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Role of Interfacial Structured Water in Membrane: Osmotic Properties of L-α-Egg Lecithin Liposomes

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Summary. The role of large amounts of membrane-bound water in regulating various functions of the membrane is not clear at present. We have investigated the effect of perturbing the interfacial water structure on the osmotic shrinkage properties, such as water permeability and extent of shrinkage of egg lecithin liposomes. Water structure was perturbed by a series of reagents which have been earlier reported to affect phase transition of dipalmitoyl phosphatidylcholine liposomes by perturbing interfacial water structure. Anomalous variations of osmotic shrinkage properties with concentration of structure maker and breaker reagents have been interpreted to arise from concentration-dependent structural transitions of the ordered water at the membrane-aqueous interface. Various modes of interaction of these reagents on interfacial structured water have been suggested. Influence of molecular size and functional groups on the molecule in actions of some structure makers and breakers were also observed.

Key Words water structure · osmotic shrinkage · egg lecithin · liposomes · anomalous · water permeability

Introduction

Water molecules are known to bind very tightly to the lipid membrane with binding energies comparable to that between water molecules in ice [40]. The hydrated membranes contain 20 to 30% bound water by weight [8, 26]. The bound water plays a major role in determining the structural integrity and stability of the membrane [39]. The bound water can be supercooled [17], behaves as nonsolvent water [23] and possesses a high degree of order through water-water and water-lipid hydrogen bonding [5, 37, 45]. High degree of structuring of water has been observed at the polar headgroup region of the membrane of *Nocardia asteroides* and *Mycobacterium smegmatis* [7]. The exact functional significance of the membrane-associated structured water is not clear at present. Permeability of lipid membranes to water and various solutes is, however, known to be affected by the presence of unstirred layer of water around the membrane [1, 6, 21, 23, 31].

We have shown previously that alteration of water structure around the polar headgroup region of the bilayer by a series of reagents (known to affect structuring of water molecules in bulk also) influences the phase transition characteristics of dipalmitoyl phosphatidylcholine liposomes [10]. Such alteration of the phase equilibrium of liposomal membrane may therefore be expected to affect its permeability to water and solutes.

Permeability of a lipid membrane depends on the physical state of the membrane as well as on its ionic environment (e.g. pH), fluidity, charge, cooperativity of lipid molecules [3, 19, 30, 34, 35, 38, 42]. Large discrepancies in permeabilities of solutes, particularly of small polar molecules from Overton's rule [23, 24, 36] suggest the necessity to consider both solution/membrane interface effects and steric factors governing the diffusion of molecules in the membrane during the process of permeation. A consideration of the above facts and a need of deeper insight into the functional role of ordered water at the membrane-aqueous interface have led us to investigate the effect of alteration of water structure by these reagents on various properties of model membrane systems. In this paper, we report the effect of altered water structure at the membrane-aqueous interface on the osmotic shrinkage properties, such as water permeability of egg lecithin liposomes as determined by the osmotic shrinkage studies.

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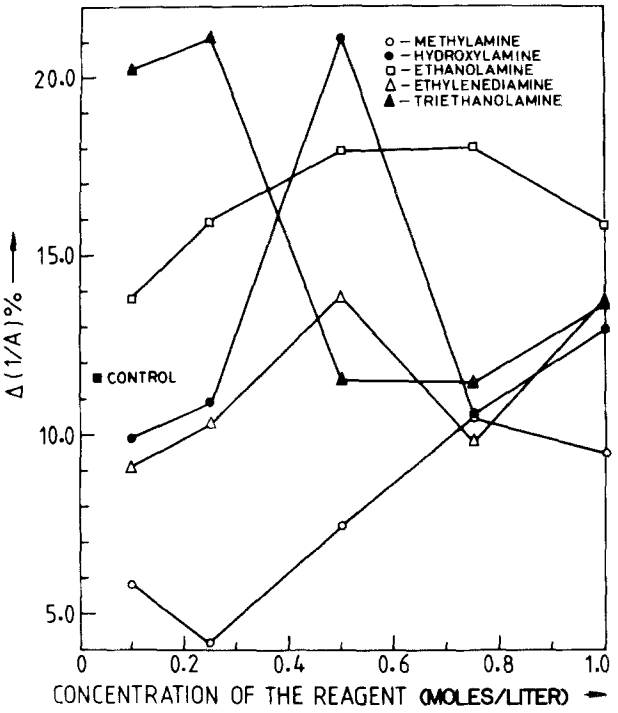

