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19 ABSTRACT

Laser Doppler velocimetry and photochemical observation of Tl(I) ion flows are used to characterize the permeation properties of ions within gramicidin channels. The laser Doppler velocimetry provides a direct experimental determination of the distribution of ion velocities within these channels. For this information, it becomes possible to deduce the mechanism for ion transport, e.g. transport via discrete sites or continuum diffusion. The photochemical studies permit an experimental determination of the fraction of the total current carried by the Tl(I) ion in mixed cation systems using a dye molecule whose fluorescence is quenched by the permeating Tl(I) ion. This ion differentiation technique is utilized for a detailed study of Tl(I) ion in the gramicidin channel. This ion is a permeant ion at large mole fractions but becomes a channel blocker at small Tl(I) ion mole fractions.

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PROGRESS REPORT ON CONTRACT N00014-87-K0301

Principal Investigator: Michael Starzak

Title: Photochemical Observation of Ion Flows in Membrane Channels

INTRODUCTION

Laser Doppler velocimetry is applied to a study of the synchronous flux of Tl(I) ions through gramicidin channels to establish the velocity distribution of ions in these channels. Such velocity distributions are prerequisite to a clear understanding of the mechanism of ion transport through these channels. For example, a discrete state model requires intrachannel velocities,

$$v_i = k_i \lambda_i$$

These distinct velocities could be determined directly from the laser Doppler spectra in the frequency domain. The laser Doppler velocimeter is calibrated and tested using electrophoretic systems in which the single particle scattering is large, e.g. large vesicles or latex spheres, or the electrophoretic motion is synchronous, e.g. bovine serum albumen in acrylamide gels or latex spheres in Nucleopore membranes.

Tl(I) ion, which is the ideal scattering ion for these studies has several anomalous properties which are studied with novel photochemical techniques as well as laser Doppler velocimetry. In mixed cation systems, Tl(I) acts as a permeant ion at large mole fraction and a channel blocker at low Tl(I) mole fraction (Neher, 1975). Similar behavior has recently been noted for calcium channels (Hess and Tsien, 1984). Ca(II) can block the movement of other divalent ions in the channel. Tl(I) ion currents do not saturate for large transmembrane potentials (Andersen and Procopio, 1980). A new photochemical technique is used to establish the percentage of total membrane current or flux carried by Tl(I) ions in such systems using the fluorescence quenching of ANTS (Moore and Raftery, 1980). Both the total current and a quenching proportional to the Tl(I) are observed simultaneously for different Tl(I) mole fractions. This study is coupled with an electrochemical study of mixed cation systems for gramicidin to elucidate a consistent mechanism for the transport process.

PROGRESS REPORT

1.LASER DOPPLER VELOCIMETRY

a)Calibration and Optimization Experiments

Since the optical alignment of the laser Doppler system is critical, the instrument was tested with systems of known particle velocity. Latex spheres were diluted in a steady flow of water through the laser probe volume, the region of interference fringes which produce the velocity dependent modulation envelop. The velocity is determined directly from the observed modulation frequency using the interference fringe spacing s which is proportional to the laser frequency and the angle of intersection of the interfering laser beams,

$$v = f\ s$$

The laser probe volume was moved perpendicular to the direction of the flow to give changes in velocity consistent with Poiseuille's law for flow in a cylinder.

b)Gel Electrophoresis Experiments

For the Tl(I)-gramicidin experiments, the small scattering from individual Tl(I) ions is compensated by their synchronous motion through a membrane whose thickness is much less than the light wavelength for scattering. An acrylamide gel was used as a model system for the Tl(I)-gramicidin system. A narrow aliquot of an aqueous solution of bovine serum albumen (BSA) was introduced into the gel and a potential was applied to permit it to move through the probe beam. The gel system permitted large potentials on the Ag-AgCl electrodes. Because BSA fluoresces after excitation at 365 nm, the width of the sample could be determined. A 1 to 10u sample width is required to produce Doppler modulation in the system. Despite the expectation that the majority of the BSA would move to the leading edge of the sample volume, only destructive interference was observed suggesting that the BSA remained homogeneous in the sample volume which proved impossible to reduce below a thickness of 1 mm as determined from fluorescence analysis of the gel after the sample had traversed the probe volume.

