

## Relation Between Ionic Channel Conductance and Conductivity of Media Containing Different Nonelectrolytes. A Novel Method of Pore Size Determination

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**Abstract.** The effects of nonelectrolytes on conductivity and viscosity of KCl solutions as well as on ion channel conductance were studied. Mobility of ions in solutions were found to solely depend on percent concentration (w/w) of the nonelectrolytes added and to be effectively independent on their chemical nature (sugars or polyglycols) and molecular size. Proportional changes in both the ion channel conductance and the conductivity of bulk solution induced by low m. w. nonelectrolytes may be used as a criterion of diffusion mechanism of ion transport through channels. The slope of the dependence of ion channel conductance on conductivity of bulk solution containing different concentrations of nonelectrolytes is a good measure of channel permeability for nonelectrolyte.

A new method of pore size determination is introduced. Results of practical application of this simple method to three types of ion channels (formed by alpha-latrotoxin, staphylococcal alpha-toxin and its N-terminal fragment) are shown. The advantages and disadvantages of the method are discussed.

**Key words:** Ionic channels — Nonelectrolytes — Pore size determination

### Introduction

The actual mechanism of ion transport through channels may be established by studying the relation between the movement of ions in bulk solution and that within the water-pore of an ion channel. Changes in ion movement in bulk solution may be induced by several method, including addition of indifferent uncharged nonelectrolytes. Studying the influence of uncharged molecules of different sizes on ion currents through channels can also supply data about geometrical parameters of water-filled pore, especially about the effective radius. So far exact values for radii of most channels have remained unknown or contradictory. The methods used

earlier were associated with numerous difficulties and limitations, and often they were inexact.

For example, the method based on the determination of channel permeability to ions of large size (Hille 1972; Cecchi et al. 1982) does not account for electrostatic interactions between passing ions and ionogenic groups of the channel though this relation can essentially change the value of the radius obtained.

Another method was built on the assumption that the conductance of an ion channel is equal to the conductivity of a simple cylinder of water solution (Benz and Hancock 1981; Colombini 1980). The length of the cylinder was usually assumed to be equal to the membrane thickness. Although the method is simple, it does not account for fundamental processes such as friction between ions and the channel wall (Antonov 1982) or repulsion of ions from the hydrophobic zone of BLM (Markin and Chizmadzhev 1974), etc. A more appropriate method was based on studying channel permeability to radio-actively labelled nonelectrolyte molecules (Holz and Finkelstein 1970). The method however was time consuming and too laborious.

In the present paper, we show the results of a systematic study of conductivity and viscosity of KCl solutions in the presence of different nonelectrolytes, as well as of ion channel conductance at the same conditions. These studies have offered the background for a new method for the determination of effective radius of water pores. The results of the first testing of this method were published earlier (Krasilnikov et al. 1988a,b; Krasilnikov and Sabirov 1989a). The method uses only electrical measurements of ion channel conductance. In addition, a test could be developed to select among the different mechanism of ion transport through the channels.

## Materials and Methods

*Staphylococcus aureus* alpha-toxin (ST) was a kind gift from Dr. K. D. Hungerer (Behringwerke Laboratories, Marburg, FRG).

N-terminal fragment of ST (F) was obtained by weak tryptic digestion with subsequent purification using preparative isoelectric focusing. Its isoelectric point was  $8.4 \pm 0.2$ . It could be shown by SDS-polyacrylamide gel electrophoresis that F contained only one band of a molecular mass (m.m.) of  $16 \pm 2$  kD (Ternovsky et al. 1991).

Black widow spider venom was extracted from the glands of spider *Latrodectus tenebrosus* with 100 mmol/l KCl solution. pH 7.5. Alpha-latrotoxin was purified on Mono-Q-column by FPLC-system (Pharmacia) as described earlier (Krasilnikov et al. 1988a).

TLC-pure phosphatidylcholine and phosphatidylserine were obtained from fresh hen eggs and ox brain, respectively, according to the method described by Bergelson et al. (1981). Cholesterol was obtained from Sigma Laboratories (Munich, FRG). Polyethylene glycols with average molecular weights ( $M_r$ ): 300 (Koch-Light); 400 and 20,000 (Schuchardt, Munich) 1000 (Austral-Preparate); 1500; 2000; 3000; 4000 and 6000 (Loba

Chemie) were used.

Planar phospholipid bilayer membranes (BLM) were prepared by opposing two monolayers on a Teflon septum with an aperture, approx. 0.7 mm in diameter, separating two buffered salt solutions (Montal and Mueller 1972) or alternatively by the method of Mueller et al. (1963) using 2–4% (w/v) lipid solution in *n*-octane.

Electrical measurements were performed under voltage clamp conditions. Currents through BLM were measured with Ag/AgCl electrodes connected in series with a voltage source and a current amplifier. The amplified signal was monitored on a storage oscilloscope and simultaneously recorded on chart paper or using a tape recorder. The compartment connected to virtual ground will be termed the "trans" compartment. The channel-forming proteins were added from a stock aqueous solutions to trans-compartment only to final concentrations sufficient to form single channels in BLM. The addition was followed by vigorous mixing of the solution using magnetic bars.

