SHORT COMMUNICATIONS

BBA 73121

Thin α-tocopherol films separating two aqueous phases

The high concentration of α-tocopherol as plasticizer and antioxidant in lipid bilayer films[1] questions the validity of a direct comparison between the phenomena observed in these model structures and those observed in biological membranes. It has been tacitly assumed that α-tocopherol is not the predominant structural component of the model membranes, though the partial molar fraction of tocopherol in the solution from which the membranes are formed is considerably greater than that of the lipid. In the work cited above, the number of tocopherol molecules in solution should amount to more than 5 times the number of lipid molecules available if the molecular weight of tocopherol is taken as approximately half that of the lipid and provided that the solute molecules do not interact to form micellar complexes. At this ratio, the question whether or not the composition of the membrane corresponds to that of the membrane-forming solution becomes academic.

α-Tocopherol membranes separating two aqueous phases can be made from solutions containing 0.02 g D-α-tocopherol (purissime, Distillation Products Industries, Rochester, N.Y.) dissolved in 1 ml chloroform–methanol (1:1, v/v; spectrograde, Fisher Scientific Co., Montreal) with 0.2 ml tetradecane (Eastman-Kodak, Rochester, N.Y.). The technique of membrane formation has been described in detail in an earlier paper[2]. The conductivity cell used consists of a small polyethylene cup with a circular hole of 1-mm diameter. This cup is held in a petri dish with metal clamps, then the "inside" and "outside" volumes are filled with phosphate buffer of pH 7.0. An aliquot of the membrane-forming solution is brushed across the hole in the inner cup and the thick film thus made is observed with a stereo-microscope under white light incident at an angle <40°. The membranes thin out during the loss of solvent into the aqueous phases and gradually develop secondary black regions. After 4–5 min, the membranes are completely black indicating that they are now less than 100 Å thick. Sinusoidal or bimodal rectangular pulses from a Wavetek Model 111 generator (Wavetek, San Diego, Calif.) are applied to the membrane across two low-resistance fiber-junction calomel electrodes at a frequency between 0.1 and 10 Hz. The voltage and the voltage drop across the membrane are continuously monitored through a high input-impedance amplifier (design: Simon Gagné, Département de Biophysique, Université de Sherbrooke) on a Hewlett-Packard Model 7030 AM X–Y recorder (Hewlett-Packard, Pasadena, Calif.) over periods of up to 6 h. Both aqueous compartments were stirred at the same rate; the temperature of the solutions on both sides of the membrane was maintained at 20°.

Black tocopherol films remained intact for periods up to 6 h without any change in resistance, though about 60 % of them broke after 5 min. They seem considerably less resistant to mechanical shock than black lecithin membranes. Their electrical resistance usually attained a constant value 5 min after they had become completely black, all membranes gave between $1 \cdot 10^7$ and $1 \cdot 10^8$ $\Omega \cdot cm^2$. The membrane capacitance was approximated from the time constants under d.c. stimuli as less than 0.6 $\mu F/cm^2$. Dielectric breakdown occurred at fields between $0.6 \cdot 10^6$ and $1.0 \cdot 10^6$ V/cm, values somewhat lower than those reported for lecithin membranes without tocopherol[3]. KCl, NaCl, LiCl, and $CaCl_2$ in concentration gradients between 2:1 and 1000:1 did not introduce potentials across the unmodified α-tocopherol films, nor did these salts in any way affect the measured resistance or capacitance.

Sodium dodecyl sulfate in final concentrations below 10 mM lowered the initially high resistance of tocopherol membranes by factors up to $1 \cdot 10^4$ within minutes. This effect of anionic detergents has been reported earlier for lecithin–tocopherol bilayers[2]. When added to the aqueous phase on only one side of the membrane, some anionic detergents produced small asymmetry potentials (<5 mV) which decayed with a time-course apparently related to the detergent's oil–water partition coefficient and, thus, the degree of restraint imposed on its diffusivity across the membrane proper. This phenomenon relates to the electrochemical behavior of asymmetric polymer membranes, *i.e.* membranes in which the charge density in one external layer differs from that of the second external layer[4]. A study of the parameters controlling the development of this potential is under way.

In the presence of a salt gradient, a cationic potential developed concomitantly with the drop in resistance induced by the detergent: the membranes become selectively permeable for cations. A system of 0.1 M KCl separated by the membrane from a 0.01 M KCl solution typically gave a potential of 53 mV, the side of the more concentrated solution being negative. At all salt gradients, the potentials measured were slightly smaller than expected from the Nernst equation indicating that tocopherol membranes are not completely impermeable to anions. Neither in the high nor in any of the low resistance states could a "dynamic" resistance change be observed on stimulation, *i.e.* the membranes do not display any points of inflection in their current–voltage characteristic.

α-Tocopherol membranes behave qualitatively in every aspect studied like lecithin membranes. Their appearance as "black" films warrants the conclusion that they are bilayer membranes when in the high resistance state. The ability to form these films between two aqueous phases suggests that α-tocopherol cannot be regarded merely as an antioxidant for the lipid molecules in the membrane. Any interpretation of the structure–function relationship in membranes containing a high concentration of this vitamin ought to take into account the possibility that tocopherol acts as a structural element. The change in surface potential and surface tension observed in monolayers with changes in the film's composition[5,6] and the changes in the osmotic permeability of bilayer films of different composition[7] indicate that the parameters governing the adsorption of *e.g.* proteins will also determine the functional properties of the membrane.

Amongst the organic substances capable of forming thin stable membranes separating two aqueous phases (rhodopsin[8]; 7-dehydrocholesterol[9]; sorbitan tristearate; and glycerol tristearate[10]), α-tocopherol is the molecule which is least surface active. With only one hydroxyl group largely responsible for the orientation of the molecule at an interface, pure tocopherol membranes appear to provide the choice system for physicochemical studies of the mutual interaction of molecules in thin films formed between two aqueous phases as well as for studies of the interaction of adsorbates with such films. Tocopherol membranes will, therefore, help define the forces in the more complex lipid–lipid or lipid–protein interaction of biological membranes.

We thank the Medical Research Council of Canada for financial support. W.D.S. holds a scholarship, M.B. a research studentship of the Medical Research Council.

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Received February 20th, 1970

Biochim. Biophys. Acta, 211 (1970) 356–358

BBA 73119

The effect of potassium permanganate on lecithin and cholesterol monolayers*

Salts of heavy metals have been used extensively as stains in electron microscopy of cell organelles and membranes. However, little is known about the interaction of these heavy metal ions with lipids and proteins in membranes. Therefore, we have undertaken a study on the interaction of heavy metal ions with phospholipid and cholesterol monolayers as a model lipid membrane. The interaction of OsO_4 and uranyl acetate with lecithin and cholesterol monolayers and liquid crystals has been reported previously[1–3].

Chromatographically pure L-α-dipalmitoyl lecithin was purchased from Mann Research Laboratory (New York, N.Y.); egg lecithin was supplied by the Sylvana Chemical Co. (Orange, N.J.). Both lecithins were chromatographically pure and gave single spots on a thin layer chromatographic plate with a chloroform–methanol–water (60:35:5, by vol.) solvent system. The fatty acid composition of egg lecithin, which contains approximately equal amounts of saturated and unsaturated fatty acids, has been reported previously[4]. High purity cholesterol was supplied by Applied

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[*] Presented at the 12th Annual Meeting of the Biophysical Society, Pittsburgh, Pa., February, 1968.