Fig. 1. Dependence of extent of shrinkage $\Delta(1/A)\%$ of egg lecithin liposomes on concentration of structure makers. Each data point is a mean of at least three observations, error limits being ± 1.3%

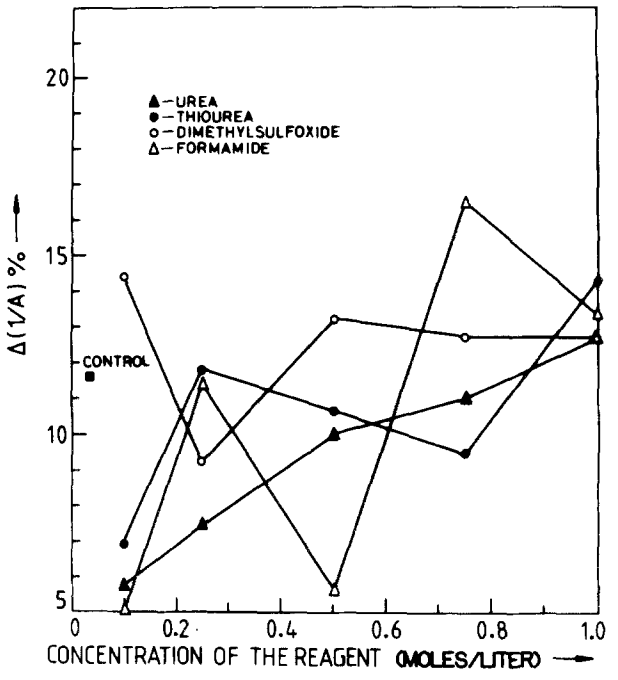

Fig. 2. Dependence of extent of shrinkage $\Delta(1/A)\%$ of egg lecithin liposomes on concentration of structure breakers. Each data point is a mean of at least three observations, error limits being ± 1.3%

Materials and Methods

Lipid

L-α-egg lecithin in hexane solution was obtained from V.P. Chest Institute, Delhi, India. This lipid gave a single spot on thin layer chromatography (TLC) (chloroform/methanol/water, 65 : 25 : 4, vol/vol) and therefore was used without further purification. Source and purity of other chemicals have been described previously [10].

Structure Makers and Breakers

A series of water-soluble amines (methylamine, ethanolamine, hydroxylamine, ethylenediamine and triethanolamine) were used as structure makers. Urea, thiourea, dimethylsulfoxide (DMSO) and formamide were used as structure breakers [*see also* ref. 10]. 1 M solutions of structure makers and breakers were made in 10 mM Tris-HCl and pH was adjusted to 7.4.

Preparation of Liposomes

Liposomes were prepared essentially as described in [3]. Required amounts of lipid in chloroform solution were taken in flat-bottom glass vials, dried in a thin film under a stream of nitrogen and vacuum-dessicated for 1 hr to remove last traces of solvent. Thoroughly acid-washed glass beads were added to the vials (1 glass bead/mg lipid approx.). Solutions of different concentrations of structure makers and breakers in 10 mM Tris-HCl buffer, pH 7.4, saturated with nitrogen, were added to the individual vials to make a final lipid concentration of 1 μM/ml. After sealing the vials under nitrogen, the lipid was dispersed gently by shaking and then in a vortex mixer for 30 sec. The resulting suspension was allowed to equilibrate at room temperature for 30 min.

