Biochimica et Biophysica Acta, 684 (1982) 127–132
Elsevier Biomedical Press

BBA 71009

TRANSPORT OF Na^+ BY MONENSIN ACROSS BIMOLECULAR LIPID MEMBRANES

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(Received July 20th, 1981)

Key words: Na^+ transport; Lipid bilayer; Monensin; pH

Transmembrane ^{22}Na fluxes across bimolecular lipid membranes are measured under two different experimental conditions: (a) the pH is the same in the two bulk aqueous solutions on either side of the membrane while the concentrations of Na^+ are different; (b) the concentrations of Na^+ are identical but pH of the two solutions are different. In this latter case, the transport of Na^+ occurs in the opposite direction to the difference of the proton concentration. In both cases, the electrical charge flux is negligible. A transport model is proposed to account for the experimental data.

Introduction

The monocarboxylic polyether antibiotics which are isolated from Streptomyces form a well defined class of carriers for alkaline and alkaline earth cations.

The molecular structure of these ionophores can be represented by a chain of tetrahydropyran and tetrahydrofuran cycles terminated on the one side by a carboxylic function and on the other by one or two hydroxyl groups. X-ray spectra of crystallized cationic complexes show a pseudomacrocylic structure centred on the cation around which are distributed [1] coordination oxygen atoms, the structure being stabilized by hydrogen bonds.

These carboxylic ionophores induce potassium fluxes through cellular membranes and at the same time, they modify the pH inside the cell [2–4]. Among these cation-carriers, monensin shows the greatest selectivity towards sodium ion [5,6]. Further it does not affect the electrical properties of lipid bilayers [7] while, in the same conditions, grisorixin and nigericin which are potassium carriers of the same family, increase the membrane conductance which depends strongly on the pH of the bathing solutions [7,8].

In this paper a study of the sodium transport by monensin, through bimolecular lipid membranes using flux measurements with ^{22}Na is presented. Two different conditions are considered: the two aqueous solutions have differing pHs but the same concentration of Na^+ or then the same pH but different Na^+ concentrations. The sodium fluxes are compared to those of the electrical charges. One can note here that this type of measurement has already been used in the elucidation of the transport mechanism of Ca^{2+} using another carboxylic ionophore A 23147 [9].

Experimental

The bimolecular lipid membranes are prepared by a technique previously described [10], the capacitance being controlled by coulostatic measurements. The area of the membrane is 1 mm^2.

The cell for the measurement of fluxes is com-

0005-2736/82/0000–0000/$02.75

posed of an inner compartment (2 ml) which contains the radioactive sodium while the volume of the outer compartment is 8 ml. The dissymmetry of the two aqueous solutions, either with respect to the pH or Na^+ concentration, is brought about after the formation of the bimolecular lipid membranes and with the addition of the radioactive tracer ^{22}Na.

The two solutions are stirred throughout the experiment. At regular intervals of time, aliquots of 0.2 ml of solution are taken from the external compartment and replaced immediately by an equal volume of the initial solution. Each sample is dissolved in 10 ml of a liquid scintillator solution and its radioactivity determined by means of a Packard Tri-Carb Liquid Scintillation Spectrometer.

During the flux determination the short circuit current is measured by means of two Ag-AgCl electrodes immersed in each of the aqueous solutions, using a picoamperemeter (Keithley 600 B).

The lipid bilayers are prepared from a 1:1 mixture of phosphatidylcholine (Supelco) and cholesterol (Sigma) dissolved in *n*-decane (Fluka purissimum), to give a solution for which the ratio of the lipids to the hydrocarbon is 1% by weight. In this bilayer forming solution, known quantities of ionophore are dissolved. The monensin used was kindly given by Dr. G Jeminet of the University of Clermont-Ferrand.

The pH of the solutions is fixed by the addition of hydrochloric acid or of sodium hydroxyde while the actual values of the media are determined using a micro glass electrode. For measurements made with symmetry of pH (pH = 8), the solutions have been buffered with tris(hydroxymethyl)-aminomethane (Tris).

The sodium chloride is a Merck Suprapur product while the ^{22}Na-enriched sodium chloride solution is obtained from the Radiochemical Center, Amersham. This radioactive isotope emits γ rays and has a period of 2.58 years.

