

BBAMEM 76147

Ion velocity distributions in gramicidin channels determined with laser Doppler velocimetry

Felipe Macias and Michael E. Starzak *

Department of Chemistry, State University of New York at Binghamton, Binghamton, NY 13902-6000 (USA)

(Received 9 February 1993)

(Revised manuscript received 8 June 1993)

Key words: Doppler scattering; Gramicidin; Intrachannel velocity; Velocity, ion; Laser Doppler velocity

Laser Doppler scattering from Tl(I) ions moving synchronously through an ensemble of gramicidin channels in a bilayer membrane gives their intrachannel velocity distribution. The observed velocity distributions are unimodal indicating that ion flow through some region or regions of the channels is relatively steady. Average intrachannel velocities range from $3.75 \cdot 10^{-2}$ m/s to $2.38 \cdot 10^{-1}$ m/s for transmembrane potentials between 10 mV and 150 mV, respectively.

Introduction

The gramicidin channel is one of the simpler channels since it contains no voltage-dependent gating and very little ion selectivity. Almost all univalent metal cations are permeant ions for gramicidin and the currents produced by these ions as they permeate this channel at a given transmembrane potential are the major experimental observables. Despite the basic simplicity of the channel, the actual ion permeation mechanism is difficult to ascertain because the single electrical observation for a given set of conditions does not provide enough information to distinguish different possible mechanisms. The mechanism can be resolved with additional information on the ion motions within the channel. Such motions could be observed as channel transport noise, but Lauger [1] has noted the difficulties which must be overcome to attain the proper frequency range using electrical measurements. Theoretical transport noise spectra have been determined for different permeation mechanisms [2,3]. However, it is not at all clear that an experimental noise spectrum of a specific type would provide an unambiguous determination of the actual transport mechanism. Sigworth has studied power spectra produced by a variety of channel processes including ion permeation [4].

Because the electrical noise necessary to extract ion motion information lies at high frequencies, it has been difficult to observe using electrochemical techniques where it must be determined by subtracting other noise

[5]. For this reason, it is advantageous to gather information on ionic motions using some alternative, non-electrical technique. In fact, if the actual ion velocities within the channel can be obtained by some alternate technique, such data, in conjunction with the electrochemical data, can be used to generate a detailed mechanism of ion motions within the channel.

The key experimental parameter for an ion within the channel under the action of an applied field is its local velocity, i.e., its velocity at each point x within the channel. For a mechanism which includes intrachannel binding sites, this local velocity is the velocity at which the ion moves between binding sites. For discrete state models of the Parlin-Eyring genre, the flux J_i between two sites is normally described by a rate constant k_i and a length parameter, λ_i

$$J_i = k_i \lambda_i c_i$$

where c_i is the concentration of ions at the i -th site in an ensemble of channels. This local flux expression is conveniently written in terms of a local velocity v_i ,

$$v_i = k_i \lambda_i$$

for motion between the sites. Thus, for both a diffusion model, where the ion moves continuously through the channel, and a discrete state model, where it moves in a series of intrasite transitions, the relevant experimental parameter is the local velocity.

The local velocities within a channel can be measured using the photophysical technique of laser Doppler velocimetry. The technique yields the distribution of velocities within an ensemble of channels, i.e., it provides the relative numbers of ions in the channels at

* Corresponding author. Fax: +1 (607) 7774478.

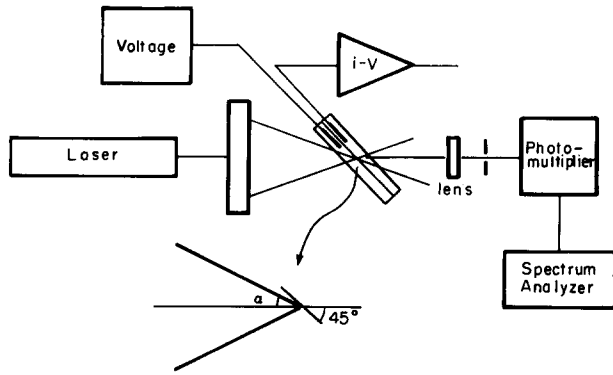


Fig. 1. Block diagram of the laser Doppler gramicidin channel system. Laser, Jodon 20 mW helium-neon; beam splitter, TSI; photomultiplier, RCA 1P21; spectrum analyzer, Hewlett Packard 3561A.

each possible velocity. It does not determine the location within the channel where the ion moves with a specific local velocity but a knowledge of the velocity distribution puts severe constraints on possible permeation mechanisms.

