MECHANISM OF ALKALI CATION TRANSPORT IN BULK MEMBRANES USING MACROTETROLIDE ANTIBIOTICS

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SUMMARY

The transport of potassium ions in a potential gradient across a bulk membrane containing macrotetrolide antibiotics is studied using ^{14}C-labelled macrotetrolide. It is shown that this transport of potassium ions across the membrane is accompanied by an equal transport of macrotetrolide within the membrane indicating that potassium ions are transported as the 1:1 macrotetrolide complex. Since exchange of ligands is shown to occur during the transport, a carrier relay mechanism accounting for all experimental results is proposed.

In a recent communication[1] we reported on the ion selective transport of potassium ions in the presence of sodium ions using macrotetrolide antibiotics[2,3,4] on an inert support in a potential gradient. These antibiotics form 1:1 complexes with alkali metal salts[5]. It has been shown by X-ray analysis[6,7] that crystalline complexes[8,9] of antibiotics having alkali cation carrier properties are built according to the following general principles[10]:

a) The external surface of the complexes is lipophilic enhancing lipid solubility of these carrier complexes.
b) The alkali cation is not hydrated.
c) The alkali cation complexes of compounds of the valinomycin group[11] (valinomycin, gramicidins, enniatins, macrotetrolides) are positively charged.
d) The alkali cation complexes of compounds of the nigericin group[11] (nigericin, monensin) are electrically neutral.

Several different mechanisms have been suggested[12] (figure 1) for the stimulating effect of the antibiotics mentioned on the alkali ion transport. There is X-ray evidence[6] that the free nonactin molecule must adopt a conformation quite different from that of the complexed molecule, for the crystal must be built from rather flat, plate-like molecules associated in closely stacked

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columns. It seemed therefore conceivable that the same structure could be adopted by associated nonactin molecules in bulk membranes.[6] If this were the case, a transfer of cations from molecule to molecule within such stacks could be realized.[6] Other evidence however seems to favour a carrier model,[12,13] although this information does not prove such a mechanism. Using labelled macrotetrolides we have now been able to show clearly that the transport in bulk membranes is controlled by a carrier relay mechanism.

EXPERIMENTAL

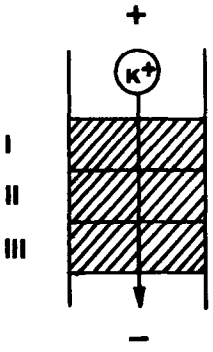
L a b e l l e d m a c r o t e t r o l i d e s: For the production of radioactive macrotetrolides the S t r e p t o m y c e s strain TÜ 10 (= ETH 7796)[2] was grown in a nutrient medium containing 4.5% soy-meal (extr.), 8% starch, 0.3% sodium nitrate, 0.4% calcium carbonate and 1% glucose. The pH of the solution before sterilization was brought to 6.5. The fermentation was carried out in Erlenmeyer flasks, each containing 50 ml of nutrient solution, at 28°C on a rotational shaker (200 rpm). A slow stream of oxygen was passed through the flasks. After 72 hours of fermentation 1% of glucose containing 0.1 mCi u-^{14}C-glucose was added and the fermentation continued for additional 24 hours. The broth was then centrifuged for one hour (3500 rpm), the sedimented mycelium washed with deionized water and freeze-dried. The mycelium from 200 ml of culture was extracted with acetone in a soxhlet apparatus and the crude extract (0.913 g) chromatographed on a column of 20 g of silicagel. From the evaporated eluate (0.818 g) the nonactin was recovered by crystallization from methanol.[2] After two recrystallizations the colourless crystals had a mp. 140-142°C (yield 270 mg). In the mother liquors the homologous macrotetrolides[3] could be found by t.l.c.

By mass spectrometry it was shown that the crystalline preparation was a mixture of approximately 75% nonactin, 23% monactin and 2% dinactin.[4] The specific radioactivity was 8750 cpm/mg.

E l e c t r o l y s i s e x p e r i m e n t s have been carried out using porous polyvinyl chloride (Porvic S, Porous Plastics Ltd., Dagenham Dock, Essex, England) as inert support for the actual membrane material which was a solution of 6 mg/ml macrotetrolide mixture in octanol-2 previously saturated with dilute hydrochloric acid of pH 3.5. Three such membranes with a diameter of 19 mm and 0.75 mm thickness (pores: 10 micron, 85% of the volume) have been stacked,

Table 1 Net Transport of Macrotetrolide Within a Bulk Membrane Connected with K^+-Transport Through the Membrane in a Potential Gradient

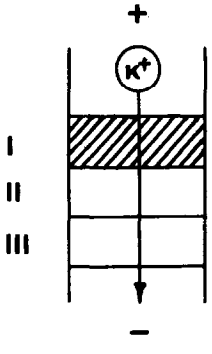
Experimental set-up



Experiment	K^+ transported through entire bulk membrane (I to III) [10^{-8} moles]	Macrotetrolide transported out of section I [10^{-8} moles]	Macrotetrolide transported into section III [10^{-8} moles]
1	9.2	- 11.5	11.3
2	11.3	- 14.1	11.8
3	8.2	- 7.0	8.7
4	10.0	- 8.4	8.5
Standard deviation	± 10 %	--	--

Table 2 Transport of Macrotetrolide from Section I to Section III of the Bulk Membrane

