Biochimica et Biophysica Acta, 641 (1981) 395—400

BBA 79148

ELECTROPHORETIC MEASUREMENTS ABOUT THE RELATION BETWEEN TRANSITION VOLTAGE AND ζ-POTENTIAL OF BIOLOGICAL MEMBRANES

A. TIPPE

Abteilung für Physiologie, Gesellschaft für Strahlen- und Umweltforschung, D 8042 Neuherberg (F.R.G.)

(Received August 25th, 1980)

Key words: Electrophoretic mobility; Surface charge; Myelin vesicle, Transition voltage; ζ-potential

Summary

The effects of inorganic cations, *n*-hexanol, saccharose and ^2H_2O on the electrophoretic mobility and ζ-potential of membrane vesicles from nerve myelin were measured and the results compared with the corresponding effects of the same reagents on the transition voltage, V_{Tr}, of the nerve axon membrane. Different cation concentrations and ^2H_2O affect both potentials, the ζ-potential and V_{Tr}, in a parallel way. Saccharose and *n*-hexanol, however, shift V_{Tr} but leave the electrophoretic mobility of the myelin vesicles unchanged. These results suggest that V_{Tr} shifts are not necessarily linked to changes in the membrane surface charge density but may also be caused by an interaction between the reagent and non-polar groups of the membrane interior.

Introduction

Fixed electrical charges which are located at the extracellular surface of the cell membrane have been determined for all mammalian cells investigated so far. The density of these charges can be changed by altering the ionic activity, ionic concentration and pH of the extracellular solution. Otherwise, such changes of ionic activity, ionic concentration and pH shift the transition voltage, V_{Tr}, of the nerve axon membrane [1] (V_{Tr} is defined as the inflection point of the chord-conductivity function). Changes in the threshold potential and voltage-dependent conductivity behavior of excitable membranes are consequences of such V_{Tr} shifts. These experimental observations are in good quantitative agreement with theoretical membrane models which are based on the assumption of fixed negative charges on the membrane surface [2,3].

However, there are non-ionic organic substances and solvent substituents which also shift the transition voltage, V_{Tr}, and influence the threshold potential of the nerve axon membrane, e.g *n*-alkanols [4] (acting as local anesthetic), saccharose [5] (substituent for ions with respect to osmolarity) and ^2H_2O [6] (solvent substituent). These effects are not covered by the theoretical models mentioned above. Thus, the questions arise: 1, whether such changes in the stationary conductivity behavior of the axon membrane are generally coupled with changes in the membrane surface charge density; and 2, whether changes of the membrane charge density due to alterations in the environmental conditions (ionic and non-ionic) are specific for the excitable membrane or reflect a behavior of biological membranes in general?

In order to try to find an answer by experiment we chose the myelin compound membrane isolated from myelinated nerve fibres as a sample of a biological membrane preparation. The electrokinetic potential of this membrane was studied as a function of different extracellular solutions which are known to effect the electrophysiological functions of the nerve axon membrane. The reasons for this choice are: 1, Single myelin compound membranes can be prepared in the native state from nerve myelin [7]. 2, These membranes form vesicles which are stable as aqueous dispersions, thus suitable to study the electrophoretic potential of the membrane surface by cell electrophoretic methods. 3, Morphology and chemical composition of this membrane are well known [8]. 4, This membrane is obtained from myelinated nerve fibre, the same biological preparation as used for the electrophysiological experiments, which might be helpful in comparing the results from both types of experiments.

Materials and Solutions

Membrane vesicles were prepared from freshly dissected nervus ischiadicus of frog (*Rana esculenta*, male) using the following method (extensively described in Ref. 7). Several nerve bundles (total weight about 18 g) were cut into small pieces and soaked in 75 ml distilled water at 4°C for 15 h. Fragments of connective tissue were removed by filtration and a standard colloidal solution of vesicles was thus obtained. The vesicles are small spheres with diameters between 2 and 8 μm. The sphere walls are formed by single myelin compound membranes as seen by electron microscopy [7].

To measure the dependency of the electrokinetic ζ-potential of the vesicles on the concentration of inorganic ions carrying different valencies, the ion concentration of the colloidal solution was adjusted to different values by adding Na^+ and/or Ca^{2+} (pH 7.1 ± 0.1). To avoid any affect by an osmotically induced shrinking of the vesicles, the ion concentration of the colloidal solution was limited to 15 mM Na^+ plus 1.8 mM Ca^{2+}.

In an additional series of experiments the effects of non-electrolytes, e.g. *n*-hexanol, saccharose and ^2H_2O, on the electrophoretic mobility of the vesicles were studied. For these experiments the ion concentration of the standard colloidal solution was adjusted to 10 mM NaCl plus 5 mM NaH_2PO_4 (buffered colloidal solution). Alkanol (*n*-hexanol-1, 5 and 10 mM) or saccharose (28 mM) were directly added to this buffered colloidal solution.