Laser Doppler velocimetry measures the velocities of ions within the channels by determining the Doppler shift produced when the radiation interacts with the moving ions. The channel length (2.6 nm) is short relative to the laser light wavelength (632.8 nm) and all the ions are moving in the same direction in this narrow spatial region. The ions can scatter radiation constructively to produce an observable signal.

Laser Doppler velocimetry has been used to measure larger single scattering centers in flow [6] and electrophoretic systems [7-9]. For the channel systems, the observable scattering is the cumulative scattering from a large number of small scattering centers (TI^+ ions) moving synchronously in a limited spatial region.

The Doppler scattering for this study of TI^+ ion in gramicidin channels was determined using a crossed beam technique [10] in which a single laser beam is split into two equal intensity halves which are then displaced equally in opposite directions from the original optical axis (Fig. 1). A lens then refocuses both

beams on the optical axis at a common point. Particles with velocity components perpendicular to the optical axis produce equal and opposite Doppler shifts to produce a net Doppler frequency difference Δf . For motion perpendicular to the optical axis, the velocity is determined from this frequency shift as

$$v = \lambda \Delta f / 2 \sin(\alpha)$$

where α is the angle between either input beam and the optical axis (Fig. 1) and λ is the laser wavelength.

A Jodon 20 mW laser beam was split, displaced and refocused on the optical axis using a TSI, Inc beam splitter and lens combination. Since the beams diverge from the optical axis after intersection, the forward Doppler scattering is collected along the optical axis. The scattered light is focused on a pinhole and directed to a 1P28 photomultiplier which acts as a non-linear detector to produce the difference frequency. Minimal extraneous noise results when the photomultiplier current is applied directly to the Hewlett Packard 3561 spectrum analyzer with a 115 kHz bandwidth.

The optical axis is centered on the bilayer membrane and channels using a micrometer-controlled hydraulic system. The normal of the membrane could be adjusted to any angle relative to the optical axis so that the actual direction of the moving particles could be determined unambiguously. The cell for the membrane was constructed from two equal halves of a cylindrical tube.

The bilayer membranes were formed from solutions of 25 mg glycerol monooleate (Sigma) in 0.9 ml of decane (Wiley). Bathing solutions were 0.1 M $\text{TI}(\text{I})\text{C}_2\text{H}_3\text{O}_2$ (TlAc) with approx. 1 mM TlCl for Ag-AgCl electrode stability. Gramicidin was added to the baths until the current reached approx. 65% of the conductance through the membrane-free orifice. A constant transmembrane potential was maintained for the 10-15 minutes required for each experiment.

Frequency difference distributions, which are directly proportional to the local velocity distributions,

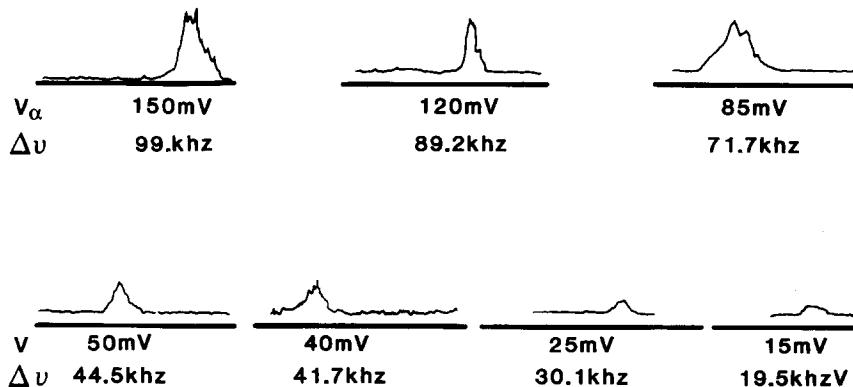


Fig. 2. Doppler difference spectra for gramicidin channels in a bilayer membrane oriented 45° to the optical axis.

