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POTENTIAL GENERATION IN BILAYER LIPID MEMBRANES IN THE NADH–FLAVIN MONONUCLEOTIDE–UBIQUINONE-6–O_2 SYSTEM

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SUMMARY

Membrane conductance and generation of transmembrane potential by the NADH oxidation reactions in the NADH–flavin mononucleotide–ubiquinone-6–O_2 system have been studied. It has been shown that in solutions of a relatively low buffer capacity at pH 5.8 in the presence of a proton carrier, a potential is generated, the value of which depends on the concentration of the reducer and amounts to 40–60 mV. In the absence of a proton carrier at pH 8, a potential arises, which suggests a transmembrane negative charge transfer. Bilayer lipid membranes have been shown to possess proton selectivity if the reaction is run at pH 3.7. At a pH higher than 5.8 the proton selectivity disappears. Schemes of potential generation in lipid bilayers in different conditions are suggested and discussed.

INTRODUCTION

In mitochondria, redox reactions may result in the generation of transmembrane potential. In model systems with lipid bilayers and micelles this process may be observed only in simplest systems, for example, I^0/I^-, Fe^{+2}/Fe^{+3}, ascorbic acid–ferro-ferricyanide [1–3]. However, recently some papers appeared demonstrating the presence of electron transfer [4] and a photoelectric effect in lipid bilayers and micelle membranes packed with pigments.

Ubiquinone-6 (Q-6) is a natural electron carrier in the respiratory chain of mitochondria. It was interesting to study the properties of this quinone as an electron carrier across an artificial phospholipid membrane. NADH was used as a reducer, as it is known to participate in electron transfer in the mitochondrial respiratory chain.

In this work, oxidation of NADH in the presence of Q-6 in lipid bilayers was studied and two major effects were revealed: the first is the generation of a transmembrane potential which seems to be due to charge transfer in redox reactions on

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the opposite sides of the membrane; the second is a decrease in the proton concentration in the unstirred aqueous layer or in the boundary layer of the lipid bilayer due to a potent oxidation of NADH by oxygen, involving Q-6 on one side of the membrane.

METHODS OF MEASUREMENT

Transmembrane potentials and lipid bilayer conductance, in which charge transfer is implemented by Q-6, were studied. NADH, capable of reducing quinone dissolved in lipids, was added to the solution on one side of the membrane. Oxygen dissolved in water served as an oxidizing agent. All the lipid bilayers were prepared by the standard method either from a mixture of lecithin with cholesterol or from bovine brain lipids and were formed over a hole in a teflon vessel [2]. Q-6 was mixed with a lipid solution in decane at a concentration of 10–20 mg/ml. The presence of Q-6 hardly affected the membrane conductance. The membrane thickness was controlled by changes in the capacitance at 0.2 cps; buffer solutions and reagents were prepared with distilled water. A magnetic stirrer was used for mixing solutions in the cell. Potentials were measured by means of AgCl electrodes connected with the solution by agar–agar bridges filled with 0.1 M KCl. The potential was controlled with a vibron electrometer, model 62A (Richmond) connected to a recorder.

RESULTS

The experiments carried out in this work have shown that, in the presence of quinone dissolved in lipid bilayers, the sign of the transmembrane potential corresponds to the transmembrane transfer of a negatively charged particle from NADH to the opposite side of membrane.

Fig. 1a (curves 1 and 2) shows the dependence of the conductance and lipid bilayers transmembrane potential in the presence of Q-6 on the quantity of NADH added. Addition of NADH without Q-6 does not cause an increase in the conductance or generation of membrane potential (curves 3, Fig. 1a, 1b). Addition of tetrachlorotrifluoromethylbenzimidazol (TTFB) or pentachlorophenol, oxidative phosphorylation uncouplers, leads to an increase in the value of the potential (Fig. 2). Addition of TTFB to lipid bilayers increases the membrane conductance by 3–4 orders. The effect of TTFB does not depend on which side of the membrane it is added. At the same time, the value of the NADH-generated membrane potential depends on the buffer capacity of the bathing solutions. It is shown in Fig. 3 that as the buffer capacity of the solution increases the membrane potential in the presence of an uncoupler goes down (curve 1, Fig. 3). In the experiments where no TTFB was added to the lipid bilayers the potential also decreases as the buffer capacity of the solution is increased (Fig. 3, curve 2). We have shown by relevant experiments that only at a sufficiently high proton concentration in the unstirred aqueous layer, a proton carrier is formed in the lipid bilayer at the expense of the NADH–Q-6–O_2 reaction.

