United States–Australia workshop on membrane biophysics

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INTRODUCTION

A workshop on membrane biophysics was convened at the East–West Center, Honolulu, HI, August 13–18, 1991. The workshop was sponsored by the National Science Foundation and the Australian Department of Industry, Technology and Commerce, with the aim of bringing together Australian and American investigators in the field of membrane biophysics. The primary themes of the workshop were lipid systems, peptide–bilayer interactions, and membrane bioenergetics, particularly electron and proton transport mechanisms. Participants included M. Baker (University of Western Australia, Perth, Western Australia, Australia), D. Cafiso (University of Virginia, Charlottesville, VA), R. Chatelier (CSIRO Division of Chemicals and Polymers, North Ryde, New South Wales, Australia), B. Cornell (CSIRO), H. Coster (University of New South Wales, New South Wales, Australia), D. Deamer (University of California, Davis, CA), O. Einarsdóttir (University of California, Santa Cruz, CA), A. Hope (Flinders University), J. Lear (DuPont Co., Wilmington, DE), W. Lucas (University of California, Davis), S. McLaughlin (State University of New York, Stony Brook, NY), P. Osman (CSIRO Division of Applied Physics), R. Pace (Australian National University, Canberra, Australian Capital Territory, Australia), J. Parker (Australian National University), A. Parsegian (National Institutes of Health, Bethesda, MD), W. Sawyer (University of Melbourne, Melbourne, Victoria, Australia), F. Separovic (CSIRO), R. Smith (University of Queensland, Brisbane, Queensland, Australia), and A. Walter (Wright State University, Dayton, OH).

BIOPHYSICAL PROPERTIES OF LIPID SYSTEMS

Surface forces

Adrian Parsegian discussed recent advances in understanding the forces acting between and within lipids and macromolecules. Such forces appear to fall into two distinct regimes. At distances <1 nm, exponentially varying "hydration" interactions can be measured that are qualitatively similar between bodies as diverse as lipid bilayers, DNA double helices, and linear polysaccharides. At greater distances there is an interplay between van der Waals, electrostatic double-layer, hydration, undulation, ion–ion correlations, and surface dipole–dipole interactions. Forces within lipid assemblies (bilayers, hexagonal phases) also produce packing stresses that must be taken into consideration to understand how the bilayer membrane acts as a host for membrane proteins.

Of particular interest to membrane biophysicists are peptides or proteins that form transmembrane polar pathways (channels) by which ions can cross an otherwise impermeable hydrocarbon phase. Parsegian described how the osmotic stress technique, extensively used for direct force measurement between membranes or between macromolecules, has now been applied to individual channel macromolecules to measure the work of transition between different functioning states. By observing the contending actions of pH, voltage, ionic strength, and especially the activity of water, one measures energies and changes in the amount of associated water when a channel opens. Channel opening water volumes appear to be large, comparable to the total aqueous volume estimated from channel conductance.

With new knowledge of interaction, packing, and conformation forces, Parsegian predicted an era of "experiment-driven" computation, wherein algorithms are calibrated against direct force and motion measurements. He argued in particular for models of molecular stability that recognize large energies of solvation.

John Parker (1) described a new instrumental approach in which a piezoelectric single cantilever spring system is used to directly measure the force between two surfaces. Static and dynamic measurements of attractive and repulsive surface forces have been compared and found to be in excellent agreement. A surface force device was also used to measure compressibility moduli, thereby providing structural information on confined mesophases such as reticular spacing and the nature and dynamics of defects (2). Work with disordered self-assembled systems, in which mica surfaces were im-

0006-3495/92/06/1454/08 $2.00

mersed in a reversed micellar system, was also discussed (3). The force was studied as a function of micellar volume fraction. At low volume fractions, the force was purely attractive van der Waals, whereas at high volume fractions the force–distance profile oscillated with a period equal to the size expected for the micelles. Such oscillations are typical for a hard sphere fluid interacting with a hard wall.