TABLE I

System parameters for the pots in Fig. 2

Potential (mV)	Frequency (kHz)	Plot range (kHz)	Amplitude (μV_{rms})
150	99.0	95–100	5.57
120	88.2	85–90	5.09
85	71.7	70–75	4.9
50	44.5	45–50	3.47
40	41.72	40–45	3.67
25	30.1	25–30	6.26
25	18.6	15–20	5.71

appear in Fig. 2 for transmembrane potentials of 150, 120, 85, 50, 40, 25 and 15 mV. The observation parameters for these spectra are listed in Table I. A unimodal distribution is observed in each case indicating a limited range of velocities within the channels. Although all the reported experiments were performed with the membrane surface at an angle of 45° to the optical axis, some experiments were done at a series of orientation angles to establish that the observed velocities involved motions normal to the membrane rather than surface waves parallel to the surface [11,12]. The observed difference frequencies and corresponding average velocities, after correction for the 45° orientation of the membrane in the cell, are listed in Table II and plotted in Fig. 3. The velocities range from $3.75 \cdot 10^{-2}$ m/s for a 10 mV transmembrane potential to $2.38 \cdot 10^{-1}$ m/s for a transmembrane potential of 150 mV. These velocities are an order of magnitude smaller than the velocities expected if ions in the channel had the mobility of the TI^+ ions in the bulk aqueous medium and an applied field equal to that for the

TABLE II

TI^+ velocities and transit frequencies for transmembrane potentials in the ohmic regime

Potential (mV)	Observations	Doppler shift (kHz)	Velocity (m/s) ($\times 10^1$)
10	2	15.6 ± 0.19	0.375
15	1	19.5	0.469
20	2	24.2 ± 1.0	0.582
25	1	30.1	0.724
30	2	32.8 ± 0.8	0.788
40	3	40.8 ± 1.3	0.981
50	2	44.5 ± 1.4	1.09
60	2	54.3 ± 0.1	1.31
70	2	61.2 ± 0.2	1.47
80	3	67.5 ± 0.5	1.64
90	3	73.5 ± 1.3	1.77
100	3	78.3 ± 0.6	1.88
110	3	83.8 ± 1.4	2.01
120	3	87.4 ± 1.2	2.10
130	3	92.3 ± 0.5	2.22
140	3	96.4 ± 0.6	2.32
150	1	99.0	2.38

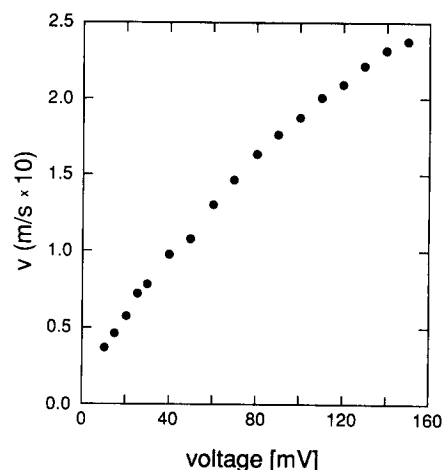


Fig. 3. Ion velocity determined from laser Doppler velocimetry versus applied membrane potential.

applied potential across a bilayer membrane with no gramicidin channels (0.3 m/s at 10 mV to 4.5 m/s at 150 mV). Although the plot of velocity versus potential approaches zero velocity as the potential goes to zero, the curvature of the plot makes it impossible to predict this intercept accurately.

