RIBOSOMAL BINDING AND ACCUMULATION OF ROKITAMYCIN IN *Bacteroides fragilis*

YOSHINORI MUTO, KAORI BANDOH, YASUNORI TANAKA, KUNITOMO WATANABE and KAZUE UENO

Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Tsukasamachi 40, Gifu 500, Japan

(Received for publication March 8, 1991)

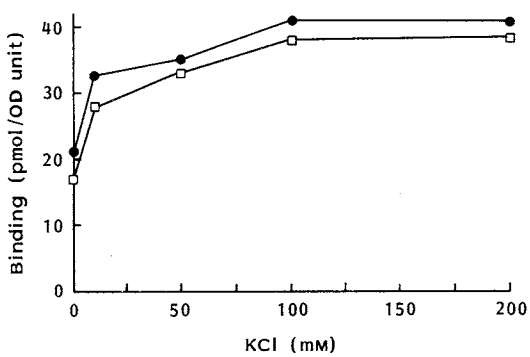
Rokitamycin, a new 16-membered macrolide antibiotic, is a potent inhibitor of bacterial protein synthesis and showed higher antimicrobial activity than other macrolide antibiotics[1]. The detailed study of ribosome-binding activities of the 16-membered macrolides indicated that the affinity of rokitamycin to ribosomes is similar to those of the other compounds[2]. This observation suggests that other factor(s), besides ribosomal affinity, might be responsible for the efficient antimicrobial activity of rokitamycin. Previously, we reported that rokitamycin is accumulated efficiently in *Bacteroides fragilis* cells by means of its hydrophobic property, and the higher permeability of the drug is related to the antimicrobial potency[3]. In this report, in order to validate the importance of drug permeability, we compared the accumulation and ribosomal binding of rokitamycin, using rokitamycin-susceptible *B. fragilis* and resistant *Escherichia coli*.

B. fragilis ATCC 25285 and *E. coli* K-12 (C-600) were grown at 35°C using Gifu Anaerobic Medium (GAM) Broth as described before[3]. MICs were determined by the agar dilution technique[4]. Ribosomes were prepared according to the method of NIRENBERG and MATTHAEI[5] and stored at −80°C. The binding of [^{14}C]rokitamycin to bacterial ribosomes was measured by the adsorption of the [^{14}C]rokitamycin-ribosome complex on Millipore filters (HAWP 0.45 μm), as described by TERAOKA[6]. The uptake of [^{14}C]rokitamycin from the extracellular medium by bacteria was measured by filtering the bacterial suspension through Millipore filter (HAWP 0.45 μm), as described in the previous paper[3]. ^{14}C-Labeled rokitamycin was kindly supplied by Toyo Jozo Co., Ltd., Shizuoka, Japan.

By the agar dilution method, MICs of rokitamycin for susceptible *B. fragilis* ATCC 25285 and resistant *E. coli* K-12 (C-600) were 0.1 and 100 μg/ml, respectively. Since the site of action of macrolides has been thought to be the ribosomal subunits[7], we, first, examined the binding of rokitamycin with both *B. fragilis* and *E. coli* ribosomes. As shown in Fig. 1, the binding of rokitamycin to ribosomes from both species was strongly dependent on the K^+ concentration in the reaction medium. Likewise, others have previously reported a monovalent cation requirement for erythromycin binding to various bacterial species[6,8]. The amount of rokitamycin bound to ribosomes reached a maximum at approximately 100 mM KCl in both *B. fragilis* and *E. coli* (Fig. 1). In addition, the maximal amount of the drug binding was essentially the same in both species. Thus, the ribosomes isolated from resistant *E. coli* behaved comparably to those isolated from susceptible *B. fragilis*. This suggests that ribosomal affinity is not related to the susceptibility to rokitamycin in these organisms. Next we examined the accumulation of rokitamycin in the bacterial suspension. Fig. 2 shows the uptake of [^{14}C]rokitamycin by *B. fragilis* ATCC 25285 and *E. coli* K-12. The profiles of time course of the drug uptake were similar for both bacteria, showing an initial rapid-uptake phase within 10 minutes, followed by a slower release phase. These observations were consistent to the previous results obtained in *B. fragilis* cells[3]. However, as shown in Fig. 2 the level of accumulation determined at 10 minutes for *E. coli* (12 nmol/mg) was several times lower than that for *B. fragilis* (63 nmol/mg). Thus, the observation suggests that the permeability of rokitamycin is decreased in resistant *E. coli*.

Fig. 1. Binding of [^{14}C]rokitamycin to ribosomes from *Bacteroides fragilis* ATCC 25285 and *Escherichia coli* K-12.