Osmotic Shrinkage Studies

Osmotic shrinkage properties were studied as described elsewhere [3]. Typically, 0.4 ml of 1 M glucose solution in 10 mM Tris-HCl, pH 7.4, was rapidly injected in 4.6 ml of the liposomal suspension. The change in turbidity with time was followed by measuring the light transmission at 662 nm using a dual-path absorption spectrophotometer (MPS 5000, Shimadzu Corp., Japan). Initial shrinkage velocity, $d(1/A)/dt\%$ and extent of shrinkage, $\Delta(1/A)\%$ were calculated from the recorder traces, as described earlier [3]. Relative osmotic permeability of water was calculated as:

$$P_W^{rel} = \frac{\text{Initial shrinkage velocity of the sample}}{\text{Initial shrinkage velocity of the control}}.$$

Osmotic shrinkage parameters of egg lecithin liposomes suspended in 10 mM Tris-HCl buffer, pH 7.4, were taken as control values for respective parameters. TLC was performed at each stage of preparation and also at the end of the experiments as described earlier. No degradation of the phospholipid could be observed.

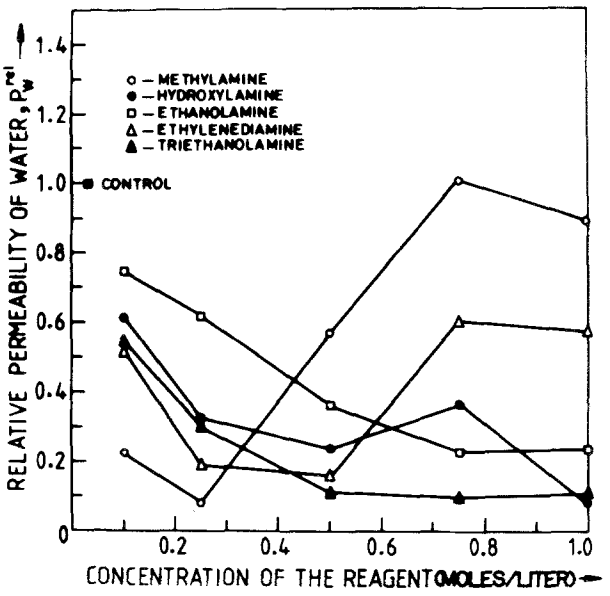

Fig. 3. Dependence of relative permeability of water P_w^{rel} of egg lecithin liposomes on concentration of structure makers. Each data point is mean of at least three observations, error limit being ± 2.5%

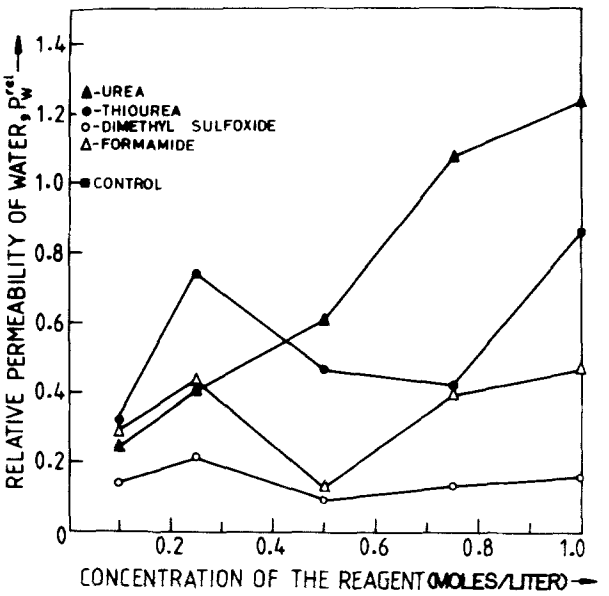

Fig. 4. Dependence of relative permeability of water P_w^{rel} of egg lecithin liposomes on concentration of structure breakers. Each data point is a mean of at least three observations, error limits being ± 2.5%

Fluorescence Studies

Binding studies with the fluorescent dye, 1-anilino,8-naphthalene sulfonate (ANS) were performed as described previously [10].

