% the poly(U)-dependent $[^{14}C]$phenylalanine incorporation into polypeptides, the cyclic derivative being a somewhat more effective inhibitor. The effect of the two antibiotics on the poly(A)-directed synthesis of polylysine was practically the same (Fig. 1B); the degree of inhibition was 82% and 85% for ERY-A and ERY-AC.

Fig. 2. Effect of erythromycin A and cyclic 11,12-erythromycin A carbonate on binding to the E. coli MRE 600 70-S ribosomes of A, phage f2 $^{14}$C-RNA and B, $[^{3}H]$Met-tRNA in the presence of phage f2 RNA. ●, ERY-A; ○, ERY-AC. For details see text.
respectively. The data for ERY-A are in close agreement with the results obtained by others with erythromycin A (Tanaka & Teraoka, 1968) and suggest a similar action of synthetic ERY-AC.

In further experiments we studied the effect of both antibiotics on binding of natural template (phage f2 RNA) and initiator tRNA to E. coli ribosomes (Fig. 2A, B.).

At the concentration which stops polylysine synthesis by more than 80% (about 0.5 nmol/100 pmol of 70-S ribosomes), the antibiotics inhibited but slightly binding of phage f2 RNA to ribosomes (Fig. 2A). Binding of fMet-tRNA to f2 RNA-ribosome complex was somewhat increased in the presence of either antibiotic (Fig. 2B). Similar results with erythromycin A were reported by Pestka (1971) and Taubman et al. (1966).

The effect of the two antibiotics on protein synthesis was studied also in the E. coli S-30 supernatant, stimulated with phage f2 RNA, and in the wheat germ S-23 supernatant containing BMV RNA. The latter was a model system for eukaryotic organisms – insensitive to erythromycin A.

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**Fig. 3.** Effect of erythromycin A and cyclic 11,12-erythromycin A carbonate on incorporation of [14C]leucine: A, in the E. coli Q13 S-30 system in the presence of phage f2 RNA, and B, in the wheat germ S-23 system in the presence of BMV RNA. For details see text.

- ●, ERY-A; ○, ERY-AC.
Both antibiotics inhibited by 79 - 82% polypeptide synthesis directed by phage f2 RNA in bacterial cell-free system, whereas the eukaryotic system was not affected even at high concentration of the antibiotics (Fig. 3A, B).

The results presented indicate that the mechanism of action of erythromycin A and cyclic 11,12-erythromycin A carbonate is very similar or identical.

REFERENCES


PORÓWNANIE MECHANIZMÓW DZIAŁANIA CYKLIczNEGO 11,12-WĘGLANU ERYTROMYCyny A I ERYTROMYCyny A

Streszczenie

Erytromycyna A i cykliczny 11,12-węglan erytromycyny A hamowały w równym lub bardzo zbliżonym stopniu syntezę polisyllazy i polifenylalaniny w bezkomórkowych układach z E. coli Q13 i MRE 600. Oba związki hamowały translocację RNA faga f2 w bezkomórkowym układzie z E. coli Q13. Badane antybiotyki nie wpływały na poziom wiązania inicjatorowego tRNA oraz RNA faga f2 do rybosomów E. coli, nie hamowały też translacji BMV RNA w bezkomórkowym układzie z zarodków pszenicy.

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