c)Vesicles and Latex Spheres

The laser Doppler system was optimized for electrophoresis experiments using latex spheres of known surface charge density and phosphatidylcholine-phosphotidylserine vesicles. With both systems, velocity increased with increasing pH. The results for the vesicles in acrylamide gel are shown in Figures 1 and 2. The velocity increased linearly with potential to give a constant mobility over the range studied. The mobility dropped sharply in each case as the pH decreased and the net negative surface charge of the vesicles decreased. For pH<7, no signals were detected suggesting the present detection system (counting fringes on a storage oscilloscope or a fast chart recorder) may be inadequate for the very low Doppler frequencies expected.

d)Membranes

To provide a low Doppler frequency model system for ion transport through the membrane, Nucleopore membranes with pore diameters of 2 u were used with charged latex spheres of 1u to probe the effects of the membrane on particle mobility. The large spheres were necessary because the thickness of the membrane (ca .1mm) made it impossible to detect synchronous flow. Because of the membrane thickness, the transmitted intensity of scattered light was significantly smaller than the intensity of scattering from the orifice without the membrane. However a decrease in Doppler frequency from 32 hz (5.4x10-5 m/sec) to 28 hz (4.9x10-5 m/sec) on addition of the Nucleopore membrane was observed. Further studies of such changes are presently underway to ascertain if the observed decrease is consistent with the particle size relative to the size of the membrane channels.

Additional experiments have been performed with both hemispherical and planar bilayers of glycerolmonooleate without gramicidin to establish background Doppler shifts and a baseline for these systems. Although some membrane surfaces produce surface waves which can be observed with laser Doppler velocimetry (Byrne and Earnshaw, 1979), such waves have not yet been observed with this system. Studies of such membranes with gramicidin channels is proceeding concurrently with studies of the electrical and photochemical properties of these ion-channel systems. Maximal stable currents are required for optimal detection.

New studies of a Eu(III)-ionomycin complex are just underway. A Eu(III)-ionomycin complex, if stable, provides both a large scattering capability and the net charge to permit it to move within the membrane under an applied potential. This system will be used to provide a high density-high scatter system for studies in the lipid bilayer membranes and provides an alternate system for studies of synchronous flow. Eu(III) was selected because the Eu(III)-ionomycin complex can be characterized with a high resolution Eu(III) ion laser spectrometer available in the laboratory.

e)Theoretical Studies

Although the velocity distributions constitute primary experimental information on the motion of ions within the channels, this information must be analyzed to determine which model is consistent with the data. To this end, the expected velocity distributions for both continuum and discrete site models with consistent kinetic paramters are being developed for comparison with the experimental velocity distribution data.

PHOTOCHEMICAL EXPERIMENTS

2)Photochemical Analysis of Tl(I) in Channels

a)Vesicle Studies

Since Tl(I) is believed to function as a permeant ion or blocking ion with gramicidin channels, a photochemical quenching technique is used to establish the number of Tl(I) ions permeating gramicidin channels in vesicles in a series of mixed cation solutions. Phosphatidylcholine-phosphatidylserine vesicles with 35 mM internal ANTS anion, whose fluorescence is effectively quenched by Tl(I) anion, are added to solutions containing Tl(I) and Na(I) ions. Initial experiments have been performed on an SLM spectrofluorometer available in the department and sought only initial (before addition of the ion gradient) and final (after quenching) levels of fluorescence. These experiments have been inconclusive and will be repeated using a stop flow system now available in the department. Calibration experiments with ANTS and Tl(I) in solution demonstrate effective quenching for 150 uM ANTS (the average concentration expected for the vesicle-solution system) and 1-35 mM Tl(I) ion.

