Short communication

Changes of the Energy Profile of Gramicidin A Ionic Channel Dependent on the Ratio of Cations of Different Species in the Flux Passing Through the Channel

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The elucidation of the mode of diffusion and migration of ions in ionic channels spanning the cell plasma membranes and cell organelles is a special problem demanding joint efforts of physicists and biologists. A general approach to this problem is to investigate a number of ionic channel properties in a wide range of electrolyte conditions. Experiments concerning model bimolecular lipid membranes with incorporated channel-forming substances represent a method of choice in this area.

Properties of ionic channels formed in lipid bilayers by pentadecapeptide — gramicidin A have been discussed in detail in recent reviews (Andersen 1984; Hladky and Haydon 1984; Urry 1985). To some extent cation selective gramicidin A channels may be considered an adequate model of the ionic channel in living cell plasma membranes, particularly K^+-channels of the giant axon and Ca^{2+} activated K^+-channels (Hodgkin and Keynes 1955; Vestergaard-Bogind et al. 1985; Eskesen and Ussing 1986). Pronounced interaction of bidirectional fluxes of K^+ ions is characteristic both of natural potassium- and model gramicidin A channels (Hodgkin and Keynes 1955; Schagina et al. 1978, 1980, 1983; Procopio and Andersen 1979; Begenishich and De Weer 1980). The interaction of bidirectional ion fluxes passing through channels is an important peculiarity of ionic movement in narrow pores. A degree of interaction of bidirectional fluxes in these channels may be determined experimentally by using tracer technique. The ratio of unidirectional fluxes of ions of i-th species is given by Ussing—Hodgkin—Keynes equation (Hodgkin and Keynes 1955);

$$\frac{\vec{\Phi}_i}{\overleftarrow{\Phi}_i} = \left[\frac{C_i'}{C_i''} \exp\left(z_i F \varphi / RT\right)\right]^{n_i},$$

where $\vec{\Phi}_i$ and $\overleftarrow{\Phi}_i$ are unidirectional ion fluxes of the species "i" with the charge z_i; C_i' and C_i'' are concentrations of ions in the solutions separated by the membrane, φ is the transmembrane potential difference, F, R and T are standard terms.

In the case of free diffusion and migration n_i should equal unity which indicates the absence of an interaction of bidirectional fluxes. Values of n_i below unity are typical of the exchange diffusion found in valinomycin treated lipid bilayers (Schagina et al. 1983). The n_i values exceeding unity are indicative of the interaction of bidirectional fluxes as a result either of single file ionic movement in the channel with multiple ionic occupancy (Schagina et al. 1983; Kohler and Heckmann 1980), or ionic movement along the channel with a fluctuating energy profile (Ciani 1984). In the latter case an assumption is made of repeated transitions from a normal to an activated state of the channel energy profile characterized by different heights of energy barriers and different depths of wells (Läuger et al. 1980).

The index n_{Na} for gramicidin A channels in lipid bilayers appeared to be between 1 and 1.2 (Procopio and Andersen 1979; Schagina et al. 1980, 1983) indicating either the absence or a very weak interaction of bidirectional Na^+ fluxes, when for Cs^+ and Rb^+ the indices appeared to be 1.4—1.6 and 1.9—2.0, respectively (Schagina et al. 1978, 1983; Procopio and Andersen 1979). The difference in n_{Na} and n_{Rb} (or n_{Cs}) values might be explained as a result of a smaller distance between two Na^+ binding sites compared to distances between binding sites for Rb^+ (or Cs^+). Strong electrostatic repulsion prevents double occupancy of the channel in the case of Na^+ ions, whereas double channel occupancy by Rb^+ is possible because of the greater distance between corresponding binding sites and thus a weaker Coulomb repulsion (Eisenman and Horn 1983; Andersen 1984).

When cations of two different species (e.g. Na^+ and Rb^+) are moving through the gramicidin A channel an increase in n_{Na} values might be expected as a result of some increase of the distance between Na^+ and Rb^+ ions (if the location of their binding sites is not altered) resulting in a smaller electrostatic repulsion and a greater probability of the double occupancy of the channel. Within the frame of this model, a decrease in n_{Rb} is expected when Na^+ ions constitute a significant portion of cationic flux passing the channel. Contrary to computer calculations based on rate constants found for Na^+ and Rb^+ crossing gramicidin A channel represented by two-site three-barrier model (2S3B) showed that, in a mixed NaCl + RbCl solution with activity of each salt equal to 0.1 mol.l^{-1}, the decrease in n_{Rb} may be only about 1 % whereas the increase in n_{Na} should reach 22 % compared to corresponding indices found for the single salt (NaCl, RbCl) solution of the same activity (Kohler 1983). Subsequently, a very careful theoretical analysis was conducted by S. Aityan (1986). This author showed that for the 2S3B model it was possible to obtain $n_{Rb} = n_{Na}$ only in a special case when energy barriers for Na^+ and Rb^+ ions leaving the channel were assumed to be equal. The latter condition is, however, not consistent with the available data (Urban and Hladky 1980). Moreover Aityan reported an

