Acta Biochimica Polonica

Vol. 47 No. 3/2000

661–673

QUARTERLY

The effect of hexadecaprenol on molecular organisation and transport properties of model membranes★✪

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Received: 26 July, 2000; accepted: 03 August, 2000

Key words: long-chain polyprenol, lipid monolayer, bilayer lipid membranes, Langmuir film studies, voltammetric studies.

The Langmuir monolayer technique and voltammetric analysis were used to investigate the properties of model lipid membranes prepared from dioleoylphosphatidylcholine (DOPC), hexadecaprenol (C_{80}), and their mixtures. Surface pressure–molecular area isotherms, current-voltage characteristics, and membrane conductance-temperature were measured. Molecular area isobars, specific molecular areas, excess free energy of mixing, collapse pressure and collapse area were determined for lipid monolayers. Membrane conductance, activation energy of ion migration across the membrane, and membrane permeability coefficient for chloride ions were determined for lipid bilayers. Hexadecaprenol decreases the activation energy and increases membrane conductance and membrane permeability coefficient. The results of monolayer and bilayer investigations show that some electrical, transport and packing properties of lipid membranes change under the influence of hexadecaprenol. The results indicate that hexadecaprenol modulates the molecular organisation of the membrane and that the specific molecular area of polyprenol molecules depends on the relative concentration of polyprenols in membranes. We suggest that hexadecaprenol modifies lipid membranes by the formation of fluid microdomains. The results also indicate that electrical transmembrane potential can accelerate the formation of pores in lipid bilayers modified by long chain polyprenols.

★75th Anniversary of Membrane Lipid Bilayer Concept.

✪This work was carried out within the research project No. 6 PO4A 014 10 supported by the State Committee for Scientific Research in 1996–1998. The financial support of the UNESCO/PAS Polish Network for Cell and Molecular Biology for Krzysztof Nowotarski is gratefully acknowledged.

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Abbreviations: DOPC, dioleoylphosphatidylcholine; C_{80}, hexadecaprenol; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography.

Polyisoprenols (polyprenols and dolichols) are found in membrane fractions of prokaryotic and eukaryotic cells (Chojnacki *et al.,* 1987). Polyisoprenols (poly-*cis*-prenols) are natural products, derivatives of the C5 isoprene unit. The occurrence of α-unsaturated polyisoprenols (polyprenols) (Świeżewska *et al.,* 1994; Wanke *et al.,* 1998) and α-saturated polyisoprenols (dolichols) (Jankowski *et al.,* 1994; Hemming 1983; Chojnacki *et al.,* 1987) in membrane fractions has been reported. Phosphopolyisoprenols function as hydrophobic carriers of glycosyl residues across membranes in glycosylation reactions (Bugg & Brandish, 1994). The behaviour of polyisoprenols in model lipid membranes has been studied using several techniques, including ESR (McCloskey & Troy, 1980; Lai & Schutzbach, 1984), NMR (de Ropp & Troy, 1985; Valterson *et al.,* 1985; Knudsen & Troy 1989), fluorescence spectroscopy (Sunamoto *et al.,* 1983; Boscoboinik *et al.,* 1985; Vigo *et al.,* 1984), X-ray scattering (Gruner, 1985), differential scanning calorimetry (Vigo *et al.,* 1984), voltammetric analysis (Janas *et al.*, 1986; 1994; 1998; 2000) and electron microscopy (Janas *et al.,* 2000). However, the mechanism of the interaction between polyprenol and phospholipid molecules is still unclear. Studies of monomolecular films and bilayer lipid membranes containing a mixture of membrane lipids are of considerable importance because of their relevance to numerous natural systems. This paper presents the investigations of monolayer and bilayer lipid membranes modified by a long chain polyprenol – hexadecaprenol (C_{80}). The molecule of hexadecaprenol is composed of 16 isoprene units with the structure: $\omega T_2C_{12}\alpha OH$, where ω is an isoprene residue, T is a *trans*-isoprene residue, C is a *cis*-isoprene residue, α is the α-saturated OH terminal isoprene residue and OH is the hydroxyl group. Monolayer techniques have been applied to the study of the interactions between dioleoylphosphatidylcholine and other lipids (Gaines, 1966; Costin & Barnes, 1975; Gruszecki *et al.,* 1999a) and this technique can be used to investigate the origin and magnitude of the molecular interactions in mixed monolayers. The aim of the present work was to study the influence of polyprenol molecules on the organisation and packing of phospholipid molecules in lipid monolayers and bilayers.

MATERIALS AND METHODS

Chemicals. DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) was purchased from Sigma. It gave a single spot on Silica Gel thin-layer plates (Merck) in chloroform/methanol/water (65:25:4, by vol.) and in chloroform/methanol/acetic acid/water (50:30:8:4, by vol.). Hexadecaprenol (C_{80}) was isolated from leaves of *Picea abies* (Chojnacki *et al.,* 1975) and purified. It gave a single spot on Silica Gel G thin-layer plates (Merck) in ethyl acetate/toluene (5:95, v/v) and on RP-18 HP thin-layer plates (Merck) in acetone. n-Decane and butanol were purchased from Aldrich and Fisher, respectively. The purity of C_{80} was verified by using a HPLC method (Wanke *et al.,* 1998).

