# ION INTERACTIONS IN ENERGY TRANSFER BIOMEMBRANES

EDITED BY
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AND S. PAPA

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PREFACE

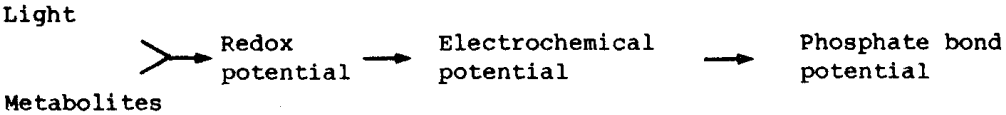
The Expert Committee on Biomaterials and Biotechnology for the European and the North American Region was founded by the General Assembly of UNESCO at its 21st Session, in 1981. The Committee comprises a Coordinating Group and four Working Groups, defined in the following scientific areas:

Group I : Proteins: source, structure and function.
Group II : Nucleic acids: the hereditary materials.
Group III : Immune materials and mechanisms.
Group IV : Membranes and transport in biosystems.

In fulfilment of one of the objectives of the Committee, which have been adopted by the General Assembly of UNESCO in 1981, namely the intensification of the exchange of scientific information on biomaterials and biotechnology, Working Group IV organized an international workshop on Ion Interactions in Energy Transport Systems, which was convened in Athens, Greece, from 8 to 12 April, 1985. Scientific papers presented at that workshop make up the chapters presented in this volume.

The present volume focusses on natural and artificial membranes that are involved in energy transduction. Several chapters are devoted to membranes and membrane components that convert and utilize light, such as the thylakoid membrane of oxygenic photosynthetic organisms (eukaryotic and prokaryotic), the chromatophore membrane of nonoxygenic photosynthetic bacteria and the purple membrane of the halophilic bacteria. Other systems examined include the mitochondrial membranes and their adenine nucleotide carrier, the plasma membrane of animal cells, and lipid bilayer vesicles, either reconstituted or not, with enzymes.

The most widespread mechanisms of biological energy transduction are those associated with the photosynthetic and the respiratory processes, which involve flow of energy among the following forms:




Intensive research, in the recent years, has succeeded in elucidating the basic mechanisms by which these transformations occur. Electrochemical potential differences established between membrane compartments having low permeability to ions play a central role in the transduction of redox energy to phosphate bond energy and have been studied and reported in depth. What distinguishes, however, the present volume from other publications dealing with these subjects is an effort to view energy transduction from the perspective of the electrostatic

properties of membrane-solution interfaces. In the physiological pH range, biological membranes carry an overall negative electric charge on their surfaces, which is due to the preponderance of ionized acidic groups over basic groups, although positively charged domains and components are known to exist. The electrostatic parameters of the membrane-solution interface, namely the surface and space charge densities, the electrostatic force field, and the electrostatic potential are influenced by the electrolyte structure of the aqueous media in contact with the membrane, and particularly by the cations present. In this way, through the electrostatic properties of the membrane-solution interfaces, electrolytes control several structural and physicochemical manifestations of membranes, such as binding of extrinsic proteins, membrane-membrane adhesion, surface area concentrations of ions, and the magnitude of electrochemical potential differences across membranes. The aim of the workshop was that of gathering together experts from various countries to report and debate their more recent studies in this particular area of biochemistry and biophysics. Judging from the contributions delivered at the workshop and published in this book, the meeting served as an unique opportunity for concerted and critical discussions of various aspects of ionic interactions with energy coupling membranes and related topics.

The workshop on Ion Interactions in Energy Transfer Systems and the volume on Ion Interactions in Energy Transfer Biomembranes would not have been possible without the support of UNESCO, IUPAB, IUB-IUPAB Bioenergetics Group and the National Research Center Demokritos of Greece. To all these organizations, and to their officials we express our gratitude.

