ELECTRICAL MANIFESTATION OF ION TRANSPORT ACROSS BLACK LIPID MEMBRANES GENERATED BY A Na^+/K^+ ATPase

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1. Introduction

The Na^+/K^+,Mg^{2+}-dependent ATPases which are located on the cellular external membranes, are responsible for the active transport of Na^+ and K^+. The free energy required for the transfer of these ions against their concentration gradient is supplied by ATP hydrolysis catalysed by the ATPase.

Flux measurements across liposome membranes using radioactive tracers [1–3] have established that, when 1 ATP molecule is hydrolysed, 3 Na^+ are carried from the inside to the outside of the cell, while 2 K^+ are carried in the opposite direction. The ATPases act as electrogenic pumps giving rise to a net flux of electrical charges.

The addition of both ATP and ATPase to the aqueous side of a black lipid membrane (BLM) [4] gave rise to either a short circuit current or to an open circuit membrane potential and at the same time a drop in membrane resistance. These results were obtained with an ATPase extracted from rat brain. ATPases from other origins however gave no electrical response. A decrease of the BLM resistance was observed [5] after the addition of bacterial membrane ATPase.

An attempt to reconstitute an electrogenic pump by incorporating a Na^+/K^+ ATPase into BLM resulted in transient electrical responses only with ATP and phospholipids bound to the ATPase [6]. Here, the problem of the electrogenic pump reconstitution is reconsidered using a Na^+/K^+ ATPase extracted from pork brain, special attention being paid to the incorporation kinetics. The electrical set up enables measurement of both the short circuit current and the membrane conductance. Several control experiments were performed to check that the observed electrical responses were not due to an artefact.

2. Materials and methods

The BLM (area 1 mm^2), were formed from a solution of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and cholesterol, in decane (Merck puriss), whose concentrations were, respectively: 13, 9, 8 and 26 mg/ml. The phospholipids were Supelco products and the cholesterol was from Merck.

The initial compositions of the two aqueous solutions on either side of the membrane were identical: 0.1 NaCl; 0.02 M KCl; 0.01 M $MgCl_2$. The aqueous solution was buffered (Tris 0.05 M; pH 7.4). The temperature of the cell was maintained at 37°C.

The Na^+/K^+ ATPase was a Sigma product extracted from pork brain by the method in [7]. Its activity, re-checked under our experimental conditions, was that given by manufacturers, i.e., 1 μmol P_i liberated . protein $unit^{-1}$. min^{-1}. The ATP solution added to the system was buffered with Tris (pH 7.4).

Two Ag/AgCl electrodes (area : 1 mm^2) were immersed in the aqueous solutions on either side of the membrane. The current was measured with a Keithley 427 fast-response current amplifier, connected to a chart recorder. A switch enabled measurement alternatively of the short circuit current and the steady state current under an applied 5 mV.

After BLM formation (its bimolecular state controlled by measuring its capacity using a coulostatic technique [10]) ATPase or ATP, or successively both were added to one of the aqueous solutions (inner compartment). The solution was then stirred. The final concentration of ATP was 0.04 M.

3. Results and discussion

Fig.1 shows the variations with time of the short

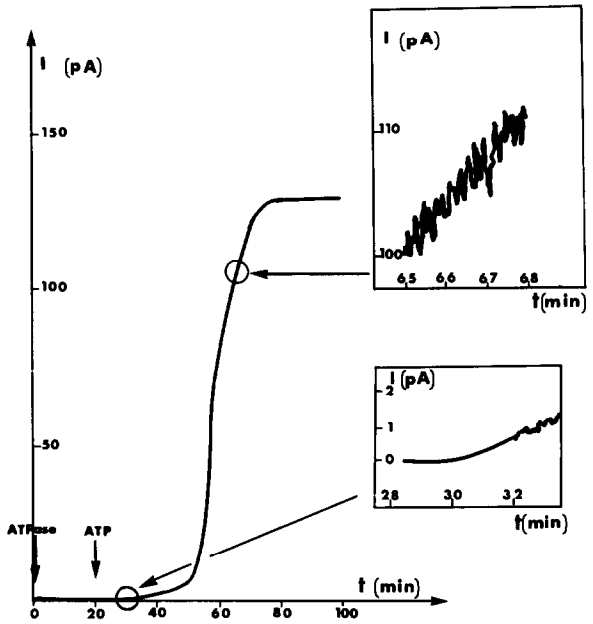

Fig.1. Variation of the short circuit current recorded after the addition of the ATPase to the innerside followed by that of ATP on the same side.

circuit current recorded after addition of ATPase to the inner compartment, followed by that of ATP on the same side. The short circuit current remained zero for several minutes. However, after a lag, it increased progressively with time and after 1 h it reached a constant ~100 pA. There was electrical noise concomitant with this.

