STIMULATION OF POLYETHER ANTIBIOTIC PRODUCTION IN STREPTOMYCETES BY HEPTAKIS-2,6-DI-*O*-METHYL β-CYCLODEXTRIN

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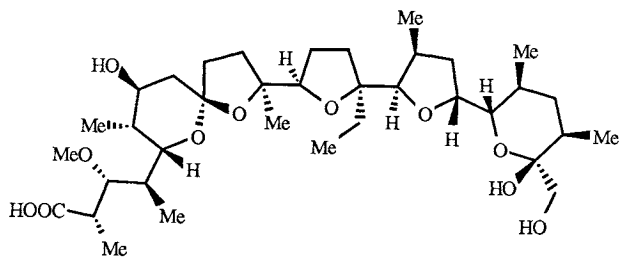
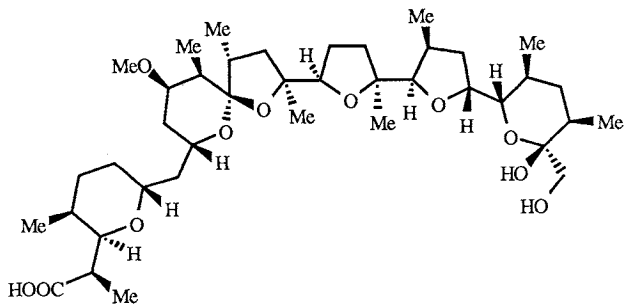
In the course of biosynthetic studies on the polyether antibiotics monensin A (**1**) in *Streptomyces cinnamonensis* A3823.5, and nigericin (**2**) in *Streptomyces hygroscopicus* V1327 and *Streptomyces violaceusniger* NRRL B-1865, we set out to determine whether cyclodextrins could be used to stimulate the cellular uptake and incorporation into these antibiotics of poorly water soluble biosynthetic precursors. As a result we discovered that heptakis-2,6-di-*O*-methyl β-cyclodextrin (DMCD) stimulates the production and isolated yield of these polyether antibiotics by up to a factor of two (unoptimised) in shaken liquid cultures.

Our initial experiments were carried out with the monensin-producing organism *S. cinnamonensis* A3823.5, grown in either a chemically defined medium[1], or in an oil-rich complex medium[2,3]. The production of monensin versus time was followed using a colorimetric assay with vanillin[2]. In the chemically defined medium[1] in a 60 ml shake culture monensin production began after 24 hours and plateaued after around 7 days shaking at 32°C. When DMCD (10 mg/ml) was added to the production medium at the start of the fermentation the rate of monensin production was increased, and the final isolated yield of monensin after extraction was about 80% higher than that obtained in the absence of the cyclodextrin (Fig. 1). This effect was not observed with several other cyclodextrin derivatives, including α-, β- or γ-cyclodextrin. A similar effect on monensin production was observed when smaller (0.03 mg/ml) and larger (up to 25 mg/ml) quantities of DMCD were added to the production medium, and in the latter case the cyclodextrin could be recovered in good yield, suggesting that DMCD acts catalytically, and is not degraded significantly by the microorganism. The weight of dry cells produced during a typical fermentation was also not influenced significantly.

The same effect was seen with *S. cinnamonensis* grown in a complex medium containing fatty acids[2,3]. Here the strong stimulatory effects on monensin production of added Fe(II) salts and



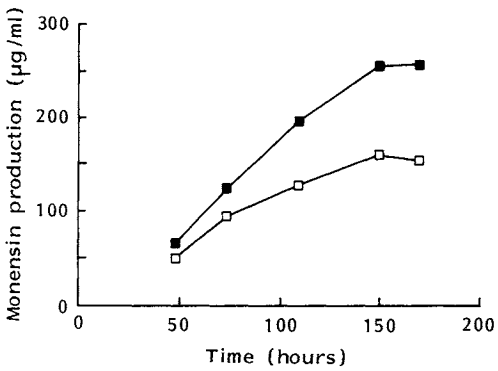
Monensin A (**1**)



Nigericin (**2**)

Fig. 1. Time course of monensin production by *Streptomyces cinnamonensis*.

□ −DMCD, ■ +DMCD.



+DMCD = monensin production in the presence of DMCD; −DMCD = monensin production in the absence of DMCD.