September, 1985

G. Papageorgiou
J. Barber
S. Papa

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# THE ELECTROSTATIC AND ELECTROKINETIC PROPERTIES OF BIOLOGICAL MEMBRANES: NEW THEORETICAL MODELS AND EXPERIMENTAL RESULTS

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## INTRODUCTION

Gouy (1) and Chapman (2) formulated the concept of the diffuse double layer by combining the Poisson and Boltzmann equations to describe the profile of the electrostatic potential in an aqueous phase adjacent to a charged surface. Stern (3) modified this concept by introducing the possibility that ions could bind to the surface. The Gouy-Chapman-Stern theory of the diffuse double layer describes adequately the electrostatic potential adjacent to a phospholipid bilayer: as the theory requires, the charges on a negative phospholipid are located in a plane at the bilayer surface.

Figure 1A illustrates the distribution of ions at a given instant in time near a negatively charged surface immersed in an aqueous solution (4). The negative charges produce an electric field that attracts counterions, ions of the opposite sign to the charge on the membrane, and repels coions, ions of the same sign to the charge on the membrane. The magnitude of the electrostatic potential is predicted to decrease with distance from the membrane as illustrated in Fig. 1B. If we assume that 23% of the lipids bear a single net negative charge and that the remaining lipids are net neutral, the average charge density is about 1 electronic charge/3 $nm^2$ because a phospholipid occupies an area of about 0.7 $nm^2$. If the concentration of salt in the bulk aqueous phase is 0.1 M, the theory predicts that the potential at the aqueous side of the membrane-solution interface is -60 mV, as illustrated in Fig. 1B. When the surface potential is not too high, the magnitude of the potential decreases in an approximately exponential manner with distance from the membrane. As indicated in Fig. 1B, the distance at which the potential falls to 1/e its value at the surface of the membrane is called the Debye length. The Debye length is predicted to be about 1 nm when the bulk concentration of monovalent ions is 0.1 M and about 10 nm when the concentration of monovalent ions is 0.001 M. Recent experiments by Israelachvili and his collaborators in Australia and by Parsegian, Rand, Lis and their collaborators in America confirm these predictions. The concentration of ions at any distance away from the membrane may be calculated from the potential illustrated in Fig. 1B via the Boltzmann relation. These concentrations are illustrated in Fig. 1C. If the concentration of monovalent ions in the bulk aqueous phase is 0.1 M and the surface potential is -60 mV, the Boltzmann relation predicts that the concentration of monovalent cations at the surface of the membrane is 1 M and that the concentration of monovalent anions is 0.01 M.

