UNCOUPLER ANTAGONISM OF VALINOMYCIN INDUCED BILAYER MEMBRANE CONDUCTANCE

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Summary

We have found that an uncoupler of oxidative phosphorylation, tetrachloro-2-trifluoromethylbenzimidazole (TTFB), can block valinomycin induced potassium ion, (K^+), conductance through bilayer membranes. Blocking is most pronounced at high K^+ concentration, ($\geq$ 0.1 M), and at a pH greater than the pK of TTFB. A blocking mechanism involving competition for membrane sites, together with association of oppositely charged species within the membrane, is proposed.

Introduction

A wide variety of compounds have been reported to modify the electrical properties of lipid bilayer membranes. Among these are the cyclic antibiotic, valinomycin,[1] which sharply increases membrane conductance in the presence of K^+ ion, and the uncoupler of oxidative phosphorylation, tetrachloro-2-trifluoromethylbenzimidazole,[2] or TTFB, which produces large pH dependent increases in membrane conductance. Neutral valinomycin facilitates K^+ transport by formation of a lipid soluble charged 1:1 complex with the ion, with conductance being described in terms of appropriate chemical equilibria between the membrane and the aqueous phases,[3] or in chemical kinetic terms.[4] The two descriptions are equivalent[5] in the ohmic or low current limit, which applies to the experiments described below. Though the uncouplers, classifiable as lipid soluble weak organic acids, are considered to enhance membrane conductance by promoting proton or uncoupler anion transport, several specific mechanisms by which they may act have been proposed.[6-9] Though differing considerably in their details, all mechanisms can account for the pronounced maximum in conductance measured versus pH, at a pH near the

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pK of the uncoupler. In the case of TTFB, however, a high residual conductance at pH > pK has also been observed[10,11] and attributed to direct transport of the anion, $TTFB^-$. The pK of TTFB is reported[12] to be 5.04.

McLaughlin[13] has recently reported bilayer membrane conductance measurements using the uncoupler 2.4-dinitrophenol (DNP), together with the macrotetralide antibiotic, nonactin, which also transports K^+ through membranes by formation of a lipid soluble 1:1 complex. His observation of sharply increased nonactin-K^+ conductance, upon addition of DNP, provide convincing evidence for the enhancement of negative membrane surface potential due to adsorption of DNP^- ions. Though our observations at low ionic strength are qualitatively similar to those of McLaughlin, we have found that valinomycin-K^+ conductance is effectively abolished by TTFB when the ionic strength of the aqueous phase is maintained at 1.0 M. These observations are detailed below and their implications are considered.

Materials and Methods

Bilayer membranes were formed by the brush technique in a conductance cell of teflon and pyrex construction. The electrical measurements employed apparatus previously described,[14] operating at voltage levels in the ohmic range of membrane conductance. Salt solutions were prepared from reagent grade materials using distilled and deionized water. Ethanolic solutions of TTFB were prepared in varying concentrations between 10^{-4} and 10^{-1} M. Small aliquoits of these were added to the cell to secure the desired concentration of uncoupler. Valinomycin was similarly introduced using 10^{-3} M ethanolic solution. Resulting ethanol concentrations in the aqueous phases never exceeded 1/2 vol. %.

The membrane lipid used was phosphatidyl ethanolamine (PE), prepared in our laboratory by solvent extraction from *E. coli*, followed by fractionation on a DEAE cellulose column. Purity of the lipid was verified by silica gel thin layer chromatography.

In a typical set of measurements involving both valinomycin and TTFB, valinomycin was added first, after the membrane was fully black. Then at least fifteen minutes were allowed for attainment of a stable conductance. Then stepwise additions of TTFB were made, increasing its concentration from 10^{-8} to 10^{-4} M. Sufficient time, usually at least five minutes, was allowed after each addition for the membrane conductance to stabilize. It was generally possible to cover the entire range of TTFB concentration with a single membrane.