Results and Discussion

Plots of initial shrinkage velocity and extent of shrinkage against glucose concentration were found to be linear for the liposomes used (*data not shown*), demonstrating that these liposomes behave as perfect osmometers even with the highest concentration of the reagents used (1.0 M). The fluorescent dye 1-anilino,8-naphthalene sulfonate (1,8-ANS) which binds to the membranes at the membrane-aqueous interface was used to monitor the binding of the reagents, perturbing the water structure, to the liposomal membrane. Through the dye binding studies, we wanted to know if direct binding of the structure maker or breaker molecules to the liposomal surface was responsible for the changes of membrane properties. However, the results of dye binding studies reveal that none of the structure makers or breakers bind directly to the liposomal surface and thus influence the phase equilibrium of the membrane by perturbing the structured water at the membrane-aqueous interface [10].

A noticeable feature of the dependence of extent of shrinkage (Figs. 1 & 2) as well as relative permeability of water (Figs. 3 & 4) on concentration of structure makers and breakers is the anomalous behavior of these parameters in the case of most of the reagents used. Each curve with a specific symbol represents the experimental parameter studied at various concentrations of the same reagent (Figs. 1 to 4). This eliminates the possibility of trend of the data being affected by differences in relative permeabilities of various structure makers and breakers and any effect these reagents may have on the aggregation and dispersion of the liposomes. Thus the effects of the structure makers and breakers on osmotic shrinkage properties of liposomes is controlled primarily by the influence these reagents exert on the phase equilibrium of the membrane and mechanism(s) of water permeation through it [4, 10]. It may be mentioned here that the method of preparation of liposomes was chosen such that no concentration gradient of the reagent existed across the liposomal membrane.

In the low concentration range (from 0.1 to 0.25 M), relative permeability of water increases for structure breakers whereas it decreases for structure makers, with concentration. This demonstrates a differential effect of the structure makers and breakers on osmotic shrinkage of egg lecithin liposomes, at low concentration.

Structure Makers

Amines can have a multitude of possible reactions with water lattice [20]. The amino group on the mol-

ecule serves an important role as a proton donor in its interaction with water [16]. In the presence of methylamine, $\Delta(1/A)\%$ and P_W^{rel} follow the same trend as concentration is changed (Figs. 1 & 3) and increase from 0.25 to 0.75 M. Marginal lowering of cooperativity of transition in DPPC liposomes in the presence of methylamine takes place in the same concentration range where increase in $\Delta(1/A)\%$ and P_W^{rel} is observed (Das & Singhal, *unpublished results*).

In contrast to methylamine, in the presence of hydroxylamine trends of variation of relative permeability of water and extent of shrinkage (Figs. 3 & 1, respectively) are opposite. We have observed that the dependence of cooperativity of transition of DPPC liposomes on hydroxylamine concentration bears a similar shape as that of P_W^{rel}, suggesting possible correlation of these two parameters (*unpublished results*). At higher concentration, hydroxylamine may affect the size distribution and probability of formation of transient, statistical, aqueous pores in the membrane [3, 4]. In the presence of ethylenediamine, relative permeability of water and extent of shrinkage exhibit concentration dependence very similar to those with hydroxylamine, suggesting similar action of these two reagents on egg lecithin membranes. The similarity in their action on osmotic shrinkage properties is interesting, keeping in view presence of multiple functional groups on these molecules. The notable similarity between variations of P_W^{rel} on the presence of ethanolamine and triethanolamine may be due to their molecular similarity. However, extent of shrinkage shows entirely different behavior in the presence of these two structure makers.

