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A Role for Ion Association and Polymer Elasticity in Bioenergetics

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A Role for Ion Association and Polymer Elasticity in Bioenergetics

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SUMMARY

This paper interprets some of the complex energy transducing reactions of lipoprotein biochemical membranes in terms of macromolecular science. The processes considered involve ion (or electron)-exchange membrane reactions. Dilation of the membrane through osmosis and electrostatic repulsion at the exchange sites are opposed by a contraction which is postulated to arise from the presence of rubberlike lipid bilayers. A change in the elasticity of the membrane alters the dilation-contraction equilibrium and modifies the ion interaction energies at the exchange sites. The elasticity is regulated by swelling interactions of the bilipids with control substances and, in some systems, by reversible cross-linking reactions. The latter can involve thioester cross-links, formed in reversible reactions with ATP, or various types of salt-links formed in association with ion concentration gradients across the membrane. Energized formation of the cross-links can oppose the dilation and change the ion selectivities reversibly. Such systems can therefore transfer energy reversibly between participants in the contractile and dilatory processes.

Such a concept can explain the operation of some membrane pumps, oxidative phosphorylation, action potentials, and some sensory receptors.

INTRODUCTION

I wish to take the opportunity afforded by this Conference to highlight

for polymer scientists the challenging and topical problem of energy transduction in polymers. This problem is of importance to water desalination and is my current research interest; some aspects of our work on the thermal regeneration of ion-exchange resins are referred to by another speaker [1]. The problem is also a major topic of current biochemical research since the energy transducing reactions of the living world occur within highly organized, lipoprotein membranes; this is a field of immense knowledge but of little comprehension.

Unlike enzymes which can be isolated, purified, and studied in solution, many of the enzymes associated with the more complex energy transducing reactions occur in assemblies of submembrane particles containing highly organized chains of reactants and cannot be studied in isolation. Failure to observe some of the classical enzyme intermediates in these membranes has led to various mechanico-chemical concepts of energy transduction [2]. This trend gained credence when new techniques indicated that such membranes undergo profound conformational changes which can be identified with various energized states of the membrane [3-6].

I wish to attempt here to interpret these complex bioenergetic processes in terms of the known behavior of cross-linked polyelectrolytes, and in so doing to highlight the likely biological importance of ion association and polymer elasticity. The concepts to be developed are consistent with many experiemntal observations, but as they are being published in detail elsewhere [7a-d], and are too extensive for inclusion in a single paper, I will simply summarize the essential principles with but little reference to the supporting data and alternative points of view.

II. STATEMENT OF THE PROBLEM

A portion of the energy derived from light acting on chloroplasts, or from the aerial oxidation in the respiratory carriers in mitochondria of certain acids and coenzymes derived from fats and carbohydrates, is converted into metaphosphate bond energy by "oxidative phosphorylation." In this process adenosine diphosphate (ADP) is phosphorylated by orthophosphate (Pi) in an endothermic reaction to form adenosine triphosphate (ATP):

$$ADP + Pi \rightleftharpoons ATP + H_2O \qquad (1)$$

Such ATP is used to energize many subsequent biological processes through reversal of reaction (1).

One device which is energized by ATP is the "sodium pump" which is found in many biological membranes. It utilizes ATP to move Na^+ ions outwardly across the membrane against their electrochemical gradient in exchange for inward movement of K^+ ions in order to create over a membrane an inwardly directed gradient of Na^+ ions and an outwardly directed gradient of K^+ ions. Energy derived from subsequent "downhill" movements of these ions through reaction sites in the membrane is utilized in nerve for the propagation of action potentials and in many membrane pumps for the movement of solutes across membranes against their concentration gradients ("active transport"). Lipids and alkali metal cations play key roles in all these systems.

This paper ponders the following basic, but unsolved, biological problems. How does energy derived from the hydrolysis of ATP or from the downhill movements of ions in trans-membrane electrochemical gradients energize membrane pumps? How do the electron-exchange reactions of the respiratory chain participate in oxidative phosphorylation? What are the roles of lipids and alkali metal cations in these processes?