Results and Discussion

Typical results are illustrated in Fig. 1. The aqueous solu-

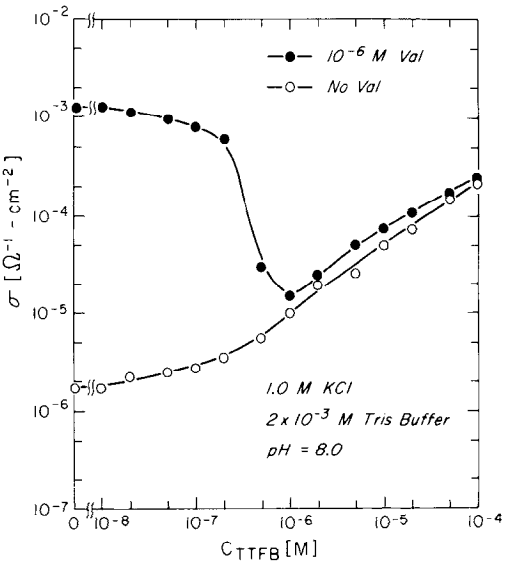

Fig. 1. The blocking of valinomycin-K^+ conductance by TTFB is illustrated (solid circles). At TTFB concentrations in excess of 10^{-6} M the conductance does not differ significantly from that observed in the absence of valinomycin (open circles).

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tions bathing the membrane contain 1.0 M K Cl and 2.0 mM tris (hydrorymethyl) aminomethane as buffer, adjusted to pH = 8.0 with H Cl. In the absence of valinomycin a monotonic increase of membrane conductance with TTFB concentration is observed. The high membrane conductance observed in the presence of 10^{-6} M valinomycin is, however, reduced upon addition of TTFB. In fact, at TTFB concentrations in excess of 10^{-6} M, the observed membrane conductance can be accounted for quantitatively in terms of conductance due to TTFB alone.

When the K Cl concentration is lowered to 10^{-3} M, however, the markedly different results illustrated in Fig. 2 are obtained. We note first that the valinomycin-K^+ conductance, in the absence of TTFB, is of the same order as (actually somewhat higher than) that observed at a K Cl concentration of 1.0 M. This observation implies the presence of a significant negative surface potential

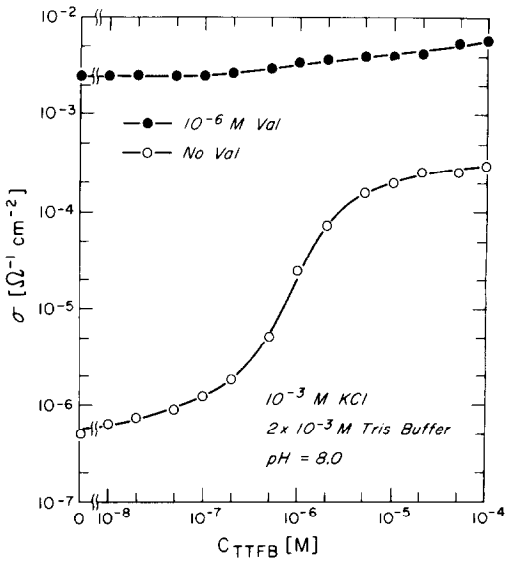

Fig. 2. Evidence of negative membrane surface potential is apparent at low ionic strength. Increasing negative potential, upon addition of TTFB, enhances conductance due to the valinomycin-K^+ complex.

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on the membranes at low ionic strength. The increase of valinomycin-K^+ conductance upon addition of TTFB indicates that the negative surface potential is enhanced by adsorption of $TTFB^-$ ions to the membrane surfaces. Such an interpretation parallels that given to the work of McLaughlin[13]. Though blocking of valinomycin-K^+ conductance is not evident in Fig. 2, its enhancement is much less pronounced than the nearly two orders of magnitude increase in nonactin-K^+ conductance observed by McLaughlin upon addition of DNP.

