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IONIC SPECIFICITY OF THE GRAMICIDIN CHANNEL AND THE THALLOUS ION

ERWIN NEHER*

Abteilung Molekularer Systemaufbau, Max-Planck-Institut für biophysikalische Chemie (Karl-Friedrich-Bonhoeffer-Institut), D 34 Göttingen-Nikolausberg, (G.F.R.)

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Summary

The thallous ion was found to interact very specifically with gramicidin channels in black lipid membranes. Although the ion itself carries current through the channel better than Na^+ or K^+, it blocks Na^+ currents at concentrations which are two orders of magnitude lower than the Na^+ concentrations.

Black lipid membranes are widely used as model systems for the study of electrical permeability. High selectivity among cations, which is common in biological systems, has so far only been reported for membranes doped with carriers, such as valinomycin. Typical channel formers such as gramicidin and alamethicin lack this property [1]. An exception to this rule has recently been demonstrated by Eisenman et al. [2] who found from membrane potential measurements in gramicidin doped bilayers that the thallous ion Tl^+ plays a special role among the monovalent cations. Its permeability ratio with respect to K^+ is approximately 50 (at a concn 10^{-2} M) as compared to ratios between 0.1 and 2 for most other monovalent cations [1].

Thallium also has some extraordinary properties with respect to biological membranes. It is the most permeable ion in the K^+ channel of squid [3] and myelinated nerve [4]. It is actively transported into human red cells in exchange for internal Na^+ and substitutes with high affinity for K^+ at the external K^+ binding sites of the Na^+ pump [5]. It also competes reversibly with tetrodotoxin and saxitoxin for binding sites in the Na^+ channel of nerve membrane [6]. Its interaction with these sites is 50 to 100 times stronger than that of Na^+ or K^+.

*Present address: Yale University School of Medicine, Department of Physiology, New Haven, Conn. 06510, U.S.A.

In the light of these findings it seemed interesting to investigate the conductance of Tl^+ in gramicidin induced ionic channels more closely.

Black lipid membranes were made from 20 mM solutions of glyceryl-monooleate in hexadecane across a 300 μm diameter hole in a polypropylene vessel as described earlier [7]. Small quantities (0.1 to 0.2 ml) of a 5 ng/ml ethanol solution of gramicidin A were added to the electrolyte (total volume: 30 ml) until discrete current steps occurred at fixed membrane voltage. The most frequent unit step size was determined for ± 20 mV and ± 15 mV membrane voltage. From these four values conductance was calculated and designated as "zero current conductance". In some cases, unit step sizes were also determined at ± 100 mV. The ratio $\Delta I/\Delta V$ (current-voltage) in these cases was designated "conductance at 100 mV". Extremely small diameter membranes between 150—300 μm diameter were prerequesite for measuring unit step sizes in the range 0.05—0.2 pA (see insert Fig. 1). The experiments were performed at room temperature (22 to 24°C). Since TlCl is not well soluble in water, acetate salts (thallous acetate and sodium acetate, suprapure from Merck) were used. NaCl or TlCl respectively was added to all solutions at a concentration of 1 mM in order to stabilize electrode potentials (silver/silver chloride sintered pellets).

Concentration-dependence of channel conductance. Zero current conductance of gramicidin-induced channels was determined as a function of concentration of Tl^+-acetate. It showed the typical saturating behaviour found for other monovalent cations [8]. This behaviour is well described by Läuger's theory of channel conductance [9], in which saturation is the result of a mutual exclusion of ions within the channel. In this theory, the concentration dependence bears a formal analogy to an adsorption isotherm. Therefore the data are plotted as double reciprocal plots in Fig. 1A. The apparent binding constant K_{Na} has a value of 6.2 M^{-1}. Fig. 1B (circles) gives the data on pure sodium acetate solutions for comparison. It is evident that Tl^+ gives a slightly higher limiting conductance than Na^+ and reaches saturation at lower concentrations. Its apparent binding constant K_{Tl} is 12.3 M^{-1}.

Surprisingly, Na^+ currents are markedly depressed by traces of Tl^+. Channel conductance as a function of NaCl concentration was determined in the presence of a constant 2 mM thallous acetate. In the double-reciprocal plot of Fig. 1B the inhibition appears to be competitive. However, a value of 800 M^{-1} is obtained for the inhibitory binding constant of thallium $K_{Tl,I}$. It should be noted that this value is almost a hundred times higher than the apparent binding constant K_{Tl} in pure Tl^+-solutions. A simple mutual exclusion mechanism would predict $K_{Tl,I} = K_{Tl}$.