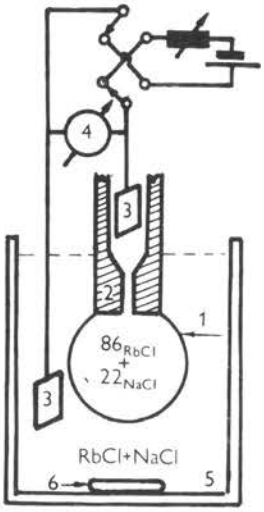

Fig. 1. Experimental set up used for tracer determination of bidirectional flux ratio. See text for details.

inability for the model to exhibit a decrease in n_{Rb} values in the presence of Na^+ ions.

An experimental assessment of changes of n_{Na} and n_{Rb} values in mixed salt (NaCl + RbCl) solutions was the aim of the present study.

Values of n_{Na} and n_{Rb} were determined by the measurement of tracer fluxes (^{22}Na and ^{86}Rb) across spherical lipid bilayers of an area up to 2 cm^2. The design of the experimental set up is displayed in Fig. 1. Spherical bilayers (1) were formed at the end of a thick-walled glass capillary (2). A transmembrane potential difference was applied using non-polarized Ag—AgCl electrodes (3) connected to a direct voltage source and measured by an electronic voltmeter (4). In the capillary and inside the lipid bilayer sphere was a mixed solution of NaCl and RbCl containing ^{22}Na and ^{86}Rb. The outside solution (5) of the same salt composition as that inside the sphere, initially devoid of radioactive isotopes, was vigorously stirred with a continuously rotating magnetic "fly" (6). This method of determination of n_i values from the data on unidirectional tracer fluxes at different transmembrane voltages (including the case when $\varphi = 0$) has been described in detail (see: Schagina et al. 1978, 1980, 1983). In this study ^{22}Na and ^{86}Rb fluxes were determined simultaneously by γ—γ coincidence and the Cherenkov effect respectively.

Stable spherical lipid bilayer membranes were formed from bulk of brain lipids with addition of cholesterol at 1 : 1 (w/w) ratio dissolved in chlorophorm-tetradecane-methanol mixture (4 : 3 : 2, v/v ratio). The concentration of lipids in the membrane forming solution was equal to 4 weight percent. Gramicidin A

("Sigma", USA), ^{22}Na and ^{86}Rb ("Isotop", USSR), NaCl and RbCl of "extra pure" grade and bidistilled water with no buffer added (pH about 6) were used in the experiments. The radioactivity of the each tracer in the solutions inside the bilayer sphere was approximately 1 MBq . ml^{-1}. Ethanol solution of gramicidin A was added to the outside electrolyte (5) up to an antibiotic concentration of 10^{-8} to 10^{-7} mol . l^{-1}.

The values of n_{Na} and n_{Rb} for gramicidin A treated membranes in mixed salt solutions of different Na : Rb molar ratios are presented in Fig. 2. The total concentration of chlorides in this series of experiments was kept constant and equal to 0.16 mol . l^{-1}. As a first approximation, the relation of both n_{Na} and n_{Rb} to molar percent of RbCl in the mixed salt solutions may be considered as linear. For all mixed salt solutions used, no significant difference between n_{na} and n_{Rb} for a given $Na^+ : Rb^+$ molar ratio (from 15 to 0.06) was found.

The data for a series of experiments on membranes in mixed salt solutions with varied total concentrations of chlorides but a constant NaCl to RbCl molar ratio (3 : 1) are presented in Table 1. Within the limits of experimental error for all three different total concentrations of chlorides, n_{Na} and n_{Rb} were found to be equal, i. e. the ratio $n_{Na} : n_{Rb}$ in neither case significantly differed from unity. It may also be noted that no significant difference existed between n_{Na} values for different total chloride concentrations at a constant NaCl to RbCl molar ratio; the same was also true for the n_{Rb} values. The data for n_{Na} and n_{Rb} obtained for membranes in 0.01 and 0.1 mol . l^{-1} single salt (NaCl, RbCl) solutions are also given in Table 1. The ratios n_{Na} to n_{Rb} found for the both single salt concentrations differed significantly from unity.

Table 1. The dependence of n_{Na} and n_{Rb} values on total salt concentrations.