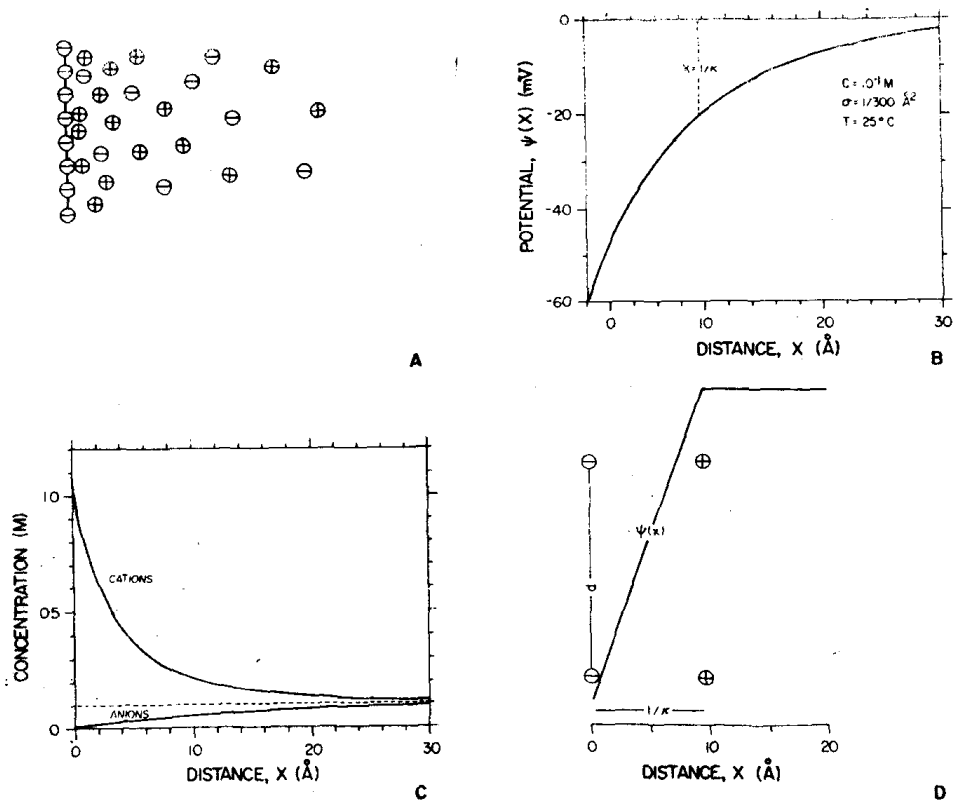


Fig. 1. (A) Schematic diagram of the distribution of ions near a negatively charged membrane. (B) The potential profile predicted by the Gouy-Chapman theory of the diffuse double layer when 20% of the lipids in the membrane bear a net negative charge. (C) The concentration of anions and cations adjacent to the membrane, as predicted by the Gouy-Chapman theory. (D) A parallel plate capacitor model of the diffuse double layer. We assume that the counterions are located a distance $1/\kappa$, the Debye length from the membrane. The average distance between the charges on the surface of the membrane is d = 1.8 nm. See text for details. The temperature was 25°C. From reference (4).

A simple analogy illustrates two important features of the diffuse double layer theory. We first note that the membrane plus any volume of fluid that extends for more than a few Debye lengths must be electroneutral. The excess number of counterions in the diffuse double layer must, therefore, be exactly equal to the number of charges on the membrane. As a crude approximation, we can consider all these counterions placed at an average distance from the membrane (Fig. 1D); this average distance is the Debye length. We are thus considering the diffuse double layer (Fig. 1B, 1C) to be analogous to a parallel plate capacitor (Fig. 1D). The analogy is only valid for low values of the surface potential. For a capacitor, the potential falls in a linear manner with distance between the plates, as illustrated in Fig. 1D. The electric field predicted by the capacitor analogy is only identical to the field predicted by the theory of the diffuse double layer at the membrane solution-interface (compare Figs. 1B and 1D), but the analogy does illustrate how the surface potential depends on the charge density and the salt concentration.

In terms of the model illustrated in Fig. 1D, increasing the charge on the membrane will increase the potential at the surface of the membrane.

The potential is predicted to increase linearly with the charge density. For low potentials the Gouy-Chapman theory also predicts the magnitude of the surface potential increases linearly with the charge density.

When the concentration of ions in the bulk phase is reduced, the Debye length or average distance of the counterions from the membrane increases. In terms of the model presented in Fig. 1D, this is equivalent to moving the capacitor plates farther apart. Thus an increase in the Debye length will produce an increase in the magnitude of the surface potential for a given charge density. This is also the behaviour predicted by the theory of the diffuse double layer. When the surface potential is large the Gouy-Chapman theory predicts that the surface potential will increase by 58 mV for a ten-fold decrease in monovalent salt concentration.

## RESULTS

The prediction that the surface potential changes by 58 mV when the monovalent salt concentration changes by a factor of ten has been tested experimentally with phospholipid bilayers and monolayers (4). The experimental results agree well with each other and adequately with the theoretical prediction. For example, the recent study by Tocanne et al. (5) demonstrates that the surface potential of a phosphatidylglycerol monolayer changes by 53 rather than 58 mV when the [NaCl] changes by a factor of ten. The surface and zeta potentials of phospholipid monolayers and bilayers change by about 27 mV when the concentration of divalent cations changes by a factor of ten (6, 7), in exact agreement with the theoretical predictions of the Gouy-Chapman-Stern theory.

Two major assumptions implicit in the theory are that the charges on the membrane are smeared uniformly over the interface and that the counterions and coions in the aqueous diffuse double layer are point charges. We recently tested these two assumptions. NMR measurements (7, 8) indicate that discreteness-of charge effects are not of great importance with phospholipid bilayers, provided the charges are at the membrane-solution interface and not buried within the membrane (9). The finite size of the cations in the aqueous phase does not appear to be of significance unless the size approaches the Debye length (10), in agreement with a simple theoretical extension of Gouy-Chapman theory (11).

