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QUARTERLY

The effect of undecaprenol on bilayer lipid membranes*

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The influence of undecaprenol on phosphatidylcholine macrovesicular bilayer lipid membranes has been studied by electrophysiological techniques. The current-voltage characteristics, ionic transference numbers, the membrane conductance-temperature relationships and the membrane breakdown voltage were measured. The permeability coefficients for Na^+ and Cl^- ions, the activation energy of ion migration across the membrane, the membrane hydrophobic thickness and the membrane Young's modulus were determined. Undecaprenol increases membrane conductance, membrane capacitance, membrane ionic permeability and membrane elastic deformability, decreases the activation energy, membrane hydrophobic thickness and membrane electromechanical stability, and does not change membrane selectivity. The formation by undecaprenyl molecules of fluid microdomains modulating membrane hydrophobic thickness is postulated. The data suggest that the behaviour of undecaprenol in membranes is regulated by transmembrane electrical potential.

Undecaprenol (C_{55}) functions as a hydrophobic carrier of glycosyl residues across a bacterial cytoplasmic membrane in the synthesis of bacterial surface glycoconjugates [1, 2]. An undecaprenyl molecule consists of a hydrophilic part — a hydroxyl group, and a relatively large hydrophobic part — a long, unsaturated, mainly of poly-*cis* configuration isoprenyl chain composed of 11 isoprene units. Undecaprenol was found in the bacterial cytoplasmic membrane as a di-*trans*, poly-*cis* prenol with the structure: ω-t_2-c_8-OH, where ω is an isoprene residue farthest from the hydroxyl group, OH is the hydroxyl group, t is a *trans*-isoprene residue, c is a *cis*-isoprene residue [1, 2]. C_{55}-Polyprenol was also isolated from plant photosynthetic tissues [3]. The preparations of C_{55}-polyprenol found in leaves of various plants contain three internal *trans*-isoprene units, though sometimes small amounts of mono-*trans*-, di-*trans*- and tetra-*trans*-components were detected [3, 4].

The behaviour of polyprenols in plasma membranes and model lipid membranes has recently been intensively studied using several techniques including EPR, NMR and fluorescence spectroscopy [5–20], X-ray scattering [13, 21], differential scanning calorimetry [10, 13], freeze-fracture electron microscopy [13], radioactive isotope techniques [22] and voltammetry [23 28].

In the present study we investigated macrovesicular bilayer membranes made of phosphatidylcholine or its mixture with undecaprenol by electrophysiological techniques. Measurements of current-voltage characteristics, ionic

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transference numbers, membrane specific capacitance, conductance-temperature relationships and membrane breakdown voltage were performed. The influence of undecaprenol on permeability coefficients for Na^+ and Cl^- ions, the activation energy of ion migration across the membrane and membrane Young's modulus were also determined.

EXPERIMENTAL

Chemicals. DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) was purchased from Sigma. It gave a single spot on Silica Gel TLC plates (Merck) in chloroform/methanol/water (65:25:4, by vol.) and in chloroform/methanol/acetic acid/water (50:30:8:4, by vol.). Undecaprenol was isolated from leaves of *Rhus typhina* [29]. It gave a single spot on Silica Gel G TLC plates (Merck) in ethyl acetate/toluene (5:95, by vol.) and on RP-18 HPTLC plates (Merck) in acetone. *n*-Decane and butanol were purchased from Aldrich and Fisher, respectively.

Membrane formation. Macrovesicular bilayer lipid membranes were formed according to the technique described previously [23] in a Teflon capillary tube in unbuffered (pH 6) aqueous solution of 0.1 M and 0.2 M NaCl (inside and outside the membrane, respectively). DOPC or DOPC/C_{55} mixtures used for membrane formation were dissolved in *n*-decane/butanol (3:1, v/v) to obtain a concentration of 10 mg of lipid per ml of solvent. The area of the macrovesicular bilayer lipid membranes was about 50 mm^2. For other details see [23].