At pH 5.8 and 200 mM buffer capacity, a pH gradient of 0.2 units results in the generation of a 5 mV additional potential of the respective signs [5]. At a lower buffer capacity, but at a more acidic pH (4.2), the pH gradient also results in a potential being formed on the membrane (Fig. 4). But in the experiments involving NADH

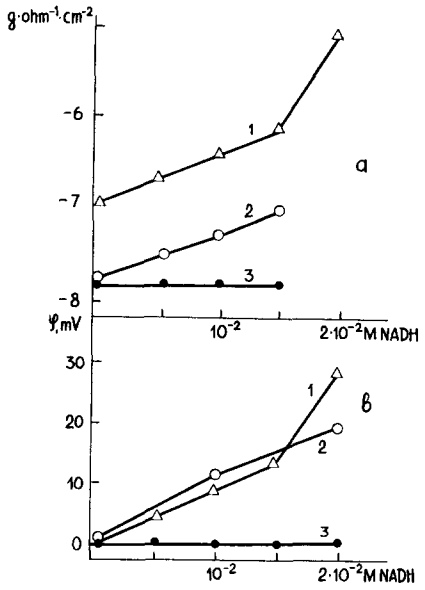


Fig. 1a. Dependence of Q-6-modified membrane conductance upon the concentration of NADH added to the solution on one side of the membrane. Buffer: 0.1 M Tris+0.1 M citrate+0.1 M NaCl, at pH 5.8. Lipid bilayers were formed: (1) from lecithin with cholesterol and Q-6; (2) from bovine brain lipids and Q-6; (3) from bovine brain lipids without Q-6.

Fig. 1b. Dependence of transmembrane potential in the lipid bilayer, modified and not modified with Q-6 on NADH concentration. (1) Modified lipid bilayer formed from lecithin with cholesterol; (2) Modified lipid bilayer formed from bovine brain lipids; (3) Non-modified membrane from bovine brain lipids.

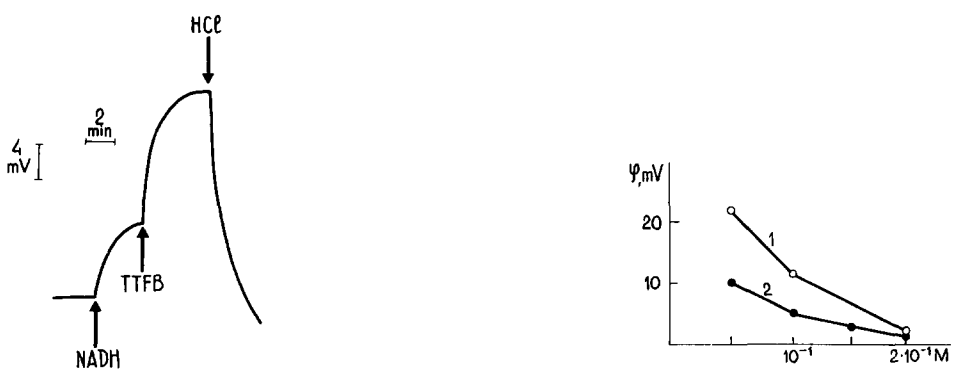
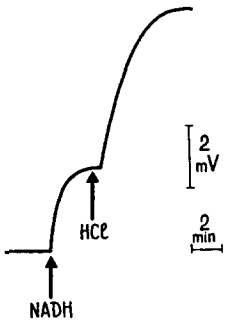


Fig. 2. Kinetics of generation of transmembrane potential on lipid bilayer with Q-6 on addition of NADH ($0.5 \cdot 10^{-2}$ M). Buffer: 0.05 M Tris+0.05 M citrate+0.05 M NaCl; pH = $5.8 \cdot 10^{-5}$ M TTFB was added, HCl and NADH were added at the same side of membrane; transmembrane Δ pH $\approx$ 0.6.