In an effort to minimize the effects of membrane surface potential we have made a series of measurements at different K^+ ion concentrations, while maintaining the ionic strength at 1.0 M by appropriate additions of Li Cl. The results are shown in Fig. 3, where it is seen that blocking of valinomycin-K^+ conductance becomes more pronounced and sets in at lower TTFB concentration as the K^+ ion concentration is increased.

The blocking which we have observed is consistent with a model of transport through bilayer membranes which envisages the binding of the permeant species to one of a fixed number of

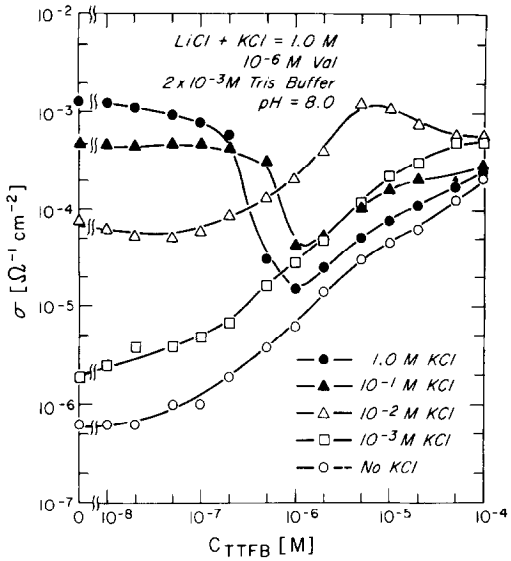

Fig. 3. Blocking at various K^+ concentrations, with total ionic strength fixed at 1.0 M, is illustrated. Blocking of valinomycin-K^+ conductance sets in at lower TTFB concentration as the K^+ concentration is increased.

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sites on or within the membrane. Such binding is assumed to be prerequisite to transport. A higher affinity of $TTFB^-$ for the sites could lead to displacement of the valinomycin-K^+ complex, and hence to the observed blocking. Such a "fixed density of sites" model was introduced by Bruner[8] in connection with the uncoupler problem. A model has also been introduced by Träuble[15] for the permeation of small solute molecules by their inclusion in "kinks" in the hydrocarbon membrane core. This model, involving a fixed number of kinks as sites, could also predict blocking.

In chemical terms the fixed sites model, applied to the system studied here, could be represented by the set of heterogeneous reactions,

$$V_{aq} + S_m \rightleftarrows V_m \quad (1)$$

$$K^+_{aq} + V_m \rightleftarrows VK^+_m \quad (2)$$

$$A^-_{aq} + S_m \rightleftarrows A^-_m \quad (3)$$

where V_{aq} is uncomplexed valinomycin in the aqueous phase, S_m represents unoccupied membrane sites, V_m is valinomycin occupying membrane sites, K^+_{aq} is potassium ion in the aqueous phase, VK^+_m is the valinomycin-K^+ complex occupying membrane sites, and A^-_{aq} and A^-_m represent uncoupler ions in the aqueous phase and on membrane sites respectively. The system would be subject to the constraint,

$$S_m + V_m + VK^+_m + A^-_m = S_o \qquad (4)$$

where S_o is a constant equal to the density of membrane sites.

Analysis indicates that blocking sets in at too low a concentration of TTFB to be accounted for in terms of this simple model. This more rapid blocking can, however, be accounted for if an association reaction of the form

$$VK^+_m + A^-_m \rightleftarrows VKA_m \qquad (5)$$

is assumed to occur. In this case the neutral complex VKA_m must be included as an additional term on the left of Eq. (4).

It is important to note, however, that the assumption of a fixed number of sites is still necessary to account for blocking, even in the presence of the association reaction. Thus if the concentrations of V_m (and hence VK^+_m) and A^-_m were fixed by simple partition equilibria with V_{aq} and A^-_{aq} respectively, then the concentration of VKA_m could simply adjust to any value required by the equilibrium constant of the association reaction. This adjustment would not modify the membrane concentrations of charged species, hence the conductance due to each would contribute additively to the total membrane conductance.

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