NEW POLYENIC ANTIBIOTICS ACTIVE AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

V. MODE OF ACTION OF ENACYLOXIN IIa

TOSHIHIKO WATANABE, TOMOKO SUZUKI and KAZUO IZAKI

The Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai 981, Japan

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During the course of studies on a series of unique polyenic antibiotics produced by *Frateuria* (formerly *Gluconobacter*) sp. W-315, we isolated enacyloxin IIa (ENX IIa); its structure was recently determined as shown in Fig. 1[1,2]. This is the first polyenic antibiotic reported which is linear in structure. Polyenic antibiotics which have lactonized structure are well known to be active against both fungi and yeasts, but not active against bacteria. Although a very few polyenic antibiotics, which are active against bacteria as well as fungi and yeasts, have been reported thus far[3,4], the mode of their action has not been clarified. ENX IIa was active against Gram-positive and Gram-negative bacteria and slightly active against fungi, but inactive against yeasts. Its action on bacteria was found to be bacteriostatic. We are interested in the mode of action of this antibiotic because it is unique in

Fig. 1. Enacyloxin IIa.

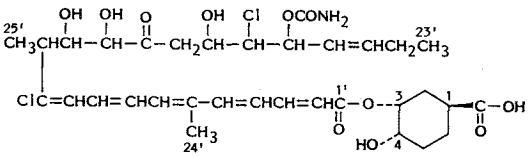
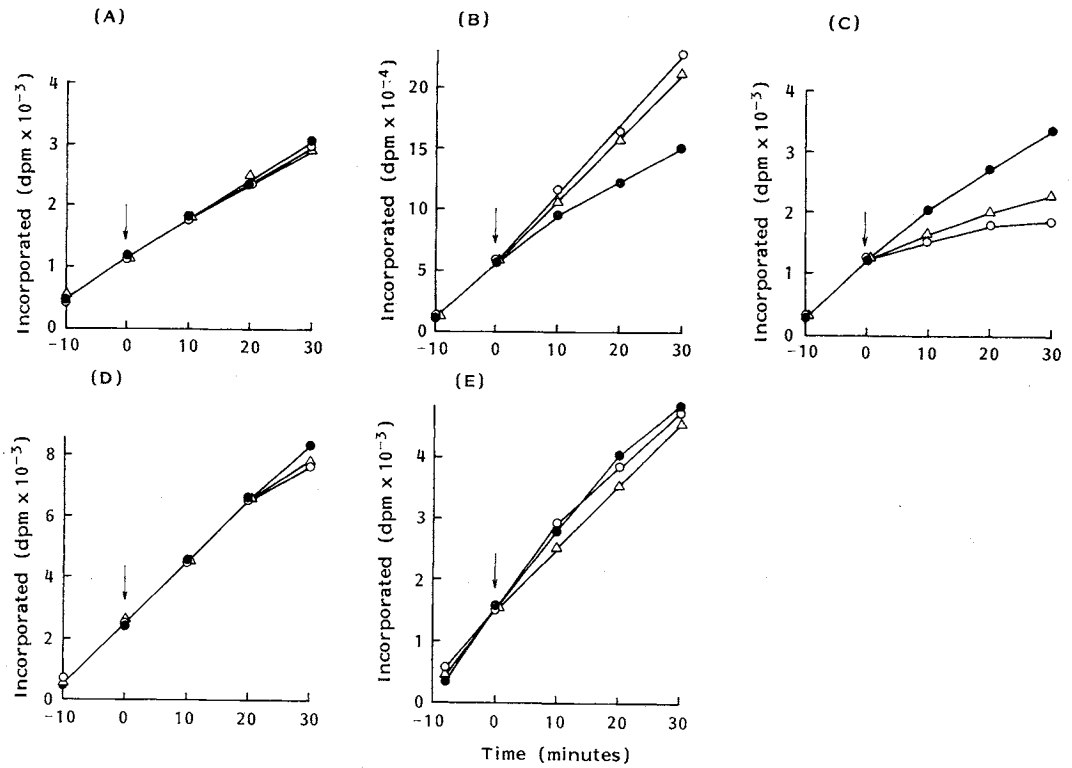


Fig. 2. Effects of enacyloxin IIa on incorporation of biosynthetic precursors into the acid-insoluble fraction of *Escherichia coli*.