Fig.2 shows the variations of steady state conductance with time under an applied 5 mV, during the same experiment. Equivalent EMF is the short circuit current–membrane resistance product. When the current starts to increase and until it reaches its stationary value, the equivalent EMF remains constant (5–6 mV).

The short circuit current direction corresponds to the passage of positive charges from the inner compartment to the outer one.

When only ATPase is added to the inner compartment, the current remains zero during ⩾4 h and the membrane conductance the same as before the addition of ATPase. These results are not consistent with those found in [4,5], namely that only the addition of membrane fragments containing ATPase increases the membrane conductance. When only ATP was added to the inner compartment the current remains negligible and the membrane conductance remains constant during a long time lag.

During an experiment the variation of the Ag/AgCl electrode potential was ⩽1 mV. The measured short circuit current can be considered as a transmembrane ion-transfer current since the total number of displaced electrical charges during an experiment was not compatible with a non-faradaïc current. Further, the current was not due to the H^+ produced by ATP hydrolysis since we have controlled that the pH remains constant [4,7] during the experiment.

Some detergents interact with lipid membranes and form ionic channels above a concentration level so that the membrane conductance is increased [8,9]. A similar effect, due to detergents possibly associated with the ATPase, was not responsible for the observed membrane conductance increase as it remained unchanged when the inner compartment contained ATPase alone.

Electrical noise was only observed in the presence of both ATPase and ATP and only when the current began to increase. This noise may be due to electrical perturbations associated with the incorporation

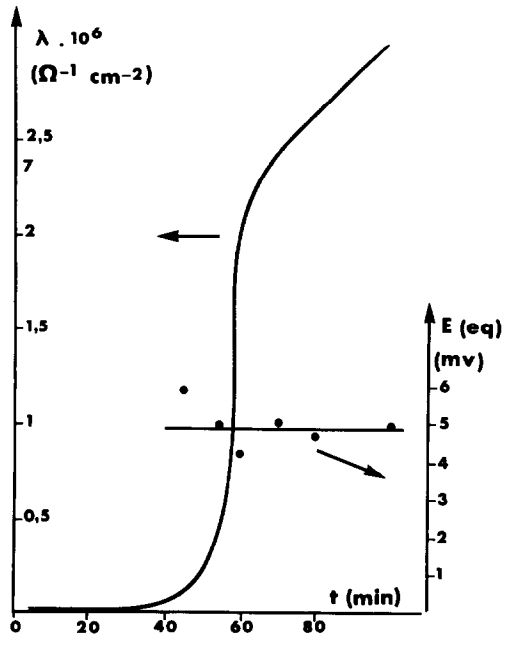

Fig.2. Variation of the steady state conductance with time under an applied 5 mV. Points (•) represent values of equivalent EMF (measured current–measured resistance product) [E(eq)].

process of ATPase into the membrane. The fact that the noise was not observed without ATP suggests that ATP–ATPase interactions are necessary for enzyme incorporation. Another possible explanation of this noise might be a statistical effect due to opening and closing of channels during the working of the ionic pump.

Membrane conductance increased gradually with time while the equivalent EMF remained constant. This is consistent with a progressive incorporation of elementary molecular pumps in the membrane. Consequently, the inner resistance of an elementary pump should be smaller than the resistance of an equivalent area of the unmodified membrane. The calculated value of the equivalent EMF corresponds to the EMF of a set of molecular pumps connected in parallel. The resulting EMF was the same as that of an elementary pump.

In the presence of ATPase, the current began to increase ~10 min after addition of ATP, the incorporation of ATPase being at least kinetically determined by its diffusion in the unstirred layer of the aqueous solution adjacent to the membrane. Further, the hydrocarbon content of the membrane decreased with time and such a change of the membrane may facilitate the ATPase incorporation into the BLM. As this incorporation is a long process, membranes studied need to have a long life time. These experiments therefore require the choice of a suitable mixture of lipids, which, in addition, constitute a favourable environment for ATPase incorporation.

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