Isolation and Characterization of a Potassium Specific Ionophore from *Streptococcus faecalis* *

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The synthesis of a potassium and rubidium specific ionophore by *Streptococcus faecalis* has been proved, using bilayer measurements. The characteristics of this substance agree with the ones, published for free mobile carriers, such as valinomycin and the macrotetrolides.

Introduction

Ionophorus antibiotics, such as valinomycin and the macrotetrolides were found to enhance potassium permeability of biological and artificial membranes [1–3]. The mode of action of these metabolites has been field of broad investigations, aiming at a better understanding of carrier mediated transport mechanisms in biological systems [4–6]. However little is known about the possible function of these antibiotics in physiological transport systems [7].

In previous papers a macrotetrolide deficient mutant of *Streptomyces griseus* has been described [8, 9]. This mutant showed a different specificity of potassium accumulation as compared to the macrotetrolide producing wild-type, indicating the participation of the macrotetrolides in the physiological potassium transport system.

We found a striking similarity between the macrotetrolide minus mutant and a class of potassium deficient mutants isolated from *Streptococcus faecalis* [10]. Therefore it is conceivable that these mutants have lost the ability to produce a potassium transport component of ionophoric nature. If this assumption is correct, a potassium specific ionophore had to be produced by the parent strain. The experiments described in this paper constitute an attempt to identify this hypothetical potassium carrier from the strain of *Streptococcus faecalis*, from which the potassium deficient mutants were derived.

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* Metabolic products of microorganisms, 168; preceeding publication: H. Achenbach *et al.*: Cyclopaldic acid from *Aspergillus duricaulis*. Arch. Microbiol., in press.

Materials and Methods

Organisms and growth conditions

Streptococcus faecalis 9790 was grown in NaTY medium as described by Zarlengo and Schultz [11], supplemented with 0.5 M NaCl. pH 7.5 was maintained by use of a pH-stat and the cells were centrifuged after 14 hours of incubation.

Extraction and purification

Lyophilized cells were extracted with acetone (3 l/100 g) in a Soxhlet apparatus for 48 hours. The acetone was evaporated *in vacuo*, the residue dissolved in chloroform and applied to a silica gel column (2.5 × 50 cm). The column was washed with chloroform and subsequently the ionophore was eluted with chloroform/methanol (95 : 5). Further purification was achieved by preparative TLC with chloroform/ethyl acetate (1 : 2) as solvent.

Analytical methods

Purification of the ionophore was analysed by BLM measurements according to previously published methods [12]. Black lipid membranes were formed from a 1% solution of L-α-phosphatidylcholine (Sigma P-8640) in *n*-decane. A teflon cell was used for membrane formation. The wall separating the two aqueous compartments contained a circular hole of 1 mm diameter. The temperature was held at 30 °C. For testing ionophoric activity, the solvent was evaporated from an aliquot of the fractions to be tested and the lipid-decane solution was added.

Results

Using the methods described in the experimental section, 300 μg of a yellowish substance were isolated from 100 g cells (dry-weight). This pre-

paration markedly increased the membrane conductance of bimolecular lipid membranes. Because of the very limited availability of pure material – the average yield of cells from 100 l of culture broth was 40 g – only few physicochemical data could be obtained. On TLC-plates the product can be stained with H_2SO_4 and J_2, but not with ninhydrine, showing a R_F-value of 0.15, using chloroform/ethyl acetate as solvent. A molecular weight of 700 – 1000 was estimated for the ionophore by elution from Sephadex LH20 with methanol. The UV-spectrum shows only end absorption at 213 nm. Work is still going on to get more information about the molecular structure of this substance.

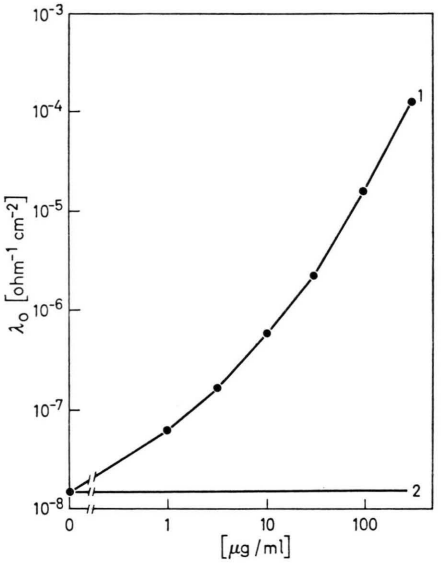

Fig. 1. Conductivity of lecithin membranes as a function of the concentration of the ionophore from *Streptococcus faecalis* ATCC 9790 with 1 M KCl in the aqueous solution. The substance was added to the membrane phase (curve 1). Conductivity in the absence of the ionophore (curve 2).