Polyelectrolytes with fixed cross-linking will be discussed first to show the relationship between cross-linking and ion binding energy in order to lay a foundation for considering membrane energy transducing systems as processes involving reversible cross-linking. This will be followed by the application of such concepts to the sodium pump and oxidative phosphorylation, and by a consideration of how lipid might influence their performance.

III. A GENERALIZED CONEPT OF BIOLOGICAL ENERGY TRANSDUCTION

Biological energy transduction is envisaged as occurring within highly specialized ion (or electron)-exchange membrane systems involving equilibria between opposing contractile and dilatory tendencies. A simple dilation-contraction equilibrium is characteristic of ion-exchange resins.

a) Ion-Exchange Resins

Ion-exchange resins dilate because of osmosis and electrostatic repulsion between neighboring exchange sites having charges of similar sign. The tendency for dilation decreases with increasing distance between the exchange sites and with increasing interaction energy between the exchange

sites and their counter ions. The latter interaction is influenced by the size, hydration energy, and valency of the exchanging ions, and by the effective dielectric constant of the medium in the vicinity of the exchange sites.

Dilation separates the exchange sites and changes the configuration of the polymeric matrix to which they are attached. The ensuing entropy change induces an opposing elastic restoring tendency. Dilation becomes increasingly difficult with increasing cross-linking of the polymeric matrix and not so extensive when the polymer is a glass rather than a rubber. Increasing the cross-linking reduces the extent of separation of the exchange sites on dilation and causes the exchanging ions to move more closely to the exchange sites so as to reduce the electrostatic potential. Consequently the electrostatic energy between the fixed and mobile charges, and the resultant ion selectivity of the resin, increases with the extent of its cross-linking. Such behavior is characteristic of strong electrolyte resins and has been treated quantitatively by Rice and his coworkers with some success [8].

When the exchange sites are weak electrolytes, their pK_a, as well as the distance between the ionized exchange sites and their counter ions, is influenced by the degree of cross-linking. Analogous principles apply to electron-exchange polymers.

The above phenomena account for energy transduction in ion (and electron)-exchange polymers. Contraction of an ionized, cross-linked, weak acid membrane occurs on adding acid, or increasing the ionic strength of a solution in contact with it, and can perform mechanical work; conversely, stretching the membrane changes its pK_a or its oxidation-reduction potential if it contains electron-exchange sites [9]. Squeezing a strong electrolyte resin or heating a mixture of acidic and basic weak electrolyte resins provide principles for energizing desalination processes [7a].

b) Ion-Exchange Systems with Variable Cross-linking

The energy transducing polymers so far considered have fixed extents of cross-linking. Larger interchanges of energy could occur if the cross-links arose from a reversible chemical reaction. Contraction from forming the cross-links in an energized reaction would then be opposed by dilation of the exchange sites until equilibrium is attained. Such a reversibly cross-linked system therefore provides means for chemical energy associated with a cross-linking reaction to change the dilation-contraction equilibrium

of a polymer, and in so doing to interact with ions associated with the exchange sites.

It is postulated [7c] that some biological processes of energy transduction utilize reversible thioester cross-links in a reaction energized with ATP (e.g., the sodium pump), or reversible salt linkages in reactions energized with ion concentration gradients across a membrane (e.g., some sugar and amino acid pumps).

In the sodium pump (Fig. 1), ATP-energized thioester cross-linking (see Section IV) is considered to contract a phospholipopeptide from an expanded configuration, where its diesterphosphate anions function as exchange sites with a preference for hydrated Na^+ ions rather than hydrated

