INCREASE IN STREPTOMYCIN PRODUCTION CAUSED BY TUNICAMYCIN

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(Received for publication March 26, 1983)

We reported previously that D-glucosamine or a close derivative was involved in the synthesis of the *N*-methyl-L-glucosamine moiety of streptomycin[1]. Since D-glucosamine is a component of the cell wall of streptomycetes[2,3], this sugar or its derivatives may be a common precursor of streptomycin and the cell wall. We studied also whether UDP-*N*-methyl-D-glucosaminephosphate was involved in the synthesis of the *N*-methyl-L-glucosamine moiety of streptomycin and whether UDP-*N*-acetylmuramyl pentapeptide was present in *Streptomyces griseus*[4]. The latter compound should be a precursor of the peptidoglycan in streptomycetes as it is in bacteria.

Using specific inhibitors of bacterial cell wall synthesis, some groups[5,6] have demonstrated that inhibition of cell wall synthesis in *S. griseus* affects the synthesis of streptomycin. However, a connection between the intermediates of cell wall synthesis and those of streptomycin biosynthesis has not been reported. We have used tunicamycin, a specific inhibitor of cell wall synthesis in bacteria, to investigate this question.

S. griseus ME936-B3 from the Institute of Microbial Chemistry, Tokyo, was used for streptomycin production. *Bacillus subtilis* IAM 1069, a streptomycin-sensitive strain, was obtained from the Institute of Applied Microbiology, Tokyo University.

D-[1-^{14}C]Glucosamine (60.8 mCi/mmol) and D-[6-^{3}H]glucosamine (38 Ci/mmol) were purchased from Radiochemical Centre, Amersham, U.K. *S. griseus* was grown in the medium previously described[4] with or without tunicamycin. In the isotopic experiments, D-[1-^{14}C]glucosamine (10 μCi, 0.16 μmol) was administered to tunicamycin-supplemented medium and D-[6-^{3}H]-glucosamine (10 μCi, 0.26 nmol) was administered to the control medium at 24 hours.

Streptomycin was determined by the agar-diffusion method with *B. subtilis* as the test organism. Streptomycin hydrochloride was isolated from culture media by the method of HUNTER and HOCKENHULL[7]. *N*-Methyl-L-glucosamine hydrochloride was then prepared from it by the method of SILVERMAN and RIEDER[3]. Nucleotides were isolated from the mycelia by the method of BLUMSON and BADDILEY[9]. UDP-*N*-acetylmuramyl pentapeptide and UDP-*N*-methyl-D-glucosaminephosphate were prepared and identified as described in a previous paper[4]. Cell wall mucopeptide was prepared by the method of PARK and HANCOCK[10].

When tunicamycin was added to culture media at inoculation, growth of *S. griseus* was not inhibited (Fig. 1), but the cell shape changed to a spherical form (Fig. 2). Production of streptomycin increased (Fig. 3). However, no increase was observed when tunicamycin was added at 24 or 48 hours after inoculation (data not shown).

The incorporation of radioactive D-glucosamine into streptomycin and its *N*-methyl-L-glucosamine moiety was somewhat increased by addition of tunicamycin to the culture media

Fig. 1. Effect of tunicamycin on the growth of *S. griseus*.

Tunicamycin was added to the culture media at 0 time at concentrations of 0 (●), 0.1 (○) and 1.0 μg/ml (△). Dry weight of mycelia was measured at 24, 48, 72 and 96 hours.

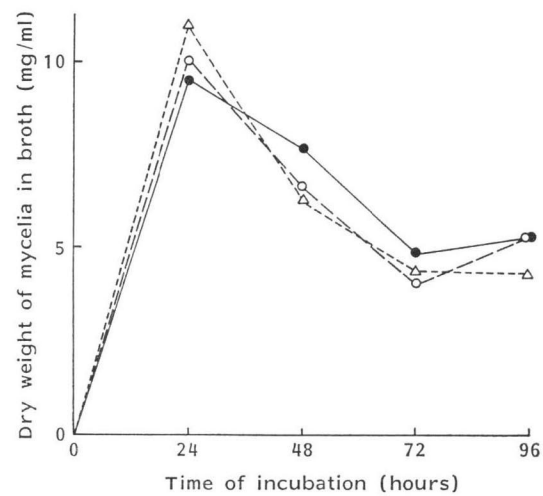


Fig. 2. Effect of tunicamycin on the morphology of *S. griseus*.

Tunicamycin was added to the culture medium at 0 time, and the photographs were taken after 24-hour growth without (a) or with (b) tunicamycin (1.0 μg/ml).