These data show that the ions move at a constant velocity in some region of the channel. The data cannot reveal, however, whether this velocity is maintained for the length of the channel or for some region within the channel, e.g., between two intrachannel binding sites. The velocity distribution is relatively narrow reflecting small velocity fluctuations as the ions move in the narrow, one dimensional confines of the channel. Velocity distributions for ions approaching or entering the channel from the bulk solution have a range of entry angles and velocity components normal to the membrane and a velocity distribution for these motions will have a low maximal amplitude and large bandwidth. Such distributions were not detected with this experimental system.

Although the current-voltage plots for TI^+ ion in gramicidin channels are linear in this potential range, a plot of observed average velocity versus potential approaches a limiting velocity, i.e., is sublinear. This suggests that the motions observed with the velocimeter are not the rate-determining velocities for ion permeation. Andersen [13] has suggested that the diffusion-controlled association step is the limiting kinetic step and this observation is consistent with these data.

If the distance of travel at constant velocity is known, the transit frequency, ν , across this region for a distance d and velocity v is

$$\nu = v/d$$

The transit frequencies determined in this manner for steady velocity through the entire channel are consis-

tent with ion transit frequencies estimated by Andersen and Procopio [14]. However, the transit frequencies calculated in this way will increase if the observed velocities occur over shorter distances in the channel. Exact transit frequencies for a given velocity can only be determined if the distance that the ion moves at this velocity is known.

A single Tl^+ in a vacuum is subject to Doppler frequency variations in the kilohertz range if the quantum effects of the Heisenberg uncertainty principle are included and this frequency uncertainty is comparable or larger than the observed bandwidths of the Doppler frequency difference spectra. However, the Tl^+ ion in the channel is not an isolated entity. The incoming photon must transfer momentum to Tl^+ ion in a water filled membrane channel. The effective moving mass from which the photon scatters is much larger than the mass of a single Tl^+ ion and the quantum uncertainty must be considerably smaller for Tl^+ in the water-filled channels. If the moving column of Tl^+ ions and water molecules defined by the channel is viewed as a long thin molecule, it may be viewed as the scattering center with all parts of this 'molecule' moving with the same velocity.

Laser Doppler spectroscopy is a new way to observe ion motions in channels and membranes which complements the data obtained using electrochemical techniques. The technique provides information not only on the synchronous motion of ions in channel but also motions of molecules, such as gating molecule analogs,

within the membrane and may ultimately be used to unravel the motions of the gating component of protein channels during channel opening.

Acknowledgements

This research was supported by the Office of Naval Research, Contract No. 1487K0301. The optical table and cell mounts were designed and constructed by J. Palmetier.

References

- 1 Luger, P. (1975) *Biochim. Biophys. Acta* 413, 1–10.
- 2 Luger, P. (1978) *Biochim. Biophys. Acta* 507, 337–349.
- 3 Frehland, E. (1978) *Biophys. Chem.* 8, 255–265.
- 4 Sigworth, F. (1990) *Biophys. J.* 57, 499–514.
- 5 Sigworth, F.J. (1985) *Biophys. J.* 47, 709–720.
- 6 Dalbiez, J.P., Tabti, K., Derian, P.J. and Drifford, M. (1987) *Rev. Phys. Appl. Phys.* 22, 1013–1024.
- 7 Haas, D. and Ware, B.R. (1976) *Anal. Biochem.* 43, 175–188.
- 8 Mohan, R., Steiner, R. and Kaufmann, R. (1976) *Anal. Biochem.* 43, 175–188.
- 9 Uzgiris, E.E. (1974) *Rev. Sci. Instr.* 45, 74–80.
- 10 Drain, L.E. (1980) *The Laser Doppler Technique*, John Wiley, Chichester.
- 11 Byrne, D. and Earnshaw, J.C. (1979) *J. Phys. D Appl. Phys.* 12, 1145–1157.
- 12 Crawford, G.E. and Earnshaw, J.C. (1987) *Biophys. J.* 52, 87–94.
- 13 Andersen, O.S. (1983) *Biophys. J.* 41, 147–165.
- 14 Andersen, O.S. and Procopio, J. (1980) *Acta Physiol. Scand. Suppl.* 481, 27–35.