ION SELECTIVE PROPERTIES OF PHOSPHOLIPID MEMBRANES

Z.Štefanac, B.Švigir and M.Proštenik

Institute for Medical Research,
Yugoslav Academy of Sciences and Arts, Zagreb, Yugoslavia,
Department of Chemistry, Faculty of Medicine,
University of Zagreb, Zagreb, Yugoslavia

Received February 16th, 1971

The potentiometric behaviour in an electrochemical cell of lecithin and sphingomyelin model membranes was determined under different conditions. The presence of Ca^{2+} ions in sample solutions reversibly changed the cationic response of the lecithin membrane to anionic. In analogous experiments the anionic response curve of the sphingomyelin membrane only showed a steeper slope. Protein adsorption did not affect the characteristics described.

Intact mitochondria accumulate potassium in the presence of ionophorous antibiotics, such as nonactin homologues[1,2] or valinomycin[3]. The development of a potassium selective liquid membrane electrode was based on these observations[4,5]. Of all the common uni- and bi-valent cations present in the cell the mitochondria accumulate immediately and stoicheiometrically only calcium. The accumulation is accompanied by the ejection of protons and alkalinization of mitochondria[6–10]. A great deal of experimental work is done in describing those properties of the Ca^{2+} transport system of mitochondria that endow it with considerable specificity, exceedingly high affinity and primacy over oxidative phosphorylation in most tissues[11]. It was assumed that there exist at least two sets of calcium binding sites: high affinity and low affinity sites. The view that high affinity Ca^{2+} binding reflects the action of a specific Ca^{2+} carrier in the membrane is supported by the fact that a Ca^{2+} binding protein was extracted from mitochondria[11]. Low affinity Ca^{2+} binding is believed to involve nonspecific anionic binding groups of membrane proteins and lipids[12].

Some properties of the binding sites of phospholipids were determined in experiments performed with a cephalincholesterol membrane model[13]. The association of Ca^{2+} with phosphate and carboxyl groups of phospholipids resulted in a drop of the membrane potential and a decrease of cation permselectivity[14].

In this work lecithin and sphingomyelin model membranes were studied with respect to the original potentiometric behaviour and Ca^{2+} induced changes. The observations that the Ca^{2+} accumulation ratio in mitochondria may vary considerably depending on potassium chloride concentration[15] induced us to perform measurements to outline a possible effect of potassium chloride concentration on the response of model membranes.

EXPERIMENTAL

EMF measurements. Sintered glass disc was used as a support for the compound investigated: a saturated solution of the compound was left to diffuse into the glass disc with simultaneous evaporation of the solvent. The formed glassy phospholipid layer was interposed as the membrane in the following electrochemical cell: Ag; AgCl, inner solution || membrane || sample | sat. Hg_2Cl_2; Hg. The EMF of the cell was measured at 25°C ± 0·1 with pH-meter Radiometer, Copenhagen, type PHM 4c. The assembly initially used was a liquid membrane potassium electrode[5]. Millipore filter wetted with a phospholipid solution in a suitable solvent showed several drawbacks as membrane. Millipore filter as such is characterized by residual selectivity in the membrane. Furthermore, it was not possible to achieve the formation of an adequate layer readily.

Materials. Animal sphingomyelin was isolated from bovine marrow and purified chromatographically (M. Proštenik). Animal lecithin and cardiolipin were gift of Dr B. Pende (Institute of Immunology, Zagreb). Synthetic products were acquired from Fluka AG, Buchs, Switzerland. Polyphosphoinositides were isolated from the bovine brain according to described procedure[16].

RESULTS

Alkalimetric titration curves of lecithin, sphingomyelin, and polyphosphoinositides were affected by the presence of calcium ions. The differentiation of acidity constants from the shifts in titration curves caused by Ca^{2+} ions was not possible. The results

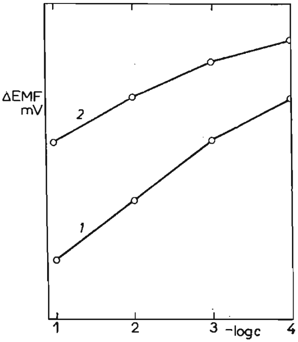

FIG. 1

EMF Differences Measured with Animal Lecithin Membrane

Inner solution: 10^{-3}M $CaCl_2$. Sample: *1* $CaCl_2$; *2* $MgCl_2$. On the EMF scale 15 mm equal 40 mV.