Electrical measurements. Two saturated silver chloride electrodes were used to apply external voltage and detect the electric potentials. Electrometers were used to measure the external resistance and the voltage distribution between the membrane. The area of the membrane, S, was determined by optical measurement of membrane dimensions. The temperature, T, was controlled by water circulating from an external bath. The capacitance of the membrane, C, was determined from recorded membrane discharge curves. To obtain values of the breakdown voltage, V_B, the applied voltage was slowly increased until membrane rapture occurred. Electrical conductance of the membrane, G, was calculated from current-voltage characteristics. The activation energy of ion migration across the membrane, E_A, was determined from Arrhenius plots of normalized conductance of macrovesicular bilayer lipid membranes. The membrane permeability coefficients for Na^+ and Cl^- ions, P_{Na^+} and P_{Cl^-}, respectively, the ionic transference numbers, the membrane Young's modulus, E, and the membrane hydrophobic thickness, h, were calculated as described in Refs. [23, 27].

RESULTS

The ionic transference numbers were nearly independent of the lipid composition of the membrane, and their ratio, t_{Na^+}/t_{Cl^-}, of 1.1 ± 0.1 was obtained at 25°C.

The behaviour of undecaprenol-phosphatidylcholine membrane as a function of applied potential was studied by performing current-voltage experiments. As presented in Fig. 1, the I/V curves were symmetric and linear for V<40 mV. The value of the curve slope increased with the increase of the percentage of undecaprenol, C_{55}, in the bilayer.

Figures 2a and 2b show, on a semilogarithmic scale, the dependence of membrane specific conductance, G_S, (Fig. 2a) and the dependence of the permeability coefficients for Na^+ ions, P_{Na^+}, and Cl^- ions, P_{Cl^-}, (Fig. 2b) on the percentage of C_{55} in the membrane. The values of G_S, P_{Na^+} and P_{Cl^-} increase with increasing percentage of C_{55} in the bilayer. The maximal rise, over 10-fold, was observed for the C_{55}/DOPC mole ratio equal to 0.2.

The normalized conductance of macrovesicular bilayer lipid membranes was measured as a function of temperature in the range of 25–42°C. Typical trends are reported in Fig. 3. An increase of normalized conductance was observed with increasing temperature. The Arrhenius plots were linear, the slope of the curves depending on the percentage of C_{55} in the bilayer.

The relationship between the value of activation energy of ion transport across the membrane, E_A, and the percentage of undecaprenol in macrovesicular bilayers is shown in Fig. 4. The values of activation energies were derived from the Arrhenius plots by the least squares fitting. The activation energy was found to be essentially independent of temperature stu-

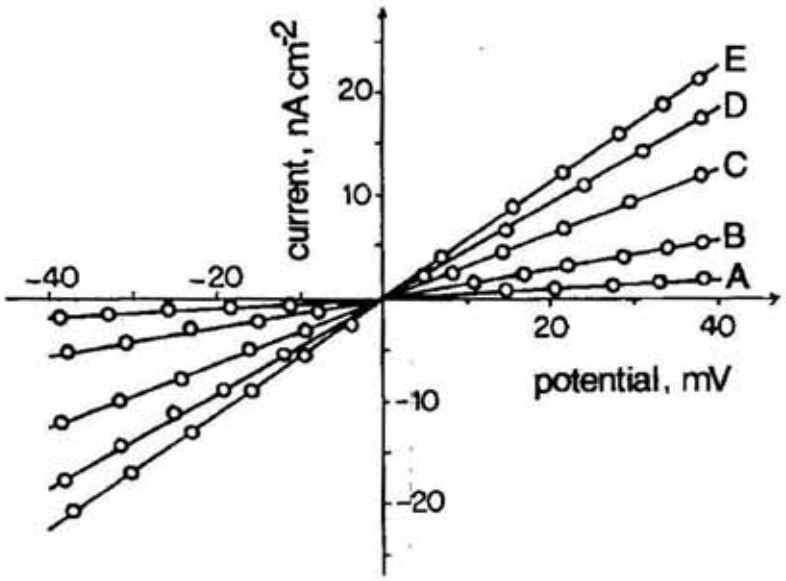

Fig. 1. Current-voltage steady-state characteristics of bilayer lipid membranes made from: DOPC (A); C55/DOPC, mole ratios: 0.01 (B), 0.04 (C), 0.08 (D), 0.2 (E). Temperature = 25 ± 1°C.