The following notation has been used with latrotoxin (LT) induced channels phosphatidylserine BLM: type 1, channels formed by fresh venom of *L. tredecimguttatus* obtained by extraction of the poison gland with 100 mmol/l KCl solution, pH 7.5. The average conductance ( $G$ ) of these channels was  $120 \pm 8$  pS; type 2, channels ( $G = 415 \pm 20$  pS) induced by the venom which had been preliminarily extracted from the gland by 10 mmol/l Tris-HCl buffer, (pH 8.0) and dried; types 3 and 4, channels ( $G = 565 \pm 25$  pS and  $G = 1110 \pm 40$  pS) induced by pure alpha-latrotoxin subjected to freeze-thaw procedure two times. Other details were described earlier (Krasilnikov et al. 1988a; Krasilnikov and Sabirov 1989a).

The viscosity of water solutions was determined using a glass capillary viscosimeter. The intrinsic viscosity ( $[\eta]$ , ml/g) was obtained from the relation between viscosity of solution (cP;  $1\text{P} = 0.1\text{Pa}\cdot\text{s}$ ) and the nonelectrolyte concentration (g/100 ml of solution). Hydrodynamic radii ( $r_h$ , Å) of nonelectrolytes were calculated by the following equations:

$$r_h = \left[ \frac{3V}{4\pi} \right]^{1/3}; \quad V = \frac{B_1 \cdot 1000}{23.5\eta_0 N_A}$$

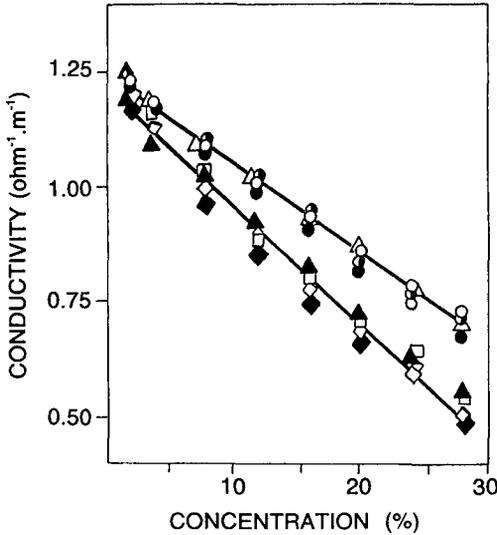
where  $V$  is the volume of molecule,  $\text{cm}^3$ ;  $\eta_0$  is the viscosity of the solvent, cP;  $N_A$  is the Avogadro's number;  $B_1$  is the slope of the viscosity vs. nonelectrolyte concentration (mol/l) dependence. Conductivity of the solutions was measured by a Radelkis OK 102/1 conductivity meter. The cation transference number ( $t_+$ ) was calculated from zero current potential in the presence of a KCl concentration gradient (40/120 mmol/l). All experiments were performed at  $25 \pm 1^\circ\text{C}$ .

## Results

### 1. Nonelectrolytes in water solution

#### 1.1 Conductivity of KCl solutions in the presence of nonelectrolytes

Upon the addition of various nonelectrolytes (ethyleneglycol, glycerine, some sugars, polyethylene glycols), to 100 mmol/l KCl solution, a strong decrease of conductivity of the initial solution was observed. An analysis of the data (Fig. 1) showed that the decrease in conductivity was determined by nonelectrolyte concentration (C,%) only. However these functions were independent on molecular



**Figure 1.** The effects of different nonelectrolytes on 100 mmol/l KCl solution conductivity. Symbols used: ○ - ethyleneglycol; ● - glycerine; ● - glucose; Δ - sucrose; ▲ - PEG 300; □ - PEG 1000; ■ - PEG 1500; ◇ - PEG 2000; ◆ - PEG 3000; ◆ - PEG 4000; ⊗ - PEG 6000; \* - PEG 20,000.

weight and/or size of the nonelectrolyte molecules. The chemical nature of nonelectrolyte had a slight effect on the nonelectrolyte concentration (w/w) conductivity relation. Hydroxyl-containing substances (ethyleneglycol, glycerine, glucose, sucrose) decreased the conductivity less than did PEGs. The relationship between the conductivity of 100 mmol/l KCl solution ( $\kappa$ ,  $\text{ohm}^{-1}\text{m}^{-1}$ ) and  $C$  for these two groups of nonelectrolytes may be expressed by the following equations:

$$\kappa_{\text{OH}} = (1.259 \pm 0.006) - (0.0204 \pm 0.0004) \times C \quad (1)$$

$$\kappa_{\text{PEG}} = (1.236 \pm 0.009) - (0.0263 \pm 0.0005) \times C \quad (2)$$

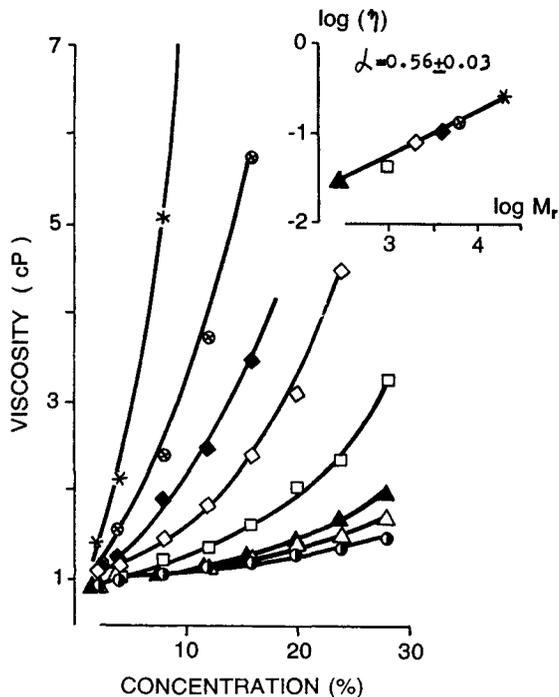
### 1.2 Viscosity of KCl solutions in the presence of nonelectrolytes