Monolayer formation and isotherm recording. The monolayers were deposited by spreading a proper volume of C_{80}/DOPC mixture in chloroform. Surface pressure was measured by the Wilhelmy method (Gruszecki *et al.,* 1999a; 1999b). Monomolecular layers at the air–water interface were formed in a 10 × 40 cm Teflon trough. The experiments were run at 21°C. Prior to isotherm recording, monolayers were equilibrated at zero pressure for 5 min to allow evaporation of chloroform. Lipid monolayers were then compressed at a speed of 0.5 mm/s. Surface pressure was measured by tensiometer PS 3 from Nima Technology and entered into computer memory. Measurement error was less than 0.1 mN/m. Deionised water was used as the subphase. The initial value of the area per molecule was 5 nm^2. The obtained data with measurement error less than 0.1 mN/m were

further elaborated by the use of Excel 5.0 worksheets (office software package, Microsoft) and mathematical calculations were performed using Mathematica 3.0 (Wolfram Research).

In the present work we analysed mixed monolayer isotherms in terms of excess free energy of mixing, ΔG^{E}_{mix} was calculated for each mixture using the following equation (Pack *et al.,* 1997; Markowitz *et al.,* 1995):

$$\Delta G^{E}_{mix} = \int_{\Pi^o}^{\Pi} A_{12} d\Pi - x_1 \int_{\Pi^o}^{\Pi} A_1 d\Pi - x_2 \int_{\Pi^o}^{\Pi} A_2 d\Pi \quad (1)$$

where A_{12} is the area per molecule in the mixed film, A_1 and A_2 are molar areas in pure films, x_1 and x_2 are molar fractions of component 1 and 2, and Π is the surface pressure. The integrals correspond to the areas under the Π-A isotherms, and Π^o is defined as the surface pressure where the monolayer components are ideally miscible. It is generally assumed that Π^o is close to zero. In practice, Π^o is commonly set to the lowest measurable surface pressure, and in this work Π^o was set to 0.4 mN/m. This method involves calculating the differences between the areas under the surface pressure–area isotherms of the mixtures and pure components at a specific surface pressure.

Experimentally it is found that many monolayers can be compressed to pressures considerably higher than their equilibrium spreading pressures. Eventually, however, it is impossible to increase the surface pressure further and the area of the film decreases if constant pressure is maintained or pressure falls in the film held at a constant area. This condition is referred to as the collapse point (collapse pressure (π_c); collapse area (A_c)) of the monolayer under the experimental conditions. When collapse occurs, molecules are forced out of the monolayer to form agglomerates of an adjacent bulk phase (Gaines, 1966).

Bilayer formation and electrical measurements. Bilayer lipid membranes in the form of hemispheres were formed according to the technique described previously (Janas *et al.,* 1986) on a Teflon capillary tube in unbuffered (pH 6) aqueous solution of 0.1 M and 0.2 M NaCl (at the inner and outer side of the membrane, respectively). DOPC or C_{80}/DOPC mixtures used for membrane formation were dissolved in n-decane/butanol (3:1, v/v) at 10 mg/ml. The area of the macrovesicular bilayer lipid membrane was about 50 mm^2. Saturated silver chloride electrodes were used to apply external voltage and detect the electric potentials. Electrometers were used to measure voltage distribution between the membrane and an external resistance. The area of the membrane, *S*, was determined by optical measurement of membrane dimensions. Temperature, *T*, was controlled by water circulating from an external bath. Electrical conductance of the membrane, *G*, was calculated from current-voltage characteristics.

Membrane permeability coefficients for Cl^- ions, P_{Cl^-}, were calculated from the following equation (Tien, 1974):

$$P_{Cl^-} = (E_{Cl^-} \times t_{Cl^-} \times G)/F(c_2 - c_1) \quad (2)$$

where c_1 and c_2 are the concentrations of NaCl inside and outside the spherical bilayer, respectively; F is the Faraday constant; E_{Cl^-} is the equilibrium potential for Cl^- ions; G is specific membrane conductance. The ratio of ionic transference numbers (t_{Na^+}/t_{Cl^-} for sodium and chloride ions, respectively) was determined from measurements of steady-state diffusion potentials (Janas & Janas, 1995). Experimental values were fitted by the Goldman-Hodgkin-Katz equation (Gamble *et al.,* 1982; Janas *et al.,* 2000):

$$\Delta V_m = V_i - V_0 = (RT/F) \ln \frac{(t_{Na^+}/t_{Cl^-})a_{Na^+,o} + a_{Cl^-,i}}{(t_{Na^+}/t_{Cl^-})a_{Na^+,i} + a_{Cl^-,i}} \quad (3)$$

which correlates the potential difference, ΔV_m, developed between the two sides of the membrane to the activities, a_{Na^+} and a_{Cl^-}, of the sodium and chloride ions, respectively, at the inner side (i) and the outer side (o) of the membrane; V_i and V_o are the electric potentials at the inner and outer side of the membrane, respectively; F is the Faraday constant.

