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Charge Transfer Mediated by Nigericin in Black Lipid Membranes*

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Abstract

Nigericin, in the concentration range (10^{-6}M or higher) at which it uncouples intact mitochondria, was found to increase the conductance of black lipid membranes (BLM) by several orders of magnitude. The dependence of the membrane conductance on pH and K^+ concentration suggests a mechanism for the transfer of charge mediated by this ionophore based on a mobile dimer with both nigericin molecules protonated and complexed with one K^+. This charged complex accounts for the uncoupling effect observed in intact mitochondria.

Introduction

Nigericin is a chemically well-characterized monocarboxylic antibiotic which forms neutral complexes with monovalent cations [1].

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It has been demonstrated in bulk-phase systems that nigericin acts as an electroneutral ionophore that exchanges K^+ for H^+ down a concentration gradient [2–4]. However, experiments performed on a variety of natural and artificial membranes indicate that this antibiotic has two effects depending on the range of concentrations utilized, 10^{-9} M–10^{-7} M and 10^{-6} M.

In intact mitochondria, low nigericin concentrations prevent the uptake of several anionic substrates [5, 6] by altering the transmembrane pH gradient [7] without apparently affecting the membrane potential. In submitochondrial sonicated particles (SMSP) and bacterial chromatophores, this ionophore uncouples oxidative phosphorylation and photophosphorylation, respectively, in the presence of K^+ and valinomycin or permeable anions such as nitrate (in SMSP), sulfate (in chromatophores), or tetraphenylboron (in SMSP and chromatophores) [8–12]. These latter effects appear to be caused by the simultaneous collapse of both components of the electrochemical H^+ gradient [13, 14], namely the membrane potential and the transmembrane pH gradient mediated by the corresponding pairs. In spheroplast particles of *Micrococcus denitrificans* [15] and protoplast sonic vesicles of *Mycobacterium phlei* [16], low nigericin concentrations, in the presence of valinomycin, uncouple oxidative phosphorylation apparently by the same mechanism which operates in SMSP and bacterial chromatophores. In chloroplasts the ionophore uncouples photophosphorylation by collapsing the pH gradient of the organelles in a K^+-dependent manner while the membrane potential is abolished by the permeation of chloride ions [17–19]. Additional experiments showing that nigericin acts as an electroneutral exchanger have been performed in microsomes, erythrocytes, *Streptococcus faecalis,* and phospholipid vesicles [3, 20, 21].

The effects described above are obtained at low antibiotic concentrations ($\leqslant 10^{-7}$ M) which do not affect either oxidative phosphorylation in intact mitochondria [22] or the ohmic resistance of BLM [3, 23]. On the other hand, concentrations of nigericin greater than 10^{-7} M uncouple oxidative phosphorylation in intact mitochondria with an absolute requirement for K^+ [24, 26]. According to the chemosmotic hypothesis for energy coupling [13, 14], the uncoupling effects of nigericin could be attributed to the collapse of the membrane potential as a consequence of the transfer of net charge across the mitochondrial cristae membrane. There are indications that nigericin ($\geqslant 10^{-6}$ M) modifies the electrical resistance of BLM [21, 27, 28]. We report here that in fact, nigericin, at concentrations in the order of 10^{-6} M, catalyzes the transfer of net charge across BLM. The dependence of membrane resistance on pH and K^+ concentrations was studied. On the basis of these results a mechanism by which nigericin transfers net charge across membranes is proposed.

Experimental Procedures

BLMs were formed and their electrical properties studied by the Mueller and Rudin method [29] as described in detail [30–32].

The membranes were formed from a solution of 2% purified (or commercial grade as indicated in each legend figure) soybean phosphatidylcholine (Sigma Laboratories), in *n*-octane (A.R. grade reagent from Matheson Coleman and Bell, Norwood, Ohio). Phosphatidylcholine was purified according the the Singleton method [33] and its purity determined as a single spot in thin-layer chromatography (TLC). The nigericin used was kindly given by Dr. Marvin Gorman (Eli Lilly Laboratories, Indianapolis, Indiana) and Dr. Julius Berger (Hoffman-La Roche Laboratories, Nutley, New Jersey). All other chemicals were reagent grade. Glass redistilled water was used throughout all experiments. The antibiotic was dissolved in ethanol and added to both compartments at room temperature (22° C) with constant stirring. After membrane formation, the membrane resistance was measured by the application of a calibrated constant current pulse and display of the resulting membrane potential. From the steady-state value of this potential, the membrane resistance (R_m) and conductance, G_0 ($G_0 = 1/R_m$) was calculated. Each point represents an average of at least six experiments. The bars indicate the standard deviation. The slope was calculated by the minimal square method. Individual experimental conditions are indicated in the figure legends.

Results and Discussion

The chemosmotic hypothesis of energy-coupling postulates that an uncoupler is a species able to catalyze net proton transfer and hence to collapse both the electrical and chemical components of the electrochemical proton gradient.