According to the Stokes-Einstein law, the ion movement and the conductivity of solutions depend on the viscosity of media, which naturally increases in the presence of nonelectrolytes (Fig. 2). However, a comparison of the experimental functions  $\sigma(C)$  and  $\kappa(C)$  (Figs. 1 and 2) made us to conclude that macroscopic viscosity of a solution does not play a major role in the change of ion movement. This is particularly clear from Fig. 3.

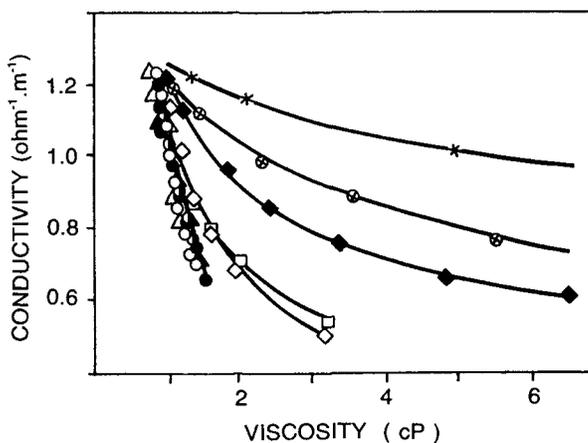
To understand the mechanism by which nonelectrolytes influence conductivity of salt solutions, data concerning macromolecule states and about their forms in the solution are necessary. These data can be obtained from viscosimetric experiments.

The increase in intrinsic viscosity of PEG solution (Fig. 2) was shown to follow the classical relation:

$$[\eta] = K \cdot M_r^\alpha \quad (3)$$



**Figure 2.** The dependence of 100 mmol/l KCl solution viscosity on the concentration of dissolved nonelectrolyte. The insert illustrates the intrinsic viscosity of PEG solution in dependence on nonelectrolyte m.w. in double logarithmic scale. For symbols see legend to Fig. 1.



**Figure 3.** The dependence of conductivity of 100 mmol/l KCl solution containing different nonelectrolytes on the viscosity of the resulting solutions. For symbols see legend to Fig. 1.

where  $K$  is the coefficient of proportionality; and  $\alpha$  is the parameter reflecting the geometry of the polymer molecule in solution.

According to the value of this parameter ( $0.56 \pm 0.03$ ), the PEG molecules are approximately spherical in salt solutions (Marshall 1981).

**Table 1.** The size of nonelectrolyte molecules ( $\text{\AA}$ ).

No	Sample	$r_h$	$r_{S-G}$	$r_{M-F}$	$r_{cr}$	$V_h/V_{cr}$
1.	Ethyleneglycol	$2.62 \pm 0.03$	3.3	—	2.81	0.93
2.	Glycerine	$3.08 \pm 0.02$	—	—	3.07	1.01
3.	Glucose	$3.70 \pm 0.10$	—	—	3.58	1.04
4.	Sucrose	$4.67 \pm 0.05$	4.6	—	4.40	1.20
5.	PEG 300	$6.0 \pm 0.2$	5.8	5.5	4.60	2.22
6.	PEG 400	$7.0 \pm 0.3$	6.8	6.3	4.99	2.76
7.	PEG 1000	$9.4 \pm 0.3$	10	10	6.70	2.68
8.	PEG 1500	$10.5 \pm 0.1$	12	12	7.45	2.57
9.	PEG 2000	$12.2 \pm 0.1$	—	—	8.53	2.93
10.	PEG 3000	$14.4 \pm 0.4$	—	—	9.76	3.21
11.	PEG 4000	$19.2 \pm 0.3$	19	18.3	10.74	5.71
12.	PEG 6000	$25.0 \pm 0.3$	29	26	12.30	8.40
13.	PEG 20,000	$51.0 \pm 0.7$	49	41.8	18.40	21.3

$r_h$  is the hydrodynamic radius determined by capillar viscosimeter, see Methods;  $r_{S-G}$ , data for hydrodynamic radii as reported by Scherrer and Gerhardt (1971);  $r_{M-F}$  statistical radii of hydration according to Mark and Flory (from Scherrer and Gerhardt 1971);  $r_{cr}$ , the crystal radii calculated as:

$$r_{cr} = [3M_r/4dN_A]^{1/3}$$

where  $M_r$  is molecular weight,  $d$  is the density of the nonelectrolyte ( $\text{g/cm}^3$ ) and  $N_A$  - Avogadro's number;  $V_h$  and  $V_{cr}$  the hydrodynamic and the crystal volume of one mole of nonelectrolyte.