Finally, measurements were carried out of the attraction between hydrophobic surfaces in the presence of di- and trivalent ions (4). In general, the magnitude of the attraction was reduced at increasing electrolyte concentrations, but the decay length remained similar and the interaction was much larger than could be accounted for by van der Waals forces. Reduction in strength is due to ion adsorption on the hydrophobic surfaces, rendering them less hydrophobic. These results cannot be reconciled with recent theoretical models that ascribe an electrostatic origin to the apparent hydrophobic attraction.

Membrane dielectric properties

David Deamer and Hans Coster discussed the bilayer barrier with respect to its dielectric properties. Permeation of a lipid bilayer by a solute is generally understood to include solvation–diffusion processes, in which the solute "dissolves" in the nonpolar bilayer phase, then diffuses to the other side. Following this reasoning, Parsegian (5) first showed that the remarkably high bilayer barrier to ionic flux could be accounted for in terms of the Born energy required to bring an ion from a high dielectric aqueous phase to the low dielectric medium of the hydrocarbon chains composing the bilayer interior. This energy, in the range of 40 kcal/mol for typical monovalent cations, is an immense barrier, and has been generally accepted as a satisfying explanation for the relative impermeability of bilayer membranes to ionic flux.

However, there are certain limitations of the Born theory in accounting quantitatively for experimental observations of ion permeation. For instance, Hauser et al. (6) calculated the rate at which sodium ions were expected to permeate lipid bilayers prepared as small liposomes. The calculated rates were compared with measured efflux rates and were found to be too slow by three orders of magnitude. To explain this discrepancy, Hauser et al. speculated that loss of sodium ions from the vesicles occurred through defects in the bilayer rather than through solvation–diffusion processes. Nichols and Deamer (7) observed that the permeability of lipid bilayers to protons was at least five orders of magnitude greater than the permeability to other monovalent cations, a result totally unexpected from Born energy considerations.

Smith et al. (8) measured the electrical impedance of bilayer membranes as a function of pH and the concentration of monovalent and divalent salts. Their results showed that the proton conductance was of the same order of magnitude as those of the alkali halide ions. Even much larger cations such as the trimethyl ammonium ion did not have substantially different conductances. Similarly, Georgallas et al. (9) recently measured permeability of lipid bilayers to lithium, sodium, potassium, rubidium, and cesium ions, thereby providing a test of Born energy predictions related to ionic diameter. Their results showed that permeability to all of these ions is essentially identical. This observation is inconsistent with Born energy calculations based on ion partitioning into the hydrophobic region of the membrane, which predict that bilayer permeability should vary by at least six orders of magnitude over the range of ionic diameters from lithium to cesium.

From these results, it follows that Born theory qualitatively accounts for the lipid bilayer barrier to ionic diffusion but does not adequately predict measured ion permeation rates. An alternative explanation is that ions permeate through rare transient defects, rather than dissolving in the bilayer phase, thus bypassing the primary Born energy barrier. Smith et al. (10) used the temperature dependence of bilayer electrical conductances to determine the activation energy per ion permeation event. The value was found to be ~ 35 kJ. Of this, ~ 17 kJ can be related to ion diffusion through an aqueous medium, and the rest may be ascribed to the Born energy of an ion partitioning into a pore (defect) in the bilayer. A pore diameter of ~ 1 nm can be estimated from these results.

Hans Coster reported on perturbations of the dielectric structure of lecithin bilayers. The molecular organization of bimolecular membranes of PC shows variations of dielectric properties relative to the central plane. When an alternating current is passed through such structures, the interfacial polarizations show a dispersion with frequency related to the overall capacitance and conductance, which can be modeled as a series combination of impedance elements representing each dielectric layer (Maxwell–Wagner dispersion). This method was used to study the effects of cholesterol inclusion in lecithin bilayers. Cholesterol decreased the characteristic frequency associated with the hydrocarbon regions, increased those associated with acetyl oxygen and glycerol bridge regions, and slightly decreased the frequency of the polar head region. The overall capacitance was decreased, whereas capacitance of the acetyl oxygen and polar head regions was substantially increased.