Composition of solution		n_{Na}	n_{Rb}	n_{Na}/n_{Rb}
NaCl mol . l^{-1}	RbCl mol . l^{-1}			
0.03	0.01	1.62 ± 0.16	1.44 ± 0.15	1.12 ± 0.23
0.12	0.04	1.42 ± 0.12	1.51 ± 0.14	0.94 ± 0.17
0.225	0.075	1.51 ± 0.14	1.52 ± 0.13	1.00 ± 0.19
0.01	0	1.22 ± 0.16	—	0.60 ± 0.12
0	0.01	—	2.04 ± 0.15	
0.1	0	1.21 ± 0.15	—	0.61 ± 0.14
0	0.1	—	1.99 ± 0.21	
		mean ± S. D.	mean ± S. D.	mean ± S. D.

The results presented indicate that the properties of the gramicidin A channels are obviously dependent on the cationic composition of solutions separated by membranes and thus on the ration of cation species in fluxes passing

through the channel. In this case the energy profile of the channel for a given cation passing through may be regarded as dependent on species of cation that had previously interacted with the channel structure. Taking into account the much shorter time needed for an ion to cross the energy barrier relative to the time spent by the ion when located in the energy well, i.e. interacting with a binding site, it seems more probable that the binding sites, rather than the barriers are in some way adjusting to the cation species.

If a fluctuating energy profile of the channel is considered rather that a static one, the adjustment of the channel to a given cation species described above may be interpreted in terms of changes in the amplitude of fluctuations of the energy well. In any case the time of ligand relaxation should be greater relative to the condition when the ion-binding site is free and ready for the next cation.

The number of Na^+ ions passing through a gramicidin A channel per second is in the order of 10^7 (Hladky and Haydon 1972). Thus, for a particular symmetric two-site three-barrier model of the channel (Hladky and Haydon 1984) transporting Na^+ ions, only one of the two sites should be occupied at a given moment. In such a case the time of an ion bound state should be in the order of 5×10^{-8} s. Correspondingly the duration of the free state of the binding sites should be of the similar value. Gramicidin A single channel conductance for Rb^+ ions is about three times higher than that for Na^+. Thus the number of Rb^+ ions transported per second should be three fold that of Na^+ and the

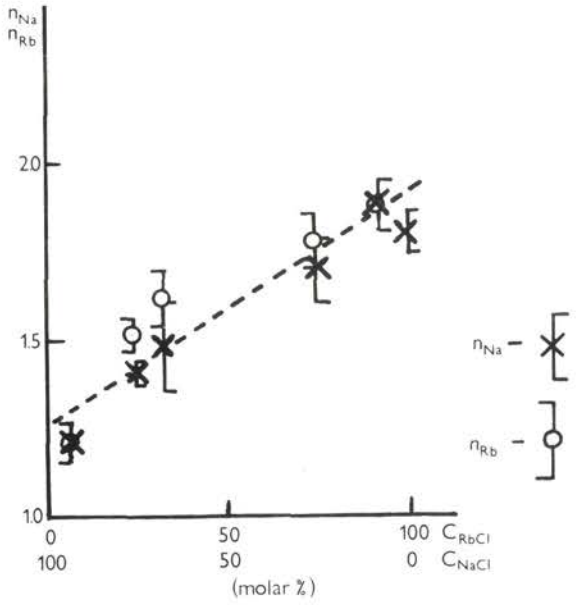

Fig. 2. Dependence of n_{Na} and n_{Rb} values on the molar % of RbCl, NaCl in mixed salt solutions at the constant total concentration of 0.16 mol . l^{-1}.

period needed for Rb^+ to pass through the channel three times shorter. Because double occupancy is possible for Rb^+ transporting channel, it is difficult to estimate free state duration of these binding sites in that case. It should probably be less than 1.5×10^{-8} s.

Results presented in Fig. 2 show that Na^+ and Rb^+ ions in mixed salt solutions have a nearly equal capability for influencing n_i values, shifting them from those characteristic for single salt (NaCl or RbCl) solutions. Therefore, the above difference in the free state duration of these binding sites in the cases of Na^+ and Rb^+ transport may not be very important and channel ligand relaxation time may be considered longer than the longest free state duration of these binding sites (i. e. greater that 5×10^{-8} s). The data obtained allow an estimate only of the lowest limit of the channel structures' "memory" about their interactions with the passing cation. This limit is in agreement with the period of so called peptide-residues libration (Urry et al. 1984; Eisenman 1987). It is therefore likely that the interaction of cations with the ligand groups in the channel somehow alters the trajectory of the peptide residues libration movement. If the restoration of the initial libration trajectory is comparatively slow, the latter process might be responsible for the "memory" of the channel for the cation species passing through the pore.

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Final version accepted June 6, 1988