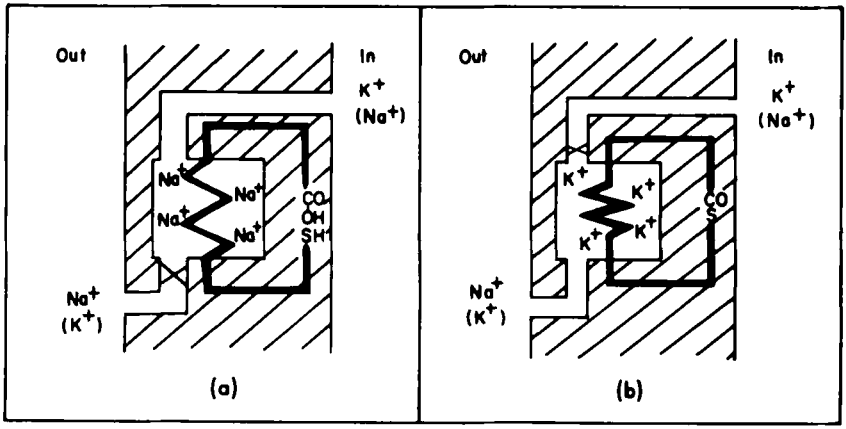

Fig. 1. Diagrammatic representation of a membrane, sodium pump model. Reaction with ATP forms thioester cross-links which contract ion-exchange sites so as to change their ion selectivity, and accessibility to the solutions separated by the membrane, from a Na^+-selective configuration (a) to a K^+-selective configuration (b).

K^+ ions, into a contracted configuration with a preference for unhydrated K^+ ions rather than Na^+ ions. It is suggested that K^+-selectivity arises from solvation of the K^+ ions by peptide keto oxygen atoms and lipid keto (or ether) oxygen atoms, which are contracted into a favorable spatial configuration. K^+ ions are preferred to Na^+ ions because of their lower hydration energies [7a].

It is postulated that in those membrane pumps which are energized by ion concentration gradients, salt bridges form reversible cross-links which

interact with the ion gradients. Sodium pumps, which maintain the ion gradients, can therefore drive such pumps indirectly. Energized cross-linking is postulated to induce a conformation in the pump where the affinity of its adsorption sites for substrate changes from a high value when the site is dilated to a low value when it is contracted.

c) Anisotropy

Energized conformational changes which alter substrate affinity cannot induce pumping unless the device is anisotropic. This can be achieved by placing the pump in an impermeable lipoprotein membrane and by providing controlled access to the solute which is to be present on both sides of the membrane but at different concentrations. A valve-like action can then be achieved as a consequence of changing pump configuration. For example, side chains could move during membrane dilation so as to block and open appropriate membrane channels to provide contact between solution on one side of the membrane and the dilated, high affinity sites, so as to permit adsorption from the more dilute solution. Movement of the "valves" would need to occur in an opposite sense during contraction so as to expose the now low affinity sites to the other side of the membrane and enable rejection of solute into the more concentrated solution. Figure 1 illustrates, in a purely diagrammatic sense, how a sodium pump might operate in such a way.

The performance of work in such pumps requires energy to be expended in cross-linking to operate "valves" and to induce contraction under opposing conditions. If the cross-linking reaction is reversible, reversal of the pump will be possible in some circumstances. Such pumps can therefore function not only for purposes of active transport but also for the reversible transfer of energy between systems which can induce a contraction and substances capable of participating in cross-linking.

These principles will be applied first to the sodium pump to show how ATP could energize thioester cross-linking to induce an exchange reaction involving uphill movement of Na^+ ions and to mitochondria to show how energy could transfer between an electron-exchange reaction site and a phosphorylation site for producing ATP. The same principle will then be extended to the mitochondrial ion pump, which pumps anions and cations by utilizing energy derived either from respiration or from ATP, and to some amino acid and sugar pumps which derive their energy from the downhill movement of Na^+ ions over a membrane.

IV. THIOESTER CROSS-LINKING REACTIONS INVOLVING ATP IN THE SODIUM PUMP AND MITOCHONDRIA

A sodium pump is energized by ATP which induces contraction [5] under normal physiological conditions. When the amount of ATP no longer suffices for contraction, the pump can reverse if the magnitude of the transmembrane ion concentration gradients is increased sufficiently to provide energy for synthesizing ATP. Contraction of the exchange sites might then result from the high concentration of external cations. Contraction will be postulated to synthesize thioesters in a mechanico-chemical reaction and to expose the exchange sites to the inner side of the membrane where the low ion concentrations expend them. As the thioester cross-links hydrolyze they provide energy for synthesizing ATP.