The Helmholtz-Smoluchowski equation relates the electrophoretic mobility of a phospholipid vesicle to the zeta potential, the potential at the hydrodynamic plane of shear, which is about 0.2 nm from the surface of the membrane in a 0.1 M salt solution (10). However, the combination of the Helmholtz-Smoluchowski and Gouy equations cannot describe the electrophoretic mobility of biological membranes. For example, the mobility of a human erythrocyte is about half the value predicted from the classical theory (12). Donath and Pastushenko (13), Wunderlich (14) and Levine et al. (12) describe the mobility of an erythrocyte in terms of structural parameters of the cell surface. These theories differ from the classical treatment in two respects: they assume that independent spherical elements (e.g. sugars) in the glycocalyx generate additional frictional forces and that the charged elements (e.g. sialic acid residues) are either spread over a volume or concentrated in a plane some distance from the surface. The additional frictional drag is predicted to decrease the electrophoretic mobility; the location of charged groups away from the surface is predicted to increase the mobiility and decrease the magnitude of the surface potential. We tested these predictions experimentally with a simple model system.

We formed membranes from mixtures of a ganglioside (e.g. $G_{M1}$, which has four neutral sugars and one charged sialic acid residue in its head group) and the zwitterionic phospholipid phosphatidylcholine (PC). When the head group of $G_{M1}$ is extended maximally, the charge is about 1 nm from the surface and the head group protrudes about 2.5 nm from the surface. These distance are comparable to the Debye length in a 0.1 M NaCl solution. Thus PC:ganglioside vesicles should be useful for testing the new theories. The results we obtained (15) with PC:$G_{M1}$ vesicles are illustrated in Figure 2.

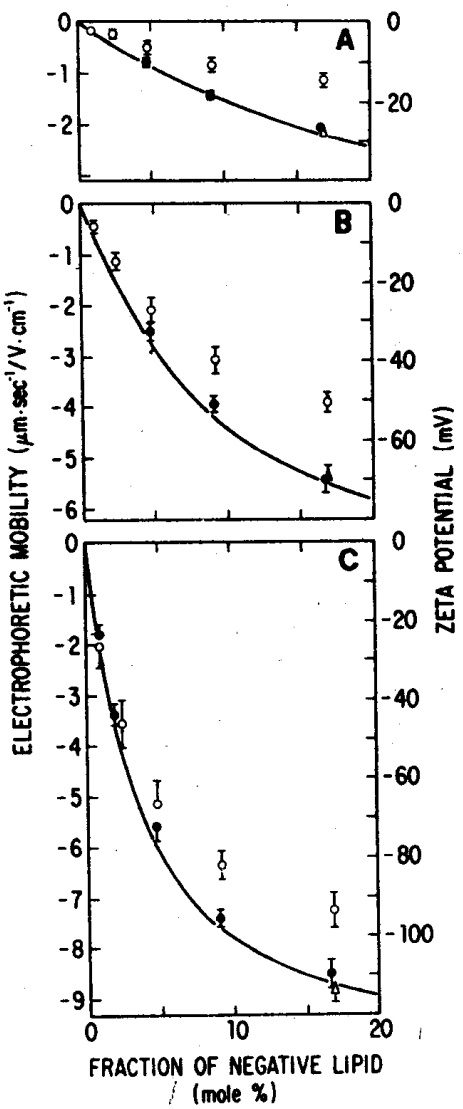


Fig. 2. Electrophoretic mobility and ζ potential of multilamellar vesicles formed from mixtures of the zwitterionic lipid phosphatidylcholine and the negative lipid $G_{M1}$ (open circles), PS (filled circles), or PG (triangles). The curves illustrate the predictions of the Gouy-Chapman-Stern theory if the intrinsic association constant of Na with the negative lipid is 1 $M^{-1}$. The error bars illustrate the standard deviations of measurements on at least 20 vesicles. The aqueous solutions contained (A) 0.1 M NaCl, (B) 0.01 M NaCl and (C) 0.001 M NACl buffered to pH 7.5 at 25°C with $10^{-2}$, $10^{-3}$, and $10^{-4}$ M MOPS, respectively. From reference (15).