The activation energy of ion migration across membrane, E_A, was determined from Arrhenius plots of normalized conductance of bilayer lipid membranes (Smith *et al.,* 1984):

$$\ln[(G/C)/(G_0/C_0)]=(E_A/R)[(1/T)-(1/T_0)] \quad (4)$$

where: $\ln[(G/C)/(G_0/C_0)]$ is the normalized conductance of the membrane; G_0 and C_0 are membrane conductance and membrane capacitance, respectively, at temperature T_0, R is the gas constant. The normalisation of membrane conductance (with respect to the membrane capacitance measured simultaneously) corrects for any variations in the bilayer conductance, which are due to variations in bilayer area or bilayer thickness.

RESULTS

Monolayer experiments

The Langmuir monolayer technique was used to investigate the interaction of hexadecaprenol with lipid films. Figure 1 shows typical surface pressure–area curves (isotherms) of monolayers prepared from pure hexadecaprenol, pure DOPC, and their mixtures in various molar fractions (0.01; 0.1; 0.5; 0.9; 0.99) spread on water and compressed at a rate of 0.5 mm/s at 21°C. By changing the molar fraction, a gradual change in the shape of the isotherms is observed. One can notice from Fig. 1 that at pressures below 20 mN/m, monolayers prepared from the molar fraction 0.01 and 0.1 are more expanded than monolayers formed from the molar fractions 0.9 and 0.99. It means that lipid packing

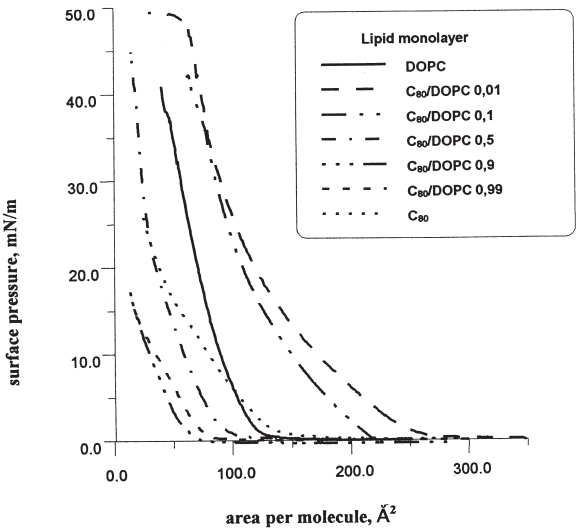

Figure 1. Collection of C_{80}/DOPC isotherms for various molar fractions [0–1].

is optimal for membranes of molar fraction equal to 0.9 and 0.99. Figure 2 shows the dependence of area per molecule, A^{π}, of the C_{80}/DOPC monolayer mixture on the molar fraction C_{80}/DOPC at constant surface pressures of 2, 4, 8 and 16 mN/m. One can see the family of maximal values of area per molecule at C_{80}/DOPC molar fraction equal to 0.01.

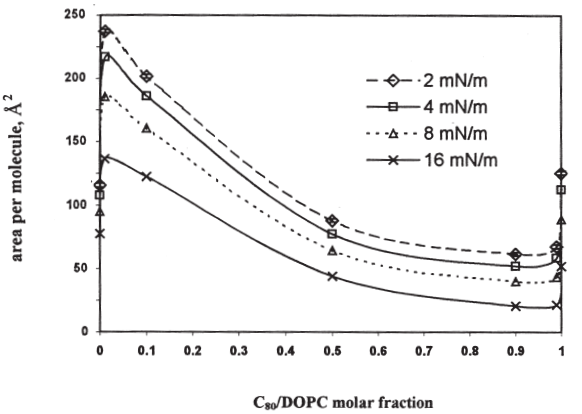

Figure 2. The dependence of area per molecule, A^{π}, of the C_{80}/DOPC monolayer mixture on the molar fraction of hexadecaprenol at constant surface pressures of 2, 4, 8 and 16 mN/m.

The family of minimal values corresponds to C_{80}/DOPC molar fraction equal to 0.9. On the basis of the results presented in Fig. 1, one

can calculate the specific molecular area, A_M, (Costin & Barnes, 1975; Gruszecki *et al.*, 1999a). The values of A_M are found by extrapolation of the linear parts of the isotherms to zero surface pressure (Fig 3A). The results are shown in Fig. 3B. For pure lipid monolayers the specific molecular area of a hexadecaprenol molecule is estimated to be 99 ± 5 Å^2 and the A_M value for a DOPC molecule is 100 ± 3 Å^2. The curve of A_M *versus* C_{80}/DOPC molar fraction reaches a minimum for the molar fraction equal to 0.5–0.6 (43 ± 3 Å^2). In addition, we can observe one maximum of 150 ± 3 Å^2 for the molar fraction equal to 0.01.