A related situation occurs in the inner mitochondrial membrane. Contraction of the membrane is induced during oxidative phosphorylation not by an ion gradient, as in a reversed sodium pump, but by reduction of a train of electron-exchange polymers; their subsequent oxidation expands the membrane and results in the production of ATP [3, 4]. In some other circumstances hydrolysis of ATP can contract and reduce the electron-exchange polymers by reversing the process. An acyl phosphate intermediate participates in phosphorylation of the sodium pump, but no such intermediate has been found during oxidative phosphorylation. Nevertheless, it will be shown how a contractile equilibrium between thioester cross-links and ATP, ADP, and Pi can account for both systems despite this fundamental difference in their phosphorylation mechanism.

a) Acyl Phosphate Intermediates and the Sodium Pump

It is established that an acyl phosphate is a key intermediate in the ATP-driven sodium and calcium pumps, and that at least one thiol is involved in each pump [10]. ATP could energize these pumps by reacting with a membrane carboxylate group R_1COO^- to form acyl phosphate:

$$R_1COO^- + ATP^{4-} \rightleftharpoons R_1COOP^{2-} + ADP^{3-} \qquad (2)$$

The phosphate bond energy of the acyl phosphate would then permit formation of a thioester cross-link by reaction with a membrane thiol R_2SH [7c]:

$$R_1COOP^{2-} + R_2SH \rightleftharpoons R_1COSR_2 + Pi^{2-} \quad (3)$$

Rupture of the cross-link would involve hydrolysis of the thioester:

$$R_1COSR_2 + H_2O \rightleftharpoons R_1COOH + R_2SH \quad (4)$$

Since the phosphorylation reaction involves a carboxylate group, ionization of R_1COOH would be necessary. This would be promoted by interaction with an alkali metal cation M^+ having a high interaction energy with R_1COO^- as a result of ion pairing:

$$R_1COOH + M^+ \rightleftharpoons R_1COOM + H^+ \quad (5)$$

M^+, being an alkali metal cation, would be bound to R_1COO^- ionically rather than covalently. Consequently, increasing amounts of M^+ would promote dissociation of the cross-link, and the resultant rise in concentration of R_1COO^- would in turn promote phosphorylation.

If R_1COO^- in the sodium pump has a high affinity for Na^+ ions, as a result of enhanced ion association in a lipid environment and presence of cavities of critical size, reactions (2) and (5) can account for the characteristic Na^+-dependent phosphorylation reaction of the sodium pump and for its activation by Na^+ ions.

b) Mitochondrial Oxidative Phosphorylation

A series of reactions in mitochondria transfers hydrogen atoms from carbohydrates and fats to coenzymes and forms some readily oxidized acids such as succinate. Subsequent oxidation of these substances (the "substrates") at a mitochondrial membrane containing a train of electron-exchange polymers (the "respiratory chain") liberates energy in a series of reactions ("respiration") as electrons flow from the reduced substrates through the respiratory chain to a terminal reaction with oxygen. The "respiratory energy" so provided can then synthesize ATP or transport substrate across the mitochondrial membrane so that it can be oxidized at its inner side [11].

Boyer [12] has proposed that contraction of the electron-exchange train results in mechanico-chemical synthesis of thioesters for conserving respiratory energy, but this raises a difficulty since his oxygen exchange studies show that subsequent hydrolysis of such thioesters are unlikely to synthesize ATP via an acyl phosphate intermediate as in the sodium pump. The apparent lack of phosphorylated intermediates in oxidative phosphorylation

has focused attention on the "chemiosmotic hypothesis" [11, 13] which proposes that protons, derived from reactions in the respiratory chain, establish a proton gradient and a potential across the membrane and drive phosphorylation of ADP by removing hydroxyl ions liberated during phosphorylation:

$$Pi^{2-} + ADP^{3-} \rightleftharpoons ATP^{4-} + OH^- \tag{6}$$

Another proposal suggests that protons derived from within the membrane drive phosphorylation by reducing the activity of water released by phosphorylation [14].