The sharp reduction in extent of shrinkage in the presence of triethanolamine may be due to intercalation of the bulky triethanolamine molecule between the polar headgroups interfering with hydrogen bonding patterns and van der Waal's interactions. At higher concentrations, extent of shrinkage increases slightly indicating fluidization of the membrane. Relative permeability of water decreases with increase in triethanolamine concentration in a fashion similar to but faster than that in the presence of ethanolamine, suggesting a greater potency of triethanolamine as a structure maker. As will be explained later, gradual increase in size of structured water clusters with concentration of ethanolamine and triethanolamine could be responsible for gradual decrease of P_W^{rel} to a constant value.

Structure Breakers

In the presence of urea, thiourea and foramide, extent of shrinkage shows a concentration dependence similar to relative permeability of water (Figs. 2 & 4) whereas for DMSO they show a trend opposite to each other. In the presence of urea, thiourea and foramide, the structuring of the liposome entrapped bulk water is presumably minimized yielding "free" water, due to the structure breaking action of these reagents. The difference between the curves in the presence of urea and thiorea reveals the importance of functional groups in determining potency for modulation of water structure. Ice-like water clusters are known to be formed in the presence of DMSO [43]. Formation of such structures at the membrane-aqueous interface and both sides of the liposomal membrane, decreasing total amount of internal volume available for shrinkage may be responsible for initial decrease of $\Delta(1/A)\%$. At a higher concentration DMSO probably replaces some water molecules from the polar headgroup region and takes part in hydrogen bonding [27, 28], causing an increase in the extent of shrinkage and marginal decrease in relative permeability of water. It may be noted in this context that biophysical activity of several small molecules, e.g. DMSO, urea, *t*-butanol, in promoting denaturation is known to depend upon their competing interaction with the interface or substrate for water molecules and functional groups present on the molecule [15, 16]. In the presence of DMSO at all concentrations relative permeability of water is much less compared to the control. This is probably due to structuring of water around DMSO [43], which prevents partitioning of water in the hydrocarbon phase and/or passage through transient statistical pores due to increased size of the cluster, as well as restricted rearrangement of hydrogen bonds in the polar headgroup region [33]. Interestingly, at higher concentrations of urea, relative permeability of water becomes more than the control, which may be due to induction of structural defects in the bilayer by urea upon its interaction with the interface.

Primarily three distinct possibilities of interaction may be visualized in the system studied by us—lipid-solute, water-solute and lipid-water. Our dye binding studies clearly negate any direct lipid-solute interaction. Water-solute interaction will conceivably modify the intrinsic structuring of bulk water and as a consequence its anomalous properties and its fitness to act as a molecular environment [18, 25]. Depending on the characteristics of the solute it may facilitate formation of water clusters, clathrate-like structures or water monomers. Water-solute interaction is known to depend on the finite molecular size of the solute and the functional group on it [14]. Such water-solute interaction leading to a change in size of water clusters in bulk water is of particular significance considering the fact that osmotic pores in lipid membranes are known to allow the passage

of molecules of diameter less than 4 Å [29, 41]. The water molecules near an interface are oriented by dipole-dipole interactions. The interface has been suggested to stabilize the structured entities of bulk water by acting mainly as a momentum sink for thermal fluctuations which may otherwise disrupt the lattice stability latently present in bulk water [11]. A modification of such structured elements due to water-solute interaction is thus expected to be reflected in lipid-water interaction. The water associated with lipid headgroup behaves as unfreezable and nonsolvent water [17, 23]. Infrared spectroscopy [40], PMR studies [8, 13], calorimetry [17], dielectric relaxation studies [9, 22] and NMR studies [7] strongly point to the existence of highly ordered membrane-associated water. Thermal anomalies in various membrane properties. e.g. BLM-resistance [44], thermal transition in ultrathin BLM [2], bi-ionic potential in polysoap and collodion-potassium oleate membranes [32], membrane potential of *Valonia utricularis* [12] have been suggested to arise from thermal transition of structured water at the membrane aqueous interface from one form to another [11]. It is conceivable that any agent which can modulate intrinsic structuring of bulk water and bring about structural transitions of ordered water at the membrane-aqueous interface would induce similar anomalies in membrane properties. We thus argue that the anomalous membrane properties observed by us (Figs. 1 to 4) reflect such an action of the structure maker and breaker reagents on the structured water at the membrane-aqueous interface, inducing concentration-dependent, order-disorder transitions. Such transitions are reflected in the membrane properties due to mutual interaction and stabilization of water and lipid molecules as discussed earlier. While we are quite aware of the complexity of such multicomponent systems, careful consideration of available facts leads us to believe this to be the most plausible interpretation of our data. The change of water structure being the sole reason for several anomalous behaviors exhibited by the nonelectrolyte solutions, even at low concentrations leads further strength to this suggestion [18]. We feel that the incapability of few of the reagents to induce anomalous behavior in the parameters studied may arise from their poor capability to perturb water structure.