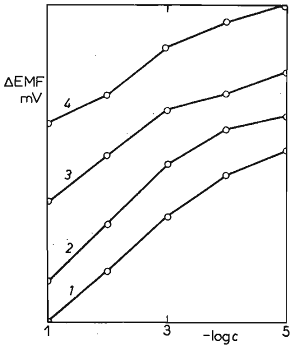

FIG. 2

EMF Differences Measured with Animal Sphingomyelin Membrane

Inner solution: 10^{-1}M-$CaCl_2$, 10^{-2}M-$MgCl_2$, 10^{-2}M-NaCl, and 10^{-2}M-KCl. Sample: *1* $CaCl_2$; *2* $MgCl_2$; *3* NaCl; *4* KCl. On the EMF scale 15 mm equal 40 mV.

of EMF measurements with these compounds as membranes in the electrochemical cell are presented graphically. The response of animal lecithin membrane in samples with decreasing $CaCl_2$ and $MgCl_2$ activities is shown in Fig. 1. Corresponding experiments with animal sphingomyelin are presented in Fig. 2 with additional data concerning NaCl and KCl samples. Samples of various salts cause potential differences given in Fig. 3 for a synthetic lecithin and in Fig. 4 for a synthetic sphingomyelin membrane. The influence of Ca^{2+} ions on the potentiometric behaviour of synthetic lecithin and sphingomyelin is demonstrated in Fig. 5 and 6 respectively. Increasing concentrations of Ca^{2+} added to samples of potassium chloride exerted an obvious diminution of the slope in the case of synthetic lecithin (Fig. 7). The response of a lecithin membrane measured in samples of variable activities of different cations in salts with different anions was in all cases a cationic response with

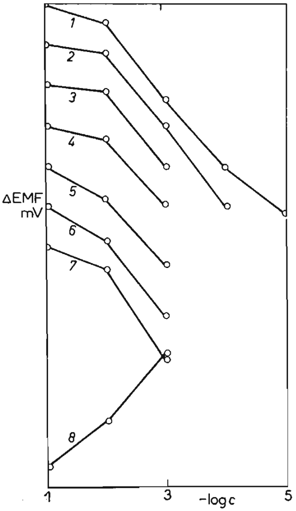

FIG. 3

EMF Differences Measured with Synthetic Lecithin Membrane

Inner solution: 10^{-3}M-KCl. Sample: *1* KCl; *2* NaCl; *3* NaJ; *4* $NaJO_3$; *5* Na_2SO_4; *6* Na_2HPO_4; *7* Na-citrate; *8* $CaCl_2$. On the EMF scale 15 mm equal 40 mV.

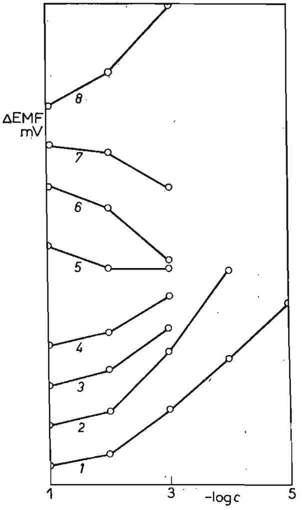

FIG. 4

EMF Differences Measured with Synthetic Sphingomyelin Membrane

Inner solution: 10^{-3}M-KCl. Sample. *1* KCl; *2* NaCl; *3* NaJ; *4* $NaJO_3$; *5* Na_2SO_4; *6* Na_2HPO_4; *7* Na-citrate; *8* $CaCl_2$. On the EMF scale 15 mm equal 40 mV.

the exception of Ca^{2+} and Mg^{2+}. Furthermore, the initially measured cationic response in a KCl sample could be reversibly changed into anionic by exposing the membrane to a Ca^{2+} solution for an hour or more. Seventy two hours after Ca^{2+} solution had been removed, the original cationic response was measured. Increasing concentrations of Ca^{2+} successively decreased the slope of the cationic response in KCl solutions. The sphingomyelin membrane, on the contrary, showed predominantly anionic response, sodium citrate, phosphate and sulphate being exceptions. Exposure of a sphingomyelin membrane to a Ca^{2+} solution only increased the slope of the original anionic response. The protein layer formed merely by adsorption on phospholipid membrane in an aqueous suspension of egg albumin presumably did not result in the formation of a lipid–protein complex and no influence on potentiometric behaviour was found. Cardiolipin and polyphosphoinositides could not be satisfactorily used for the formation of an adequate membrane.

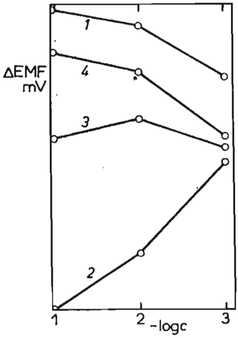

FIG. 5

Measurements Performed with Synthetic Lecithin Membrane

1 10^{-1}–10^{-3}M-KCl, measured immediately. 2 10^{-1}–10^{-3}M-KCl, measured after conditioning during 1 hour in 10^{-1}M-$CaCl_2$. *3* 10^{-1}–10^{-3}M-KCl, measured 24 hours after curve *B*; during this period the membrane was immersed in 10^{-3}M-KCl. *4* 10^{-1}–10^{-3}M-KCl, measured 3 days after curve *C*; in the meantime the membrane was immersed in 10^{-3}M KCl. On the EMF scale 6·5 mm equal 10 mV.

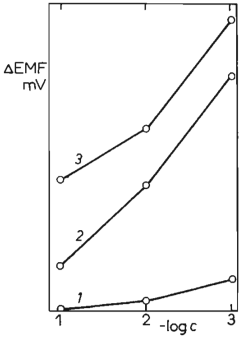

FIG. 6

Measurements Performed with Synthetic Sphingomyelin Membrane

1 10^{-1}–10^{-3}M-KCl, measured immediately, 2 10^{-1}–10^{-3}M-KCl, measured after exposure of the membrane to the 10^{-1}M solution of $CaCl_2$ for 1 hour. *3* 10^{-1}–10^{-3}M-KCl, measured 24 hours after curve *B*; in the meantime the membrane was immersed in 10^{-3}M-KCl. On the EMF scale 6·5 mm equal 10 mV.