The viscosimetric investigations allowed us to calculate both the equivalent hydrodynamic radii of nonelectrolytes and the value of their molar volume in solution. Our results are given in Table 1 together with data published by other authors (Scherrer and Gerhardt 1971). We could establish that the hydrodynamic radii of PEG ( $r_h$ ) showed a hyperbolic nonlinear dependence on the molecular weight of the polymer. This dependence can be expressed as:

$$r_h = 5.08 + 3.7 \cdot 10^{-3}M_r - 7.03 \cdot 10^{-8}M_r^2 \quad (4)$$

The average deviation of experimentally obtained data from the theoretical curve was approx.  $0.65 \text{ \AA}$ .

### 1.3 Accessibility of polymer molecules to dissolved ions

A priori, several relations could be expected to exist between ions and polymer molecules in water solution. These possibilities included the whole volume of hydrated polymer being inaccessible to ions, or ions being able to reach the entire volume (except the volume of the linear polymer molecule itself) of water of the hydrated polymer or its part. The real situation can be established by measuring the respective ion concentration in polymer-free bulk solution. Three methods were used to approach this problem. First, polymer was separated from salt solution by ultrafiltration with subsequent determination of ion concentration in polymer-free solution. Then, the ion concentration in polymer-salt solution was determined using K-selective electrode and two standard additions of stock KCl solutions aliquots. Also, alpha-staphylo toxin modified BLM was used. In this case, BLM separated two KCl solutions, one of them additionally containing some amount of PEG. The difference in KCl concentrations between these two solutions was determined as an additional concentration of KCl that had to be added into the polymer-free solution to obtain zero trans-membrane potential.

All the data obtained suggested that the ion concentration in 20% PEG (2000–20,000) solution really was approximately 30% higher than that calculated based on the entire volume of the solution.

Obviously, a part of solution volume is occupied by the nonelectrolyte molecules. The crystal volume of PEG is known to be independent on the molecular weight of polymers, and occupies  $\sim 16\%$  of volume ( $d = 1.28 \pm 0.03 \text{ g/cm}^3$ ) in 20% solution. As a result, the ion concentrations in polymer-free solution is expected to increase by up to 19%. This value is smaller than the experimentally obtained ion concentration in polymer-free solution, the difference being only 10%. This result was independent on  $M_r$  of PEGs, although the portion of volume filled by hydrated polymer rose continuously along with the increasing molecular weight of PEG, reaching e.g. 42% for 20% solution of PEG 1000 and 90% for 20% PEG 4000. Therefore, the ion concentration of both the water phase of the hydrated PEG molecule and the inter-polymer solution were effectively identical. This allows the suggestion that almost the entire water phase of PEG molecules is accessible to ions. Actually, in similar condition the PEG molecule resembles a porous sphere filled with the water-KCl solution.

This result explained well the independence of the polymer – KCl solution conductivity on the nonelectrolyte molecule size in the presence of standard polymer concentrations (%). Probably, the ion mobility was influenced by microviscosity of the environment (affected by hydration of monomeric chain of polyglycols) much more than by macroviscosity which depends on the configuration of the polymer chain. Thus, the ion movement in the polymer solutions was not determined by macromolecules as distinct particles. This conclusion agrees with data obtained by

Toryanic (1987). He showed that the self-diffusing coefficient of water molecules in PEG-solutions was a function of the polymer concentration and temperature, but not of PEG molecular mass.

Thus, the spherical shape of PEG molecules in water solutions and the independence of ions movement on the polymer chain size makes PEG molecules a useful tool for studying mechanisms of ion movement through ion channels and for determination of effective radii of the water pores.

## ***2. Effects of nonelectrolytes on ionic current through single channels***

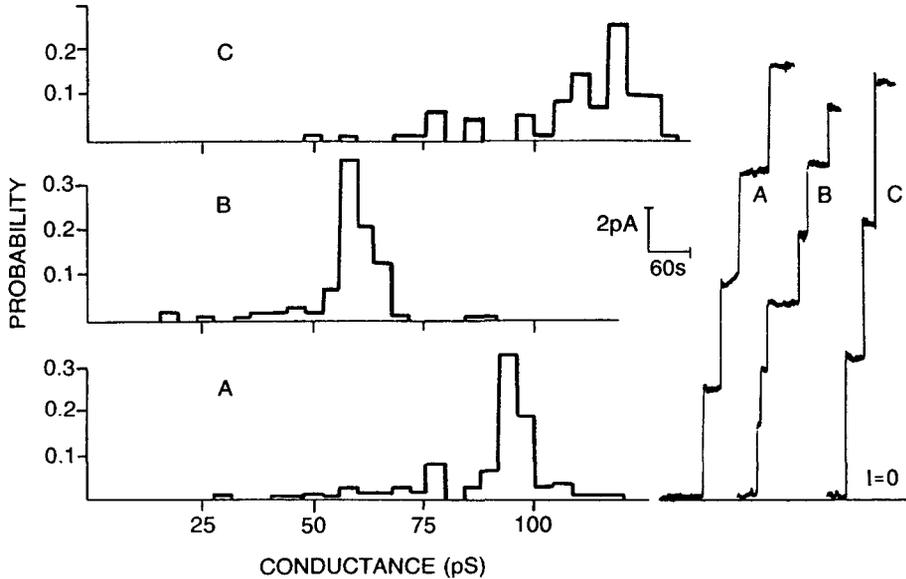
Three types of ion channels were used in the present experiments. It could be established that: (i) the proportionate change in both the ion channel conductance and the conductivity of bulk solution induced by low m.w. nonelectrolytes can be employed as a criterion of the diffusion mechanism of ion transport through channels; (ii) the slope of ion channel conductance vs. conductivity curve for bulk solutions containing different concentrations of nonelectrolytes is a good measure of channel permeability for the nonelectrolyte; (iii) the hydrodynamic radius of a nonelectrolyte molecule located in transition zone (between limited nonelectrolyte permeation and impermeability) can be taken as the effective radius of the ion channel water pore.

