Communications to the editor

MECHANISM OF ACTION OF GLOBOMYCIN

Sir:

As reported previously,[1,2] globomycin, a new cyclic peptide antibiotic, is active against certain Gram-negative bacteria (*e.g. Escherichia coli, Klebsiella pneumoniae* and *Shigella*), but inactive against *Pseudomonas, Proteus*, and Gram-positive bacteria. Formation of spheroplasts was observed when *E. coli* was grown in the presence of globomycin, indicating that it inhibits bacterial cell wall synthesis.

In the present paper we wish to present evidence that the mechanism of action of globomycin involves an inhibition of the prolipoprotein processing enzyme that converts prolipoprotein to lipoprotein in the outer membrane of Gram-negative bacteria. Globomycin does not inhibit macromolecular synthesis (DNA, RNA, protein, peptidoglycan or lipid) and also does not affect the level of ATP in cells of *E. coli* B. However, when we analyzed the synthesis of envelope *vs.* cytoplasmic proteins labeled with [^{14}C]-arginine, after separation by differential centrifugation,[3] we found inhibition by globomycin of synthesis of both cytoplasmic and envelope proteins, markedly the latter (Table 1).

Among the envelope proteins, synthesis of the bound form of lipoprotein, as measured by the method of TANAKA and his coworkers,[4] was

Table 1. Effects of globomycin on the synthesis of envelope protein

GLM conc.	Cytoplasm	Envelope	Bound form lipoprotein
0 μg/ml	11,300 (cpm/mg protein) 100%	10,530 100%	100%
5 μg/ml	10,520 93.1%	9,280 88.1%	44.5%
25 μg/ml	9,660 85.5%	7,360 69.9%	42.3%

Exponentially growing *E. coli* B cells were labeled with 10 μCi of [^{14}C]-arginine in the presence or absence of globomycin (GLM). The labeling of envelope and cytoplasmic proteins was measured after differential centrifugation. The bound form of lipoprotein was measured by the method of TANAKA and his coworkers.[4]

Fig. 1. SDS polyacrylamide gel electrophoresis of the cell envelope fraction from mixed cells of *E. coli* B labeled with [^{3}H]-arginine or [^{14}C]-arginine.

Exponentially growing cells of *E. coli* B were labeled independently in M9 medium with 100 μCi of [^{3}H]-arginine in the presence of 5 μg/ml of globomycin or 10 μCi of [^{14}C]-arginine in the absence of globomycin. After 45 minutes of incubation at 37°C, cells were mixed and envelope fraction was isolated by differential centrifugation. Envelope fraction was solubilized with 10 mM sodium phosphate buffer, pH 7.1, containing 1% SDS, 1% 2-mercaptoethanol and 0.005% bromophenol blue at 70°C for 20 minutes. The solubilized envelope fractions were subjected to 7.5% SDS polyacrylamide gel electrophoresis. Capital letters indicate envelope protein peaks. Cytochrome c arrow indicates the position of cytochrome c (MW=12,000).

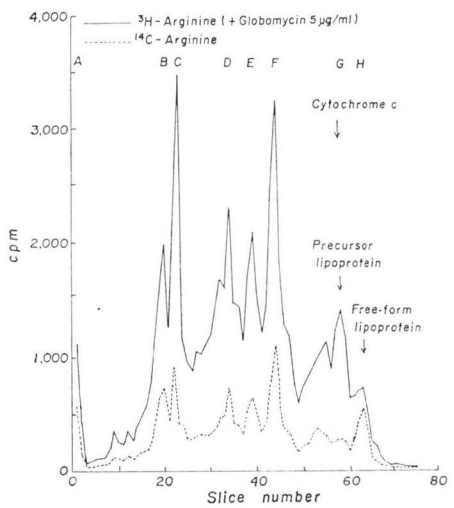


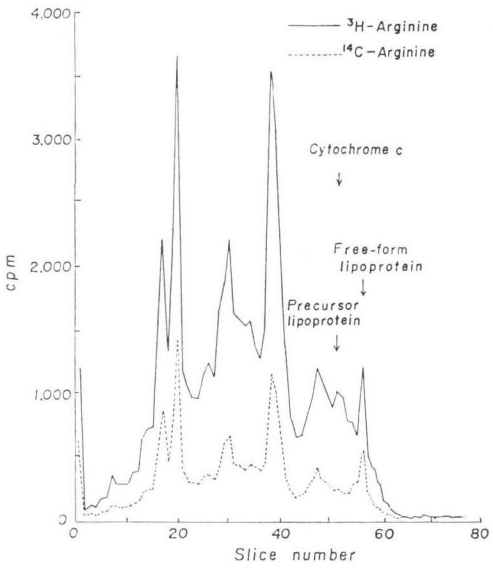
Table 2. Effect of globomycin on the synthesis of lipoprotein

Peak	A (origin)	B	C	D	E	F	G	H
Relative value	0.634	0.880	0.784	1.00	1.05	0.997	1.792	0.425

Relative values of $\frac{[^{3}H]/[^{14}C] \text{ of the peak}}{[^{3}H]/[^{14}C] \text{ of peak D}}$ were calculated from Fig. 1.