DISCUSSION

The presence of high phospholipid concentration in the zone of provisional calcification[17], the fact that phospholipids are essential for active transport processes and for the activity of a number of mitochondrial enzymes[18], and the indirect evidence that unsaturated phospholipids are involved in active transport of calcium across some biological membranes[19], illustrate the importance of the contribution of phospholipid Ca^{2+}-binding sites to the integral ion transport mechanism.

The Ca^{2+} induced reversible change of cephalin membrane potential[14] as well as the reversal of potentiometric response of lecithin membrane effected by Ca^{2+} ions are not to be discussed separately. Healy and coworkers[20] showed that the charge on quartz at pH 7 can be reversed by 10^{-4}M Ca^{2+}. Besides, calcium is able to reverse the electrophoretic mobility of a manganate(IV) suspension at pH 7 (ref.[21]). In all these cases the data obtained on the association of Ca^{2+} illustrate the formation of moderately strong surface complexes. On the basis of available information it is not yet possible to distinguish whether the surface complexes are held together by covalent bonds or whether the surface interaction is characterized by an ion-pair association or by simple penetration of the Ca^{2+} ions into the adequately structured compact layer, adjacent to the solid surface.

These specific Ca^{2+} interactions represent a basis of related processes which characterize potential generating mechanisms, the selective permeability of biological membranes and the behaviour of ion selective membrane electrodes.

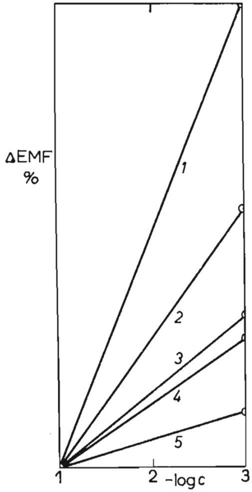

FIG. 7

Measurements Performed with Synthetic Lecithin Membrane

1 Decrease in EMF value from 10^{-1} to 10^{-3}M-KCl taken as 100%. Decrease in EMF value from 10^{-1} to 10^{-3}M-KCl in % relative to A in the presence of: *2* 10^{-4}M-$CaCl_2$; *3* 5 . 10^{-4}M-$CaCl_2$; *4* 10^{-3}M-$CaCl_2$; *5* 10^{-2}M-$CaCl_2$. The whole EMF scale equals 100%.

REFERENCES

1. Graven S. N., Lardy H. A., Johnson D., Rutter A.: Biochemistry *5*, 1729 (1966).
2. Graven S. N., Lardy H. A., Rutter A.: Biochemistry *5*, 1735 (1966).
3. Moore C., Pressman B. C.: Biochem. Biophys. Res. Commun. *15*, 562 (1964).
4. Štefanac Z., Simon W.: Microchem. J. *12*, 125 (1967).
5. Pioda L. A. R., Stankova V., Simon W.: Anal. Letters *2*, 665 (1969).
6. Lehninger A. L., Carafoli E., Rossi C. S.: Advan. Enzymol. *29*, 259 (1967).
7. Rossi C. S., Azzone G. F.: Azzi A.: European J. Biochem. *1*, 141 (1967).
8. Chance B., Mela L.: J. Biol. Chem. *241*, 4588 (1966).
9. Lynn W. S., Brown R. H.: Arch. Biochem. Biophys. *114*, 260, 271 (1966).
10. Addanki S., Cahill F. D., Sotos J. F.: Science *155*, 1678 (1967).
11. Lehninger A. L.: Biochem. J. *119*, 129 (1970).
12. Reynafarje B., Lehninger A. L.: J. Biol. Chem. *244*, 584 (1969).
13. Tobias J. M., Agin D. P., Pawlowski R.: J. Gen. Physiol. *45*, 989 (1962).
14. Van Breemen C.: Biochem. Biophys. Res. Commun. *32*, 977 (1968).
15. Carafoli E., Gamble R. L., Rossi C. S., Lehninger A. L.: J. Biol. Chem. *242*, 1199 (1967).
16. Dawson R. M. C., Eichberg J.: Biochem. J. *96*, 634 (1965).
17. Ali S. Y., Sajdera S. W., Anderson H. C.: Proc. Nat. Acad. Sci. US *67*, 1513 (1970).
18. Fiehn W., Hasselbach W.: European J. Biochem. *13*, 510 (1970).
19. Balzer H., Makinose M., Fiehn W., Hasselbach W.: Naunyn-Schmiedebergs Arch. Pharmak. Exp. Pathol. *260*, 456 (1968).
20. Healy T. W., James R. D., Cooper R.: Advan. Chem. Ser. *79*, 62 (1968).
21. Stumm W., Huang C. P., Jenkins S. R.: Croat. Chem. Acta *42*, 223 (1970).