(A) [2-^{14}C]Thymidine, (B) [5,6-^{3}H]uridine, (C) L-[*U*-^{14}C]amino acid mixture, (D) [2-^{3}H]glycerol, (E) *meso*-[^{3}H]-2,6-diaminopimelic acid dihydrochloride. *E. coli* W-3110 for (A), *E. coli* RDE 5 for (E) and *E. coli* K-12 for (B), (C) and (D) were used, respectively. Arrows indicate the time of ENX IIa addition. ● None, △ 5 μg/ml, ○ 20 μg/ml.



chemical structure. Cells of *Escherichia coli* K-12, *E. coli* W-3110 (Thy$^-$) or *E. coli* RDE 5 (DAP$^-$) were aerobically grown in 10 ml of nutrient broth in a 30-ml test tube at 30°C and various radioactive precursors were added to the cultures when absorbancy (OD 660 nm) of the cultures reached to 0.1. After 10 minutes, 1% $NaHCO_3$ solution and two different concentrations of ENX IIa dissolved in the same $NaHCO_3$ solution (5 μg or 20 μg at final concentration) were added to each tube as shown in Fig. 2. The incubation was continued for 40 minutes. At time intervals of 10 minutes, 1 ml fractions were withdrawn from the control and the ENX IIa-treated cultures, and their reactions were terminated by addition of 1 ml of 10% TCA. The precipitates formed were collected on a Millipore filter (HAWP; pore size 0.45 μ) and were washed with 5% TCA. To solubilize the precipitates, they were placed with the filter in a glass counting vial and 0.5 ml of 5% sodium dodecyl sulfate was added. The vials were incubated at 37°C overnight. Radioactivity was counted by a liquid scintillation spectrometer (Aloka LSC-903) using the toluene-Nonion system[5]. Radioactivity of the labeled precursor incoporated into the precipitates was corrected from the ratio of the increase of OD of the control culture to that of the ENX IIa-treated culture. As shown in Fig. 2(C), ENX IIa markedly inhibited the incorporation of a mixture of ^{14}C-amino acids (L-[*U*-^{14}C]amino acid, 2.1×10^3 Bq/milliatom carbon) into TCA-insoluble fraction of *E. coli* K-12 depending on the concentration of ENX IIa. This result was confirmed by the experiments using two more labeled amino acid preparations, *i.e.* [*U*-^{14}C]casein hydrolysate (Daiichi Pure Chemicals, Tokyo) and L-[2,3-^{3}H]phenylalanine (1.7×10^6 MBq/mmol) (data not shown). When other labeled compounds such as [2-^{14}C]-thymidine (1.8×10^2 MBq/mmol), [5,6-^{3}H]uridine (1.6×10^6 MBq/mmol), [2-^{3}H]glycerol (5.3×10^3 MBq/mmol) and *meso*-[^{3}H]diaminopimelic acid dihydrochloride (5.6×10^4 MBq/mmol) were used as precursors, no inhibitory action of ENX IIa was observed. It is noteworthy that ENX IIa stimulated uridine incorporation significantly although it was not studied in details. Consistent with the results of the inhibition of amino acid incorporation into the TCA insoluble fraction of the cells, ENX IIa markedly inhibited poly U-dependent incoporation of L-[^{3}H]phenylalanine into the protein using the cell-free S-30 fraction of *E. coli* K-12 as shown in Table 1[6]. From these results, the primary site of action of ENX IIa seems to be protein synthesis.

Table 1. Effect of enacyloxin IIa on polyU-dependent incorporation of [^{3}H]phenylalanine into proteins by S-30.

Components	[^{3}H]Phenylalanine incorporated (dpm/mg protein)	Ratio
Complete	29,853	1.00
Complete − ATP, PEP, pyruvate kinase	136	0
Complete − GTP	13,795	0.46
Complete − poly U	3,934	0.13
Complete + ENX IIa (5 μg/ml)	17,205	0.58
Complete + ENX IIa (20 μg/ml)	5,970	0.20
Complete + chloramphenicol (20 μg/ml)	26,819	0.90

One ml of reaction mixture contains 40 μmol of Tris-HCl (pH 8), 10 μmol of magnesium acetate, 40 μmol of ammonium chloride, 0.2 μmol of GTP, 0.8 μmol of ATP, 4 μmol of PEP, 40 μmol of mercaptoethanol, 0.8 μmol of spermidine, 100 μg of pyruvate kinase, 600 μg of tRNA, 60 μg of poly U, 200 μl of S-30 (2.32 mg of protein), 0.25 μg of L-phenylalanine and 1.8×10^4 Bq of L-[2,3-^{3}H]phenylalanine.

The reaction was carried out at 30°C for 30 minutes and then terminated by TCA. TCA insoluble materials on the filter was counted.

This mode of action is quite different from that of usual lacotonized polyene antibiotics which are considered to break down cell membranes of yeasts and fungi. More detailed mode of action studies of ENX IIa will be reported later.

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