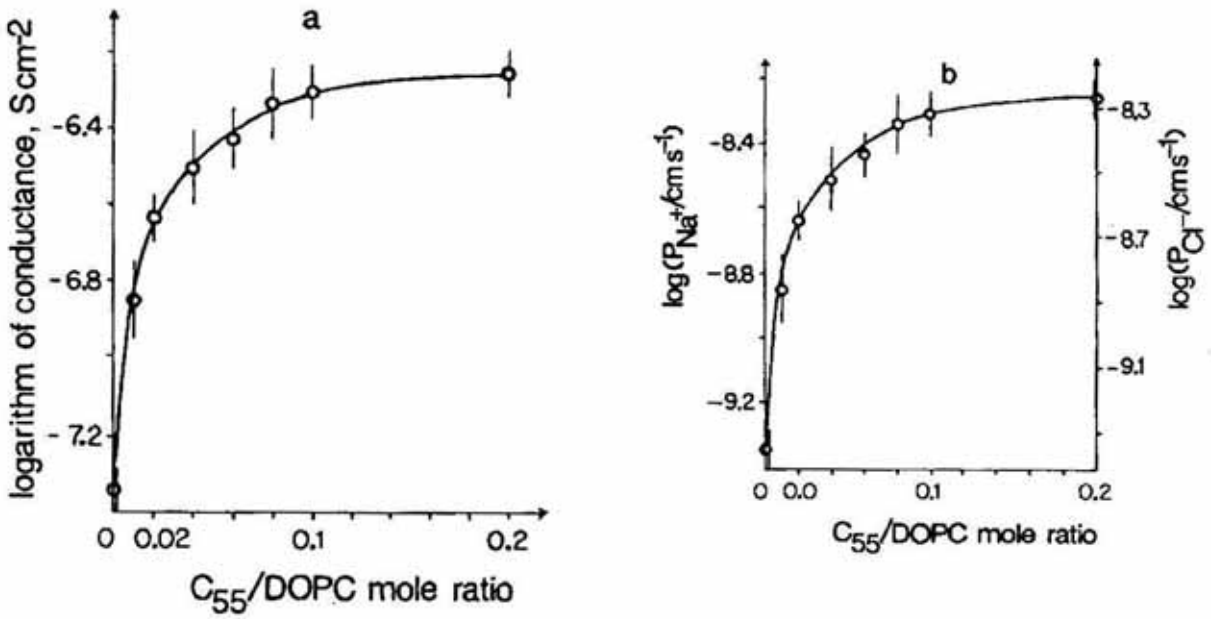

Fig. 2. Ionic conductance (a) and ionic permeability coefficients P_{Na^+}, P_{Cl^-} (b) versus the C55/DOPC mole ratio.

Each point represents the mean value (± S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25 ± 1°C.

died (not shown). The maximum drop, in comparison with the DOPC bilayer, in the value of the activation energy was obtained for the C55/DOPC mole ratio equal to 0.2. The E_A value obtained for the DOPC bilayer was 47.2 ± 3.2 kJ × mol^{-1}.

The values of the membrane specific capacitance and the membrane hydrophobic thickness of bilayer lipid membranes formed from various mixtures of DOPC and C55 are reported in Fig. 5a and Fig. 5b, respectively. Unlike the specific capacitance, the membrane hydrophobic thickness decreased with the increase of the percentage of C55 ir. the bilayer. The maximum drop, over 12% in comparison with DOPC bilayers, was observed for the mole ratio C55/DOPC equal to 0.2. The hydrophobic