Fig. 3. Dependence of the transmembrane potential on the concentration of buffer solution: citrate–Tris–NaCl, 1 : 1 : 1, pH = 5.8, NADH ($0.5 \cdot 10^{-2}$ M). (1) Lipid bilayers with Q-6 and TTFB; (2) Lipid bilayers with Q-6 without TTFB.



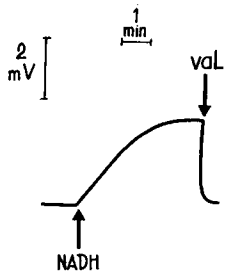


Fig. 4. Kinetics of generation of transmembrane potential in buffer: 0.06 M citrate+0.06 M NaCl, pH 4.2. NADH and HCl were added at the different sides of the membrane. (HCl shifts pH to 4.)

Fig. 5. Removal of the potential from NADH by adding K^+ with valinomycin ($2 \cdot 10^{-6}$ M). Buffer: 0.1 M Tris+0.05 M NaCl+0.05 M KCl, pH 8.4.

and Q-6, where the buffer capacity is low (50 mM), at pH 5.8 the pH gradient does not change the potential on the membrane formed from bovine brain lipids.

Experiments in the alkaline pH region (pH 8.4) have shown that the pH gradient does not cause a Nernst potential at a high buffer capacity. The transmembrane potential generated on lipid bilayer in these conditions may be eliminated by adding valinomycin, as seen in Fig. 5, and FCCP.

It is known that in the respiratory chain of mitochondria between NADH and Q-6 there is a flavoprotein involved in the electron transfer from NADH to Q-6.

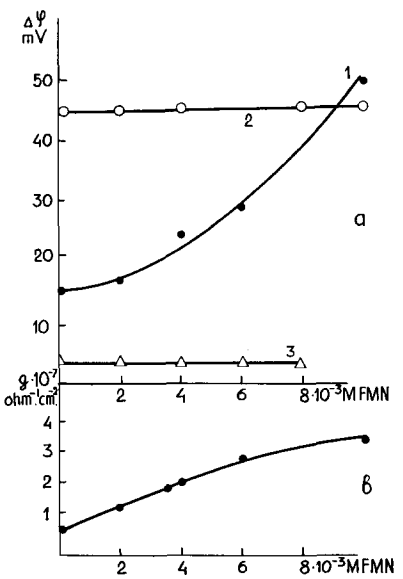


Fig. 6. The dependence of the transmembrane potential (a) and the conductance (b) in Q-6-modified lipid bilayers on the concentration of added FMN in the presence of $2 \cdot 10^{-2}$ NADH added to the same side of the membrane as FMN, (1); with 10^{-5} M TTFB, (2); non-modified membrane, (3). Buffer: 0.1 M citrate+0.1 M NaCl; pH 5.8.

Therefore it was logical to work with a model system in which the potential was generated in the following chain: NADH–FMN–Q-6–O_2. Addition of NADH and FMN to the system without Q-6 does not result in the appearance of e.m.f. in the membrane (curve 3, Fig. 6a). In the presence of Q-6, relatively low concentrations of FMN increase the conductance and the transmembrane potential on lipid bilayers when NADH is added (curves 1, Fig. 6a, 6b). The potential is eliminated by adding valinomycin. FMN added to the functioning NADH–Q-6–O_2 system in the presence of an uncoupler does not change the value of the potential generated on lipid bilayers (curve 2, Fig. 6a).

DISCUSSION

Generation of potential on lipid bilayers modified by coenzyme Q-6 is controlled by redox reactions on the membrane/electrolyte interface. In the system studied no linear dependence between the NADH concentration logarithm and the value of potential arising on the lipid bilayers is observed (Fig. 1). This demonstrates that the system of redox reactions participating in electron transfer is not in the equilibrium state at all.