Results

In the absence of a carrier, and in the case where either the pH or the Na^+ concentration are different, the quantity of ^{22}Na which is found in the external compartment is always smaller than the amount detectable by the spectrometer, even when experiments are carried out for periods longer than three hours.

In the presence of monensin, with an identical Na^+ concentration in the two aqueous solutions and a pH dissymmetry, the amount of ^{22}Na in the external compartment increases with time. A typical curve is shown in Fig. 1. This was obtained with a solution of NaCl $2.5 \cdot 10^{-2}$ M and pHs of 10 and 5, respectively, in the internal and external solutions. One notes that a steady flux is reached 20 to 30 min after the modification of the pH of the aqueous solutions. The measured short circuit current remains approximately constant with a value of 3–6 pA throughout this period.

Fig. 2 shows that for the same Na^+ concentration but differing pHs on either side of the membrane, the steady-state flux is directly proportional to the concentration of monensin in the bilayer forming solution.

With the same pH difference between the two aqueous solutions, the monensin concentration in the membrane-forming solution being kept constant ($5 \cdot 10^{-3}$ M), the Na^+ flux increases with the concentration of Na^+ which here is the same on both sides. The steady-state values of the Na^+ flux are respectively 0.23, 0.37 and 0.67 nmol $\cdot$ cm^{-2} $\cdot$ s^{-1} when the NaCl concentrations of the aqueous

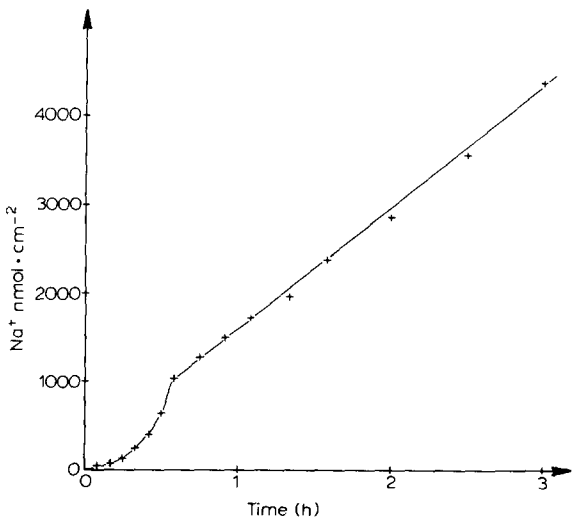

Fig. 1. Variation with time of the number of Na^+ ions crossing the bimolecular lipid membrane. NaCl, $2.5 \cdot 10^{-2}$ M; monensin, $5 \cdot 10^{-3}$ M; ΔpH, 5.

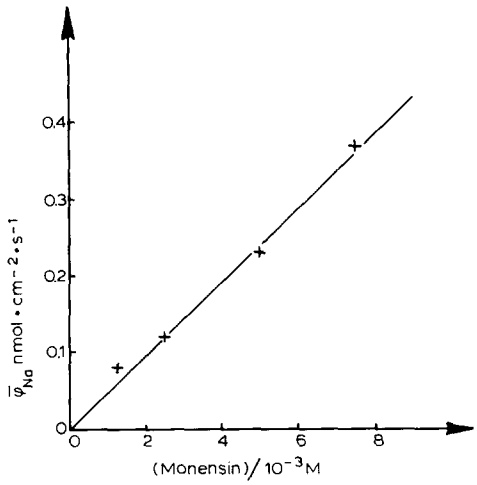

Fig. 2. Variation of the Na^+ flux with the concentration of monensin. NaCl, 10^{-2} M; ΔpH, 5.

solutions are 10, 25 and 50 mM.

Fig. 3 shows the variation with time of the number of Na^+ ions which appear in the more acidic solution when the pH of the other solution