### ***2.1 The anion selective channel induced by *S.aureus* alpha-toxin (ST)***

ST is a toxic protein with m.m.=33 kD and pI 8.4. It has several kinds of biological activity (Thelestam and Blomquist 1988). All the effects of ST are connected with its ability to form ion channels in lipid matrix of plasma membranes. ST also induces the trans-membrane channels in BLM. The maximum of the conductance distribution of these channels was reported to be approx.  $100 \pm 4$  pS (100 mmol/l KCl, pH 7.5). The cation transference number was  $0.26 \pm 0.2$  (Krasilnikov et al. 1981; Krasilnikov and Sabirov 1989b; Menestrina 1986).

It was established that nonelectrolytes with small hydrodynamic radii changed the conductance of ion channels stronger than did molecules with large radii. The efficiency of the influence was proportional to the percent concentration.

With ethylene glycol concentration in BLM bathing solution raising from 10% to 30%, the conductance of single ST-channels decreased linearly in parallel to the change in conductivity of 100 mmol/l KCl solution (Fig. 1). The effect of 20% ethyleneglycol on conductance of ST-channels is shown in Fig. 4. Similar results were obtained with other low m.w. nonelectrolytes such as glycerin, glucose or sucrose. It should be noted that the molecules of all the nonelectrolytes tested had small hydrodynamic radii. The plots of single channel conductance against conductivity of bulk solution containing the nonelectrolyte are clearly linear and similar (Fig. 5). In characterizing the effects of nonelectrolytes on the conductance



**Figure 4.** Amplitude histogram of conductance fluctuations of voltage-clamped (50 mV) phosphatidylcholine-cholesterol (3:1, w:w) membranes in basic solution (100 mmol/l KCl, 5 mmol/l Tris-citric acid buffer, pH 7.5) (A), in basic solution plus 20% ethyleneglycol (B), or 20% PEG 4000 (C). Original single channel recordings under the corresponding conditions are shown on the right. The line  $I=0$  indicates zero current.

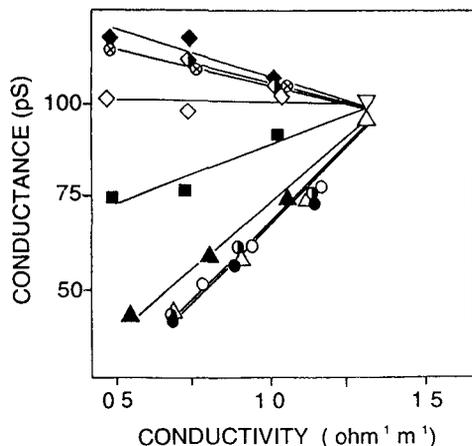
of ion channel ( $G$ ) it is convenient to use parameter  $\nu$ , defined as follow:

$$\nu = dG/d\kappa : G_0/\kappa_0 \quad (5)$$

where  $dG/d\kappa$  is the slope of the dependence of  $G$  on conductivity of KCl solutions ( $\kappa$ ) upon the addition of different concentrations of each distinct nonelectrolyte;  $G_0$  and  $\kappa_0$  are the ion channel conductance and the conductivity of bulk solution in absence of nonelectrolytes.

For the solutions of low molecular weight nonelectrolytes the numerical value of  $\nu$  was close to unity. It follows that the ion channel conductance changed in parallel with the conductivity of bulk solution. Two conclusions can be drawn from this. First, the nonelectrolytes tested permeate easily through ST-channel. Secondly, the mechanism underlying the ion transport through ST channel is free diffusion, since ion movements both in water pore and in bulk solution were identical.

PEG with higher m.w. (300 – 2000) decreased the slope of the dependence of  $G$  on  $\kappa$  to zero. With PEGs of m.w. equal to or exceeding 3000 slightly negative values were obtained (Fig. 5).