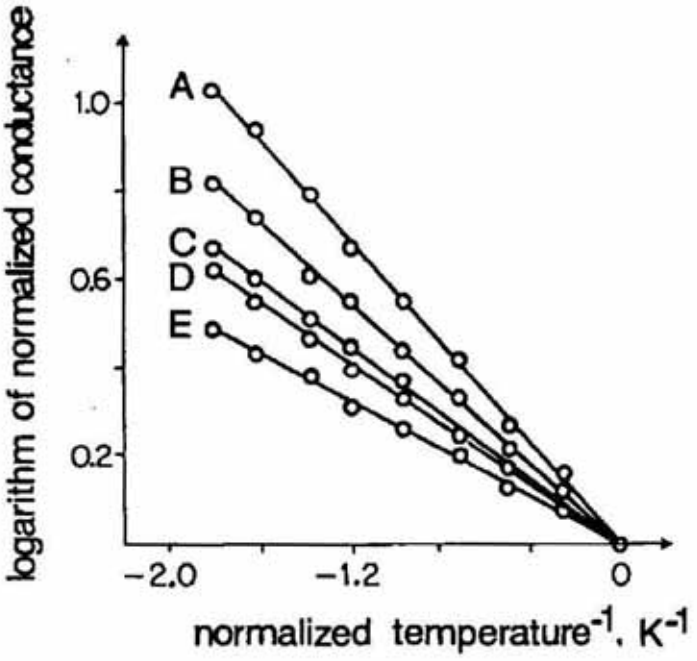

Fig. 3. Arrhenius plots of normalized conductance of macrovesicular bilayer lipid membranes made from: DOPC (A); C55/DOPC, mole ratios: 0.01 (B), 0.04 (C), 0.08 (D), 0.2 (E).
Logarithm of normalized conductance was calculated as: $\ln[(G/C)/(G_o/C_o)]$; where G, C represent membrane conductance and capacitance, respectively, for the temperature studied; G_o and C_o are the conductance and capacitance, respectively, at 298 K. Normalized temperature^{-1} was calculated as: temperature^{-1}/K^{-1}/–/298 K/$^{-1}$.

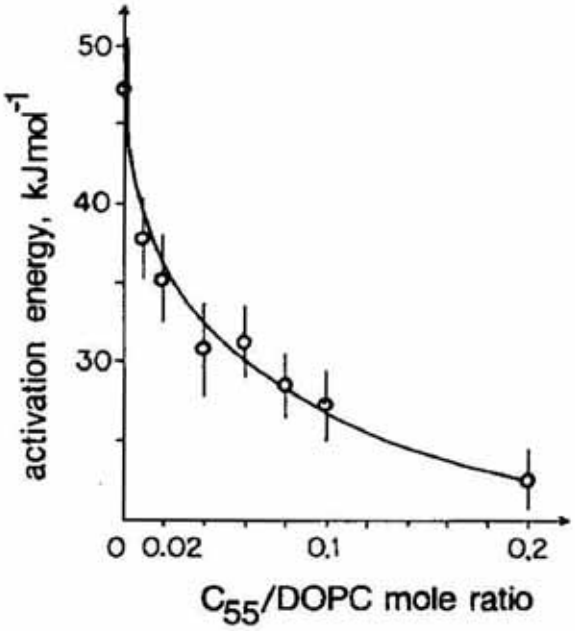

Fig. 4. Activation energy of ion migration versus the C55/DOPC mole ratio.
Each point represents the mean value (± S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25 ± 1°C.

thickness obtained for the DOPC bilayer was 5.2 ± 0.12 nm.

Figures 6a and 6b illustrate the effect of undecaprenol on the breakdown voltage, V_B, and the Young's modulus of the membrane, E, respectively. Both the membrane electromechanical stability (proportional to the value of V_B) and the membrane elastic deformability (inversely proportional to the value of E) were modulated by the presence undecaprenol in the membrane. The value of membrane breakdown voltage decreased from 195 ± 10 mV for DOPC bilayers to the value of 154 ± 8 mV for the bilayer prepared from the C55/DOPC mixture at 0.2 mole ratio. The decrease in the value of Young's modulus, i.e. the increase in membrane elasticity was observed with the increase of the percentage of C55 in the membrane. The value of Young's modulus decreased from 7.2 ± 0.34 N × m^{-2} to the value of 5.8 ± 0.26 N × m^{-2} with increasing C55/DOPC mole ratio.

DISCUSSION

In order to get some insight into the possible role of undecaprenol in the membrane phenomena, model lipid membranes in the form of C55/DOPC macrovesicular bilayers were formed and several functional properties of the membranes were studied. Since the main phase transition temperature of DOPC bilayers is below –20°C [30] and undecaprenyl chains contain double bonds, mainly in *cis* conformation, the investigated membranes were, in our experimental conditions, at the liquid-crystalline state. No phase transitions of α-saturated polyprenol/DOPC bilayers could be detected between 12°C and 82°C [13].