Stimulation of monensin production in shake cultures (60 ml) in chemically defined medium[1] caused by DMCD.

fatty acids such as methyl oleate have already been well documented[4], but monensin production was almost doubled in the presence of 2 mg/ml DMCD. To investigate whether a similar effect on polyether antibiotic production is seen in other organisms, the nigericin (**2**) producers *Streptomyces hygroscopicus* V1327 and *Streptomyces violaceusniger* NRRL B-1865 (previously *Streptomyces albus* NRRL B-1865[5]) were tested. For both strains, a vegetative medium[5] (glucose 3% w/v, corn steep solids 0.5%, soy flour 0.5% and $CaCO_3$ 0.5%) pH 7.0 was inoculated with spores. After shaking at 30°C for 30 hours a 5% inoculum was transferred to the production medium, which for both strains was soy flour (2% w/v) plus mannitol (2%) pH 7.5. In both strains nigericin production began after about 5 days shaking at 30°C, and plateaued after about 21 days at *ca.* 0.2 and 1.0 mg/ml, respectively. The nigericin was extracted from the mycelium and purified as the sodium salt. A strong stimulatory effect on nigericin production was seen with 0.2 mg/ml (or more) of DMCD, and the isolated yield was improved by up to 70% over that obtained in the absence of DMCD.

These experiments reveal a consistently high stimulation of polyether antibiotic production by DMCD in three different streptomycetes grown in a variety of different media. This suggests that the effect may be a general one on polyether biosynthesis in this genus of microorganism. As far as we are aware, a similar effect of DMCD on polyketide antibiotic production has not been described previously. Cyclodextrins, and DMCD in particular, are known to stimulate fatty acid synthesis[6] catalysed by fatty acid synthase from *Mycobacterium phlei*, and to stimulate the growth of *Bordetella pertussis*[7], which is inhibited by free fatty acids. The effect on fatty acid biosynthesis is thought to arise from the ability of cyclodextrins to bind the hydrophobic portion of palmitoyl-CoA[8], thereby relieving negative feedback inhibition of the fatty acid synthase. Based on this analogy, we suggest that DMCD may also exert its influence on polyether antibiotic production by binding to the partially reduced polyketide chains synthesised by the polyketide synthases active in polyether biosynthesis[9]. These polyketide chains are thought to comprise fatty acid derivatives, which contain ketone, alcohol, alkene, and fully reduced alkane functionality[10]. The hydrophobic nature of these molecules should facilitate their binding to the internal cavity of DMCD. Similar reduced polyketide chains are produced during the biosynthesis or other polyketide antibiotics *e.g.* macrolide, polyene, and related antibiotics, so DMCD may also have a stimulatory effect on the biosynthesis of these other polyketide antibiotics in streptomycetes. This effect of DMCD on polyether (polyketide) antibiotic biosynthesis may be valuable for increasing antibiotic yields in batch fermentations, and especially for the isolation of new metabolites which are otherwise produced in only very small amounts.

Acknowledgments

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References

1) Day, L. E.; J. W. Chamberlin, E. Z. Gordee, S. Chen, M. Gorman, R. L. Hamill, T. Ness, R. E. Weeks & R. Stroshane: Biosynthesis of monensin. Antimicrob. Agents Chemother. 4: 410~414, 1973
2) Haney, M. E., Jr. & M. M. Hoehn: Monensin, a new biologically active compound. I. Discovery and isolation. Antimicrob. Agents Chemother. -1967: 349~352, 1968
3) Ashworth, D. M.; D. S. Holmes, J. A. Robinson, H. Oikawa & D. E. Cane: Selection of a specifically

blocked mutant of *Streptomyces cinnamonensis*: Isolation and synthesis of 26-deoxymonensin A. J. Antibiotics 42: 1088～1099, 1989

4) STARK, W. M.; N. G. KNOX & J. E. WESTHEAD: Monensin, a new biologically active compound. II. Fermentation studies. Antimicrob. Agents Chemother. -1967: 353～358, 1968
5) DAVID, L.; H. L. AYALA & J.-C. TABET: Abierixin, a new polyether antibiotic. Production, structural determination and biological activities. J. Antibiotics 38: 1655～1663, 1985
6) MACHIDA, Y.; R. BERGERON, P. FLICK & K. BLOCH: Effects of cyclodextrins on fatty acid synthesis. J. Biol. Chem. 248: 6246～6247, 1973
7) IMAIZUMI, A.; Y. SUZUKI, S. ONO, H. SATO & Y. SATO: Heptakis(2,6-*O*-dimethyl)β-cyclodextrin: a novel growth stimulant for *Bordetella pertussis* phase I. J. Clin. Microbiol. 17: 781～786, 1983
8) BERGERON, R.; Y. MACHIDA & K. BLOCH: Complex formation between mycobacterial polysaccharides or cyclodextrins and palmitoyl coenzyme A. J. Biol. Chem. 250: 1223～1230, 1975
9) ROBINSON, J.: Polyketide synthase complexes: their structure and function in antibiotic biosynthesis. Philos. Trans. R. Soc. Lond. (Biol.) 332: 107～114, 1991
10) CANE, D. E.; W. D. CELMER, J. W. WESTLEY: Unified stereochemical model of polyether antibiotic structure and biogenesis. J. Am. Chem. Soc. 105: 3594～3600, 1983