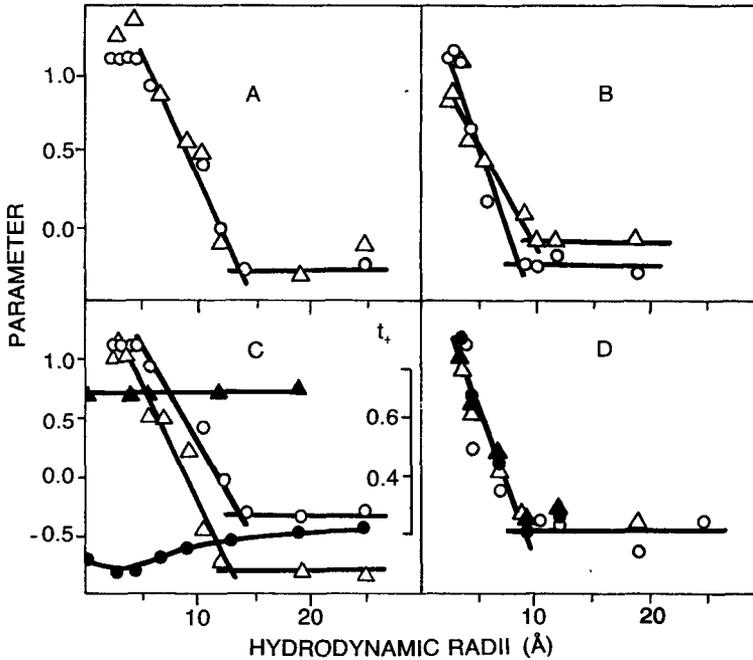


**Figure 5.** Single-channel conductance of ST channel as a function of electrical conductivity of bulk solution containing different nonelectrolytes. For conditions see Figure 4. Symbol X stands for media without any nonelectrolytes. Other symbols as Figure 1.

The samples of ST-induced current steps in presence of some nonelectrolytes and the distribution of conductance amplitudes of these ST channels are shown in Fig 4. In presence of PEG 4000, current through a single channel was actually larger than in pure water-salt solution. Moreover, it should be noted that the conductivity of bulk solution was considerably lower than that of pure KCl solution (Fig 1).

The following explanation can be offered for the effects obtained. When the size of the nonelectrolyte approaches the size of the channel water pore the friction between the pore wall and the nonelectrolyte molecules passing through the channel becomes significant. This will decrease the effective nonelectrolyte concentration inside the channel and, correspondingly, increase both the ion mobility within the pore and the channel conductance. As soon as the hydrodynamic radius of the nonelectrolyte molecules exceed the size of the channel water pore the composition of the solution inside the pore will be identical with that of the nonelectrolyte-free part of the bulk solution. Thus, a situation occurs in the water pore of the ion channel similar to that with the bulk solution containing no nonelectrolyte. However, the channel conductance may be somewhat larger than that of nonelectrolyte-free solution because the activity of ions in nonelectrolyte-containing solutions was larger (by 30% in 20% solutions of PEGs, as shown in part 1). Due to this the slope of the dependence of  $G$  on  $\kappa$  may become negative when the bulk solution contains impermeable nonelectrolytes. This was actually observed (Fig 5). This data analysis allowed the suggestion that the numeric value of the slope can be used as the channel permeability criterion for each distinct nonelectrolyte.

Then the three different parts of real function of  $\nu$  on the hydrodynamic radius of nonelectrolyte ( $\nu = 1$ ,  $0 < \nu < 1$  and  $\nu \leq 0$ ) will correspond to three



**Figure 6.** Values of parameter  $\nu$  for some channels, as a function of the hydrodynamic radii of nonelectrolytes. The basic solution contained 100 mmol/l KCl, 5 mmol/l Tris-citric buffer. *A*) ST channels:  $\circ$  - pH 7.5, data presented on Fig. 4 were used;  $\Delta$  - pH 6.0,  $\nu$  was determined by rapid method, membranes were formed of phosphatidylcholine:cholesterol mixture (1:1; w:w) by method of Montal and Mueller (1972). *B*) LT channels G170 ( $\Delta$ ) and G75 ( $\circ$ ) induced by fresh venom; BLM was formed according to Mueller et al. (1963) using a 4% solution of phosphatidylcholine:cholesterol mixture (3:1; w:w) in *n*-octane. Data taken from Fig. 7. *C*) ST ( $\circ$ ) and F channels ( $\Delta$ ); pH 7.5. The membranes were formed using a 4% solution of phosphatidylcholine:cholesterol mixture (4:1; w:w) in *n*-octane.  $\bullet$  and  $\blacktriangle$  - the cation transference number for these channels respectively (left scale). Parameter  $\nu$  for F channels was calculated by rapid method. *D*) LT channels in phosphatidylserine membranes formed according to Mueller et al. (1963). For pH and KCl concentration see Methods. Symbol  $\circ$ ,  $\bullet$ ,  $\Delta$  and  $\blacktriangle$  indicate channel type 1, type 2, type 3 and 4 (see in Methods). Parameter  $\nu$  was calculated by the rapid method.