Lipid micelles

Anne Walter reported on micellar structures in lipid–water systems. Surfactant–lipid mixtures can assume either a micellar or bilayer configuration, depending on the mole fraction of surfactant. Transitions among the structures entail concerted rearrangements of these amphiphilic molecules and are a function of surfactant activities, temperature, ionic strength, the presence of other amphiphiles, and the chemical nature of the lipid with respect to polar heads and nonpolar hydrocarbon chains. The structural pathway between lipid vesicles and small mixed micelles (SMM) was investigated with cryotransmission electron microscope (11, 12). As surfactant was added to unilamellar vesicles, the average diameter of the vesicles changed toward a characteristic size for each surfactant. Long flexible cylindrical mixed micelles (CMM) were observed between the SMM and lamellar transition, and their existence was correlated with changes in light scattering. The solution cleared at somewhat higher surfactant concentrations, corresponding to the conversion of CMM to SMM. The latter structures evolved both by increase in surfactant to lipid ratios and by decrease in the total amount of material per micelle. These relatively simple systems underscore the complexity of behaviors that occur with mixtures of amphiphiles in aqueous solution and may provide insights to the means by which lipid modulators work in more complex biological systems.

Microemulsions

Bill Sawyer described investigations of microemulsions, defined as surface monolayers of phospholipid enclosing a core of neutral lipid. Such particles are models for lipoproteins, and the physical state of the lipids and their repartitioning from the core compartment affects the activity of hydrolytic enzymes and lipid exchange proteins that interact with the emulsion and initiate the interconversions of lipoprotein classes. Fluorescent and spin-labeled fatty acids were used to examine the fluidity and lipid order at several depths in the monolayer. Below the phase transition of the lipid, the emulsion monolayer is more fluid than in vesicle bilayers composed of the same phospholipid. Above the phase transition, the bilayer is more fluid. A broadening of the phase transition of the surface monolayer was observed and attributed to the disruption of the lipid packing at the monolayer–core interface.

Partitioning of cholesteryl ester between the core and surface compartment was also studied. A possible mechanism for the action of lipases and lipid transfer proteins is that they do not penetrate the emulsion surface but instead use as substrate small amounts of neutral lipid in the surface monolayer. Such a mechanism requires that neutral lipids repartition from the compartment once they are removed from the monolayer. Incorporation of unesterified cholesterol in the surface affects this process by moving cholesteryl ester from the surface to the core compartments.

Thin film technology

Murray Baker discussed recent studies of monolayers of alkanethiols on gold substrates. The monolayers self-assemble on clean gold surfaces that are exposed to alkanethiols in solution or in the vapor phase. By use of straight-chain alkanethiols with appropriate terminal functional groups, these self-assembled monolayers provide a means of controlling the properties of surfaces. For example, monolayers prepared from undecanethiol (terminal methyl group) are very hydrophobic, whereas monolayers prepared from 11-hydroxyundecanethiol (terminal hydroxyl group) are hydrophilic. Mixed monolayers, prepared by immersion of gold substrates into solutions containing both alkanethiols, have intermediate hydrophobicities (13).

Mixed monolayers containing a hydrophobic component (a methyl-terminated alkanethiol) and a hydrophilic component (alpha hydroxyl-maltose- or hexa-(ethylene glycol)-terminated alkanethiol) serve as well-defined systems that can be used to study the adsorption of proteins to surfaces. Adsorption of RNAse, pyruvate kinase, fibrinogen, lysozyme, and chymotrypsinogen onto the surfaces of these monolayers decreased as the proportion of the hydrophilic component in the monolayer increased (14). The mechanism of adsorption of proteins to these surfaces is unknown.

Self-assembled monolayers have potential application in electronic devices. In one recent demonstration (15), mixed monolayers of a ferrocenyl thiol and a quinone thiol adsorbed onto a gold electrode were used as voltammetric sensors of pH. Cyclic voltammetry experiments were performed in aqueous solutions, and the potential of the ferrocene–ferricinium couple was found to be independent of pH, whereas that of the quinone–hydroquinone couple varied with pH. The monolayer on the gold electrode was stable even in 10 M $HClO_4$, so that the device provides a means for measuring pH in highly acidic media.