b) Planar Bilayers

Because only concentration gradients are possible with the vesicle systems, a planar bilayer system for the study of Tl(I) ion permeation in mixed cation systems has also been constructed to allow electrical potential-induced ion fluxes. The membrane is formed on a teflon support in a cell with quartz windows for both the exciting beam and the fluorescence. The net current through the membrane and the net photomultiplier current generated by the ANTS fluorescence are detected with current to voltage converters and directed to A/D converters in a Horizon NorthStar computer. The membrane currents are integrated to give a net ion flow; the ratio of fluorescence change to integrated current provides a direct measure of the fraction of current carried by Tl(I) after the system is calibrated using a system containing only Tl(I) ion. The system is presently under test using the cell without a membrane to establish total times for the transport of Tl(I) ion to the chamber containing the ANTS. The chamber, as originally designed, had no provision for solution mixing. This now appears essential for optimal quenching efficiency.

c) Tl(I) Currents in Mixed Ion Systems

Although studies on the anomalous effects of the Tl(I) ion have involved single channel studies (Neher, 1975) or studies on small membranes (Andersen and Procopio, 1980), the photochemical analyses require large currents for detection. This required verification of some of these experiments for a large ensemble of gramicidin channels. Because TlCl is extremely insoluble, it has generally been necessary to work with TlAc and a trace of TlCl to maintain the stability of the electrodes. With .75 M TlAC and 1 mM TlCl, some TlCl does precipitate indicating the Cl^- concentration in the bath is even lower. For the major ion transport required for the photochemical analysis experiments, this low Cl^- concentration produces significant variations in the observed ion currents. To alleviate this problem, Fe-FeOAc electrodes were studied as a possible acetate sensitive electrode of second order. Although FeOAc is indeed insoluble, a necessary prerequisite for such electrodes, the precipitate does not plate the electrodes effectively. This alternative electrode system is still under investigation. The electrical experiments are proceeding with the Ag-AgCl electrodes.

d) Theoretical

The model currently used to explain the blocking effect of the Tl(I) ion at low mole fractions postulates a large association constant for the ion at a channel binding site. An alternate model, similar to those now proposed for the Ca(II) channel suggests that Tl(I) ion can only be displaced from this site by a second Tl(I) ion. Some of the electrical experiments now underway should ascertain whether such selective displacement constitutes a viable alternative to the simple binding models and will explore the special chemical properties of Tl(I) (and Ag(I) ion) which permit it to function in this unique manner.