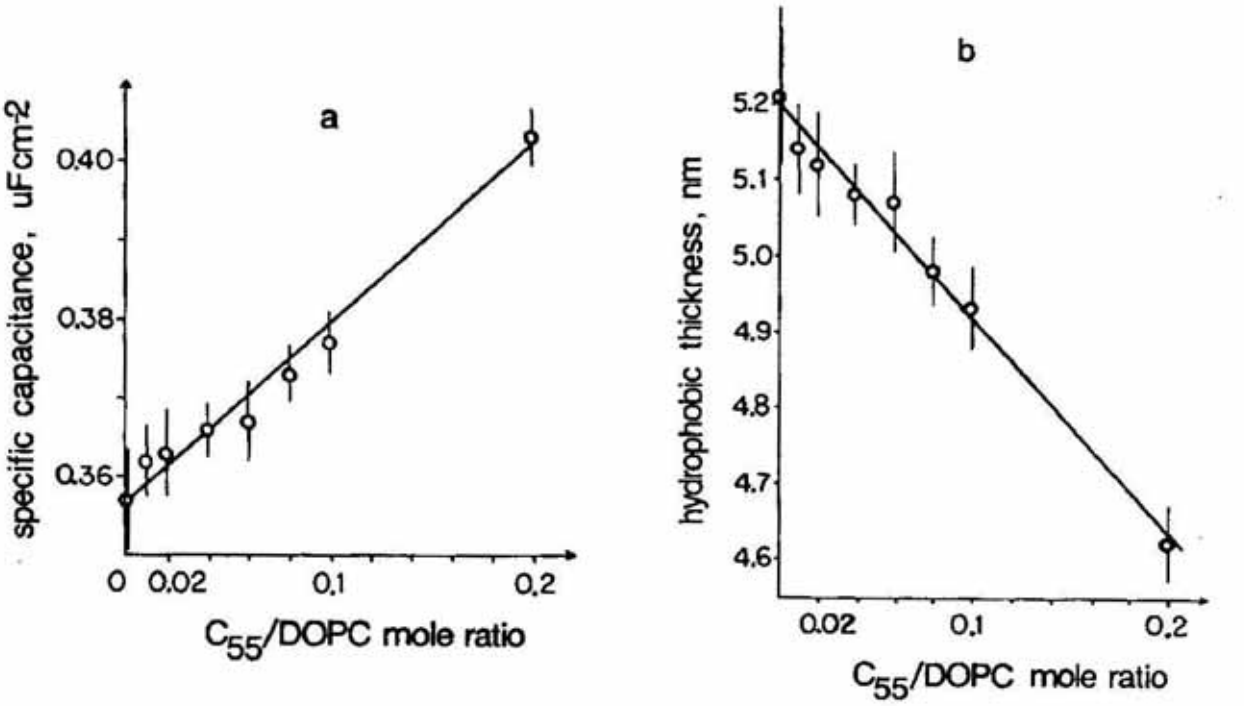

Fig. 5. Membrane specific capacitance (a) and membrane hydrophobic thickness (b) versus the C_{55}/DOPC mole ratio.
Each point represents the mean value (± S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25 ± 1°C.

The macrovesicular bilayers prepared from undecaprenol/DOPC mixtures exhibited much higher permeability for ions in comparison with DOPC bilayers, and the obtained

