Effect of γ-butyrolactone derivative on an artificial membrane

U. Gräfe, R. Schlegel, P.A. Grigoriev+ and W. Römer

Central Institute of Microbiology and Experimental Therapy, GDR Academy of Sciences, PO Box 73, DDR-6900 Jena, GDR and *+Institute of Biophysics, USSR Academy of Sciences, Pushchino na Oke 142292, USSR*

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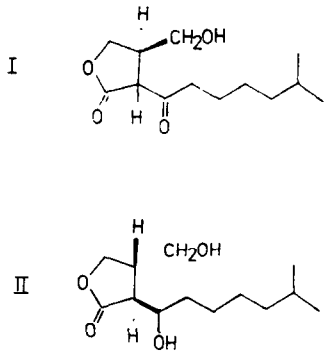
trans-2-(6′-Methylheptanol-1′-yl)-3-hydroxymethyl-4-butanolide (II) was shown to increase the passive flux of cations such as Co^{2+} through a black lipid membrane made from ox brain phospholipids. This membranotropic effect appears to be involved in the activity of II towards blocked mutants of streptomycetes 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 nd 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 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.



Scheme 1

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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

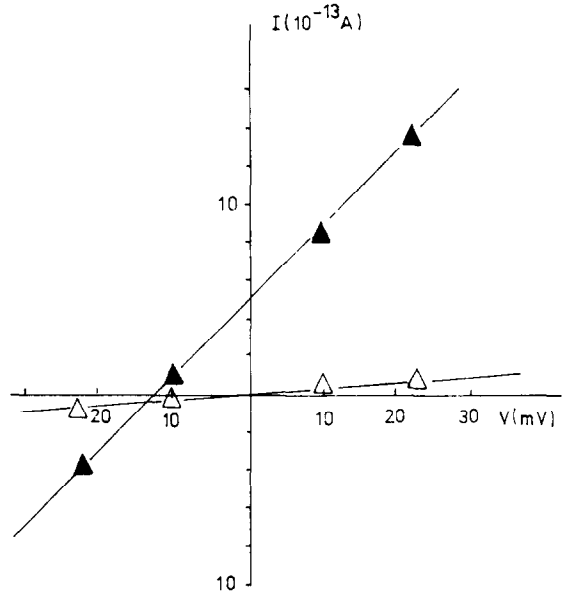

Fig.1. Influence of II on the conductance of the artificial bilayer (current *(I)*-voltage *(V)* curve). (△) No addition of II, (▲) 5×10^{-4} M II in the outer volume, 10^{-3} M II in the inner volume, 2 mM $CoSO_4$ in the buffer.

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

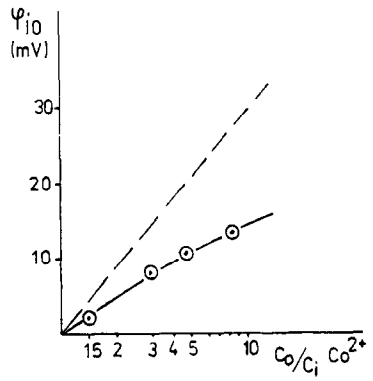

Fig.2. Zero current potentials in/out (φ_{io}) as a function of salt concentration in the inner (C_i) and the outer (C_o) solutions. (—⊙—) C_i = 2 mM $CoSO_4$ (inner volume) and C_o = 2-20 mM $CoSO_4$ (outer volume); (– – –) Ideal curve for high Co^{2+} selectivity.

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

REFERENCES

[1] Khokhlov, A.S. (1982) in: Overproduction of Microbial Products (Krumphanzl, V. et al. eds) pp. 96–109, Academic Press, New York.
[2] Kleiner, E.M., Onoprienko, V.V., Pliner, S.A., Soifer, V.S. and Khokhlov, A.S. (1977) Bioorg. Khim. 3, 424–426.
[3] Gräfe, U., Schade, W., Eritt, I., Fleck, W.F. and Radics, L. (1982) J. Antibiot. 35, 1722–1723.
[4] Eritt, I., Gräfe, U. and Fleck, W.F. (1982) J. Basic Microbiol. 22, 91–96.
[5] Gräfe, U., Reinhardt, G., Krebs, D., Eritt, I. and Fleck, W.F. (1984) J. Gen. Microbiol. 130, 1237–1245.
[6] Gräfe, U. Riesenberg, D. and Eritt, I. (1985) J. Basic Microbiol. 25, 279–283.
[7] Grigoriev, P.A., Schlegel, R., Thrum, H. and Ermishkin, L. (1985) Biochim. Biophys. Acta 551, 229–237.
[8] Gräfe, U., Eritt, I., Hänel, F., Friedrich, W., Roth, M., Röder, B. and Bormann, E.J. (1985) in: Regulation of Secondary Metabolite Formation, pp. 225–247, VCH, Weinheim.