We have shown that concentrations of nigericin greater than 10^{-7} M in the presence of K^+ uncouple oxidative phosphorylation in intact mitochondria [24–26]. It is conceivable that nigericin exists in a given molecular form, complexed with metal cations, capable of collapsing the membrane potential. Several alternatives having nigericin as an electrically charged entity can be envisaged:

1. The anion nigericin $(N)^-$ is the permeant species. Uncouplers, such as CCCP (*m*-chlorocarbonylcynidephenylhydrazone), behave in a similar manner [34].
2. Nigericin is protonated and complexed with one K^+ ion $(NHK)^+$.
3. Nigericin is dissociated and complexed with two K^+ ions. This

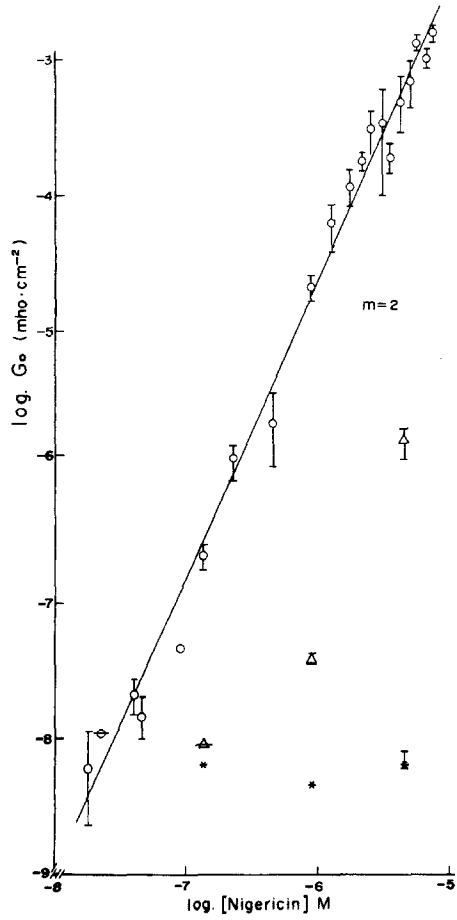

Figure 1. The dependence of the electrical conductance of BLM on nigericin concentration. The buffer medium contains: 5×10^{-2}M Tris–HCl at pH 8.0 and salts as indicated: ○ 100 mM KCl, △ 100 mM NaCl, * 100 mM choline chloride. The phospholipid was commercial grade.

alternative appears unlikely since the internal core of the antibiotic is not large enough to clathrate two ions.

4. A dimer consisting of one undissociated antibiotic molecule and another one dissociated $(N_2H)^-$, as is the case with uncouplers such as the DNP (2,4-dinitrophenol) [35, 36] and DTFB (5,6-dichloro-2-trifluoromethylbenzimidazole) [36].

5. A dimer with both nigericin molecules protonated and complexed

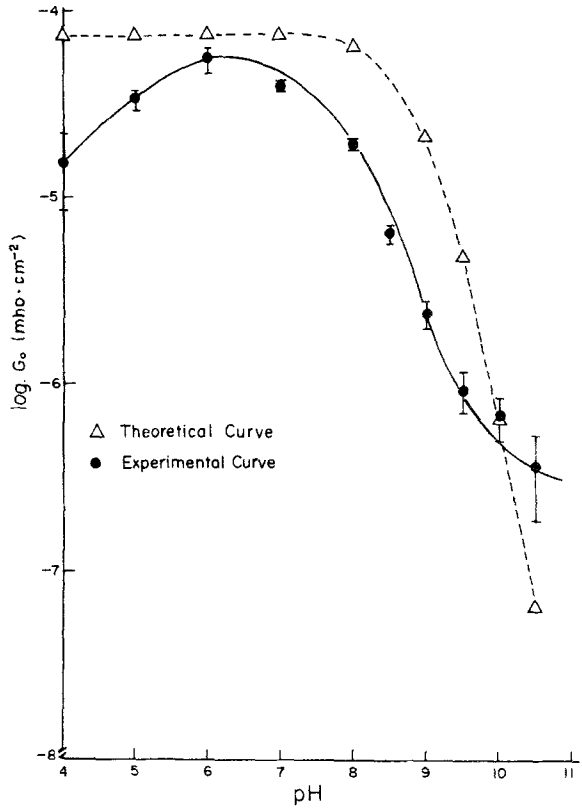

Figure 2. Dependence of conductivity upon pH at a fixed nigericin concentration of 1.8×10^{-6}M. The total amount of K^+ was 100 mM. The buffer medium from pH 4.0–8.5 was the following: 5 mM citric acid, 5 mM K_2HPO_4 adjusted with triethanolamine (TEA); from pH 9.0–10.5 was: 5 mM histidine and 5 mM K_2HPO_4 adjusted with KOH. The phospholipid was chromatographycally purified.

with one K^+. There is no report of this type of carboxylic antibiotic [31, 32, 34–37].

Our approach has been to rule out four alternatives, retaining the most probable one.