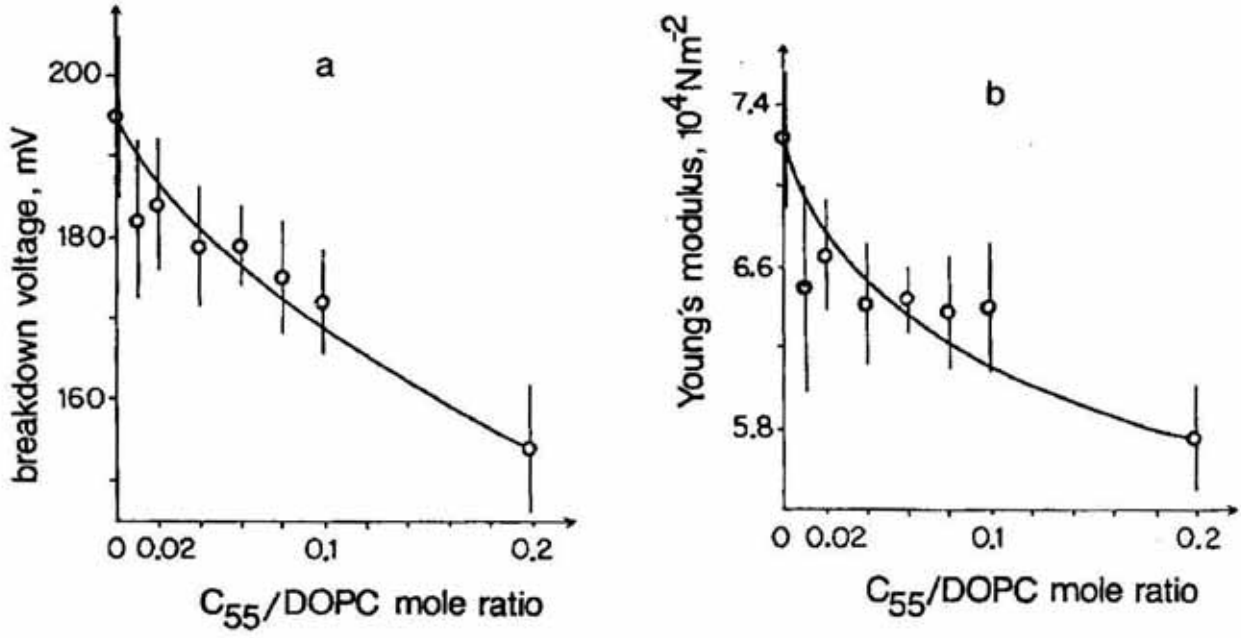

Fig. 6. Membrane breakdown voltage (a) and membrane Young's modulus (b) versus the C_{55}/DOPC mole ratio.
Each point represents the mean value (± S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25 ± 1°C.

values of activation energy of ion transport were much lower than those for DOPC bilayers. The linearity of current-voltage characteristics for all types of the studied membranes and similar selectivity of these membranes indicate that the increase in membrane permeability and decrease in the activation energy are not compatible with formation of carriers or selective channels by undecaprenyl molecules [31].

The aggregation of spin-labelled polyisoprenols in phospholipid membranes was observed even at relative concentrations not exceeding 0.005 [6]. These aggregates can modulate the permeability and stability of polyisoprenol-phospholipid membranes. Our investigations show that undecaprenol substantially decreases the energy barrier for ion migration through membranes, giving rise to an increase of ionic conductance. Polyisoprenols promote the TEMPOcholine leakage from liposomes composed of phosphatidylethanolamine /phosphatidylcholine mixture, but not from liposomes composed only of phosphatidylcholine. Here we report on the increase of ion permeability in the presence of transmembrane potential, observed in C_{55}/DOPC bilayers containing no phosphatidylcholine. A strong destabilization of phosphatidylethanolamine bilayers but not the phosphatidylcholine ones, in the presence of α-saturated polyprenols was detected by Valtersson *et al.* [13] on studying the phase transition of the bilayers. In our experiments undecaprenol decrease substantially the value of the breakdown voltage of the membranes, which reflects the destabilizing effect of undecaprenol on the phosphatidylcholine bilayers in the presence of transmembrane potential. These phenomena point to the importance of transmembrane potential in the dynamics of undecaprenol in membranes.

The activation energy of ion transport was found to be essentially independent of temperature (not shown). This indicates that the influence of temperature on the aggregation behaviour of C_{55} and DOPC molecules in the membrane is negligible, which is consistent with the observation that temperature dependence of clustering of polyisoprenols in model membranes is minimal [6].

We report a correlation between the percentage of undecaprenol in the bilayer and the value of membrane Young's modulus. The greater the modulus the lower the membrane elastic deformability. Thus, as shown in Fig. 6, undecaprenol induces an enhancement of membrane elasticity, which in turn can facilitate membrane fusion, as reported for polyisoprenols [7, 14].

α-Saturated polyprenols were previously found to increase the motional freedom of bilayer lipid membranes [9, 10, 12–14, 17, 18] and plasma membranes [15, 19, 20]. The change in hydrophobic thickness of a bilayer can be regarded as an indicator of the change in the phospholipid fluidity caused by other amphiphilic molecules [23]. We report a considerable decrease of hydrophobic thickness when undecaprenol is present in phosphatidylcholine bilayers. The data support the idea of fluidizing effect of polyprenols on membranes.

The existence of polyisoprenol clusterings in phospholipid bilayers has been demonstrated by McCloskey & Troy [6]. We suggest the existence in C_{55}/DOPC bilayers of undecaprenol-rich microdomains which can form cavities in the membrane plane. The microdomain can be stabilized by hydrogen bonds between hydroxyl group of C_{55} and the ester oxygens of DOPC. These microdomains can modulate the permeability and stability of undecaprenol-phospholipid membranes. The function of such a microdomain in biological membranes can depend on its ability to form local changes in membrane thickness and membrane fluidity corresponding with hydrophobic thickness and environment requirements of an integral membrane protein located in this domain, in accordance with the mattress model [32] of lipid-protein interactions in membranes.

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