Fig. 2. SDS polyacrylamide gel electrophoresis of the cell envelope fraction from the mixed cells of *E. coli* B labeled with [^{3}H]-arginine or [^{14}C]-arginine.

Envelope fraction obtained from Fig. 1 was incubated at 37°C for 4 hours in 10 mM sodium phosphate buffer, pH 7.1, before solubilization.



strongly inhibited. Various protein components are present in the cell envelope. Therefore, further analysis of the labeled envelope by SDS polyacrylamide gel electrophoresis was performed to determine whether synthesis of specific proteins is inhibited by globomycin. The results indicate that globomycin inhibited synthesis of the proteins [origin (A)] and [peak H] and that simultaneously an unknown protein [peak G] with a molecular weight of approximately 12,000 (which migrates to the same position as cytochrome c) accumulated (Fig. 1 and Table 2). The protein [origin (A)] was identified as the bound form of lipoprotein in accordance with the results of INOUYE and his coworkers.[5] In an experiment in which the cells were doubly labeled with [^{3}H]-arginine and [^{14}C]-histidine, the peak H protein was found to lack histidine among its amino acid constituents and to migrate to the top of the gel using the same electrophoretic conditions as described above. The protein of peak H was, therefore, considered to be identical with the free form of lipoprotein.[3]

The unknown protein of peak G was converted to the free form of lipoprotein when the globomycin-treated cell envelope was further incubated

Fig. 3. SDS polyacrylamide gel electrophoresis of the cell envelope fraction from the mixed cells of *E. coli* B labeled with [^{3}H]-arginine or [^{14}C]-arginine.

Envelope fraction obtained from Fig. 1 was incubated at 37°C for 4 hours in 10 mM sodium phosphate buffer, pH 7.1, containing 50 μg/ml of globomycin before solubilization.

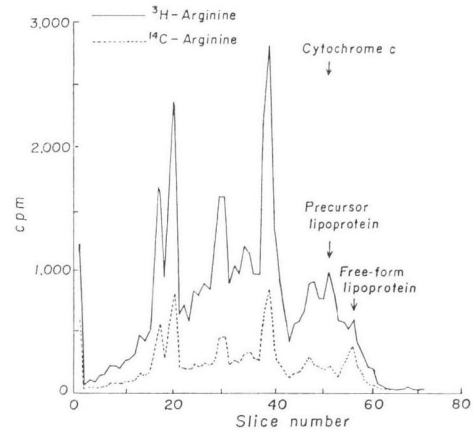
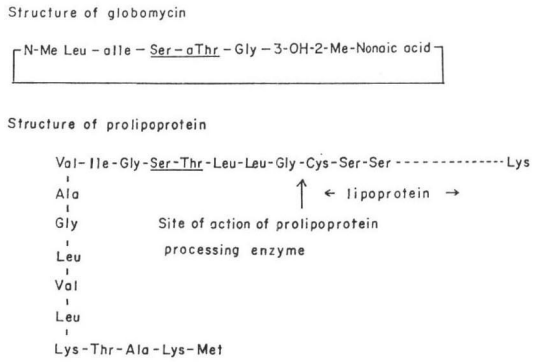


Fig. 4. Resemblance of amino acid sequence in the structures of globomycin and prolipoprotein at the sites underlined and other neighboring amino acids.



in the absence of globomycin (Fig. 2). However, approximately a 70% inhibition of the disappearance of the peak G protein was observed by the addition of 50 μg/ml of globomycin to the incubation mixture (Fig. 3).

From these data, we conclude that globomycin acts by an inhibition of a prolipoprotein processing enzyme which converts prolipoprotein to lipoprotein. As a consequence there is an accumulation of prolipoprotein in the envelope.

It is of interest to find that the amino acid sequence of prolipoprotein, reported by INOUYE and his coworkers,[6] is somewhat analogous to

that of globomycin (Fig. 4).[7] Details of further investigations on the mechanism of action of globomycin will be reported elsewhere.

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