Ron Chatelier presented a technique for covalently attaching membrane components to any solid surface. The attachment technique uses a radiofrequency glow discharge plasma to deposit, onto a solid surface, a thin film containing chemically reactive groups. Proteins may then be immobilized on this functionalized surface using bivalent reagents. The technique was illustrated with a plasma struck in an acetone vapor. The treatment placed a polymeric film containing various oxygen-containing groups on the surfaces of plastic and metal substrates, as shown by FTIR and x-ray photoelectron spectroscopy. Approximately 7% of the total carbon was in the form of C=O groups. These groups were then reacted with a dihydrazide (H_2N–HN–OC–R–CO–NH–NH_2) to generate reactive hydrazides on the surface. The success of this reaction scheme was verified using a novel "crossover derivatization" technique: treatment of the C=O groups with the dihydrazide increased the reactivity of the surface toward a fluorinated aldehyde but decreased its reactivity toward a fluorinated hydrazine. The crossover derivatization technique partly overcomes the problems of nonspecific binding that plague surface reactions. The hydrazide groups on the surface may be reacted with glycoproteins, the sugar moieties of which have been oxidized to aldehydes by use of periodate. The resulting surfaces might be used in biosensors or to alter the biocompatibility of medical devices.

Lipid multilayers

Peter Osman described a series of solid-state nuclear magnetic resonance (NMR) studies of electric field effects up to 1.6 MV/m on aligned, hydrated, multilamellar lipid bilayers. These bilayers were sandwiched between stacks of metalized glass cover slips interconnected to produce a capacitor with effective areas up to 20 cm^2 and plate separations from 3 to 100 μm. The lipid dispersion acted as a dielectric possessing up to 1,500 bilayers between each pair of slides. Impedance spectra of the multilamellar stacks were capacitative but with a conductive component an order of magnitude greater than anticipated for an assembly of fully sealed bilayers. The membrane stack was excited using a power source limited to 4 mW/ms, up to 400 V, pulsed for 1–10-ms duration. Each application of potential was followed by a pulse of opposite amplitude and duration to neutralize any net flow of charge within the sample. The effect of this electric field on the induction of the La–H_{II} phase transition in phosphatidylethanolamine was studied with ^{31}P NMR, and fields of 1 MV/m were found to reduce the transition temperature by 10°C.

BIOPHYSICAL PROPERTIES OF PEPTIDE–BILAYER SYSTEMS

Gramicidin

Bruce Cornell discussed a series of recently published studies of the ion channel, gramicidin A. These investigations included the insertion and interconversion of gramicidin into its conductive state (16), the location of ions within the channel (17–19), the effects of modifying the gramicidin structure on the conductivity of the channel (20, 21), and the contribution of membrane distortion to channel formation (22–24).

Cornell also reported results of research undertaken with Ron Pace, Peter Osman, Burkhard Raguse, and Lionel King. This work involved the synthesis of a novel family of gramicidins in which biotin was attached to the ethanolamine moiety, permitting its aggregation within a lipid bilayer membrane after the addition of streptavidin. Impedance spectroscopy showed a decrease in the conductance of the membrane when streptavidin was added. A model that was based on the disruption of the channel-forming dimer of gramicidin A was presented accounting for this effect.

Solid-state NMR studies of membrane-incorporated polypeptides; the ion channel gramicidin A; and a fusogen, the 23-mer NH_2-terminus of gp41, were described by Frances Separovic. The determination of the secondary structure of integral membrane proteins has been restricted because of the difficulty of obtaining protein–lipid cocrystals for x-ray diffraction measurements. The structural determination of gramicidin A in membrane bilayers was carried out using solid-state NMR spectroscopic techniques (25, 26). The determination of the orientation of the Gly_2–Ala_3 peptide plane was illustrated using a full-order parameter analysis of ^{13}C, ^{15}N, and ^{2}H NMR data. By analysis of the reduced ^{13}C chemical shift anisotropy of labeled analogues of gramicidin A, the orientation of the tryptophan side chains was found to be consistent with high resolution NMR results.