If thioesters are indeed intermediates in the processes linking respiratory oxidation-reduction reactions with those of oxidative phosphorylation, how can energy derived from thioester hydrolysis be conserved for the phosphorylation of ADP without acyl phosphate formation? The following reaction sequence suggests one possibility [7d]:

Reduction and contraction of a redox complex in the respiratory chain could provide an entropy change which enables a thioester cross-link to form between an attached polymer chain R_a, containing a carboxylate group R_aCOO^- associated with a protonated membrane base BH^+, and another attached thiol R_bSH:

$$R_aCOOHB + R_bSH \rightleftharpoons R_aCOSR_b + B + H_2O \tag{7}$$

In the contracted state, R_a could be highly strained and this strain could be retained when the thioester hydrolyses and the liberated carboxyl group attaches to an adjacent phosphorylation site:

$$R_aCOSR_b + H_2O \rightleftharpoons R_aCOOH + R_bSH \tag{8}$$

Phosphorylation would then result as R_aCOOH accepts OH^- ions during the phosphorylation of ADP (reaction 6):

$$R_aCOOH + OH^- \rightleftharpoons R_aCOO^- + H_2O \tag{9}$$

Formation of R_aCOO^- would release the strain in R_a and provide an entropy contribution for phosphorylation. R_aCOO^- and B then react with a proton from the medium to reform R_aCOOHB and complete the cycle:

$$R_aCOO^- + B + H^+ \rightleftharpoons R_aCOOHB \tag{10}$$

In such a mechanism, energy for phosphorylation may be provided by the removal of OH^-, by a gain in entropy of R_a and, if R_aCOOHB were to bind water strongly in a lipid environment, by removing the water liberated during phosphorylation. All three mechanisms may be involved as they are not mutually exclusive.

Our current investigations of the thermal regeneration of a mixture of weak base and weak acid ion-exchange resins shows how the efficiency of proton transfer between thioester hydrolysis and phosphorylation could be substantially enhanced if R_aCOO^- were to form an ion pair with BH^+ in a lipid environment of low dielectric constant.

The titration curves of homofunctional carboxyl and primary amine (curve A, Fig. 2) ion-exchange resins have a steep slope due to increasing electrostatic interactions between neighboring charged exchange sites as their ionization increases. When one or more alkyl substituents are incorporated into the amino groups (curves B and C, Fig. 2), or when

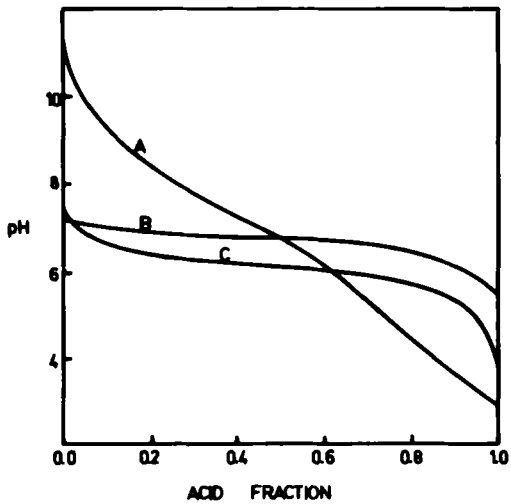

Fig. 2. Titration curves of some homofunctional, cross-linked, weak base resins: A, polyvinylamine; B, poly(N-t-butylaminoethyl methacrylate) and C, poly(vinylbenzyldiethylamine).

hydrocarbon groups are introduced near carboxyl groups, we observed a marked flattening of the titration curve [15]. This indicates increased pH buffering capacity and reduced interaction between neighboring sites. The phenomena can be accounted for by enhanced ion pairing resulting

from a reduction of the dielectric constant of the medium in the vicinity of the exchange sites [16].

c) The Mitochondrial Ion Pump

Mitochondria can utilize respiratory energy to move cations over an inner membrane and protons in the opposite direction [2]. The process occurs at the expense of phosphorylation and probably drives auxiliary processes for the transport of substrate anions across the membrane at specific carrier sites [11].

