COMMUNICATION TO THE EDITOR

Liposidomycin B inhibits *In Vitro* Formation of Polyprenyl (pyro)phosphate *N*-Acetylglucosamine, an Intermediate in Glycoconjugate Biosynthesis

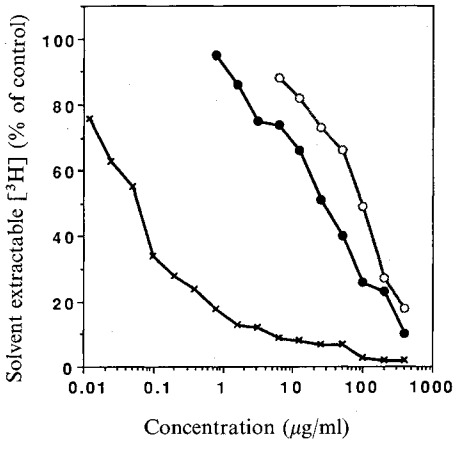
Sir:

The emergence of bacterial strains resistant to current antibiotics highlights the need to explore new targets for antibiotic action. Peptidoglycan biosynthesis is one of the well-precedented targets for therapeutic antimicrobial agents. Phospho-*N*-acetylmuramyl-pentapeptide translocase (also called translocase I) catalyzes the first reaction in the lipid cycle in bacterial peptidoglycan biosynthesis, but remains an unexploited target for therapeutic drugs, as many β-lactam antibiotics have been synthesized as inhibitors of transpeptidase[1]. Four groups of antibiotics have been reported to inhibit this translocase, *i.e.* tunicamycin[2], amphomycin[3], mureidomycins[4], and liposidomycins[5]. Lipid intermediates participate in biosyntheses of glycoprotein, and teichoic and teichuronic acids in addition to peptidoglycan. Therefore, it is important to determine the effect of translocase I inhibitors on the formation of these lipid intermediates in development of therapeutic agents. Tunicamycin and amphomycin inhibit translocase I, but they also inhibit lipid-linked intermediate formation in mammalian glycoprotein biosynthesis[6,7]. Mureidomycin A inhibits translocase I reaction *in vitro*, with a 50% inhibitory concentration (IC_{50}) of 0.01 μg/ml[4]. But, dolichyl pyrophosphate (Dol-*PP*) *N*-acetylglucosamine formation *in vitro* is relatively resistant to its action (IC_{50} of 100 μg/ml), and the growth of mammalian cells is not severely affected at 1000 μg/ml. Liposidomycins potently inhibit *Escherichia coli* translocase I *in vitro* with an IC_{50} of 0.03 μg/ml[5], but they are not toxic in mice[8]. Effects of liposidomycins on lipid intermediate formation have not been reported for biosynthesis of glycoconjugates other than peptidoglycan. Present study revealed that liposidomycin B inhibits *in vitro* formation of lipid intermediates in biosyntheses of glycoprotein and teichoic/teichuronic acid, and its activity is compared with that of tunicamycin and mureidomycin A.

Fig. 1 shows the effects of test compounds on Dol-*PP*-*N*-acetylglucosamine formation in rat liver microsomes. A dose-dependent inhibition of Dol-*PP*-*N*-acetylglucosamine formation was observed, and the IC_{50} values of liposidomycin B, tunicamycin and mureidomycin A are 20, 0.05 and 100 μg/ml, respectively. Dol-*P*-mannose and Dol-*P*-glucose, in addition to Dol-*PP*-*N*-acetylglucosamine, participate in lipid-linked oligosaccharide formation in glycoprotein biosynthesis. Liposidomycin B and mureidomycin A did not affect the formation of both lipid-linked sugars at the highest concentration tested, 400 μg/ml, but tunicamycin inhibited Dol-*P*-glucose formation with an IC_{50} value of 5 μg/ml (data not shown).

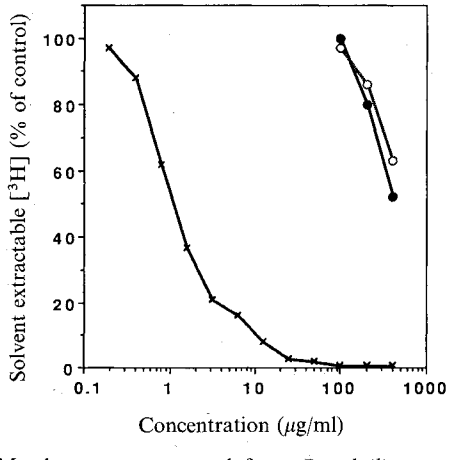
Undecaprenyl (pyro)phosphate *N*-acetylglucosamine formation in the *Bacillus subtilis* membrane was dose-dependently inhibited by liposidomycin B, tunicamycin, and mureidomycin A, and their IC_{50} values were 400, 1, and more than 400 μg/ml, respectively (Fig. 2). The IC_{50} values of the three antibiotics for undecaprenyl (pyro)phosphate *N*-acetylglucosamine formation were higher than those for Dol-*PP*-*N*-acetylglucosamine formation, and a 20-fold difference was demonstrated

Fig. 1. Effects of liposidomycin B (●), tunicamycin (×), and mureidomycin A (○) on Dol-*PP*-*N*-acetylglucosamine formation in rat liver microsomes.