Figure 4 shows the limiting molecular areas, A_∞, as a function of C_{80}/DOPC molar fraction (Rolland *et al.,* 1996). The A_∞ values correspond to the areas occupied by the molecules at the surface pressure equal to 0.4 mN/m. The maximal A_∞ value equal to 276 Å^2 was obtained for the C_{80}/DOPC molar fraction 0.01, and the minimal A_∞ value equal to 83 Å^2 for molar fraction 0.99. From eqn. 1 we have calculated the excess free energy of mixing, ΔG^E_{mix}, for the surface pressure range 0 to 75 mN/m. The results are shown in Fig 5. The excess free energy of mixing has the minimal value equal to –4.5 ± 1 kJ/mole for C_{80}/DOPC molar fraction 0.9 and maximal value equal to 8.2 ± 1 kJ/mole for C_{80}/DOPC molar fraction 0.01. By definition, the excess free energy of mixing for pure lipid monolayers equals 0 kJ/mole. Figure 6A represents the values of collapse pressure, π_C, for pure C_{80}, pure DOPC and mixed C_{80}/DOPC monolayers. The collapse pressure of the lipid monolayers of various C_{80}/DOPC molar fractions vary from 49.9 ± 1 mN/m for pure DOPC monolayer to 26.5 ± 1 mN/m for pure C_{80} monolayer. The maximal value equal to 49.1 ± 1 mN/m for π_C was obtained for C_{80}/DOPC molar fraction of 0.01 and the minimal value equal to 15.3 ± 1 mN/m for C_{80}/DOPC molar fraction of 0.99. These results indicate a greater stability of DOPC in comparison with C_{80} monolayers. Figure 6B shows the collapse area A_C as a function of the C_{80}/DOPC molar fraction. The collapse area varies from 41.3 ± 5 Å^2 for a pure DOPC monolayer to 22.6 ± 5 Å^2 for a pure C_{80} monolayer. The maximal value equal to 63 ± 5 Å^2 of π_C was obtained for C_{80}/DOPC molar fraction of 0.1 and the minimal one equal to 10.2 ± 5 Å^2 for C_{80}/DOPC molar fraction of 0.5–0.6.

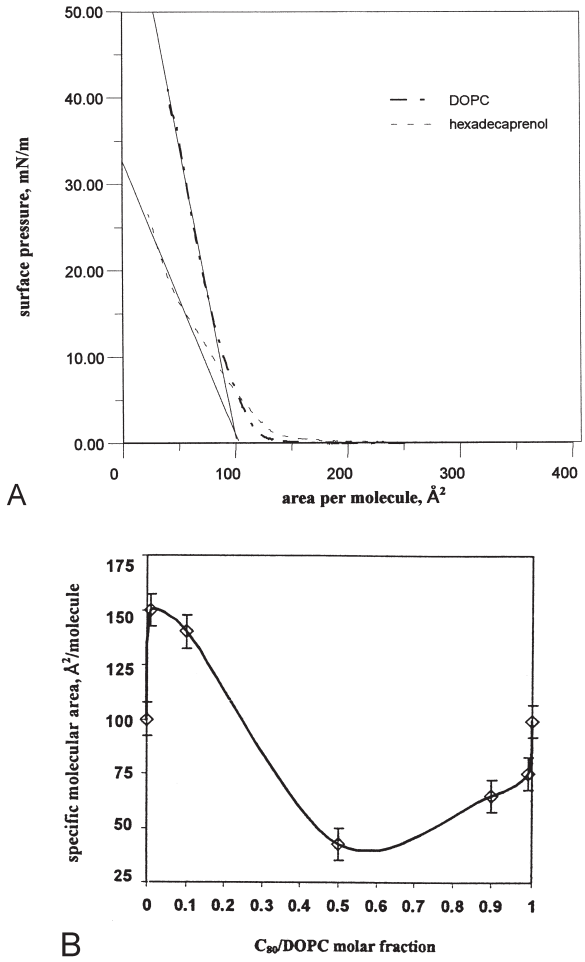

Figure 3A. Isotherms of compression of monomolecular layers of hexadecaprenol and DOPC at the air water interface.

Specific molecular areas A_M are found by extrapolation of the linear parts of the isotherms to zero surface pressure. The A_M values shown are averages of three experiments.

B. Specific molecular area, A_M, as a function of molar fractions C_{80}/DOPC [0–1].

The value of A_M was linearly extrapolated from the linear parts of the isotherms to zero surface pressure.

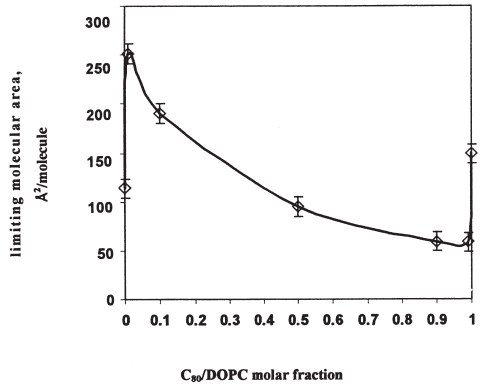

Figure 4. Limiting molecular area, A_∞, as a function of molar fraction C_{80}/DOPC [0–1].

The values of A_∞ were taken for surface pressure of 0.4 mN/m.

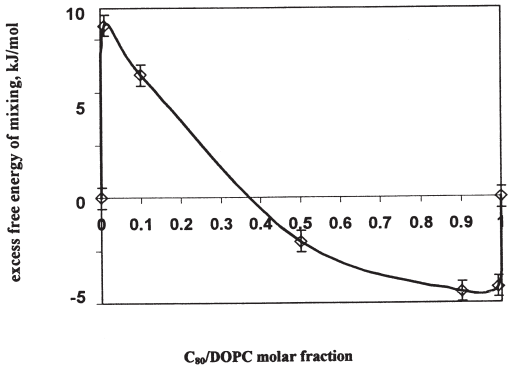

Figure 5. Excess free energy of mixing, ΔG^{E}_{mix}, as a function of molar fraction C_{80}/DOPC [0–1].