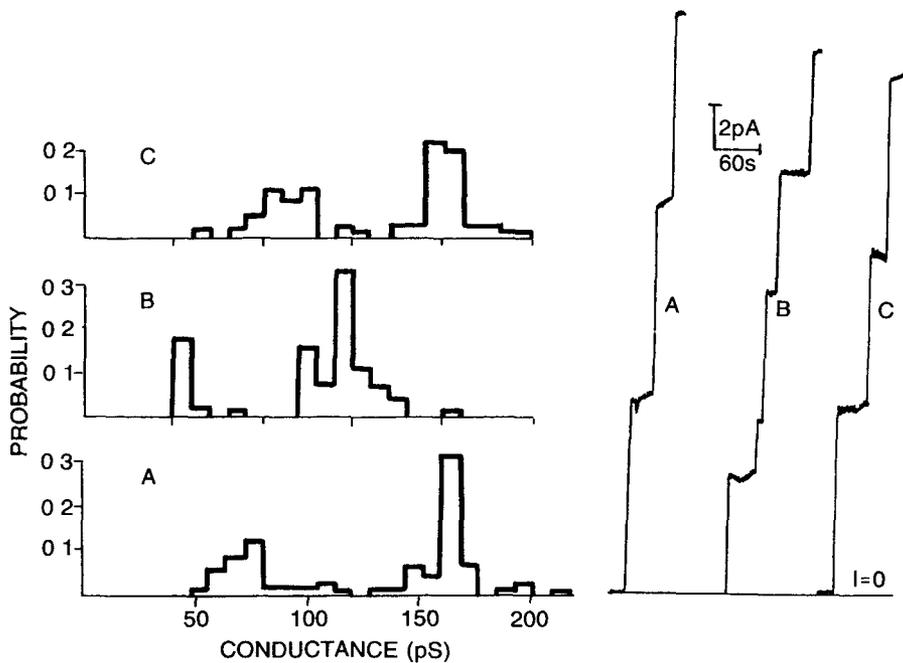
types of channel permeability to nonelectrolytes: high, limited and no permeability. Thus, the effective radius of a water pore may be determined as a minimal size of an impermeable nonelectrolyte molecule, i.e. the transition zone from limited permeation of a nonelectrolyte to an impermeable one. The error of the estimation of a pore radius can be calculated as standard deviation of nonelectrolytes radii from  $r_h = f(\nu)$ . Usually the error was in the range of 0.5–1.0 Å.

Using this method to determine the water pore size of the ST-channel (Fig 6 A) yielded  $13.5 \pm 0.5 \text{ \AA}$  as the effective radius. This value is approximately equal to that of the effective radius of ST-induced water pore in human erythrocyte membrane ( $13 \text{ \AA}$ , Krasilnikov et al 1988b). Our results are in good agreement with earlier electrooptic investigations ( $10\text{--}15 \text{ \AA}$ , Fussle et al 1981 and  $12.5 \text{ \AA}$ , Olofsson et al 1988).

## 2.2 The cation-selective channel induced by *Latrodectus tredecimguttatus* alpha-toxin, and its water pore

Alpha-latrotoxin (LT) was usually obtained from *L. tredecimguttatus* venom. It is a neurotoxic protein with  $M_r$  of about 130 kD and  $pI$  5.6. LT was able to induce ion channels in BLM. The LT channels have an almost ideal cation selectivity. The cation transference number ( $t_+$ ) was  $0.98 \pm 0.02$  at pH 7.5. As was shown earlier, the conductance of LT channel depended on the conditions of purification and on the lipid composition of BLM, and it varied between 100 pS and 1100 pS in identical bathing solution (Krasilnikov et al 1988a, Krasilnikov and Sabirov 1989a).

Salt extract (100 mmol/l KCl, 5 mmol/l Tris-HCl, pH 7.5) of venom glands stored at  $-24^\circ\text{C}$  was used. Channels induced by this extract were effectively iden-



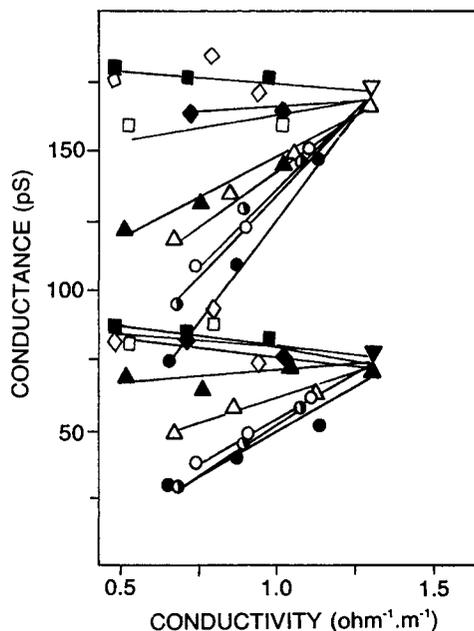
**Figure 7.** Histogram of LT channels conductances measured in the presence of some nonelectrolytes. The voltage was  $-50 \text{ mV}$ . For other conditions see Fig 4.

tical to channels induced by fresh venom produced by the spider at the biting moment (Krasilnikov and Sabirov 1989a). Usually, these preparations were observed to generate two major pools of ion channels in phosphatidylcholine BLM. One pool had a conductance ( $G$ ) of  $170 \pm 14$  pS (G170), and that of the other one was  $75 \pm 6$  pS (G75) (Fig. 7). An addition of the different nonelectrolytes into water phase changed the LT channel properties (see properties of ST channels). The original current steps generated by LT, and the corresponding distributions of conductance amplitudes for these LT-channels under slightly different conditions are also shown in Fig. 7. The slope of the dependence of both channel pools conductances on the conductivity of bulk solution was positive if the solution contained nonelectrolyte with a low m.w., and it was weakly negative if the solution contained nonelectrolyte with a high m.w. (Fig. 8).

The dependence of parameter  $\nu$  on the hydrodynamic radius of the nonelectrolytes (Fig. 6B) resembled that for ST channel except for  $\nu = 1$  where no clear-cut plateau appeared. According to our data (Fig. 6B) the radii of LT-channels (G170 and G75) were slightly different, reaching  $9.8 \pm 0.9$  Å and  $8.8 \pm 0.8$  Å respectively.