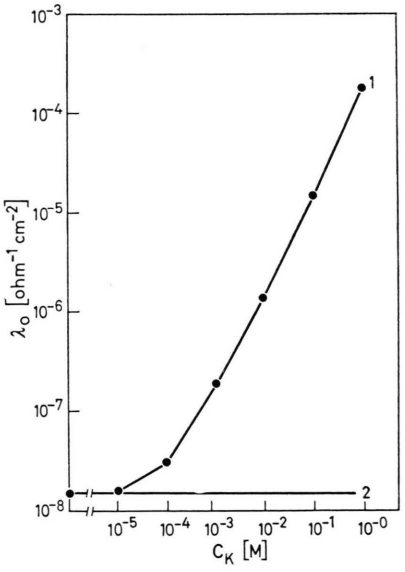

Fig. 2. Conductivity of lecithin membranes as a function of KCl concentration in the aqueous phase. The ionic strength was held constant at 1 M with LiCl. 500 µg/ml in the membrane phase (curve 1). Conductivity in the absence of the ionophore (curve 2).

In Fig. 1 the membrane conductance is plotted as a function of the concentration. The substance was dissolved in the solution from which the membrane was formed. The data indicate a nearly linear relationship between membrane conductance and a carrier concentration up to 500 µg/ml.

The conductivity also depends on the potassium concentration (Fig. 2). The ionic strength was held constant in these experiments with LiCl. LiCl does not influence membrane conductance in the presence of ionophore, as is shown by the data without potassium.

Further evidence for a selective increase in the potassium permeability of membranes is presented in Table I. Biionic potentials observed at membranes, separating solutions of two different salts are typical for ionophores [1, 13]. The selectivity coefficients K_{AB}, calculated from these potentials show a selectivity of the substance for potassium and rubidium similar to that of valinomycin or the macrotetrolides. Divalent cations do not influence the membrane conductance in the presence of the ionophore.

Table I. Ionic selectivity data. Membranes mere formed from lipid, containing 100 µg ionophore/ml. The membranes were separating 0.05 M solutions of the ion A from aequimolar solutions of the ion B. Ion B was Li^+ in all experiments. E_{AB}, observed biionic potential. The selectivity coefficients K_{AB} are derived from E_{AB} by: $K_{AB} = [A]/[B] \exp E_{AB} F/R T$.

Ion A	Biionic potential E_{AB} [mV]	Selectivity coefficient K_{AB}
Li^+	0	1.0
Na^+	4	1.2
K^+	104	60.0
Rb^+	110	73.0
Cs^+	5	1.2

Discussion

The synthesis of a potassium and rubidium specific ionophore by *Streptococcus faecalis* has been proved, using bilayer measurements. The bimolecular lipid membrane technique, first employed by Mueller and coworkers [14] has become an established method for measuring ionophoric activity, because of its high sensitivity and resemblance to natural membranes. Two models for ion transport have been established by this technique.

1. The transport of ions by mobile carriers with high selectivity, such as valinomycin and the macrotetrolides [15, 16].

2. An unspecific transport of ions through channels, integrated into the membrane. This type is represented by the antibiotic grammicidin A [17].

The following effects, induced by the metabolite isolated from *Streptococcus faecalis* were measured:

1. A linear relationship between membrane conductance and ionophore concentration (Fig. 1).

2. A linear relationship between membrane conductance and alkaline ion concentration (Fig. 2).

3. Selectivity for potassium and rubidium (Table I).

These findings agree with the ones, published for free mobile carriers [13, 16]. For the Streptococcus ionophore we therefore favour this carrier mechanism, instead of the pore mechanism. Final conclusions however need further information on the chemical structure of this metabolite. A peptide, however, like valinomycin or grammicidin can be excluded, because hydrolysates of the substance are ninhydrine negative as well as the native ionophore.

The participation of this metabolite in physiological potassium transport of *Streptococcus faecalis* also needs further examination. It should be mentioned however, that the substance can only be isolated from cells, grown in a medium with high sodium concentrations. Furthermore tr_{K8}^--mutants, derived from this strain, do not grow under these conditions [10], and no synthesis of this ionophore can be detected in this mutant. This is suggestive, that the carrier is part of a high affinity potassium transport system, the synthesis of which is strongly regulated by external salt concentrations. This strong regulation and the high effectivity of the ionophore also may explain the low yield of substance, produced by the cells. In this view the inhibition of growth of tr_{K8}^--mutants by sodium is caused by the lack of this transport component. Whether the pheno-type of the parent strain can be reconstituted by adding the isolated carrier to mutant cells is subject of our current investigation.

Mutants of the same type have been isolated from *E. coli* [18], and we have evidence, that ionophoric activity also can be isolated from the corresponding wild-type of *E. coli*.

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