The vesicle solution containing ^2H_2O as solvent was obtained by soaking the

nerve bundle segments in ^2H_2O (99.75%, Merck). This colloidal ^2H_2O vesicle solution was adjusted to 10 mM NaCl plus 5 mM NaH_2PO_4 using the corresponding H_2O solutions. The final ^2H_2O concentration in these experiments was therefore reduced to about 85—90%.

Methods

The electrophoretic mobility of myelin vesicles (U_m) was determined using a cytopherometer [9] equipped with a phase-contrast microscope. The measurements were performed at 25°C and 0.1 ÷ 1.5 mA utilizing a rectangular electrophoresis chamber of the dimensions 35 × 14 × 0.7 mm. The time which the particles needed to cover a distance of 34 μm was determined alternatively in the two directions with respect to the large dimension of the chamber by changing the polarity of the d.c. field. Each mobility value represents the average of 30 ÷ 50 single measurements. The ζ-potentials were calculated according to the Helmholtz-Smoluchowski equation applying viscosity values which were determined experimentally for each colloidal solution (Low shear 2 viscosimeter, Contraves).

Results

1. Ion concentration. The electrophoretic mobility U_m of myelin vesicles decreases by increasing the ion concentration of the surrounding aqueous solution. Measured values for U_m at different ion concentrations in the solution are listed in Table I together with the corresponding ζ-potentials. The value U_m for the buffered colloidal solution containing 10 mM NaCl and 5 mM NaH_2PO_4 as a reference value was measured several times to check for systematic errors — no such error was observed. The relative error of about 5% for U_m is purely statistical, being calculated from the standard deviation of the single velocity measurement.

2. n-Hexanol. In the experiments where two different *n*-hexanol concentrations are added to the colloidal solution we observed no change in the mobility of the myelin vesicles (see Table II) although the applied hexanol concentrations are sufficient to shift the transition voltage V_{Tr} of the axon membrane [4].

TABLE I

ELECTROPHORETIC MOBILITY U_m AND ζ-POTENTIAL, ζ_m, OF MYELIN VESICLES FOR DIFFERENT CATION CONCENTRATIONS

Values for U_m are expressed as 10^{-4} cm^2/V per s.

Na^+ (mM)	0 mM Ca^{2+}		0.9 mM Ca^{2+}		1.8 mM Ca^{2+}	
	U_m	ζ_m (mV)	U_m	ζ_m (mV)	U_m	ζ_m (mV)
5	3.63	45.6	2.20	27.7	1.68	21.1
10	2.80	35.2	1.98	24.9	1.65	20.7
15	2.22	27.9	1.74	21.9	1.42	17.8

TABLE II

ELECTROPHORETIC MOBILITY, U_m, OF MYELIN VESICLES FOR DIFFERENT REAGENTS

Colloidal solution	U_m (10^{-4} cm^2/V per s)	Variance (%)
Standard buffered solution	2.93	5.0
+ 5mM *n*-hexanol-1	2.88	5.5
+ 10 mM *n*-hexanol-1	2.99	6.0
+ 28 mM saccharose	2.82	7.7
Standard buffered ^2H_2O solution	2.59	8.0

3. Saccharose. The electrophoretic mobility of the myelin vesicles was not changed by adding saccharose to the external solution (Table II), although the transition voltage V_{Tr} will be shifted by Ringer solutions containing the same amounts of saccharose.

4. ^2H_2O. A decrease (approx. 12%) of the electrophoretic mobility of myelin vesicles was obtained if H_2O is replaced by ^2H_2O in the surrounding solution (see Table II). This decrease cannot simply be explained by the reduced specific conductivity of ions such as Na^+ and Ca^{2+} in ^2H_2O with respect to H_2O because this effect was allowed for in the calculation of the electric field effective on the vesicles in the measuring chamber. Our experimental result, therefore, suggests that ^2H_2O lead to a decrease of the surface charge density of the myelin membrane.

Discussion

Each of the four reagents investigated (cation concentration, *n*-alkanol, saccharose and ^2H_2O) influence the transition voltage V_{Tr} of the nerve axon membrane, whereas only two (cation concentration, ^2H_2O) of them affect the ζ-potential too. This observation suggests that the molecular mechanism underlying the observed V_{Tr} shifts might be grouped into: 1, mechanism which cause transformations of the lipid-protein structure of the membrane by changing the