Such membrane pumping can be accounted for by a reaction which is but a variant of reactions (4) and (5) which have been suggested for Na^+ ion stimulation of the sodium pump [7d]. It is proposed that, in some circumstances, a cation M^+ can react with R_aCOOH when it is not attached to the phosphorylating site, and so competes with phosphorylation:

$$R_aCOOH + M^+ \rightleftharpoons R_aCOOM + H^+ \qquad (11)$$

Subsequent energized thioacylation then liberates M^+ and an equivalent amount of alkali at the inner side of the membrane where thioacylation occurs:

$$R_aCOOM + R_bSH \rightleftharpoons R_aCOSR_b + MOH \qquad (12)$$

The acidic substrate carrier sites are postulated to involve a membrane weak base which reacts with a proton and substrate from the outer solution to form a membrane salt of the base and enables substrate to cross the membrane. Alkalinity arising from cation accumulation regenerates the free base carrier site and releases the substrate anion at the inner side of the membrane. The net result is then energized transport of substrate and cations over the membrane.

In the light of the above mechanisms for oxidative phosphorylation and mitochondrial ion transport, it is possible to interpret a complex series of observations in terms of competitive reactions between the participants [7d].

V. MEMBRANE PUMP CROSS-LINKING SYSTEMS ENERGIZED BY ION CONCENTRATION GRADIENTS

Possible membrane cross-linking mechanisms involving alkali metal cation

gradients for energizing some membrane pumps can be considered within three categories [7c].

a) Cross-Linking Involving Na^+ and Cl^- Ions

A simple reversible cross-linking system would be one where a salt linkage B_1A, formed between a membrane base B_1^+ and a membrane anion A_1^-, reacted with Na^+ and Cl^- ions:

$$B_1A_1 + NaCl \rightleftharpoons B_1Cl + A_1Na \tag{13}$$

Decreasing the concentration of Na^+ and/or Cl^- ions on one side of the pump membrane would promote cross-linking. Such a cross-linking mechanism can account for the operation of an amino acid pump in pigeon red cells.

b) Cross-Linking Involving Na^+ Ions and Protons

If the protonated form of a membrane weak base B_2 were to form reversible salt linkages B_2HA_2 with a membrane anion A_2^- having a high affinity for Na^+ ions, the following cross-linking reaction could occur between B_2 and A_2^-:

$$B_2 + H^+ \rightleftharpoons B_2H^+ \tag{14a}$$

$$B_2H^+ + A_2Na \rightleftharpoons B_2HA_2 + Na^+ \tag{14b}$$

Protons would be released through reduced ionization of B_2H^+ if dilation, resulting from rupture of the cross-link, caused B_2H^+ to enter an environment of reduced polarity. Such a cross-linking reaction therefore need involve only an exchange of Na^+ ions and protons. If A_2^- were a weak electrolyte, the curve relating the extent of cross-linking, as reflected by its activity, with pH would show a maximum. Such cross-linking may be involved in an amino acid pump in cell nuclei.

c) Cross-Linking Involving Na^+ and K^+ Ions

Many sugar and other pumps utilize both the Na^+ and K^+ ion concentration gradients which are established over biological membranes by sodium pumps. It is suggested that their dilation-contraction mechanism may

involve an ion-exchange system which is Na^+-selective when dilated, but which is K^+-selective when contracted, as in a sodium pump. If this were so, K^+ ions could contract attached, high affinity substrate adsorption sites into a state of low affinity. Both Na^+ ions and substrate would co-operate in inducing dilation.

VI. LIPID BILAYERS AND RUBBERLIKE MEMBRANE BEHAVIOR

Current biochemistry highlights the importance of conformational changes in energy transducing membranes [2]. This suggests that entropy is likely to be an important component in the free energy changes of such membranes. Section III has discussed the relationship between the entropy changes of swelling ion-exchange resins with different cross-linkings and their ion-selectivities. Subsequent sections have developed the thesis that such effects can be magnified and exploited for energy transduction through reversible cross-linking reactions.

It will now be proposed that membrane conformational changes might be modified and controlled by interactons between membrane polymers and substances, such as swelling solvents, whose presence determines the magnitude of the membrane entropy changes by influencing the rotational freedom of segments of the polymers. Such interactions could then affect both the ion-exchange and cross-linking reactions in the membrane.

The mechanical properties of polymers reflect their entropy changes and are influenced profoundly by chain stereochemistry, by temperature, and by swelling solvents. It is suggested that all these phenomena have their counterparts in biological membranes [7a, 7b].