Ross Smith described the use of solid-state NMR spectroscopy to study the structure, orientation, and dynamics of the ionophoric peptides, gramicidin A, and melittin in oriented lipid multilayers (27, 28). For this purpose an extensive set of peptides labeled with ^{13}C either in the peptide bond carbonyl groups or the amino acid side chains was synthesized. Using these methods, it was possible to follow changes in the behavior of the peptide channels in the presence of ions (25) and with changes in the state of the lipid bilayer (29). The conformation and orientation of the peptide was unaffected by variation of the lipid hydrocarbon chain length from C-10 to C-18, by introduction of unsaturated lipids, or by cooling the lipid below its gel-to-liquid crystalline transition temperature. The three D-leucine carbonyl groups that are located near the bilayer surface appear to be perturbed when the ion channel is occupied by sodium ions. Side chain–labeled analogues have been used to determine the order of the tryptophan indole rings, which are critical for the conductivity of the channel.

Smith also discussed controversies regarding the state of aggregation of melittin in membranes, its location with respect to the lipid, and the mechanisms by which it promotes both ion transport and cell lysis. Experiments on melittin-PC multilayers have shown that the lipid remains well oriented and lamellar at high peptide/lipid ratios and demonstrated the molecular perturbations in the lipid that result from the presence of the peptide. Above the phase transition temperature, the peptide is well ordered and appears to rotate on an axis normal to the lipid bilayer. The results obtained with the ^{13}C-labeled melittin analogues are consistent with a transbilayer orientation for the peptide.

Synthetic peptides

Although gramicidin is probably the best understood ion channel, it is a unique structure and is not an ideal model for the biological membrane channels. The latter appear to be composed of transmembrane aggregates of alpha helices that provide an aqueous transmembrane channel. However, such channels are extraordinarily complex, typically with 2,000 or more amino acid residues. Jim Lear reported recent investigations aiming to study structure–function relationships in systems with fewer structural complexities.

On the basis of relatively simple geometric and chemical considerations, amphiphilic helical peptides of 21 amino acid residues containing only leucine and serine were designed and chemically synthesized in sequences representing each of eight possible combinations of the heptad repeats: LSLLLSL and LSSLLSL (30). When these were incorporated into planar bilayer membranes with 1 M HCl as electrolyte, each peptide produced a distribution of single-channel conductance states with voltage-dependent opening frequencies and different average open lifetimes and conductances. Four clearly distinguishable conductance states were found to be reproducibly associated with specific peptide sequences. All conductances were cation selective, and two appeared to be highly selective for protons. The number of "gating charges" for each channel type, calculated from the voltage dependence of single-channel current probability distribution functions (pdf), together with independent evidence from fluorescence experiments (31), suggests that channels form by a helical dipole–driven rotation of the peptide $-CONH_2$ terminals into the membrane followed by aggregation into transmembrane multimers. The voltage dependence of the single-channel pdf showed a positive correlation with the single-channel conductance. This is consistent with the idea that the different conductance states reflect differences in aggregation numbers. Moreover, the observation of different distributions of channel conductances and open lifetime average for different peptide sequences suggests that apparently minor amino acid sequence changes such as explored here can produce significant observable effects on aggregate stabilities.

Alamethicin

Alamethicin is a linear 20–amino acid peptide that exhibits a strongly voltage-dependent ion conductance in planar bilayer systems. Because it is relatively small and can be synthetically manipulated, this peptide provides a useful model for investigating voltage-dependent gating and membrane protein electrostatic interactions. David Cafiso discussed several models that have been proposed to account for the voltage-dependent conduction of alamethicin. Voltage-dependent spin probes and electrical measurements in liposome vesicles eliminated one of the proposed models. Frequency-dependent ^{13}C spin lattice relaxation and nuclear Overhauser effects were obtained for alamethicin in solution. A fit of these data to models for molecular motion shows that alamethicin behaves as a rigid rod under conditions where it is likely to show more conformational flexibility than in a membrane; this fit also excludes segmental flexibility in the alamethicin peptide.

An examination of the state of aggregation of alamethicin using spin-labeled analogues shows that the peptide is essentially monomeric over a wide range of concentrations in lipid vesicles. Furthermore, the cooperative binding of alamethicin is not a function of aggregation, but is due to other physical or electrostatic interactions in the interface. Given the strong evidence that alamethicin forms aggregates in order to produce transmembrane channels, the fraction of total alamethicin in an active state in the bilayer may be very small. This finding has important implications for studies aimed at examining the active structure and voltage-dependent mechanism of alamethicin conduction.