The structures maker and breaker reagents may have various probable modes of action on the interfacial structured water. Membrane may be partially dehydrated and may become more rigid due to the competition between these reagents and lipid polar headgroups for binding to water molecules or due to a cosolvent action of these reagents at a higher concentration where the reagent molecules may physically replace the water molecules from the interface. Dehydration of the membrane is known to make it more rigid and decrease its fluidity [17, 24, 39], which in turn decreases the membrane permeability. In the case of some reagents, e.g. DMSO, the water clusters or clathrate-like structures formed around the reagent molecules, may be stabilized at the membrane-aqueous interface by hydrogen bonding, and alter the fluidity of the membrane, consequently changing the membrane permeability.

The above discussion may be summarized as follows: our fluorescent dye binding data demonstrates that the structure maker and breaker reagents used in this study are capable of influencing the phase equilibrium without binding directly to the liposomal membrane, by altering the water structure at the membrane-aqueous interface. The anomalous concentration dependence of water permeability and extent of shrinkage of egg lecithin liposomes suggest the perturbation of the highly ordered water at the polar headgroup region by these reagents. A change in water structure at the membrane-aqueous interface leads to a change of water permeability and osmotic shrinkage properties of the membrane by altering its phase equilibrium. The specific size, shape and functional group of a molecule seems to exert some influence on the water permeability of the lipid membranes.

Enhancement or disruption of intrinsic structuring of the liposome-entrapped bulk water by the structure makers and breakers probably contributes potentially to the concentration dependence of water permeability and osmotic shrinkage properties of egg lecithin liposomes.

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References

1. Andreoli, J.E., Troutman, S.L. 1971. An analysis of unstirred layers in series with 'tight' and 'porous' lipid bilayer membranes. *J. Gen. Physiol.* **57**:464–478
2. Bean, R., Chan, H. 1969. Thermal transitions in conductivity of ultra-thin membranes. *In:* The Molecular Basis of Membrane Function. D.C. Tosteson, editor. pp. 133–146. Prentice-Hall, Englewood Cliffs, New Jersey
3. Blok, M.C., Van Deenen, L.L.M., De Gier, J. 1976. Effect of gel to liquid-crystalline phase transition on the osmotic behaviour of phosphatidylcholine liposomes. *Biochim. Biophys. Acta* **433**:1–12
4. Blok, M.C., Van Der Neut-Kok, E.C.M., Van Deenen, L.L.M., De Gier, J. 1975. The effect of chain length and lipid phase transitions on the selective permeability properties of liposomes. *Biochim. Biophys. Acta* **406**:187–196