Papers

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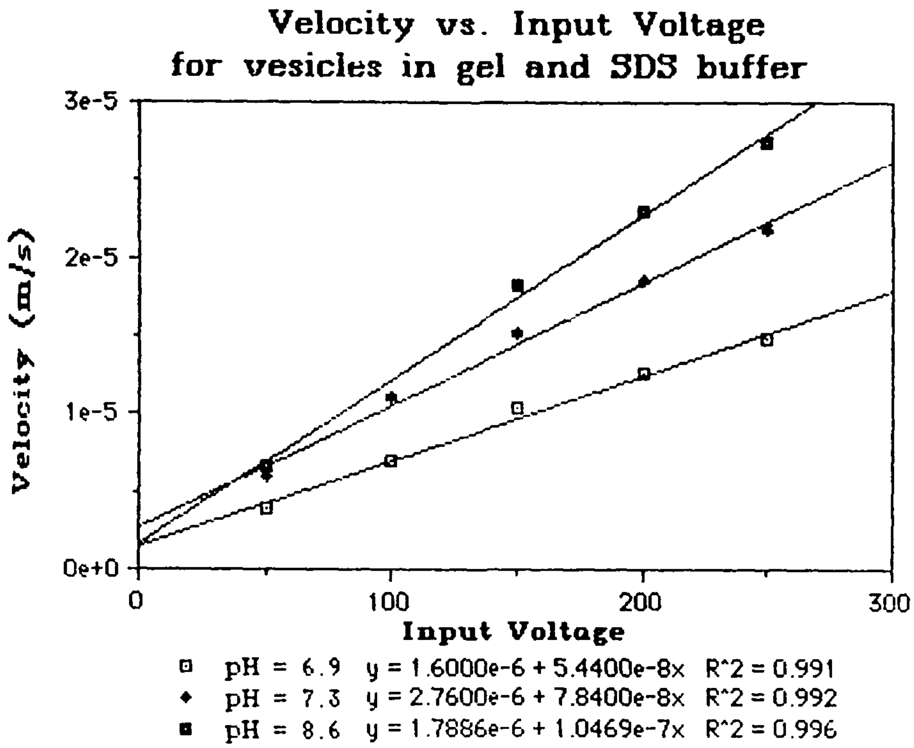
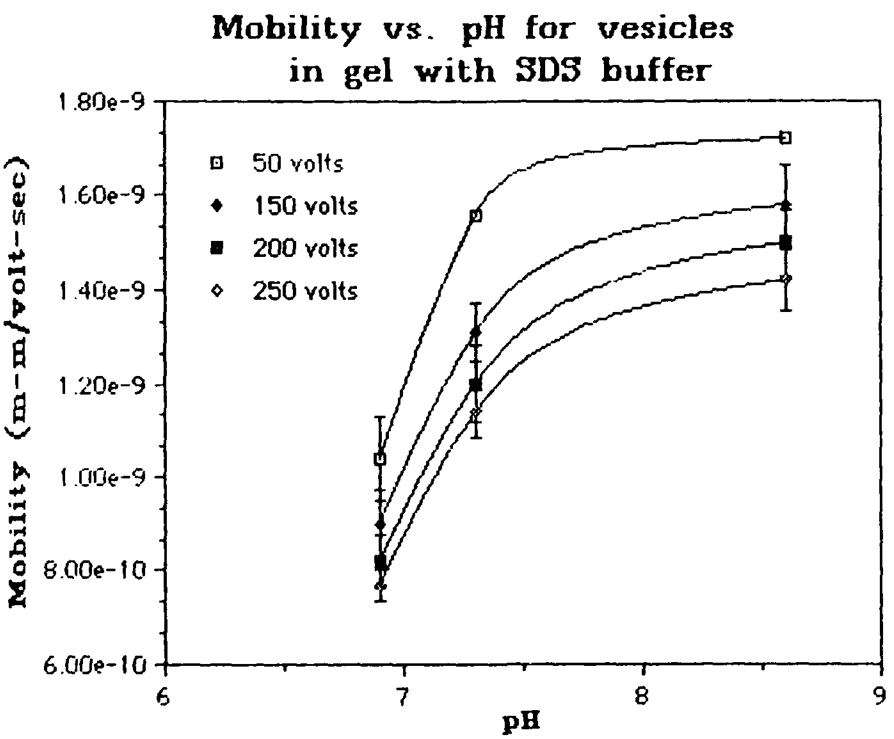

Velocity vs. Input Voltage
for vesicles in gel and SDS buffer
Velocity (m/s)
3e-5
2e-5
1e-5
0e+0
0
100
200
300
Input Voltage
pH = 6.9 y = 1.6000e-6 + 5.4400e-8x R^2 = 0.991
pH = 7.3 y = 2.7600e-6 + 7.8400e-8x R^2 = 0.992
pH = 8.6 y = 1.7886e-6 + 1.0469e-7x R^2 = 0.996


Mobility vs. pH for vesicles
in gel with SDS buffer
Mobility (m-m/volt-sec)
1.80e-9
1.60e-9
1.40e-9
1.20e-9
1.00e-9
8.00e-10
6.00e-10
50 volts
150 volts
200 volts
250 volts
6
7
8
9
pH