ΔG^{E}_{mix} was calculated for surface pressure of 75 mN/m. The values of ΔG^{E}_{mix} were calculated according to eqn. 1 (see Materials and Methods).

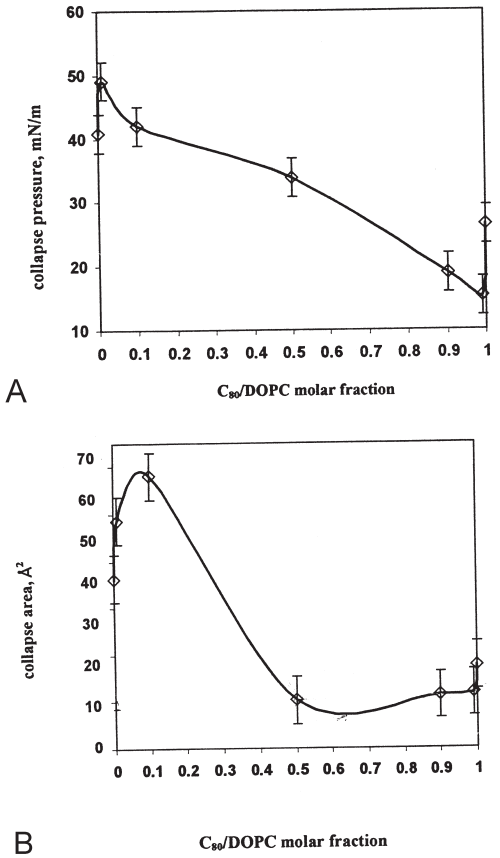

Figure 6. A. Collapse pressure, π_C, for pure C_{80}, pure DOPC and molar fraction of C_{80}/DOPC (0–1). B. Collapse area, A_C, for mixed lipid monolayers made from C_{80}/DOPC at various molar fractions [0–1].

Bilayer experiments

The behaviour of hexadecaprenol/DOPC membranes as a function of applied potential was studied by performing current-voltage experiments. As presented in Fig. 7A, the curves are symmetric and linear for values in the potential range −20 to +20 mV. The value of the slope increases with the increased percentage of hexadecaprenol in the membrane. The dependence of membrane specific conductance, G_S, on the percentage is shown on a semilogarithmic scale in Fig. 7B. The values of G_S increase with the increasing percentage of hexadecaprenol (C_{80}) in the membrane. The maximal rise, up to $G_{S\,max} = (6.8 \pm 1.1)\times10^{-7}$ S cm^{-2}, was observed for the C_{80}/DOPC mole ratio equal to 0.2. The value of membrane conductance obtained for DOPC bilayers equals $(4.8 \pm 0.9)\times10^{-8}$ S cm^{-2} and is in accordance with the value $(4 \pm 1)\times10^{-8}$ S cm^{-2} reported by Gamble *et al.* (1982). The normalized conductance of bilayer lipid membranes was measured as a function of temperature in the range of 25–42°C. Typical trends are reported in Fig. 8A. An increase of normalized conductance is observed with increasing temperature. The Arrhenius plots are linear, the slope of the curves depending on the percentage of C_{80} in the bilayer. The relationship between the value of activation energy of ion transport across the membrane, E_A, and the percentage of hexadecaprenol in macrovesicular bilayers is shown in Fig. 8B. The values of activation

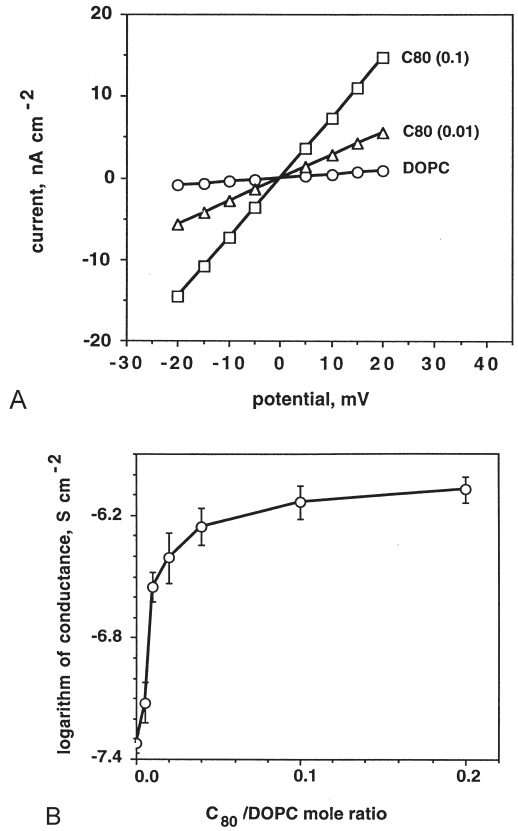

Figure 7A. Current-voltage, I/*V*, steady-state characteristics of bilayer lipid membranes made from: DOPC (•); C_{80}/DOPC mole ratio 0.01 (Δ); C_{80}/DOPC mole ratio 0.1 (•).

Experiments were performed at 25 ± 0.1°C.