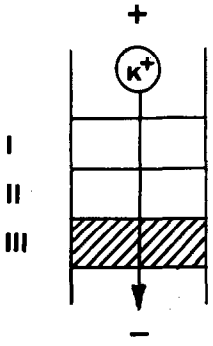
Experimental set-up



Experiment	K^+ transported through entire bulk membrane (I to III) [10^{-8} moles]	Macrotetrolide transported from section I to III *) [10^{-8} moles]
1	11.8	1.7
2	11.5	3.0
3	12.2	3.0
4	11.8	1.6
Standard deviation	± 10 %	± 0.8

*) Corrected for diffusion

Table 3 Macrotetrolide Transported in Opposite Direction to K^+-Transport (Section III to I)

Experimental set-up	Experiment	K^+ transported through entire bulk membrane (I to III) [10^{-8} moles]	Macrotetrolide transported in opposite direction to K^+ transport*) (III to I) [10^{-8} moles]
	1	11.2	1.2
	2	11.9	1.1
	3	12.3	0.0
Standard deviation		± 10 %	± 0.8

*) Corrected for diffusion

forming a triple membrane of 2.25 mm thickness. This composite bulk membrane was mounted as described earlier[1)8)] exposing an area of 80 mm^2 (10 mm diameter) to the inner and the outer solutions (0.1 M aqueous potassium chloride, pH 3.5). For the electrolysis a platinum electrode was inserted in each of the compartments and during one hour a voltage of 30 V was applied. The amount of potassium ions transported through the bulk membrane during electrolysis corresponds to 42% of the current-time integral, as determined earlier.[1)]

In one series of 4 experiments all three sections of the composite bulk membrane have been impregnated separately with labelled macrotetrolide solution of identical specific radioactivity. The results are presented in table 1.[14)]

In a second series of experiments the antibiotic in only one of the sections of the bulk membrane was labelled, while the total concentration of antibiotics in all three sections of the bulk membrane was identical (tables 2 and 3).

M e a s u r e m e n t o f r a d i o a c t i v i t y : The amount of macrotetrolide transported from one section of the membrane to the other was determined

by measurement of the radioactivity contained in the separate sections of the composite bulk membrane by liquid scintillation counting. The samples were prepared by extracting each membrane section with 1 ml methanol and diluting with 15 ml scintillator (7‰ Butyl-PBD in toluene).

RESULTS AND DISCUSSION

The results are given in tables 1-3. The data presented in table 1 show that the transport of potassium ions across the membrane is obviously connected with a transport of macrotetrolide molecules, in contrast to the stack mechanism (figure 1). In agreement with the 1:1 complex formation, one potassium ion is accompanied by one molecule of antibiotic (table 1). The concentration gradient between section I and III at the end of a typical experiment is approximately 20%.

In a free carrier or pore mechanism the macrotetrolide molecules should be transported directly across the entire bulk membrane since there occurs no exchange of ions between the ligands once they are complexed. Therefore the increase of the radioactivity in section III should be the same, whether the macrotetrolide in section II and III is labelled or not. This is clearly not the case. When

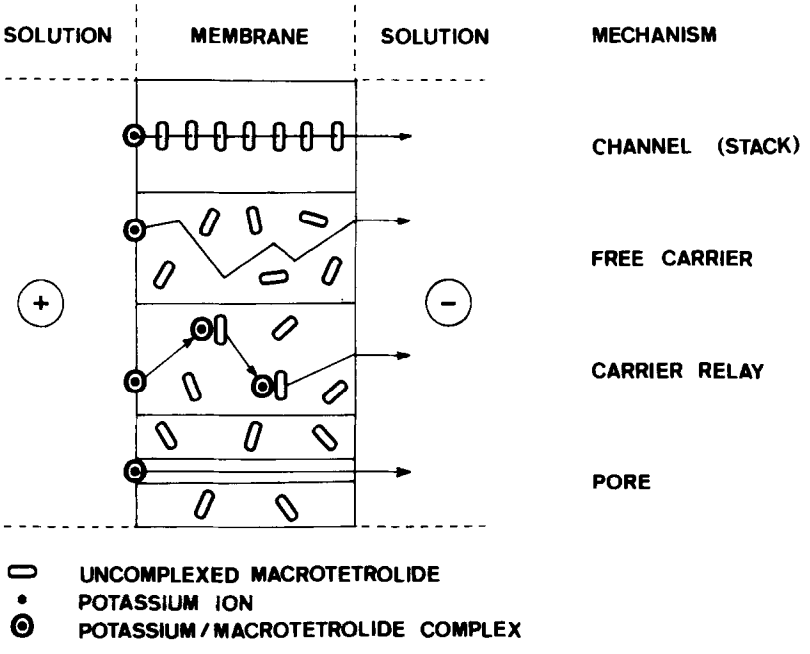


Figure 1: Transport Mechanisms

only section I is labelled (table 2) there is a significantly smaller increase of radioactive macrotetrolide concentration in membrane III compared with the experiments with uniform labelling in all three sections (table 1), indicating an exchange of ions within the unlabelled carrier in membrane II. Therefore a free carrier or pore mechanism can be excluded. The results in table 3 confirm that within the limit of error there is no observable transport of macrotetrolide in the direction opposite to K^+ transport, as expected by all the proposed mechanisms. All the results presented are consistent only with the carrier relay mechanism which controles the transport of K^+ in the bulk membranes studied. In principle a similar mechanism can be assumed for the alkali cation transport in bilayers and biological membranes. However, since these are much thinner (<100 Å) than the bulk membranes studied and since the average distance between two macrotetrolide molecules is comparatively large (∿ 60 Å in our experiments) the exchange of ions becomes negligible, and the carrier relay mechanism should degenerate into a free carrier mechanism.

A more detailed discussion of the implication of these results as well as transport data using other antibiotic carriers will be given in Helvetica Chimica Acta (Switzerland).

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