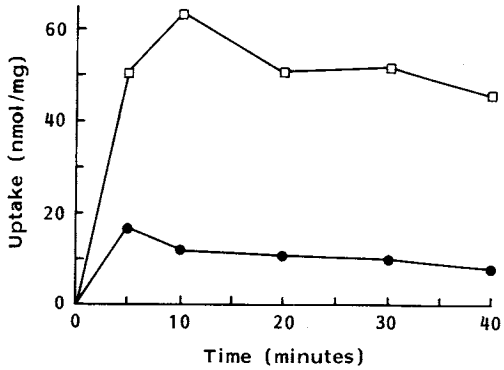
□ *B. fragilis*, ● *E. coli*.



The incubation mixture contained 2.0 OD_{260} units of each ribosome preparation and the indicated amount of KCl.

Fig. 2. Accumulation of rokitamycin by *Bacteroides fragilis* ATCC 25285 and *Escherichia coli* K-12.

□ *B. fragilis*, ● *E. coli*.



Uptake of rokitamycin was measured in an anaerobic glove box using [^{14}C]rokitamycin.

In general, macrolides are intrinsically inactive against Gram-negative bacteria[9]. However, some anaerobic Gram-negative rods such as *B. fragilis* are usually susceptible to lower concentrations of macrolide antibiotics[10]. This is especially true for rokitamycin[11]. In the present study, we indicated that the increased susceptibility to rokitamycin in *B. fragilis* was due to the increased permeability of the drug to the cells, and not due to the change of ribosomal affinity for the drug. Furthermore, the previous results from this laboratory[3] demonstrated that the antimicrobial activity of three macrolides (erythromycin, josamycin, rokitamycin) against *B. fragilis* correlates well with the extent of drug accumulation in the bacteria. These findings strongly suggest that increased permeability of rokitamycin plays an essential role in the antimicrobial action of the drug against *B. fragilis* cells.

References

1) Morohoshi, T.; M. Toriya, S. Yokoiyama, H. Sakakibara, J. Murase & K. Hayano: Bacteriological evaluation of a new macrolide antibiotic, rokitamycin (TMS-19-Q). *In* Recent Advances in Chemotherapy. Antimicrobial Section 2. *Ed.*, J. Ishigami, pp. 1425~1426, University of Tokyo Press, 1985

2) Ōmura, S.; H. Tanaka, J. Inokoshi, H. Sakakibara & T. Fujiwara: Binding of [^{3}H]tetrahydroleucomycin A_3 to *Escherichia coli* ribosomes and the effect of 3″-*O*-acyl derivatives of leucomycins on the binding. J. Antibiotics 35: 491~496, 1982

3) Muto, Y.; K. Bandoh, K. Watanabe, N. Katoh & K. Ueno: Macrolide accumulation by *Bacteroides fragilis* ATCC 25285. Antimicrob. Agents Chemother. 33: 242~244, 1989

4) Kesado, T.; K. Watanabe, Y. Asahi, M. Isono & K. Ueno: Comparative antibacterial activities of 7α-methoxy cephalosporins and 7β-methoxyiminoacetamido cephalosporins against *Bacteroides fragilis*. Antimicrob. Agents Chemother. 25: 131~133, 1984

5) Nirenberg, M. W. & J. H. Matthaei: The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring of synthetic polyribonucleotides. Proc. Natl. Acad. Sci. U.S.A. 47: 1588~1602, 1961

6) Teraoka, H.: A reversible change in the ability of *Escherichia coli* ribosomes to bind to erythromycin. J. Mol. Biol. 48: 511~515, 1970

7) Mao, J.C.-H. & R. G. Wiegand: Mode of action of macrolides. Biochim. Biophys. Acta 157: 404~413, 1968

8) Mao, J.C.-H. & M. Putterman: The intermolecular complex of erythromycin and ribosome. J. Mol. Biol. 44: 347~361, 1969

9) Godfrey, A. J. & L. E. Bryan: Intrinsic resistance and whole cell factors contributing to antibiotic resistance. *In* Antimicrobial Drug Resistance. *Ed.*, L. E. Bryan, pp. 113~145, Academic Press, 1984

10) Quentin, C.; B. de Barbeyrac, N. Dubosc-Marchenay & C. Bébéar: Susceptibility of anaerobic bacteria to eight macrolides and related drugs. *In* Recent Advances in Chemotherapy. Antimicrobial Section 2. *Ed.*, J. Ishigami, pp. 1511~1512, University of Tokyo Press, 1985

11) Watanabe, K.; N. Kato, K. Sawa, M. Aoki & K. Ueno: Antimicrobial activity of TMS-19-Q (3″-propionylleucomycin A_5) against anaerobic bacteria and *Ureaplasma*. Chemotherapy (Tokyo) 32 (Suppl. 6): S54~S59, 1984