For Tl^+ a pronounced change in the I-V relation from sublinearity to supralinearity is seen with increasing concentration; for Na^+ this is not so obvious.

Single channel conductance is dependent on mol fraction. To study Na^+/Tl^+ interaction further, channel conductance was measured at a 1 M total salt concn with the ratio $Tl^+ : Na^+$ being varied symmetrically in both chambers, by analogy with the procedure first used by Eisenman et al. in analyzing the complex behavior of thin glass films [10]. Fig. 2 shows a pronounced depression in the curve relating unit channel conductance to mol fraction of Tl^+.

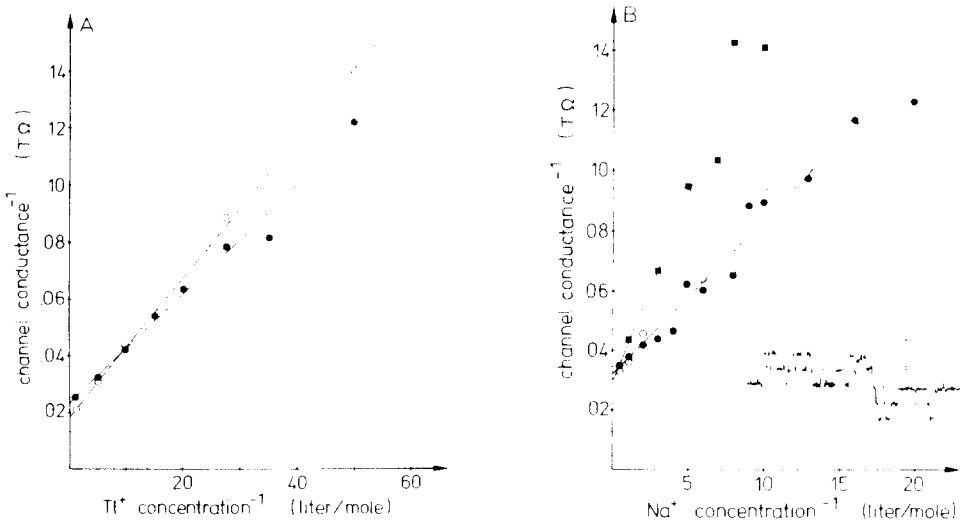

Fig. 1. Double-reciprocal plot of the conductance of gramicidin-induced single channels versus salt-concentration. Closed symbols are values of "zero current conductance". Open symbols refer to conductance at 100 mV applied potential. The solutions are symmetrical on both sides of the membrane. In part A the salt is thallous-acetate; in part B the salt is pure sodium acetate for circles and it is sodium acetate plus a constant amount of 2 mM/liter thallous acetate for squares. Analysing "zero current conductance", part A suggests a binding constant [9] for Tl^+ of $K_{Tl} = 12.3\ M^{-1}$; from B a value of K_{Na} of $6.2\ M^{-1}$ is obtained. The scatter in the data points for diluted solutions does not originate from inaccuracy in the determination of conductance values but from differences between different membranes and also slow drifts. These drifts were not observed, when salt concentrations were in the saturating range. In order to document the accuracy of the data, an original current trace is included as an insert in part B. Salt concentration is 62.5 mM sodium acetate. Voltage is switched from −15 mV at about the midpoint of the recording. These conditions are among the most unfavourable ones encountered during this investigation (smallest channel sizes). Mean channel sizes of ± 0.13 pA are determined from this record at ± 15 mV. The vertical bar represents 0.25 pA; total duration of this segment is 140 s.

