Sizing of an ion pore by access resistance measurements

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INTRODUCTION

The characteristic dimensions of a molecular assembly forming conducting transmembrane pore are among the most important parameters for its structural models. Here we describe a novel approach that incorporates a concept of an ion channel access resistance. The approach is based on the actual measurement of the access resistance contribution to the total channel resistance with the use of viscosity altering water-soluble polymers. Addition of polymers varies the magnitude of the single-channel current by several mechanisms, one of which is the increase in the channel access resistance. Separating different polymer effects by independent measurements we were able to obtain absolute values of access resistances and channel radii by the analysis of a current through the channel in several conductive states of alamethicin channel and the open state of voltage dependent anionic channel from outer membrane of mitochondria (VDAC) reconstituted in lipid bilayers.

RESULTS AND DISCUSSION

Resistance of an ion channel R_t measured in a bilayer reconstitution experiment includes contributions R_{ac} from the access areas along the convergent current paths from the bulk electrolyte to the mouth of the channel (Fig. 1 A):

$$R_t = R_{ch} + 2R_{ac}, \qquad (1)$$

where R_{ch} is the resistance of the channel proper. For the circular pore opening of a given radius r in the solution of resistivity ρ the access resistance can be written in the form (1–2):

$$R_{ac} = \rho/4r. \qquad (2)$$

Indeed, the total resistance of the channel contains information about pore dimensions, but a direct evaluation of pore radius from R_t is complicated by the

uncertainty in the value of R_{ch}. Unfortunately, the mechanisms of an ion transport within the ion channel are still poorly understood and theoretical evaluation of R_{ch} even in the detailed structural models is quite problematic.

Using water-soluble polymers of different molecular weight and physical properties we were able to separate contributions from the channel proper and the access resistance into the total resistance of the channel. In principle, addition of water-soluble polymers can change the total channel resistance due to following reasons. First, polymers of sufficiently small size can penetrate the channel, "block" the current paths, and increase the channel resistance proportionally to the polymer partition into the channel. Second, the polymer addition can influence dissolved salt activity, thus changing salt concentration inside the channel and, therefore, resistance of the channel proper. Third, a nonelectrogenic polymer increases the bulk solution resistivity inducing an increase of the channel access resistance (Fig. 1 B).

The measurement of the access resistance contained several steps that allowed separation of the effects listed above. The planar bilayer membranes were prepared at room temperature by Montal-Mueller technique from solutions of L-Alpha-Diphytanoyl-Lecithin from Avanti Polar Lipids, Inc. (Pelham, AL). Polyethylene glycols (PEGs) of different molecular weights from Aldrich Chem. Co., Inc. (Milwaukee, WI) and dextran 17,900 from Sigma Chemical Co. (St. Louis, MO) were added to 1 M aqueous NaCl solutions (buffered at pH 6.2) to obtain needed weight/weight concentrations.

The absence of any significant partition of the polymers, used for the access resistance evaluation was verified by measuring the dependence of the polymer-induced channel conductance change on polymer weight (3). Sufficiently large polymers, namely PEG 3,400; PEG 17,000; and dextran 17,900 were chosen for the access resistance evaluation.