The addition of nigericin to BLM in the presence of K^+ leads to a net increase in the electrical conductance measured. Such an increase is negligible when K^+ is substituted in the medium by choline chloride. The dependence of the membrane conductance on ionophore concentration is presented in Fig. 1. The circles designate the conductance measured in the presence of K^+ after the application of a voltage of 50 mV. The

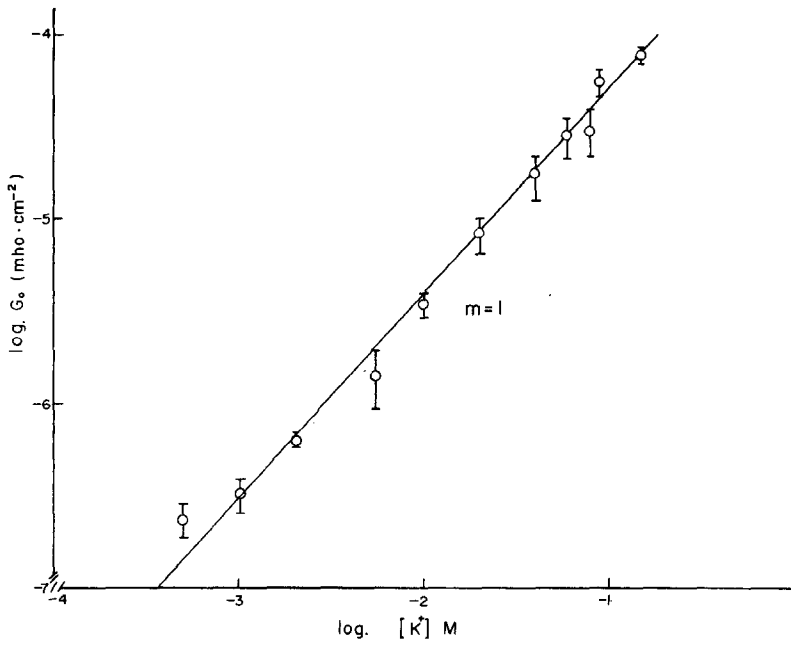

Figure 3. The dependence of the conductance mediated by nigericin on different concentrations of K^+. Nigericin was 1.8×10^{-6} M. The solution buffer: 5 mM H_3PO_4 adjusted with TEA at pH 6.0. Different media were prepared with the corresponding concentrations of K^+. The phospholipid was chromatographycally purified.

log–log plot of conductance (G_0) vs. nigericin concentration exhibits linear behavior with a slope of 2, indicating that G_0 depends on the square of the nigericin concentration. This result suggests that the species which catalyzes K^+ movements across the membrane is a dimer. The experiment rules out the first three alternatives which imply that nigericin acts as a monomer.

We now turn to the question: Is the dimer formed by one dissociated nigericin molecule and another undissociated one or by two protonated nigericin molecules? Thus, the dependence of the membrane conductance on the pH of the bathing solution, at constant nigericin and K^+ concentrations, was studied. The experiment is illustrated in Fig. 2. It is shown that the maximal conductance reached is at a pH lower than the pK of nigericin (which is known to be 9.1 [3]), indicating that both antibiotic molecules are protonated. The line through the triangles in Fig. 2 illustrates the theoretical curve obtained as follows: the concentration of nigericin protonated at different pH was calculated knowing the pK of nigericin. From the log–log plot of the conductance vs. nigericin concentration, the (NH) obtained as mentioned can be

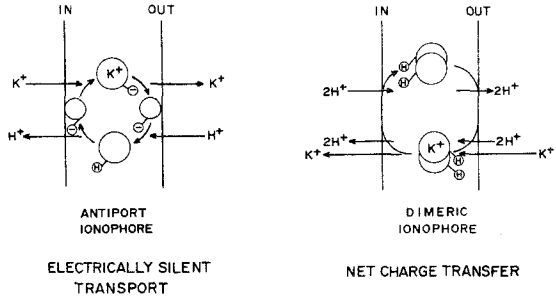

Figure 4. Mechanism of ion fluxes and uncoupling mediated by high concentrations of nigericin in intact mitochondria.

interpolated to obtain the conductance value expected. As can be seen, the experimental and theoretical curves are in close agreement, suggesting that the two molecules of nigericin which form the translocating complex are undissociated.

Therefore, the fifth alternative is the only one left. Further support for this proposal is derived from the experiment illustrated in Fig. 3, where the conductance in the presence of a constant nigericin concentration and at a given pH is plotted in a log–log manner against various concentrations of K^+. The linear dependence on K^+ concentration with a slope of 1 indicates that only one K^+ is involved in the transferring complex.

The fact that there is a second-power dependence of membrane conductance on nigericin concentration, a first-power dependence of the conductance on the K^+ concentration, and a maximal conductance at a pH lower that the pK of nigericin indicates that the last of the five mechanisms proposed is the only one which exhibits the experimentally found characteristics. It appears clear, therefore, that a complex consisting of two protonated nigericin molecules and one K^+ ion can account for the net transfer of charge across the membrane. From these considerations, we suggest that the uncoupling effect observed in intact mitochondria, at high concentrations of nigericin, is due both to the dimer which collapses the membrane potential and to the monomer that abolishes the transmembrane pH gradient, as postulated in the coupling scheme of Mitchell (Fig. 4). Experiments on selectivity and characterization of this complex are in progress.

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