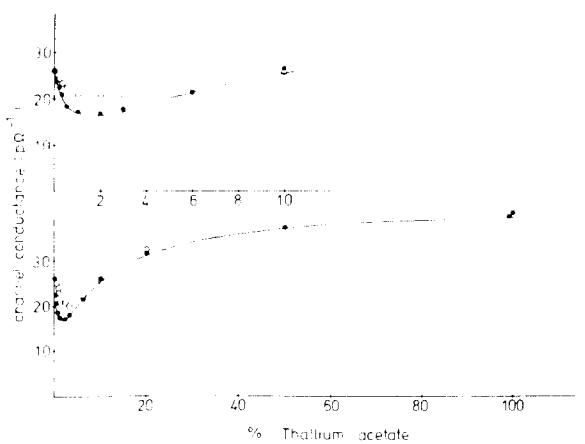

Fig. 2. Plot of the conductance of gramicidin induced single channels versus mole-fraction of Tl^+ at 1 M total concentration of thallous acetate plus sodium acetate; symmetrical conditions on both sides of the membrane ●-●, zero current conductance; o-o, conductance at 100 mV. The upper part is an expanded view of the lower one. The thin continuous line in each plot is calculated according to

$$\lambda = \lambda_{o,Na}/[1+(1K_{Tl,I}c_{Tl})/(K_{Na}c_{Na})] + \lambda_{o,Tl}/[1+(1+K_{Na,I}\cdot c_{Na})/(K_{Tl}\cdot c_{Tl})]$$

with $\lambda_{o,Na}$, $\lambda_{o,Tl}$, K_{Na}, K_{Tl} and $K_{Tl,I}$ taken from Fig. 1. $K_{Na,I}$ had to be taken as zero. When setting $K_{Na,I} = K_{Na}$ deviations up to 50% occurred.

Analogous behavior was reported for the membrane of starfish egg cell [11].

Extending Läuger's theory [9] of channel conductance to the case of two cations present symmetrically on each side of the membrane, one arrives at

$$\lambda = \lambda_{0,Na} \frac{1}{1 + (1 + K_{Tl} \cdot c_{Tl})/(K_{Na} c_{Na})} + \lambda_{0,Tl} \frac{1}{1 + (1 + K_{Na} \cdot c_{Na})/(K_{Tl} c_{Tl})} \quad (1)$$

where λ is the conductance of the channel, $\lambda_{0,Na}$ and $\lambda_{0,Tl}$ are maximum conductances obtainable at high concentrations of the respective ion, K_{Na} and K_{Tl} are binding constants of Na^+ and Tl^+ to the pore, and c_{Na} and c_{Tl} are ion concentrations. This expression is obtained by replacing the quantity P (probability of occupancy of a pore) in Eqns 40 and 41 of ref. 9 by its equilibrium value as derived from Eqns 5 and 6 of the same reference. A monotonically increasing curve would be obtained in Fig. 2 under these conditions, since $\lambda_{Tl} > \lambda_{Na}$. Obviously Eqn 1 cannot be fitted to the data. Considering, however, that the term $(1+K_{Tl}c_{Tl})$ in the first part of Eqn 1 represents the inhibitory effect of Tl^+ on the Na^+ contribution to the current, it seemed interesting to replace K_{Tl}, in this expression by $K_{Tl,I}$ as derived from Fig. 1B. An accurate fit to the data can be obtained (see solid line in Fig. 2) by inserting the values for $\lambda_{0,Na}$, $\lambda_{0,Tl}$, K_{Na}, K_{Tl}, and $K_{Tl,I}$ from Fig. 1 into Eqn 1 with the additional assumption that there is no inhibitory effect of Na^+ on the Tl^+ contribution to the current (K_{Na} in the second term of Eqn 1 was set to zero).

This result is a strong indication that a simple mutual exclusion mechanism among ions in a channel does not apply to the action of Tl^+. On the other hand, the specific alterations in Eqn 1 which are necessary to fit the data may provide some hints for selecting a better model: If it is assumed that Tl^+ binds specifically to the lipid, creating a surface charge, all cation concentrations would be reduced at the interface in the presence of small amounts of Tl^+. Such a mechanism would predict the observed effects but was ruled out by the experimental finding that the presence of divalent, negatively charged ions (2 mM Na_2SO_4) did not increase the channel conductance in 20 mM Tl^+ acetate solutions. If the proposed mechanism would apply, sulfate-ions should have a strong screening effect under these circumstances.

Alternatively a mechanism involving a structural change of the channel, depending on mol fraction, might apply, similar to that suggested by studies on ion-sensitive glasses [10].

The results reported here are the consequence of a highly selective interaction between Tl^+ and an ionic channel (or the membrane in which it is embedded). This is the only specific interaction between an ion and a well defined channel so far reported for model membranes.

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