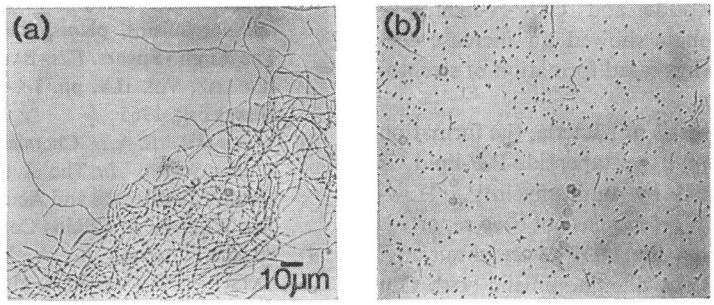
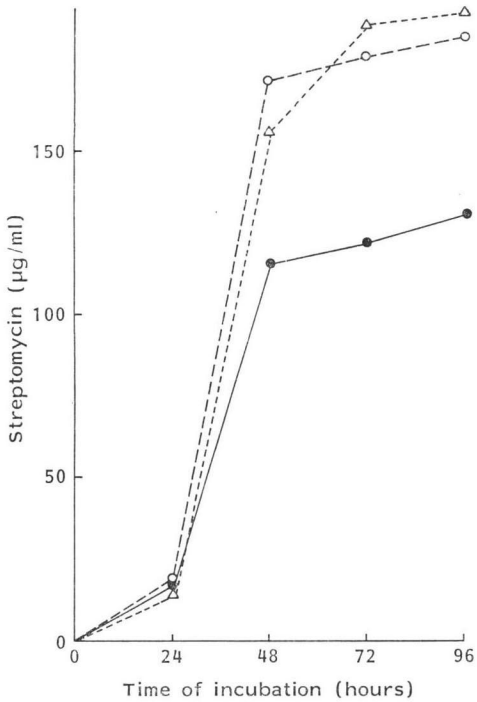


Fig. 3. Effect of tunicamycin on the formation of streptomycin.

Tunicamycin was added to the culture media at 0 time at the concentration of 0 (●), 0.1 (○) and 1.0 μg/ml (△). Streptomycin in the media was assayed at 24, 48, 72 and 96 hours.



(Table 1). The stimulatory effect of tunicamycin on the production of streptomycin corresponded with the enhanced incorporation of D-glucosamine into streptomycin. On the other hand, the incorporation of D-glucosamine into the cell wall mucopeptide was decreased by addition of tunicamycin to the culture media (Table 1). When

Table 1. Effect of tunicamycin on the incorporation of D-glucosamine into streptomycin, its *N*-methyl-L-glucosamine moiety and cell wall mucopeptide.

	Ratio of incorporation (+Tunicamycin/ −tunicamycin)
Streptomycin	1.26
N-Methyl-L-glucosamine moiety	1.35
Mucopeptide	0.53

Tunicamycin (0.1 μg/ml) was added to the culture media at 0 time. After 24 hours D-[1-^{14}C]-glucosamine (10 μCi) was administered to the media with tunicamycin and D-[6-^{3}H]glucosamine (10 μCi) was administered to the media without tunicamycin. After further incubation for 24 hours, the culture media were combined and the ^{14}C/^{3}H ratio in the compounds was measured. The ^{14}C/^{3}H ratio of glucosamine administered was set at 1.0.

Table 2. Effect of tunicamycin on the incorporation of D-glucosamine into sugar nucleotides of mycelia.

	Ratio of incorporation (+Tunicamycin/ −tunicamycin)
UDP-*N*-acetylmuramyl pentapeptide	1.44
UDP-*N*-methyl-D-glucosaminephosphate	1.31

Tunicamycin (0.1 μg/ml) was added to the culture media at 0 time. After 24 hours D-[1-^{14}C]glucosamine (10 μCi) was administered to the media with tunicamycin and D-[6-^{3}H]glucosamine (10 μCi) was administered to the media without tunicamycin. After further incubation for 6 hours, the culture media were combined, and the ^{14}C/^{3}H ratio in the compounds was measured. The ^{14}C/^{3}H ratio of glucosamine administered was set at 1.0.

the effect of tunicamycin on the incorporation of radioactive D-glucosamine into sugar nucleotides in the mycelia was determined, UDP-*N*-acetylmuramyl pentapeptide and UDP-*N*-methyl-D-glucosaminephosphate showed an increase corresponding to the increased formation of streptomycin (Table 2).

In cell wall synthesis of bacteria, the formation of *N*-acetylmuramyl pentapeptide-P-P-lipid is selectively inhibited by tunicamycin[11]. However, its action in *S. griseus* has not been reported. We have found that the UDP-*N*-acetylmuramyl pentapeptide contains diaminopimelic acid, Glu and Ala in the pentapeptide moiety[4]. As all these amino acids and muramic acid are components of the cell wall of streptomycetes[2,3], UDP-*N*-acetylmuramyl pentapeptide may also be a precursor of the cell wall in these organisms. Because tunicamycin enhanced the incorporation of D-glucosamine into UDP-*N*-acetylmuramyl pentapeptide and decreased the incorporation of D-glucosamine into mucopeptide in the mycelia, it may inhibit the formation of *N*-acetylmuramyl pentapeptide-P-P-lipid in streptomycetes as in bacteria. Also, the incorporation of D-glucosamine into UDP-*N*-methyl-D-glucosaminephosphate, a possible precursor of *N*-methyl-L-glucosamine moiety of streptomycin, was increased by tunicamycin. When cell wall synthesis in *S. griseus* is inhibited by tunicamycin, D-glucosamine would be utilized for the production of streptomycin.

Acknowledgments

We thank Drs. H. UMEZAWA and K. HOTTA, the Institute of Microbial Chemistry, Tokyo for the streptomycin-producing strain, and the Institute of Applied Microbiology, Tokyo University, for *B. subtilis*.

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