Note that in a 0.1 M NaCl solution, where the Debye length is about 1 nm, the electrophoretic mobility of the PC:$G_{M1}$ vesicles is about half the mobility of PC:phosphatidylglycerol (PG) or PC:phosphatidylserine (PS) vesicles of equivalent composition. The mobility of the PC:PG and PC:PS vesicles can be described by the combination of the Gouy-Chapman-Stern and Helmholtz-Smoluchowski equations (curves, Fig. 2). We also studied membranes containing the divalent gangioside $G_{D1a}$ and trivalent ganglioside $G_{T1}$. We could describe these results with a combination of the the nonlinear Poisson-Boltzmann and Navier-Stokes equations by assuming that the sugar moieties exert hydrodynamic drag and that the sialic acid groups are located at some distance from the vesicle surface. The theoretical predictions depend strongly on the thickness of the head group region and the location of the charges but are essentially independent of the location of the shear plane and the Stokes radius of the monosaccharide residues. We obtained a reasonable fit to the mobility data by assuming that the ganglioside head groups are 2.5 nm thick and that the fixed charges on the gangliosides are located in a plane 1.0 nm from the surface of the bilayer. We tested the latter assumption by measuring the surface potentials of PC:ganglioside bilayers using three techniques: conductance measurements with planar membranes, $^{31}P$ nuclear magnetic resonance with sonicated vesicles and TNS fluorescence measurements with sonicated vesicles. The results were consistent with our assumptions.

## CONCLUSION

The electrostatic potential produced by charges on phospholipids can be well described by the Gouy-Chapman-Stern theory of the diffuse double layer and the electrokinetic properties of phospholipid vesicles can be described by combining this theory with the Helmholtz-Smoluchowski equation. However, the electrostatic and electrokinetic properties of biological membranes are more complex. Several authors have combined the Poisson-Boltzmann and Navier-Stokes equations to describe these properties. Our experimental results support this approach.

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# EFFECT OF THE SURFACE POTENTIAL ON MEMBRANE ENZYMES AND TRANSPORT

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The surface charge of biological membranes is produced by dissociable groups of membrane constituents, mainly phospholipids and proteins. The surface potential ( $\psi_o$ ) is related to the surface charge density ( $\delta$ ) according to the Gouy-Chapman equation which, when simplified for a symmetric electrolyte, is

$$\psi_o = \frac{2RT}{z\,F} \operatorname{arc\,sinh}[\,\delta\,(8RT\,\epsilon_o\,\epsilon_r\,C)^{-\frac{1}{2}}] \qquad (1)$$

where R is the gas constant, T is the absolute temperature, F is the Faraday constant, C denotes the concentration of the electrolyte, and z is its valency, $\epsilon_o$ is the permittivity of the vacuum, and $\epsilon_r$ is the relative permittivity (dielectric constant) of the medium. Because of electric attraction or repulsion, the concentration of ions in the immediate vicinity of the membrane surface ($C_o$) is different from that in the bulk solution ($C_\infty$), as described by the Boltzmann distribution

$$C_o = C_\infty \exp(-zF\,\psi_o/RT) \qquad (2)$$

Since the surface of natural membranes at pH close to neutrality is, in general, negative, the concentration of anions is decreased, whereas that of cations is increased, as compared to bulk concentrations. It has been shown[1,2] for soluble enzymes immobilized on charged solid supports that their kinetics depend on the charge of the support and that of the substrate molecule. The present contribution summarizes studies from the author's laboratory showing that kinetics of enzymes located in biological membranes are also altered by changing the surface potential of these mem-

branes. Evidence is also presented that transport processes through membranes can be affected by the surface potential as well.

The surface potential of natural and artificial membranes can be manipulated by (1) screening by high electrolyte concentrations, (2) changing pH of the medium, (3) ionized amphiphiles, (4) di- and polyvalent ions with high affinity towards membranes, (5) changing phospholipid composition of the membranes, and (6) chemical modification of membrane proteins. These changes of the surface potential can easily be monitored e.g. by using the fluorescent membrane probe, 8-anilino-1-naphthalene sulfonate (ANS).[3,4]

