

Effect of γ -butyrolactone derivative on an artificial membrane

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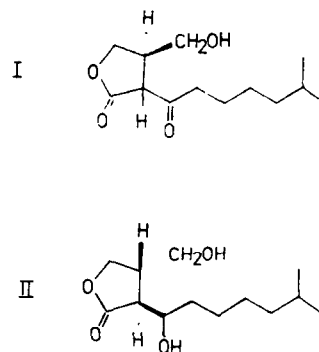
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trans-2-(6'-Methylheptanol-1'-yl)-3-hydroxymethyl-4-butanolide (II) was shown to increase the passive flux of cations such as Co²⁺ through a black lipid membrane made from ox brain phospholipids. This membraneotropic effect appears to be involved in the activity of II towards blocked mutants of streptomycetes as an autoregulator of cytodifferentiation.

Cytodifferentiation (Streptomycetes) Autoregulator γ -Butyrolactone derivative Membrane effect
Passive ion flux

1. INTRODUCTION

Streptomycetes are known to produce derivatives of γ -butyrolactone such as A-factor (2-(6'-methylheptanoyl)-3-hydroxymethyl-4-butanolide(I) [1,2] and *trans*-3-(6'-methylheptanol-1'-yl)-3-hydroxymethyl-4-butanolide (II, absolute stereochemistry not determined) [3]. I or II (for structures see scheme 1) are needed as endogenous regulatory molecules for normal cytodifferentiation of some strains of *Streptomyces griseus* [1,4]. Thus mutant strains incapable of producing I or II did not form both aerial mycelium and antibiotics such as streptomycin or daunomycin but responded to the administration of low amounts of these autoregulators to the growth medium with full reconstitution of sporulation and/or secondary metabolism [4,5]. Though the morphological and biochemical changes induced by I or II in the pertinent blocked mutants of *S. griseus* have been studied [5,6], the initial site of action of these inducers of cytodifferentiation still remains to be elucidated. It has been suggested that I chelates cations essential for cytodifferentiation, particularly the cobalt, and transports them through the cytoplasmic



Scheme 1

membrane as a lipophilic carrier [6]. This interpretation implies that II could be oxidized to I as a kind of prodrug. In fact, both I and II represent bipolar molecules which possess a polar γ -butyrolactone head and a nonpolar aliphatic tail but an interference with biological model membranes has not yet been investigated. We report here the results of our *in vitro* studies attesting to the remarkable effect of an autoregulator of cytodifferentiation of streptomycetes such as II on passive fluxes of cobalt ions.

2. MATERIALS AND METHODS

2.1. Preparation of autoregulator II

II was prepared as purified material by fermentation of *S. viridochromogenes* in 500 l fermentors. Isolation and purification by solvent extraction from the culture broth and subsequent chromatographic procedures have been described elsewhere [3].

2.2. Purity of II

The purity of II thus obtained was 95%, at least, as checked by bioassay using *S. griseus* JA 5142/86 as an indicator organism [4] and physicochemical methods (MS, IR, NMR) as well. The material displayed no antibiotic activity against *Bacillus subtilis*, used as test germ during agar plate diffusion assay.

2.3. The black lipid membrane model

A black lipid membrane model was employed as described in detail [7]. The preparation of the lipid bilayer was carried out by use of a 10:1 mixture of phospholipids extracted from ox brain and cholesterol which were dissolved together in *n*-heptane (20 mg/ml). The buffer concentration was 100 mmol KCl/l in both the inner and outer compartment, at pH 5.3.

2.4. Operation

Throughout the pertinent experiments, 2–10 mmol CoSO_4 /l were admixed to the inner volume. The effect of autoregulator II on the conductance of the bilayer membrane was measured after the concomitant addition of 5×10^{-3} mol/l to the inner volume and of 5×10^{-4} mol/l to the outer volume.

2.5. Measurement of conductance

A common measuring bridge equipped with a standard electronic device was used for the measurement of conductance [7].

3. RESULTS AND DISCUSSION

Fig.1 demonstrated the influence of II on the conductance of the bilayer membrane. In the presence of 2 mmol CoSO_4 /l in the inner volume, addition of II (5×10^{-4} mol/l in the outer volume; 5×10^{-3} mol/l in the inner volume) increased the conductance by approx. 500-times compared with

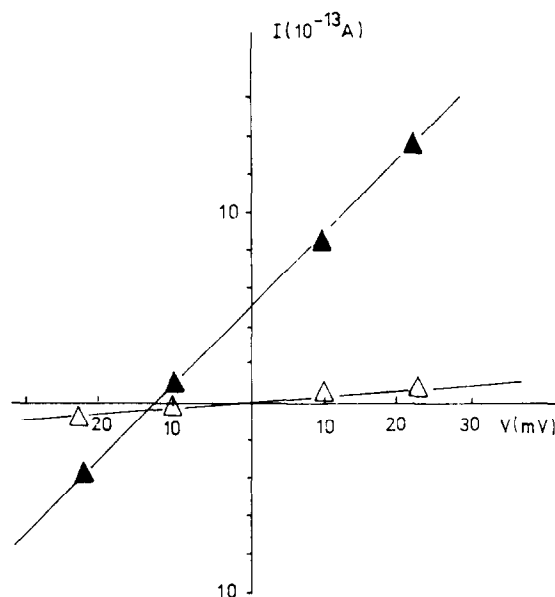


Fig.1. Influence of II on the conductance of the artificial bilayer (current (*I*)-voltage (*V*) curve). (Δ) No addition of II, (▲) 5×10^{-4} M II in the outer volume, 10^{-3} M II in the inner volume, 2 mM CoSO_4 in the buffer.

the control experiments in which the addition of II was omitted. When the zero current potentials were measured in the presence of the same concentrations of II in the inner and outer volume under a gradient of CoSO_4 in the inner volume (2–20 mmol/l), a considerable deviation from the linear shape of the curve (a characteristic of a high-ion selectivity) was observed (fig.2).

As a generalization, these findings suggest rather low-ion selectivity of II in mediating increased passive ion fluxes through artificial membranes. It thus seems reasonable to propose that autoregulators of cytodifferentiation in streptomycetes such as II cause disturbances in the molecular organization of the lipid bilayer of the cytoplasmic membrane due to their detergent-like insertion as a bipolar molecule. Moreover, it can be assumed that the membranotropic activity of II is involved in the biological effect towards *Streptomyces* mutants blocked in sporulation and antibiotic biosynthesis [8]. Thus, oxidation of II within the cells of such blocked mutants could deliver the true autoregulator I which possesses probably a much higher cation selectivity as a transport vehicle due

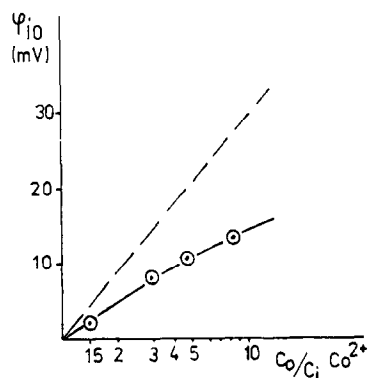


Fig.2. Zero current potentials in/out (φ_{io}) as a function of salt concentration in the inner (C_i) and the outer (C_o) solutions. (—○—) $C_i = 2$ mM CoSO_4 (inner volume) and $C_o = 2$ –20 mM CoSO_4 (outer volume); (---) Ideal curve for high Co^{2+} selectivity.

to the presence of a β -ketolactone structure. But experiments using pure I have to be carried out with artificial membranes in future.

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