5. Boggs, J.M. 1980. Intermolecular hydrogen bonding between lipids: Influence on organization and function of lipids in membranes. *Can. J. Biochem.* **58:**755–770
6. Cass, A., Finkelstein, A. 1967. Water permeability of thin lipid membranes. *J. Gen. Physiol.* **50:**1765–1784
7. Cerbon, J. 1970. The influence of pH and temperature on the limited rotational freedom of the structured water and lipid hydrocarbon chains of natural membranes. *Biochim. Biophys. Acta* **211:**389–395
8. Clifford, J., Pethica, B.A., Smith, E.G. 1968. A nuclear magnetic resonance investigation of molecular motion in erythrocyte membranes. *In:* Membrane Models and the Formation of Biological Membranes. L. Bolis and B.A. Pethica, editors. pp. 19–42. North-Holland, Amsterdam
9. Coster, H.G.L., Simons, R. 1970. Anomalous dielectric dispersion in bimolecular lipid membranes. *Biochim. Biophys. Acta* **203:**17–27
10. Das, S., Singhal, G.S. 1981. Effect of change of water structure on the phase transition of liposomes of dipalmitoyl phosphatidylcholine. *Int. J. Quant. Chem.* **20:**495–504
11. Drost-Hansen, W. 1971. Structure and properties of water at biological interfaces. *In:* Chemistry of the Cell Interface. H.D. Brown, editor. Part B, pp. 1–184. Academic, New York
12. Drost-Hansen, W., Thorhaug, A.K. 1967. Temperature effects in membrane phenomena. *Nature (London)* **215:**506–508
13. Finch, E.D., Schneider, A.S. 1975. Mobility of water bound to biological membranes: A proton NMR relaxation study. *Biochim. Biophys. Acta* **406:**146–154
14. Franks, F. 1966. Solute-water interactions and the solubility behaviour of long chain paraffin hydrocarbons. *Nature (London)* **210:**87–88
15. Glasel, J.A. 1970. Deuteron magnetic relaxation studies on the solution properties of some denaturing agents and surfactants. *J. Am. Chem. Soc.* **92:**372–375
16. Glasel, J.A. 1970. Participation of water in conformational changes of biopolymers as studied by deuteron magnetic relaxation. *J. Am. Chem. Soc.* **92:**375–381
17. Hauser, H. 1975. Lipids. *In:* Water—A Comprehensive Treatise. F. Franks, editor. Vol. 4, pp. 209–303. Plenum, New York
18. Horne, R.A. 1969. Marine Chemistry: The Structure of Water and the Chemistry of the Hydrosphere. Wiley-Interscience, New York
19. Inoue, K. 1974. Permeability properties of liposomes prepared from dipalmitoyl lecithin, dimyristoyl lecithin, egg lecithin, rat liver lecithin and beef brain sphingomyelin. *Biochim. Biophys. Acta* **339:**390–402
20. Jeffrey, G.A., Jordan, T. H., McMullan, R.K. 1967. Clathrate hydrates of some amines. *Science* **155:**689–691
21. Jung, O.Y., Carlson, L.M., Balzer, C.J. 1973. Characteristics of the permeability barrier of human erythrocyte ghosts to nonelectrolytes. *Biochim. Biophys. Acta* **298:**101–107
22. Kaatze, U., Henze, R., Pottel, R. 1979. Dielectric relaxation and molecular motions in C14-lecithin-water systems. *Chem. Phys. Lipids* **25:**149–177
23. Katz, Y., Diamond, J.M. 1974. Nonsolvent water in liposomes. *J. Membrane Biol.* **17:**87–100
24. Kjellander, R. 1978. Water—A structural element in model membrane systems: An approach to some structural changes in the lecithin-water system. *J. Colloid Interface Sci.* **66:**303–312
25. Klotz, I.M. 1970. Water: Its fitness as molecular environment. *In:* Membranes and Ion Transport. E.E. Bittar, editor. Vol. 1, pp. 93–122. Wiley-Interscience, London
26. Ladbrooke, B.D., Chapman, D. 1969. Thermal analysis of lipids, proteins and biological membranes. A review and summary of some recent studies. *Chem. Phys. Lipids* **3:**304–367