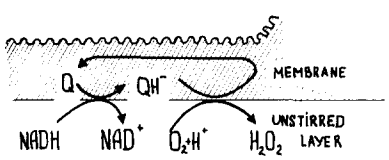
The redox reactions taking place on the interface of the membrane with an aqueous solution may be presented in the following way [6]:

$$NADH + Q\text{-}6 \rightarrow NAD^+ + Q\text{-}6\text{-}H^- \quad (1)$$

$$Q\text{-}6\text{-}H^- + H^+ + O_2 \rightarrow Q\text{-}6 + H_2O_2 \quad (2)$$

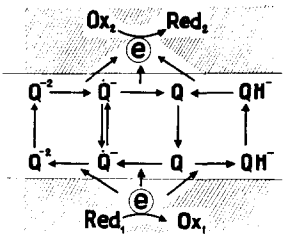
It cannot be ruled out that not only H_2O_2, but also lipid peroxides (RCOOH) are formed in the course of NADH oxidation. The latter may be directly involved in the mechanism of membrane potential generation.

The increase of the potential on the lipid bilayer as a result of TTFB or pentachlorophenol being added to the membrane (Fig. 2), after which the selective permeability towards H^+ ions increases, is indicative of a pH gradient being formed in the unstirred layer in the course of redox reaction (2) (Scheme I).



Scheme I.

This idea is supported by the fact that the membrane potential generated in the NADH–Q-6–O_2 system in the presence of TTFB decreases as the buffer capacity of the solution is increased (Fig. 3, curve 1). In the absence of the uncoupler the results were the same (Fig. 3, curve 2); this fact points to the identical nature of the potentials. In these conditions the reduced form of Q-6 probably plays the role of a proton carrier. The proton conductance of Q-6-modified membranes as well as phenols modified ones is a function of pH. In solutions of low buffer capacity, the proton conductance can be revealed only in an acidic medium at pH = 4.2, see Fig. 4, but not at pH 8. Under such conditions potential generation on the lipid bilayer may be



Scheme II.

represented as Scheme II. At pH 8.4 in Q-6-modified membranes, a small potential arises at the expense of electron exchange reactions involving ubiquinone-6. As was to be expected, in accordance with Scheme II, the pH gradient of bathing solutions does not result in an additional transmembrane potential being generated, which points to the absence of a proton carrier (Q-6–H_2) in the membrane. The electron exchange nature of the NADH-dependent potential may be proved by the following experiments: $8 \cdot 10^{-3}$ M NADH was added into one of the compartments, containing 10 mM Tris, 100 mM NaCl (pH = 8.7) and separated by the Q-6 modified membrane. Then $2.5 \cdot 10^{-2}$ M $Fe(CN)_6^{3-}$ was added into the other compartment. It was found that the membrane potential was 5 mV before and 35 mV after ferricyanide addition. This potential was shown to be abolished by TTFB. No membrane potential was found without Q-6.

As was pointed out above, when FMN is added to the functioning NADH–Q-6–O_2 system, it increases the membrane potential which may be eliminated by valinomycin. Without Q-6 no such effect is observed (Fig. 6, curve 3). These data seem to rule out the possibility of FMN being involved in transmembrane electron transfer from NADH without the participation of Q-6.

In a non-equilibrium system the effect of FMN may be accounted for by the fact that FMN, being lipid-insoluble, increases the rate of heterogenous Q-6 reduction (Fig. 6) which occurs at the interface*.

Summing up the results obtained, it may be stated that ubiquinone-6 may be involved in both proton and electron transfer across the membrane in the redox reactions occurring on the membrane/electrolyte interface. At the same time, potential generation in lipid bilayers detected in the presence of a proton carrier where redox reactions occur only on one side of the membrane, may be interpreted as indicating that Mitchell's scheme of potential generations, in which respiratory chain carriers are localized across the membrane, is not the only possible mode of charging the membrane by the respiratory chain.

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* Reduced form of Q-6 in the system (Fig. 6) may act both as an electron or proton carrier.

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