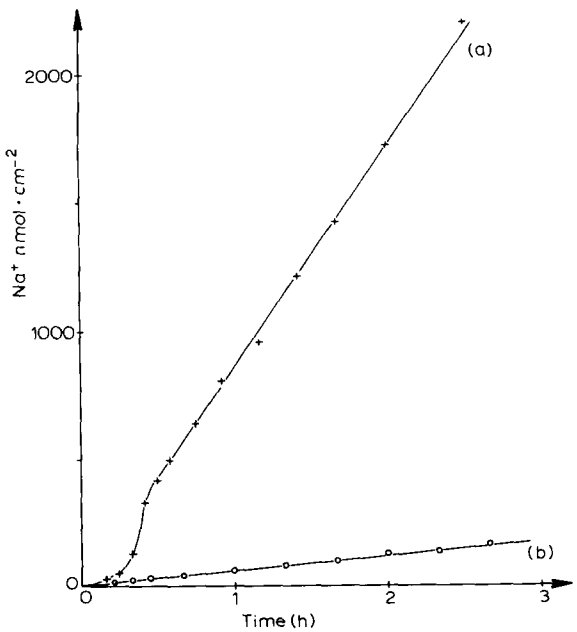

Fig. 3. Variation with time of the number of Na^+ ions crossing the bimolecular lipid membrane. NaCl, 10^{-2} M; monensin, $5 \cdot 10^{-3}$ M. (a) ΔpH, 5; $\bar{\phi}_{Na} = 0.23$ nmol · cm^{-2} · s^{-1}. (b) ΔpH, 1; $\bar{\phi}_{Na} = 0.02$ nmol · cm^{-2} · s^{-1}.

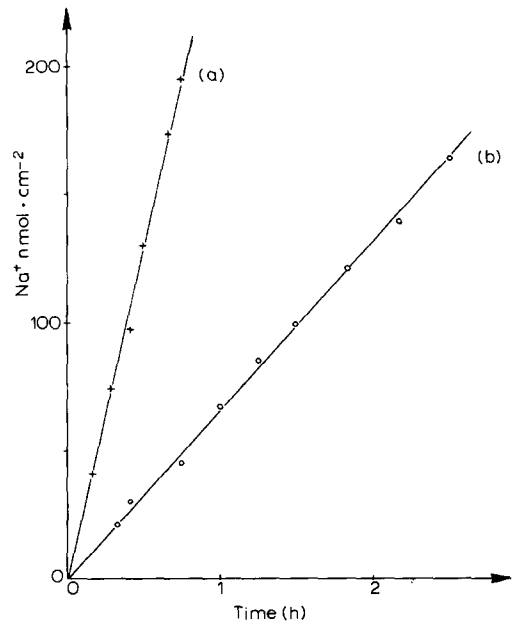

Fig. 4. Variation with time of the number of Na^+ ions crossing the bimolecular lipid membrane. pH, 8; monensin, $5 \cdot 10^{-3}$ M. (a) ΔC_{Na}, $4 \cdot 10^{-2}$ M; $\bar{\phi}_{Na} = 0.072$ nmol · cm^{-2} · s^{-1}. (b) ΔC_{Na}, $1 \cdot 10^{-2}$ M; $\bar{\phi}_{Na} = 0.018$ nmol · cm^{-2} · s^{-1}.

is either 10 (curve a) or 6 (curve b); the Na^+ and monensin concentrations being the same in both cases. Measurements were also carried out with solutions having the same pH on both sides (pH 8) but only one containing Na^+ ions. The amount of these ions appearing in the solution which initially did not contain any was determined and Fig. 4 shows the variation with time of the amount of these ions when the concentration in the other solution was either 10^{-2} M or $4 \cdot 10^{-2}$ M and that of monensin in the membrane-forming solution $5 \cdot 10^{-3}$ M.

Discussion

The standard transport model is similar to the one proposed for the alkaline cation transport by neutral carriers such as valinomycin and nonactin [11,12].

In the membrane the carboxylic carrier can exist in three different forms: the anionic C^-, protonated CH, and complex with a cation M^+, CM form. All three forms are located at the two

interfaces between the membrane and the solutions. Heterogenous reactions of proton exchange and cation complexation and decomplexation occur at each interface between the ionophore in the bimolecular lipid membrane and the ions H^+ or M^+ in the planes of closest approach of the adjoining aqueous solutions. In principle, each of these forms C^-, CH and CM can undergo a translocation reaction between the two sides of the membrane.

Under the two experimental conditions of this study ($\Delta pH \neq 0$, $\Delta C_{Na^+} = 0$ and $\Delta pH = 0$ and $\Delta C_{Na^+} \neq 0$), the flux of electrical charges is very small as compared to the measured Na^+ flux. In all measurements, the short-circuit current at the steady state is always smaller than 6 pA. As the membrane area is 10^{-2} cm^2, the resulting flux of charged species is smaller than $6 \cdot 10^{-15}$ $mol \cdot cm^{-2} \cdot s^{-1}$, while the measured flux of Na^+ ranging about 10^{-10} $mol \cdot cm^{-2} \cdot s^{-1}$.