Biological lipoprotein membranes probably contain some bilipids whose reaction with detergents enables the membranes to be broken into organized, submembrane particles. Recent x-ray studies show the presence of bilipids in at least some membranes. Spin labeling also shows the presence in some membranes of liquid hydrocarbon regions which are probably the hydrocarbons of membrane bilipids [7a, 7b].

It is suggested that, in the specialized lipoprotein energy transducing structures, the polar groups of lipid bilayers are important not because of the surface activity they impart, but because they enable the lipid to become ionically, or covalently, bonded to the membrane and because they provide ion-exchange sites. One end of the bilipid may be bound to a rigid, supporting membrane structure while the other end may be attached

to a flexible peptide or other polymer. In a relaxed, contracted configuration the hydrocarbons of the bilipid are likely to assume a liquidlike state above a critical temperature (Fig. 3a). Dilation of the flexible structure, as a result of its containing ion (or electron)-exchange sites, would then stretch the attached hydrocarbons (Fig. 3b) whose increased orientation would increase with release of heat (cf. stretching rubber). Such a loss of entropy on dilation then induces an opposing contraction which increases on heating [7a, 7b]. Bilipid, rubberlike elasticity therefore could provide the restoring force which is essential if conformational changes are to be reversible mechanically (cf. the use of springs in machinery).

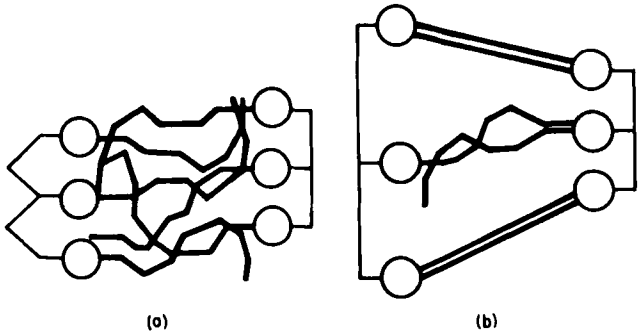

Fig. 3. Diagrammatic representation of a lipoprotem membrane bilayer in a contracted state with liquidlike hydrocarbon regions (a) and in an expanded state with orientated lipid hydrocarbons (b).

If small amounts of lipid-soluble substances are added to the bilayer and enhance the rotational freedom of segments of the hydrocarbon chains by reducing interpolymer interactions, the increased difference in entropy between the relaxed and strained chains will enhance the elasticity and tend to stabilize the contracted membrane. This is the phenomena of "general anaesthesia." Further additions of such substances will eventually dissolve the chains and rupture the bilayers. This is "lysis" [7a, 7b].

Some other substances will hinder the rotational freedom of the bilayer hydrocarbons and by reducing the elasticity will favor dilation. These are the membrane "labilizers." But if such effects are large enough, or if the membrane is chilled to below its glass transition temperature, the bilayers will condense into a rigid glass which inhibits dilation. This is the anaesthesia induced by some, but not all, steroids and by chilling [7a, 7b].

If a mixture of membrane labilizers and stabilizers is present within the

bilayers, they will oppose each other's effects just as they do in monolayers. This is one form of drug antagonism and may also be relevant to hormonal control by steroids [7a, 7b].

All such interactions that can change the entropy of the bilayers will in turn influence the interaction energies and ion selectivities of ion (or electron)-exchange reactions associated with dilation; the effect is analogous to the influence of cross-linking on the ion selectivity and pK_a of an ion-exchange resin. If reversible cross-linking reactions are also associated with the exchange reactions, they too will be influenced by such lipid interactions.

The above principles can be applied to the lipophosphopeptide structure that has been invoked above to explain the ion selectivity changes of the sodium pump and that is based on the discovery of such a peptide in nervous tissue by Heald [17]. Action potentials can be accounted for qualitatively by a similar structure, but without the cross-linking mechanism [7b]. In such polymers a phosphatidylserine peptide provides a polyelectrolyte with cation-exchange properties attached to lipid hydrocarbons which in turn can serve to bind the structure in a flexible manner to a membrane by forming bilipids with anchoring membrane hydrocarbons.

