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 cinnamomensis 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. cinnamomensis A3823.5, grown in either a chemically defined medium1) or in an oil-rich complex medium2,3). The production of monensin versus time was followed using a colorimetric assay with vanillin2). In the chemically defined medium1) 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. cinnamomensis grown in a complex medium containing fatty acids2,3). Here the strong stimulatory effects on monensin production of added Fe(II) salts and
fatty acids such as methyl oleate have already been well documented40, 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 VI327 and Streptomyces violaceusniger NRRL B-1865 (previously Streptomyces albus NRRL B-18655) were tested. For both strains, a vegetative medium5) (glucose 3% w/v, corn steep solids 0.5%, soy flour 0.5% and CaCO₃ 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 synthesis9) catalysed by fatty acid synthase from Mycobacterium phlei, and to stimulate the growth of Bordetella pertussis9), 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-CoA8), 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 biosynthesis9). These polyketide chains are thought to comprise fatty acid derivatives, which contain ketone, alcohol, alkene, and fully reduced alkane functionality10). 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.

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References