B. Specific membrane conductance, *G*, *versus* C_{80}/DOPC mole ratio.

Values of membrane conductance were derived from the linear parts of I/*V* curves by least-squares fitting. Each point represents the mean value ± S.D. obtained for 6–8 different macrovesicular bilayer lipid membranes.

energies were derived from the Arrhenius plots by least squares fitting according to eqn. 4. For lower concentrations of hexadecaprenol, a pronounced decrease of E_A is observed. For higher concentrations of C_{80} in the membrane, the value of E_A increases slightly. The E_A value decreases from 48 ± 3 kJ/mole for DOPC bilayers to the value of minimal activation energy, $E_{A\,min}$, equal to 23 ± 2 kJ/mole for bilayers prepared from mixtures at C_{80}/DOPC mole ratio 0.1 and increases slightly for higher concentrations of hexadecaprenol. Smith *et al.* (1984) reported activation energy equal to 35 ± 2 kJ/mole for a lecithin bilayer. Ionic transference numbers, calculated according to eqn. 3, were nearly independent of the lipid composition of the membrane and the ratio (t_{Cl^-}/t_{Na^+}) of about 1.5 was obtained at 25°C. The dependence of the membrane permeability coefficient for chloride ions, P_{Cl^-}, on the percentage of

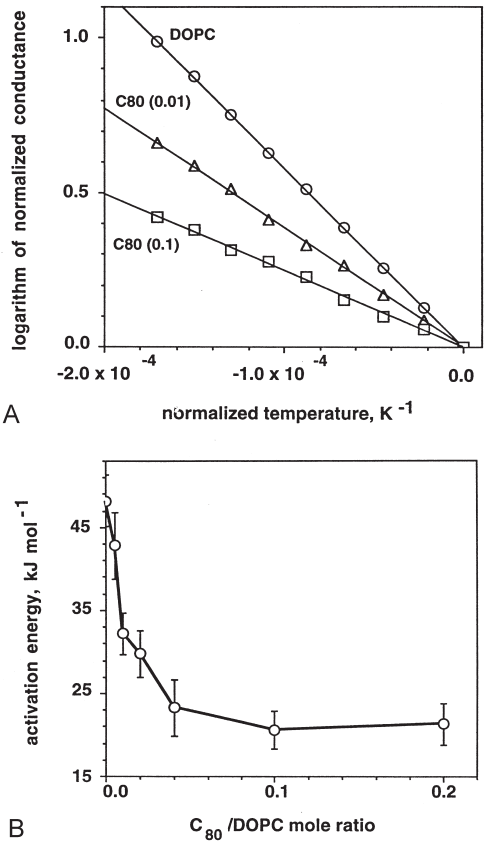

Figure 8A. Arrhenius plots of normalized conductance of macrovesicular bilayer lipid membranes.

Membranes were made from: DOPC (•); C_{80}/DOPC mole ratio 0.01 (Δ); C_{80}/DOPC mole ratio 0.1 (•). Logarithm of normalized conductance was calculated as $\log[(G/C)/(G_0/C_0)]$, where G and C represent membrane conductance and membrane capacitance, respectively, at 298 K. Normalized temperature was calculated as: (K^{-1})-$(298\ K)^{-1}$.

B. Activation energy, E_A, of ion migration across the membrane *vs* C_{80}/DOPC mole ratio.

Values of activation energies were derived from Arrhenius plots by least-squares fitting according to eqn. 4 (see Materials and Methods). Each point represents the mean value ± S.D. obtained for 6–8 different macrovesicular bilayer lipid membranes.

hexadecaprenol in the bilayer is shown on a semilogarithmic scale in Fig. 9. The value of P_{Cl^-}, calculated according to eqn. 2, increases with the increasing percentage of C_{80} in the bilayer. The value of P_{Cl^-} equal to $(4.2 \pm 0.7) \times 10^{-11}$ cm/s is obtained for a DOPC bilayer. The value of this coefficient for lipid bilayers modified by long chain polyprenols is higher than for DOPC bilayers and the maximal rise, about 30-fold, is observed for

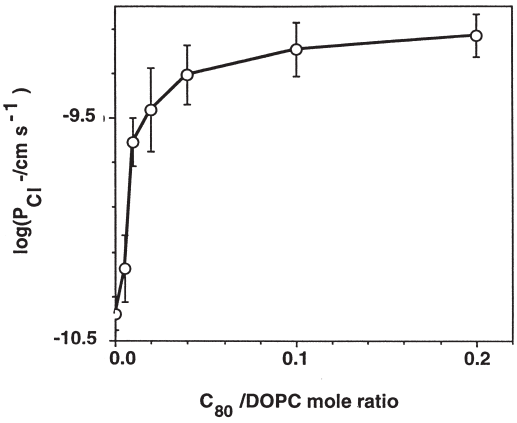

Figure 9. Membrane permeability coefficient for chloride ions, P_{Cl^-}, *vs* C_{80}/DOPC mole ratio.

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 P_{Cl^-} were calculated according to the eqn. 2, the values of t_{Cl^-} were calculated according to eqn. 3 (see Materials and Methods).

C_{80}/DOPC mole ratio equal to 0.2. For lower concentrations of the polyprenol in the membrane, the rate of increase of P_{Cl^-} is considerable. For higher concentrations of C_{80} in the membrane, a slight increase in the value of P_{Cl^-} is observed in comparison with DOPC bilayers.