This result shows that while Na^+ is carried from one side of the membrane to the other, under the 1 : 1 complexed form, the return passage of the carrier from the second to the first interface takes place only in the protonated form. Nevertheless under a pH gradient, and for the same Na^+ concentration in both aqueous solutions, the interfacial concentration of C^- is much higher on the basic than on the acidic side. Further, when the pH is 8 on both sides, the number of free C^- is greater at the interface in contact with the less concentrated solution of Na^+. In the last case a flux of electrical charges carried by C^- could be expected during the transport of Na^+ as there exists a difference between the two interfacial concentrations of C^-.

The electrical conductance measurement of symmetrical systems [7] have shown that even in presence of Na^+, the monensin increases only very slowly the membrane conductance and this is even true at higher pH. These two results suggest that the translocation rate constant of C^- is much smaller than the rate constant of the neutral forms CH and CM. This is probably due to a greater standard adsorption enthalpy of the carrier in the anionic form as compared to the others because of the hydratation of the ionized group. It is a well known fact that protonated fatty acids are less soluble in water than their ionized forms. With the Na^+ complexation a structure change occurs with the formation of a pseudo macrocycle and this may well diminish the interactions between the water molecules and the carboxylic groups. Such a change is also borne out by the results of surface tension measurements between water and decane in the presence of an other carboxylic ionophore, grisorixin, dissolved in decane (Gros, M. and Gavach, C., unpublished data). An important decrease in the surface tension is observed when the ionophore passes from the protonated to the anionic form. This result confirms the stronger adsorption of the carboxylic ionophore in its anionic form as compared to the non ionized molecule. Therefore the translocation of C^- is not involved in the transmembrane transport of Na^+.

As the total electrical flux is zero, at the steady state, protons and cations are exchanged at both interfaces. At each interface, the number of Na^+ ions passing from the aqueous solution to the membrane per unit time, is equal to that of the protons passing in the opposite direction from the membrane to the aqueous solution.

Under the two different conditions, i.e. different pHs and the same Na^+ concentration on both sides and different Na^+ concentrations but similar pHs, the transport of the Na^+ can be represented schematically by the simple model in Fig. 5. At the steady state, the Na^+ flux is proportional to the monensin concentration in the membrane-forming solution (Fig. 2). This result confirms the 1 : 1 complex formation.

One can note that when the pH value is 5 on one side and 6 on the other (Fig. 3, curve b), or when the pH is 8 in both sides with dissymmetrical Na^+ concentrations (Fig. 4) the flux has a constant value while, when the pH is 10 on one side and 5 on the other, it fluctuates at the beginning (Figs. 1 and 3, curve a) only afterwards reaching a constant value. It is impossible to state whether this variation of the flux is due to a change of the interfacial concentration of the different forms of the carrier or to a modification of the nature of the bilayer as for example the possible hydrolysis of the lipids at higher pH.

As the actual interfacial concentration of monensin in the membrane is unknown, one cannot determine the value of the rate constant of the different steps. Nevertheless the lowest limit of the

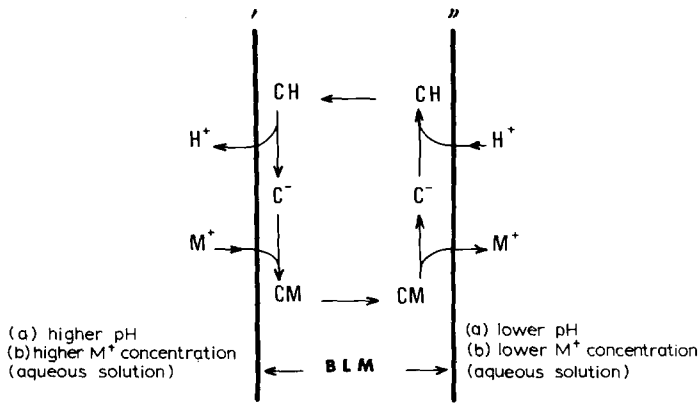

Fig. 5. Schematic representation of the transport model under the two different conditions. (a) pH dissymmetry with the same Na^+ concentration in both sides. (b) Different Na^+ concentrations with a symmetry of pH.