Stuart McLaughlin noted that many proteins bind to membranes by interacting with acidic lipids (32, 33). Examples from the calcium–phospholipid second-messenger system include protein kinase C (34–36) several of its substrates (37) and phospholipase C (38, 39). These proteins contain clusters of basic residues that, at least in the case of neuromodulin (37), act as phospholipid binding sites. The Hill equation is often used to describe the binding of ligands, including phospholipids (35), to proteins and to assess the degree of cooperativity. However, a simple theoretical analysis predicts that even if basic residues on proteins act as independent binding sites for acidic lipids, the electrostatic potential produced by the lipids and the reduction of dimensionality (40) that occurs when the protein binds to the membrane will produce apparent cooperativity of binding (i.e., a Hill coefficient greater than unity will be observed). This prediction was tested by measuring the binding of basic peptides to membranes. The data could be described with a simple Gouy–Chapman mass action analysis (41–43).

MEMBRANE BIOENERGETICS: ELECTRON, PROTON, AND ION TRANSPORT

Cytochrome oxidase

The third major area of the conference concerned integrated membrane functions, particularly those concerning the relationship between electron transport, proton transport, and the active transport of other ions. A central question of membrane bioenergetics is the mechanism by which electrons move within electron transport enzymes involved in respiration and photosynthesis and how oxygen interacts with the metal atoms typically associated with such enzymes. One approach to this question is to use gas molecules such as carbon monoxide (CO) as probes of the oxygen-binding site. 'Olöf Einarsdóttir described her multichannel transient ultraviolet (UV)-Vis spectroscopic studies on electron transfer in cytochrome oxidase, a key enzyme in aerobic energy production. The electron transfer was studied on timescales of nanoseconds to milliseconds by photolysing CO from the mixed valence carboxy enzyme (a^{3+} $Cu_A{}^{2+}$ $a_3{}^{2+}$ –CO Cu_{B^+}). This causes a back flow of electrons from the cytochrome a_3/Cu_B site to cytochrome *a* and/or Cu_A. The time-resolved UV-Vis difference spectra revealed a new transient in the visible region centered at ~515 nm ($\epsilon \sim 8{,}000$ M/cm). This transient ($t_{1/2} \sim 2$ microseconds) has not previously been observed in cytochrome oxidase electron transfer dynamics (Einarsdóttir, O., T. D. Dawes, and K. E. Georgiadis, manuscript submitted for publication). Simultaneously, there is an absorbance increase in the UV region centered at ~390 nm. The absorbance change at 515 nm can be partially attributed to reduction of cytochrome *a* ($\epsilon \sim 3{,}000$ M/cm). The position and shape of this band and the extinction coefficient of the additional contribution ($\epsilon \sim 5{,}000$ M/cm) is consistent with a charge transfer transition involving copper (II). Time-resolved infrared studies have shown that photodissociated CO binds to Cu_{B^+} before equilibrating with CO in solution (Einarsdóttir, O., T. D. Dawes, and K. E. Georgiadia, manuscript submitted for publication). The CO subsequently rebinds to the ferrous cytochrome a_3. The electron transfer is on the same time scale as the dissociation of CO from Cu_{B^+} (44). The transient UV-Vis spectral changes are interpreted as charge transfer accompanying the oxidation of Cu_{B^+} to $Cu_B{}^{2+}$ upon electron transfer to cytochrome *a*. Therefore, Cu_{B^+} may have a general role as a "ligand shuttle," a conclusion with important implications in ligand binding, electron transfer mechanisms, and proton pumping by cytochrome oxidase.