DISCUSSION

Lipid membranes modified by hexadecaprenol (C_{80}) exhibit different electrical, transport and mixing properties from DOPC monolayers and bilayers. The behaviour of hexadecaprenol–lecithin in monolayers was studied by performing Langmuir monolayer technique experiments. Surface pressure–area isotherms were measured for pure C_{80}, DOPC films and their mixtures. The changes of specific molecular area, A_M, free energy of mixing, ΔG^E_{mix}, and area per molecule at constant surface pressure, A^{π}, show that hexadecaprenol can modify the structure of lipid aggregates. Similar conclusions were presented for xanthophyll–lecithin monolayers (Tomoaia-Cotişel *et al.,* 1987; N'soukpoé-Kossi *et al.,* 1988). Negelmann *et al.* (1997) noted that the limiting molecular area value represents the beginning of observable intermodular forces between adjacent molecules in the monolayer. The behaviour of mixed monolayers can be considered in terms of excess free energy of mixing ΔG^E_{mix}. This interesting phenomenon, represented in Fig. 5, indicates two opposite processes: van der Waals interactions between polyprenol chains, and hydrogen interactions between polyprenol hydroxyl group and the oxygen atom of a water molecule. In the case of a two-component monolayer, the hydrogen bond can be additionally formed between the hydroxyl group of hexadecaprenol and the phosphate group of a DOPC molecule. Our research indicates that for high molar fractions of polyprenols van der Waals forces predominate. This means that the DOPC molecules are monodispersed and surrounded by several C_{80} molecules each. One can notice that a negative deviation from the rule of additivity is usually interpreted as pointing at a mutual interaction in a two-component system, decreasing the area occupied by molecules in the mixture and lowering the excess of free energy in a monolayer. Positive deviation from the straight line in this case seems to reflect a phenomenon of molecules packed in a monolayer indicating that even small additions of C_{80} disturb the layer of DOPC. The increase of the area per molecule seems to be the cause of the existence (in the mixture) of the border of phases not occupied by molecules. As can be noticed in the isothermic isobars of Fig. 2, two ex-

tremes are shown. The curves for surface pressures 2, 4, 8 and 16 mN/m exhibit a maximum of mean area per molecule of the monolayer at molar fraction of C_{80} equal to 0.01. This indicates that van der Waals interactions between polyprenyl chains prevail over the attractive interactions between polyprenol and phosphatidylcholine molecules for low molar fractions of hexadecaprenol. It seems that polyprenol molecules form small aggregates in the "sea" of DOPC molecules. We observed that under appropriate conditions the area per molecule of mixed lipid films could increase. The changes depend on the molar fraction of C_{80} in the membrane indicating that C_{80} molecules modify the organisation of phospholipid monolayers. The minima in the area per molecule *vs* molar fraction plots corresponding to the molar fraction of C_{80} equal to 0.9 may be understood in terms of the appearance of the most packed state of the monolayer caused by the aggregation or association of hexadecaprenol molecules with their neighbours. Another interesting parameter characterising C_{80}–DOPC interactions is the miscibility of mixed monolayers (Gaines, 1966). When two components of monolayers are immiscible, mixed monolayers of various compositions will all collapse at the same surface pressure. If components are miscible, collapse pressure will vary with composition. The collapse pressure and collapse area represented in Fig. 4A and 4B show that components of the C_{80}/DOPC mixed monolayers are miscible.

The family of local minima from Fig. 2 corresponding to the molar fraction of C_{80} equal to about 0.9 may be understood as illustrating the most packed state of the monolayer and thus aggregation or association of its molecules. This aggregation corresponds to each molecule of DOPC having nine molecules of hexadecaprenol as its neighbours. This could lead to the conclusion that molecules of DOPC during mixing enforce packing of nine C_{80} molecules around one DOPC molecule and that the process lowers the area per molecule in the monolayer. Figure 10 presents a hypothetical model of the mixing behaviour of polyprenol and DOPC in the monolayers investigated. The polyprenyl chain is much more flexible than DOPC acyl hydrocarbon chains since it is mainly in *cis* configuration. Therefore the changes of specific molecular area of mixed C_{80}/DOPC monolayers can come mainly from changes of specific molecular area of polyprenyl molecules. The changes of A_M for hexadecaprenol show both the modulation of the A_M value of C_{80} by the surrounding molecules and the tendency to aggregation of C_{80} molecules at low molar fractions.