Rat liver microsomes were added with drugs of indicated concentrations and UDP-[^{3}H]*N*-acetylglucosamine, and lipid-linked sugar was extracted as reported previously[6].

Fig. 2. Effects of liposidomycin B (●), tunicamycin (×), and mureidomycin A (○) on undecaprenyl (pyro)phosphate *N*-acetylglucosamine formation in the *B. subtilis* membrane.



Membrane was prepared from *B. subtilis* as reported previously[4], and lipid-linked *N*-acetylglucosamine formation was measured as described in the legend to Fig. 1.

with both liposidomycin B and tunicamycin between the two activities. The cause for the difference in the two activities waits for a further clarification.

Tunicamycin inhibited glycoprotein biosynthesis and multiplication of enveloped viruses such as Newcastle disease virus and vesicular stomatitis virus, but liposidomycin B and mureidomycin A did not at all at the highest concentration tested, 400 μg/ml (data not shown). Such a resistancy to the action of liposidomycin B at a cellular level has been reported with bacteria. Liposidomycin B exerts a strong inhibitory activity *in vitro* against translocase I[5] and, in contrast, the antibacterial activity of liposidomycin B is limited[8], and the reason has yet to be determined. Taken together these results obtained by *in vitro* and *in vivo* assays using microbial and mammalian systems, liposidomycin B seems to be hardly transported into cells, but not ruling out other possible explanations.

Present study revealed that liposidomycin B inhibits formation of lipid intermediates in glycoconjugate biosynthesis at high concentrations compared with its reported action against translocase I. BRANDISH *et al.* compared the salient features of inhibition of translocase I activity by tunicamycin, liposidomycin B, and mureidomycin A, and found that tunicamycin is a reversible inhibitor and, in contrast, liposidomycin B and mureidomycin A are both slow-binding inhibitors and suggested similarities in the mechanisms of action of the latter two antibiotics[9]. The potent and selective inhibition of translocase I reaction in bacterial peptidoglycan biosynthesis by liposidomycins and mureidomycins at low concentrations could contribute to the rational design of novel therapeutic agents.

Acknowledgments

This work was supported in part by a grant for Biodesign Research Program from the Institute of Physical and Chemical Research to A. T. and M. M.

MAKOTO MUROI[a]
KEN-ICHI KIMURA[b]
HIROYUKI OSADA[a]
MASATOSHI INUKAI[c]
AKIRA TAKATSUKI[a,*]

[a]The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan
[b]Snow Brand Milk Products Co., Ltd., Ishibashi-machi, Shimotsuga-gun, Tochigi 329-05, Japan
[c]Sankyo Co., Ltd., 1-2-58, Hiromachi, Shinagawa-ku, Tokyo, Japan

(Received October 1, 1996)

References

1) BUGG, T. D. H. & C. T. WALSH: Intracellular steps of bacterial cell wall peptidoglycan synthesis: enzymology, antibiotics and antibiotic resistance. Natural Products Rep. 9: 199~215, 1992
2) TAMURA, G.; T. SASAKI, M. MATSUHASHI, A. TAKATSUKI & M. YAMASAKI: Tunicamycin inhibits the formation of lipid intermediate in cell-free peptidoglycan synthesis of bacteria. Agric. Biol. Chem. 40: 447~449, 1976
3) TANAKA, H.; R. OIWA, S. MATSUKURA & S. OMURA: Amphomycin inhibits phospho-*N*-acetylmuramyl-pentapeptide translocase in peptidoglycan synthesis of *Bacillus*. Biochem. Biophys. Res. Commun. 86: 906~908, 1979
4) INUKAI, M.; F. ISONO & A. TAKATSUKI: Selective inhibition of the bacterial translocase reaction in peptidoglycan synthesis by mureidomycins. Antimicrob. Agents Chemother. 37: 980~983, 1993
5) KIMURA, K.; N. MIYATA, G. KAWANISHI, Y. KAMIO, K. IZAKI & K. ISONO: Liposidomycin C inhibits phospho-*N*-acetylmuramyl-pentapeptide transferase in peptidoglycan synthesis. 53: 1811~1815, 1989
6) TAKATSUKI, A.; K. KOHNO & G. TAMURA: Inhibition of biosynthesis of polyisoprenol sugars in chick embryo microsomes by tunicamycin. Agric. Biol. Chem. 39: 2089~2091, 1975
7) KANG, M. S.; J. D. SPENCER & A. D. ELBEIN: Amphomycin inhibits the incorporation of mannose and GlcNAc into lipid-linked saccharides by aorta extracts. Biochem. Biophys. Res. Commun. 82: 568~574, 1978
8) ISONO, K.; M. URAMOTO, H. KUSAKABE, K. KIMURA, K. IZAKI, C. C. NELSON & J. A. MACCLOSKEY: Liposidomycins: novel nucleoside antibiotics which inhibit bacterial peptidoglycan synthesis. J. Antibiotics 38: 1617~1621, 1985
9) BRANDISH, P. E.; K. KIMURA, M. INUKAI, R. SOUTHGATE, J. T. LONSDALE & T. D. H. BUGG: Modes of action of tunicamycin, liposidomycin B, and mureidomycin A: inhibition of phospho-*N*-acetylmuramyl-pentapeptide translocase from *Escherichia coli*. Antimicrob. Agents Chemother. 40: 1640~1644, 1996