The influence of the polymers on dissolved salt (NaCl) activity was examined by a sodium-selective electrode. It was shown that dextran does not change ion activity. In this case, the increase in the total channel resistance is
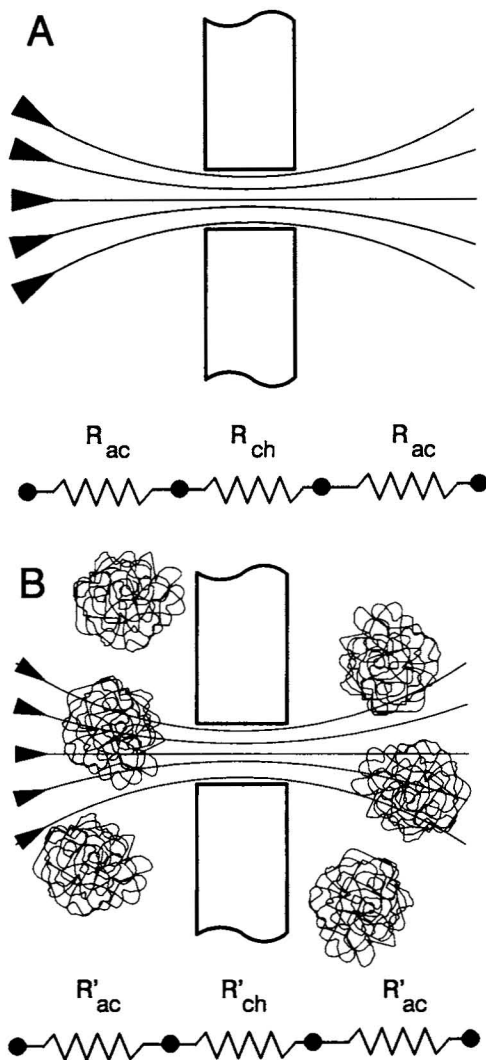
FIGURE 1 (A) Total channel resistance is a sum of the resistance of the channel proper and access resistances at both sides of the channel. (B) Addition of water-soluble polymers that do not penetrate the channel increases the access resistances due to electrolyte viscosity increase, and can change resistance of the channel proper due to electrolyte activity change.

induced only by the increase in the access resistance affected by the increase in bulk solution resistivity $\Delta\rho$:

$$\Delta R_{\mathrm{t}} = 2\Delta R_{\mathrm{ac}} = \Delta\rho/2r, \qquad (3)$$

giving radius of the channel and the absolute value of access resistance through Eq. 2.

Addition of PEGs increases salt activity in the electrolyte as a result of binding of several water molecules per chain and consequently competing with the ions in electrolyte for the water molecules. Interpretation of measurements for PEGs and similar water-binding poly-

mers should take into account a decrease in the resistance of the channel proper that can be characterized by the ratio $R_{\mathrm{ch}}/R'_{\mathrm{ch}}$ calculated from the ion activity measurement data. From Eqs. 1 and 2, the channel access resistance can be written in the form:

$$2R_{\mathrm{ac}} = \frac{\rho R'_{\mathrm{t}}}{\rho'} \frac{R_{\mathrm{ch}}/R'_{\mathrm{ch}} - R_{\mathrm{t}}/R'_{\mathrm{t}}}{R_{\mathrm{ch}}/R'_{\mathrm{ch}} - \rho/\rho'}, \qquad (4)$$

where symbols with an asterisk stand for the values measured or calculated in the experiments with the polymers.

Results of the channel radius evaluation obtained from the experiments with solutions of 15 and 20% (w/w) polymer concentrations are presented in Table 1.

TABLE 1 Channel radii calculated from measured access resistance values

	VDAC	Alamethicin		
	Open state	Level 1	Level 2	Level 3
Total channel resistance R_{t}, MOhm	285	1,490	617	380
Access resistance $2R_{\mathrm{ac}}$, MOhm	53 ± 3*	98 ± 9	60 ± 3	54 ± 4
Channel radius‡, Å	9.8 ± 0.5*	6.3 ± 0.6	10.3 ± 0.6	11.4 ± 0.8

*Obtained from experiments with dextran only. ‡Calculated ignoring ionic hydration radius.

Channel radii can also be estimated in a traditional way using the total channel resistance. The sources of potential errors in estimating the channel radius from the total channel resistance measurements are well known (4). Most evident are the channel length, "bulk resistivity" of electrolyte within the channel, and deviations in the structure of the pore from cylindrical geometry. We think that evaluation of the pore radius using the access resistance measurements is much more reliable because it is based on considerations of transport phenomena outside the ion pore.

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