translocation constant of the protonated form can be estimated from the results obtained under a pH dissymmetry. If one assumes that the number of monensin molecules in the protonated form is zero on the side where the pH of the solution is 10, and that the maximum ratio of the number of monensin molecules over the number of phospholipid molecules is higher than 1/5, it is possible to estimate the maximum number of monensin molecules in the protonated form on the side where the pH is 5, knowing the values of the molecular areas of these molecules. These values have been determined from balance film measurements (Van Mau, N., unpublished data). The maximum value of surface concentration of monensin is $4 \cdot 10^{-11}$ $mol \cdot cm^{-2}$. From this estimation and recalling that the maximum measured flux is $0.67 \cdot 10^{-9}$ $mol \cdot cm^{-2} \cdot s^{-1}$, the turnover rate of the monensin in the protonated form is higher than 14.

In Fig. 4, one notes that in spite of the high value of the pH, the Na^+ flux increases with the Na^+ concentration in the concentrated side. This means that the rate of Na^+ transport is not limited by the diffusion of protons from the bulk to the membrane in the less concentrated solution.

Wulf and Pohl [13] have measured, under similar conditions, the flux of Ca^{2+} transported by the carboxylic carrier A23187. They have found that the flux reaches a saturation value which increases with the ionophore concentration.

From the data in Fig. 4, one deduces that the Na^+ flux is $7.2 \cdot 10^{-11}$ $mol \cdot cm^{-2} \cdot s^{-1}$ when the pH is 8 on both sides and the difference of Na^+ concentration is $4 \cdot 10^{-2}$ M. According to the proposed transport model, the steady-state flux has the same value as the proton flux which diffuses in the less concentrated solution from the bulk to the membrane solution interface.

Assuming as did the above authors, that the thickness of the diffusion layer is 10^{-3} cm and taking for the diffusion coefficient of the proton the value of $2.25 \cdot 10^{-5}$ $cm^{+2} \cdot s^{-1}$, the calculated proton flux limited by the diffusion should be $2.25 \cdot 10^{-13}$ $mol \cdot cm^{-2} \cdot s^{-1}$ if the proton concentration in the aqueous layers adjoining the membrane is the same as in the bulk. This paradox cancels if one recalls that, in this case, the solutions are buffered and the transport of proton in the unstirred layers is therefore accomplished by the buffer molecules.

Acknowledgements

The authors wish to thank Dr. G. Jeminet for his gift of the monensin used and gratefully to acknowledge financial assistance from the Delegation Générale à la Recherche Scientifique et Technique of France (Contract No. 650.1952) and from the Centre National de la Recherche Scientifique (R.C.P. No. 80/605).

References

1 Pinkerton, M. and Steinrauf, L.K. (1970) J. Mol. Biol. 49, 533–546

2 Pressman, B.C. (1968) Fed. Proc. 27, 1283–1288
3 Feinstein, M.B., Henderson, E.G. and Sha'afi, R.J. (1977) Biochim. Biophys. Acta 468, 284–295
4 Lichtshtein, D., Dunlop, K., Kaback, H.R. and Blume, A.J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2580–2584
5 Smith, J.B. and Rozengurt, E. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 5560–5564
6 Choy, E.M., Evans, D.F. and Cussler, E.L. (1974) J. Am. Chem. Soc. 96, 7085–7090
7 Sandeaux, R., Seta, P., Jeminet, G., Alleaume, M. and Gavach, C. (1978) Biochim. Biophys. Acta 511, 499–508
8 Toro, M., Gomez-Lojero, C., Montal, M. and Estrada, O.S. (1976) Bioenergetics 8, 19–26
9 Kafka, M.S. and Holz, R.W. (1976) Biochim. Biophys. Acta 426, 31–37
10 Gavach, C. and Sandeaux, R. (1975) Biochim. Biophys. Acta 413, 33–44
11 Stark, G., Ketterer, B., Benz, R. and Laüger, P. (1971) Biophys. J. 11, 981–994
12 Laüger, P. and Stark, G. (1970) Biochim. Biophys. Acta 211, 458–466
13 Wulf, J. and Pohl, W.G. (1977) Biochim. Biophys. Acta 465, 471–485