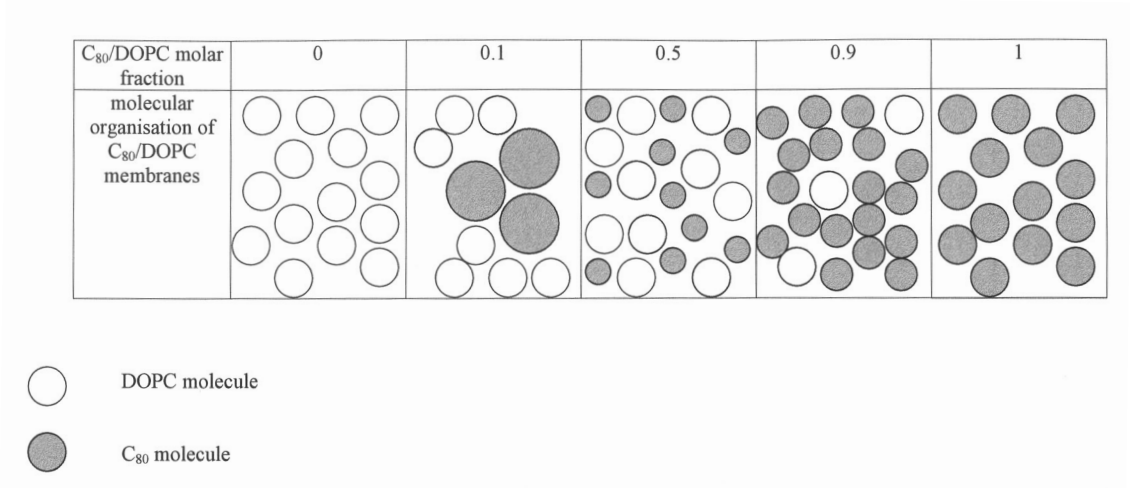

Figure 10. Schematic drawings of a model of the molecular organisation of polyprenol and DOPC in the monolayers investigated.

With respect to the electrical properties, the measurements showed that hexadecaprenol increases membrane specific conductance which is consistent with the data for dotriacontaprenol (C_{160}) (Janas *et al.,* 2000). Macrovesicular lipid bilayers prepared from hexadecaprenol/DOPC mixtures exhibited much higher permeability for ions in comparison with DOPC bilayers and the values of activation energy of ion transport were much lower than those for DOPC bilayers. The perfect linearity of current-voltage characteristics for hexadecaprenol-containing DOPC bilayers indicates that the increase in membrane permeability and decrease in activation energy are incompatible with the formation of carriers or selective channels by hexadecaprenol molecules. Aggregation of spin-labelled polyisoprenols in phospholipid membranes was observed even at relative concentrations not exceeding 0.005 (McCloskey & Troy, 1980). These aggregates can modulate the permeability and stability of polyisoprenol-phospholipid membranes. Our investigations show that hexadecaprenol substantially decreases the energy barrier for ion migration through membranes, giving rise to an increase of ionic conductance. Lai & Schutzbach (1984) showed that dolichol promoted membrane leakage in the absence of transmembrane potential in liposomes composed of phosphatidylethanolamine and phosphatidylcholine but not in liposomes composed of phosphatidylcholine only. A strong destabilisation of phosphatidylethanolamine bilayers, but not the phosphatidylcholine ones, in the presence of α-saturated polyprenol (dolichol) was detected by Valterson *et al.* (1985) on studying the phase transition of the bilayers. These experiments were also performed in the absence of transmembrane potential. In contrast to their observation, we report an increase of ion permeability in the presence of transmembrane potential, observed for C_{80}/DOPC bilayers containing no phosphatidylethanolamine.

The activation energy of ion transport was found to be essentially independent of temperature. This indicates that the influence of temperature on the aggregation behaviour of C_{80} and DOPC molecules in the membrane is negligible, which is consistent with the observation that the temperature dependence of clustering of polyisoprenol molecules in model membranes is minimal (McCloskey & Troy, 1980). α-Saturated polyprenols were previously found to increase the motional freedom of bilayer lipid membranes (de Ropp & Troy, 1984; 1985; Vigo *et al.,* 1984; Knudsen & Troy, 1989) and plasma membranes (Wood *et al.,* 1986; 1989; Schroeder *et al.,* 1987). McCloskey & Troy (1980) have demonstrated the existence of polyisoprenol clustering in phospholipid bilayers. We suggest the existence in C_{80}/DOPC bilayers of hexadecaprenol-rich microdomains, which can form transmembrane pores. The microdomain may be stabilized by hydrogen bonds between hydroxyl group of C_{80} and the ester oxygen of DOPC. These microdomains can modulate the permeability and stability of hexadecaprenol–phospholipid membranes. As analysed in the paper of Smith *et al.* (1984) the decrease in the activation energy of ion migration across lipid bilayers is related to the increase in the radius of the transmembrane pore. For the value of activation energy of 18 kJ/mole, the authors estimated the minimum pore radius to be about 1 nm. For C_{80}/DOPC bilayers, with the activation energy for ion transport about 2-fold lower, the minimal pore radius can be estimated to be around 2 nm. The action of C_{80} seems therefore to affect the formation of these pores and to increase their size. The function of polyprenyl microdomains in biological membranes may also depend on their ability to induce local changes in membrane thickness and membrane fluidity corresponding to the hydrophobic thickness and environment requirements of an integral membrane protein located in such domain, in accordance with the mattress model (Mouri-

tsen & Bloom, 1984) of lipid–protein interactions in membranes.

In conclusion, the results of monolayer and bilayer investigations show that some electrical, transport and packing properties of lipid membranes change under the influence of hexadecaprenol. The results indicate that: hexadecaprenol modulates the molecular organisation of the membrane; the specific molecular area of polyprenol molecules depends on the relative concentration of polyprenols in membranes; hexadecaprenol can modify lipid membranes by the formation of fluid microdomains. The results also indicate that electrical transmembrane potential can accelerate the formation of pores in lipid bilayers modified by long chain polyprenols.

The authors would like to thank Prof. Tadeusz Chojnacki and Dr. Ewa Świeżewska from the Department of Lipid Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, for stimulating discussions.

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