27. Lyman, G.H., Papahadjopoulos, D., Preisler, H.D. 1976. Phospholipid membrane stabilization by dimethylsulfoxide and other inducers of Friend leukemic cell differentiation. *Biochim. Biophys. Acta* **448:**460–473
28. Lyman, G.H., Preisler, H.D., Papahadjopoulos, D. 1976. Membrane action of DMSO and other chemical inducers of Friend leukemic cell differentation. *Nature (London)* **262:**360–363
29. Macey, R.I., Karan, D.M., Farmer, R.E.L. 1972. Properties of water channels in human red cells. *In:* Biomembranes—Passive Permeability of Cell Membranes. F. Kreuzer and J.F.G. Slegers, editors. L.A. Manson, series editor. Vol. 3, pp. 331–340. Plenum, New York
30. Micelli, S., Galucci, E., Lippe, C. 1978. Nonelectrolyte permeability through black lipid membranes with different surface charge. *Arch. Int. Physiol. Biochim.* **86:**755–759
31. Naftalin, R.J. 1971. The role of unstirred layers in control of sugar movements across red cell membranes. *Biochim. Biophys. Acta* **233:**635–643
32. Nelson, S.S., Blei, I. 1966. Model Membrane Studies Related to Ionic Transport in Biological Systems. Office of Saline Water, Research and Development Progress Report 221, U.S. Govt. Printing Office, Washington, D.C.
33. Nichols, J.W., Deamer, D.W. 1980. Net proton-hydroxyl permeability of large unilamellar liposomes measured by an acid-base titration technique. *Proc. Natl. Acad. Sci. USA* **77:**2038–2042
34. Oku, N., Nojima, S., Inoue, K. 1980. Selective release of nonelectrolytes from liposomes upon perturbation of bilayers by temperature change or polyene antibiotics. *Biochim. Biophys. Acta* **595:**277–290
35. Papahadjopoulos, D., Nir, S., Ohki, S. 1971. Permeability properties of phospholipid membranes: Effect of cholesterol and temperature. *Biochim. Biophys. Acta* **266:**561–583
36. Petersen, D.C. 1980. Water permeation through the lipid bilayer membrane: Test of the liquid hydrocarbon model. *Biochim. Biophys. Acta* **600:**666–677
37. Phillips, M.C., Finer, E.G., Hauser, H. 1972. Differences between conformations of lecithin and phosphatidylethanolamine polar groups and their effects on interactions of phospholipid bilayer membranes. *Biochim. Biophys. Acta* **290:**397–402
38. Redwood, W.R., Haydon, D.A. 1969. Influence of temperature and membrane composition on the water permeability of phospholipid bilayers. *J. Theor. Biol.* **22:**1–8
39. Schneider, A.S., Middaugh, C.R., Oldewurtel, M.D. 1979. Role of bound water in biological membrane structure: Fluorescence and infrared studies. *J. Supramol. Struct.* **10:**265–275
40. Schneider, M.J.T., Schneider, A.S. 1972. Water in biological membranes: Adsorption isotherms and circular dichroism as a function of hydration. *J. Membrane Biol.* **9:**127–140
41. Solomon, A.K. 1972. Properties of water in red cell and synthetic membranes. *In:* Biomembranes—Passive Permeability of Cell Membranes. F. Kreuzer and J.F.G. Slegers, editors. L.A. Manson, series editor. Vol. 3, pp. 299–330.

42. Stein, W.D. 1967. The Movement of Molecules Across Cell Membranes. Academic, New York
43. Szmant, H.H. 1975. Physical properties of dimethyl sulfoxide and its function in biological systems. *In:* Biological Actions of Dimethylsulfoxide. S.W. Jacob, and R. Herschler, editors. *Ann. N.Y. Acad. Sci.* **243:**20–23
44. Thompson, T.E. 1964. The properties of bimolecular phospholipid membranes. *In:* Cellular Membranes in Development. M. Locke, editor. pp. 83–96. Academic, New York
45. Yeagle, P.L., Hutton, W.C., Huang, C., Martin, R.B. 1975. Head group conformation and lipid cholesterol association in phosphatidylcholine vesicles: A ^{31}P (^{1}H)-nuclear Overhauser effect study. *Proc. Natl. Acad. Sci. USA* **72:**3477–3481

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