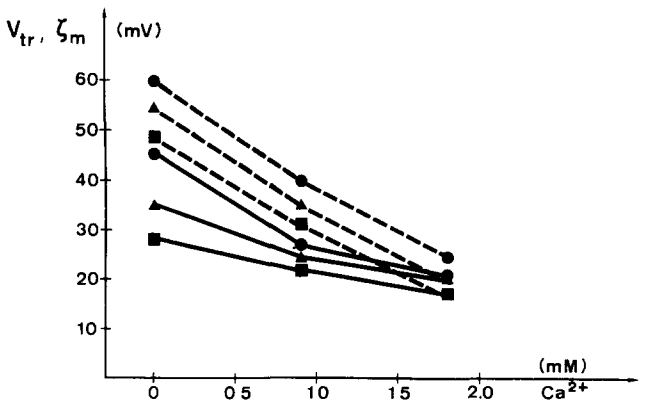

Fig. 1. Dependency of transition voltage V_{Tr} (solid line) and membrane ζ-potential (dotted line) on the extracellular ion concentration. ●, 5 mM Na^+; ▲, 10 mM Na^+; ■, 15 mM Na^+.

membrane surface potential; and 2, mechanism which cause such transformation by direct interaction between reagent and non-polar groups of the bilayer constituents.

In the case of the concentration series of monovalent and divalent inorganic ions, the results show a close correlation between the electrokinetic ζ-potential of the myelin vesicles and V_{Tr}. The two potentials are in the same order of magnitude and the dependencies of both the ζ-potential and V_{Tr} on cation concentration are parallel (see Fig. 1). These experimental facts are in agreement with the adsorption model of the cation-membrane interaction [3]. This model assumes an electro-adsorptive interaction between fixed membrane surface charges and the cations in the bulk solution. By changing the surface charge density this interaction will control structural transformations within the membrane. Such coupling between structural transformations and surface potential could explain the V_{Tr} shifts (group 1).

Studies about the effect of *n*-alkanols on the thermotropic phase transition of phospholipid bilayers [10] have indicated that the *n*-alkanols interact with the aliphatic part of the phospholipids in the bilayer, thereby leaving the charge density of the membrane surface unchanged. Transformations of the membrane structure due to this interaction are most likely not correlated with the membrane surface potential. Our result, no change of U_m by adding *n*-hexanol, supports this assumption of an interaction mechanism between alkanol and the hydrophobic interior of the membrane and agrees with X-ray diffraction experiments about the organisation of *n*-alkanes in lipid bilayers [11]. The mechanism of the V_{Tr} shifts produced by *n*-alkanols, therefore, seems to belong to group 2.

Saccharose customary is applied as a substitute for ions to control the molarity of solutions which are used in physiological investigations. Any influence of such uncharged compounds on the transition voltage V_{Tr}, as observed for the excitable membrane of myelinated nerve [5], is expected not to be coupled to the membrane surface potential. The close chemical relationship between saccharose and alkanols rather suggests an saccharose-membrane interaction mechanism which belongs to group 2. The result of our experiments supports this interpretation.

This agreement between theoretical models about the measured behavior of V_{Tr} and experimental results on the electrophoretic mobility of myelin vesicles suggests that, at least with regard to the different reagents investigated, excitable and non-excitable biological membrane behave in the same way (unit membrane concept, see Ref. 12). According to this conclusion the result of the experiments reported here demonstrate that shifts of the transition voltage V_{Tr} are not necessarily coupled with changes of the membrane surface potential.

Acknowledgement

I wish to thank Professor Dr. G. Ruhenstroth-Bauer of Abteilung für experimentelle Medizin, MPI für Biochemie, D 8033 Martinsried, for his interest and permission to use the facilities of his laboratory. I am very grateful to Dr. S. Bamberger for permanent aid in carrying out the electrophoresis experiments.

References

1 Müller-Mohnssen, H. and Barske, H. (1974) Am. J. Physiol. 226, 844—854
2 Stern, O. (1924) Z. Elektrochem. 30, 508—516
3 Gilbert, D.L. (1971) in Biophysics and Physiology of excitable Membranes (Adelman, W.J., ed.), pp. 359—378, VNR-Company, New York
4 Tippe, A. (1980) Biochim. Biophys. Acta 598, 200—205
5 Barske, H. (1971) Thesis, Technische Universiteit, Munchen
6 Müller-Mohnssen, H. and Albrecht-Bühler, G. (1969) Pflüg. Arch. 305, 65—75
7 Albrecht-Bühler, G. (1971) Thesis, Technische Universiteit, München
8 Schnapp, B. and Mugnaini, E. (1975) in Golgi Centennial Symp. Proc. (Santini, M., ed.), pp. 209—233, Raven Press, New York
9 Fuhrmann, G. and Rohenstroth-Bauer (1965) in Cell Electrophoresis (Ambrose, E.J., ed.), pp. 22—41, Churchill, London
10 Jain, M.K. and Wu, N.H. (1977) J. Membr. Biol. 34, 157—201
11 McIntosh, T.J., Simon, S.A. and MacDonald, R.C. (1980) Biochim. Biophys. Acta 597, 445—463
12 Robertson, J.D. (1960) Prog. Biophys. 10, 343—418