The electrical activity associated with action potentials can be accounted for qualitatively by membrane permeability changes arising from dilation of the membrane from a contracted Ca^{2+}-K^+ configuration to a dilated Na^+ selective configuration as a result of removing Ca^{2+} ions by an excitation current [7b]. General anaesthetics within the bilayers of such structures would inhibit dilation by enhancing the entropy loss on dilation. Other substances, such as DDT and some cyclopropane insecticides shaped like wedges [18], could prop open and stabilize the Na^+-configuration of such structures. Such effects of DDT would be opposed by labilizing substances such as anaesthetics, heat, or Ca^{2+} ions. If one component of such a bilipid layer in olfactory receptors is carotene, adsorption by the carotene of odors having appropriate shapes and interaction energies could change the elasticity and therefore the ion selectivity of the associated membrane action potential sites.

In the related sodium pump structure, anaesthetics could inhibit dilation in a similar manner [7c]. In the visual receptors of eyes, light changes a membrane potential created by a sodium pump [19]. In a dark reaction, a carotene side chain in the retinal component of rhodopsin is put into a highly strained, sterically hindered cis-conformation [20]. Light photoisomerizes the structure to a mobile, trans-configuration which then influences the sodium pump. If the carotene were to form a bilayer with the lipid in the sodium pump, a change from a glass to a rubberlike state

in the bilayer would change the ion-selectivity of the sodium pump and the membrane potential [7c].

In mitochondria, attachment of lipid to the electron-exchange sites provides opportunity for the regulation of oxidation-reduction potentials through lipid interactions with steroids for example. The inhibitory effects of general anaesthetics on oxidative phosphorylation could be due to their interaction with polymer segments of R_a so as to relax it by breaking the bonds holding it in a strained configuration [7d].

Since it appears that lipophosphopeptides contain all the necessary features to account for action potential and related membrane phenomena, it would seem worthwhile to subject such little studied substances to more intense scrutiny and to seek possible relationships with carotenes in some sensory receptors.

VII. CONCLUSIONS

It is concluded that lipid fulfills the following main functions within energy transducing membranes.

Lipid provides the generally impermeable barrier necessary for the establishment of trans-membrane concentration gradients. It provides a medium of low dielectric constant to promote ion association for influencing ion selectivity, pH buffering, and pK_a control mechanisms. It can participate in glass-rubber transitions to provide varying elastic restoring tendencies for the control of membrane conformational changes. Bilipid interactions between the hydrocarbon chains of lipids attached to separate submembrane particles provide means for holding the particles together in a specific configuration, but with retention of higher chain mobility for participation in particle entropy changes than would be possible if the particles were bound covalently. The polar structures of lipids provide anchoring points for attaching hydrocarbon chains to polymers and their phosphate groups provide built-in cation exchange sites.

Alkali metal cations are utilized in some membrane processes to enhance the stability of reactant anions because they form ionic rather than covalent bonds. K^+ ion is likely to be favored over Na^+ ion for participation in ionic reactions within lipid membranes because it is more compatible with lipid, since lipid oxygen dipoles can replace its water of hydration more easily.

Membrane configurational changes provide means for regulating the selectivity, pK_a, and oxidation-reduction potentials of ion (or electron)-exchange sites within the membranes, for the opening and closing of

membrane channels, and for changing the affinities of substrate adsorption sites. Reaction with ATP, or downhill movements of ions along their electrochemical gradients over membranes, can energize such configurational changes through reversible cross-linking reactions.

Because even the properties of simple ion-exchange resins can be quantitatively described only in an approximate fashion, there is little immediate prospect of developing the above concepts in a rigorous, quantitative manner, particularly as details of the participating structures are largely unknown. Although quantitative models of complex biological processes can be made, and indeed are the current fashion, they often involve so many empirical parameters that such an approach is of doubtful utility when seeking molecular understanding. By highlighting the possible significance of well-known biochemical structures it is hoped that the above hypothesis, though qualitative, may be of practical utility for guiding chemical studies of the complex biological membrane processes and of lipophosphopeptides. It may also be useful in designing simple model polymeric systems to establish elements of the concept in a rigorous fashion.

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