It has been observed[4,5] that enzymes reacting with anionic substrates, like arylsulfatase C (EC 3.1.6.1), glycerol-3-phosphate dehydrogenase (EC 1.1.99.5), and glucose-6-phosphate phosphatase (EC 3.1.3.9), are inhibited by factors making the membrane more negative and activated by those shifting the membrane surface potential to less negative values. Opposite relationships occur for enzymes reacting with cationic substrates, e.g. acetylcholinesterase (EC 3.1.1.7), monoamine oxidase (EC 1.4.3.4), and dimethylaniline oxidase (EC 1.14.13.8).[4-6] In these studies apparent $K_m$ values of the enzymes were altered, wherease, in general, no significant change of the maximum reaction rate at saturating substrate concentration ($V_{max}$) was observed. This is in agreement with the assumption that mem-

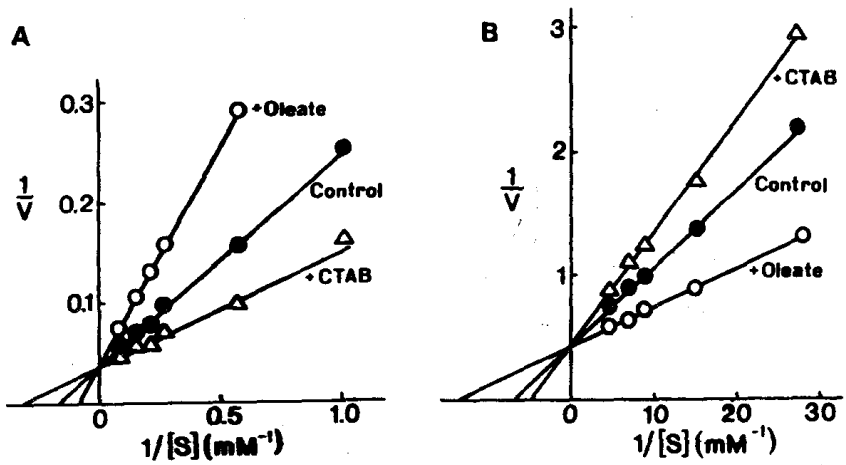


Fig. 1. Effect of ionic amphiphiles on the kinetics of glycerol-3-phosphate dehydrogenase in mitochondria from insect muscles (A) and monoamine oxidase in mitochondria from rat liver (B). Double reciprocal plots. Oleate and cetyltrimethylammonium bromide (CTAB) were added at concentrations of 68 μM and 36-39 μM, respectively. From Wojtczak and Nałęcz.[4]

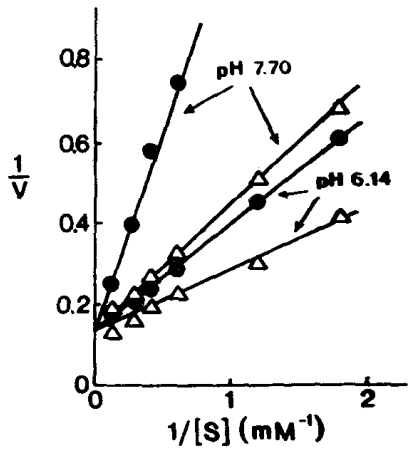


Fig. 2. Effect of pH and cationic surfactant cetyltrimethylammonium bromide (CTAB) on glycerol-3-phosphate dehydrogenase in insect muscle mitochondria. Double reciprocal plots. ●, Control; Δ, 34 μM CTAB. From Wojtczak and Nałęcz.[4]

brane-located enzymes sense the local concentration of their substrates at the membrane surface rather than bulk concentrations. Examples of the effect of ionic amphiphiles, pH, and phosphorylation of membrane proteins are shown in Figs. 1, 2, and 3, respectively. These effects disappear when the membranes become solubilized in nonionic detergents and re-appear on re-incorporation of the enzymes into liposomes.[4-8] In the latter case apparent $K_m$ also depends on the phospholipid composition of liposomes, being increased by negatively charged phospholipids for enzymes reacting with anionic substrates and decreased for those reacting with cationic substrates[7] (Table 1).

Assuming that the change of apparent $K_m$ results only from a change of the local concentration of the substrate at the membrane surface, the change of the surface potential can be calculated from the following equation[4]

$$\Delta\psi_o = \frac{R\,T}{z\,F} \ln \frac{K_m''}{K_m'} \qquad (3)$$

where $K_m'$ and $K_m''$ are apparent Michaelis constants before and after the