### 2.3 A simplified method for the determination of ion channels water pore radius

This rapid method for the size determination of transmembrane channels water pores is also based on the estimation of ion channel conductance. However, this method requires but a single fixed concentration of different nonelectrolytes. The



**Figure 8.** Single-channel conductance of LT channels as a function of the electrical conductivity of bulk solution containing different nonelectrolytes. For conditions see Fig. 7, same symbols as in Fig. 1 and 5.

method is based on both the linear relationship between  $G$  and  $\kappa$  and the independence of  $\nu$  on nonelectrolyte concentration. The use 20% solutions of nonelectrolyte was convenient. Higher nonelectrolyte concentrations (especially of old polymers with high m.w.) may widely interfere, with a decreasing BLM stability along with changes of channel properties (e.g. the rate of channel transitions between open and closed states and its cation-anion selectivity may be changed). Using lower concentrations of nonelectrolytes was associated with a decreased precision of channel radius estimations. This rapid method also makes use of the parameter  $\nu$  expressed as:

$$\nu = (G_0 - G_{20})/(\kappa_0 - \kappa_{20}) : (G_0/\kappa_0) \quad (6)$$

Where  $\kappa_{20}$ ,  $\kappa_0$  are electric conductivities of 100 mmol/l KCL solution with and without nonelectrolyte (20%);  $G_{20}$ ,  $G_0$  are ion channel conductances in the same solutions respectively.

All procedures and analyses used by this simplified method were basically identical with those used by the original method. The degree of precision of the determination of effective pore radius is effectively very close for both methods, but data usually can be obtained more quickly with the simplified method.

Results of the application of the rapid method are shown in Fig. 6D. Obviously, the water pore radii of all the different types of LT channels studied (phosphatidylserine BLM: 100 pS; 460 pS; 540 pS; 1100 pS) were effectively of identical size ( $9.4 \pm 1 \text{ \AA}$ ). Thus, cluster organization is very probable for LT-channels in BLM (Krasilnikov et al. 1988a; Krasilnikov and Sabirov 1989a). Similar values were obtained for radii size of this channel in uncharged BLM (see under 2.2).

This rapid method was used by us to study channels induced by N-terminal fragment (F) of *S. aureus* alpha-toxin (Fig. 6C). The radius of F-channel water pore was estimated to be  $13.1 \pm 0.7 \text{ \AA}$ . The numerical value of this pore radius did not differ from that induced by the entire toxin molecule (see under 2.1).

A comparative analysis of the phenomena illustrated in Fig. 6 showed the numerical value of  $\nu$ , corresponding to impermeable nonelectrolytes, to vary between zero (for LT-channels) and minus 0.8 (for F-channels). The latter value may suggest a higher activity of KCl in the polymer-free part of the used solution (see under 1) associated with a higher channel conductance. By their weak influence on ion channel properties nonelectrolytes likely raise the value of  $\nu$  as can be observed with some channels.

## Discussion

According to our data, the diffusion mechanism is operative in ion transport through all channels studied, including ST channel, LT channel and F channel. Some of the channel properties, such as current-voltage characteristics (which may

be nonlinear and asymmetric) ionic selectivity, and saturation of conductance upon increasing ion concentration (Krasilnikov et al. 1988a,b; Ternovsky et al. 1991) suggest that the energetic profile of ions crossing the channels was not homogeneous. Note that diffusion equations accounting for heterogeneous energetic profile of the channel were successfully applied to ST channel (Krasilnikov and Sabirov 1989b). An analogical description may suit other ion channels.

It should be stressed that the suggested method is actually simple in laboratory use. However, it presents a few difficulties which interfere with a correct explanation of results. A major problem is the dispersion of PEGs m.w. of commercial preparations (Scherrer and Gerhardt, 1971). In theory, this is expected to displace the dependence of  $\nu$  on nonelectrolyte m.w. towards larger values thus overestimating the pore radius size. However, both these effects were found to be within one standard deviation of the ion channel radii estimates.

Another problem is the assumption of hydrophilic nonelectrolytes being inert. This assumption does not hold since dipole-dipole, charge-dipole and other interactions between functionally important groups of the channel and nonelectrolyte molecule exist. It were small changes in selectivity observed in the presence of nonelectrolytes in water solution, for ST channels only, that prompted a similar suggestion. However, the effect observed on ST channel being weak and the influence of nonelectrolytes on the selectivity of other channels examined being almost invisible, made us to conclude that the discussed interaction did not have any strong influence on the estimated value of the water pore size obtained by this method.

Thus, despite some difficulties the values obtained by this method for water pore radii obviously exactly match the real size of the ion channel and are reliable. Our method has two major advantages: (i) it uses the uncharged molecular instruments and (ii) only direct electrical measurements of channel conductance are needed. This is very convenient when single ion channels are to be investigated. The test of the mechanism of ion transport through the channel and the method of pore radii estimation can be expected to be useful for studying many other types of ion channels.

It should be noted that although nonelectrolytes undoubtedly interact with membrane lipids, there was no effect of this interaction observed on capacity and conductance of nonmodified BLM. These effects thus can be ignored and need not be accounted for.

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