Photosystem II and oxygen evolution

Ron Pace described electron spin resonance (ESR) studies on the manganese-containing oxygen-evolving site within plant photosystem II. This site binds water and contains up to four Mn atoms, but the number directly involved in water oxidation is as yet unresolved. One intermediate state (S2) of the catalytic site is ESR active and may be trapped at low temperatures. The relative temperature dependencies (5–20°K) of the two characteristic S2 state ESR signals, one centered at $g = 2$ with an extensive Mn nuclear hyperfine pattern, and the other around $g = 4$ and featureless, were consistent with the signals arising from a ground and first excited states, respectively, of an antiferromagnetically exchange-coupled Mn III, Mn IV heterodimer (45). Recent work was described in which the $g = 2$ hyperfine signal was modeled in terms of a two-Mn–bridged site with one Mn atom in a highly anisotropic ligand environment. Further studies at Q band frequency and on one-dimensionally oriented samples at X band frequency lend support to a two-Mn interpretation of the active site structure, suggesting that ligand oxidation may contribute to the charge accumulation (four equivalents) known to occur during enzyme turnover.

Cytochrome *b*/*f* complex

Alex Hope discussed the kinetic characterization of coupled electron and proton transport reactions in and around the chloroplast cytochrome *b*/*f* complex. This system is a plastoquinol/plastocyanin oxidoreductase that resembles the mitochondrial and photosynthetic bacterial cytochrome b/c1 complex in apparently exhibiting direct coupling (in space and time) between the proton and electron transfers. It differs from the cytochrome oxidase type of proton pump, which appears to have indirect coupling. Some outstanding questions relating to the cytochrome *b*/*f* complex include the following: 1) Does a Q-cycle or semiquinone cycle better describe the occurrence, extent, and speed of proton and electron transfers? 2) Do transmembrane electric fields influence events occurring in the complex? 3) What is the sequence of proton and electron release from the plastoquinol as it is oxidized?

Hope described a series of experiments on the kinetics of the oxidations and reductions and proton translocations that occur in reponse to a single-turnover flash under defined conditions. Both isolated thylakoids and a reconstituted system of isolated membrane complexes, together with appropriate cofactors, have been used. The resulting data, together with predictions of kinetics from models such as the Q-cycle, were treated by the inverse method to find a set of optimized kinetic parameters (rate coefficients) that satisfy the condition of minimum global error between the experimental data and model data. A typical comparison of such data showed that under reducing conditions, a Q-cycle model was able to predict the speed and extent of the redox responses of cyt *b*, cyt *f*, and plastocyanin; the kinetics of proton deposition; and the slow electrochromic band-shift signal that followed a single-turnover flash.

In this particular model, plastoquinol is oxidized at a cyt *b*/*f* complex by the concerted, simultaneous donation of one electron to the Rieske center and of the other to cyt b563 (high potential) shared with a plastoquinone at the stromal side of the complex, the two protons being simultaneously released to the lumen. Electron paramagnetic resonance signals attributed to the plastiquinone radical anion so formed have recently been described (46). Dissipating the thylakoid electric potential difference caused by charge separation after a flash (by addition of nonactin + K^+) had no effect on the kinetics of the cytochromes or plastocyanin.

Proton transport in *Chara* membranes

Bill Lucas discussed spatial and temporal control over membrane transport in terms of his investigations of the internodal cells of the freshwater alga, *Chara corallina*. These giant cells establish alternating acidic and alkaline regions along their surface in response to illumination. The time-dependent activation of the plasma membrane transport system was studied with a new experimental system involving a combination of an extracellular vibrating probe and a current–voltage clamp, which enabled simultaneous measurement of extracellular current during current–voltage analysis (47).

Results from these experiments indicated that acid and alkaline bands are formed by the operation of a hydrogen ion transporter, which had characteristics of a class II transport system. In the active mode the transporter functions as a proton-ATPase, with a reversal potential of -450 mV, and, therefore, transport stoichiometry of 1 H^+ per ATP hydrolysed. In the passive mode the transport operates as a proton channel, giving rise to net H^+ influx in the alkaline regions along the cell surface.

The cellular control system regulating the operation of this transporter establishes regions of local autonomous control (48) that appear to be regulated by a master switch involving the firing of the action potential. It was also shown that agents that elicit a perturbation in the state of the microtubules of these *Chara* cells cause the spatial pattern to collapse, indicating cytoskeletal involvement in regulating transport at the plasma membrane (49).

Received for publication 17 January 1992 and in final form 28 January 1992.

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