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THE ROLE OF POLYPRENOL IN MODULATION OF PHYSICAL PROPERTIES OF MODEL MEMBRANES

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The influence of hexadecaprenol on molecular organisation and transport properties of model lipid membranes (monolayers and bilayers) was investigated. Current-voltage characteristics were measured. Dependence of area per molecule at constant surface pressures, deviations from the rule of additivity, membrane permeability coefficient for sodium ions was determined. The data show that the presence of polyprenol in lipid membranes modulates their molecular organisation and transport properties.

INTRODUCTION

This paper presents the investigations of monolayer and bilayer lipid membranes modified by a long chain polyprenol. Polyisoprenols (poly-*cis*-prenols) are natural products, derivatives of the C5 isoprene unit. The molecule of hexadecaprenol (C_{80}) 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. The occurrence of α-unsaturated polyisoprenols (polyprenols) (Świeżewska, Sasak, Mańkowski, Jankowski, Vogtmann, Krajewska, Hertel, Skoczylas & Chojnacki, 1994; Wanke, Chojnacki & Świeżewska, 1998) and α-saturated polyisoprenols (dolichols) (Jankowski, Świeżewska, Sasak & Chojnacki, 1994; Chojnacki, Świeżewska & Vogtmann, 1987) in membrane fractions has been reported. Phosphopolyisoprenols function as hydrophobic carriers of glycosyl residues across membranes in glycosylation reactions (Bugg & Brandish, 1994). Monolayer techniques have been applied to the study of the interactions between DOPC and other lipids (Gaines,, 1966; Gruszecki, Sujak, Strzałka, Radunz & Schmid, 1999a) and this technique can be used to investigate the molecular interactions in mixed monolayers. The effect of polyprenol on the permeability of a model membrane system – bilayer lipid membranes (BLMs) is reported. The results are discussed in light of the aggregation behaviour and the intramolecular clustering of a polyprenol molecule. The aim of the present work was to study the influence of polyprenol molecules on the organisation of phospholipid molecules in lipid monolayers and bilayers.

MATERIALS AND METHODS

Chemicals

DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) was purchased from Sigma. Hexadecaprenol (C_{80}) was isolated from leaves of *Picea abies* (Chojnacki, Jankowski, Mankowski & Sasak, 1975) and purified. It gave a single spot on Silica Gel G TLC plates (Merck) in ethyl acetate/toluene (5:95 v/v) and on RP-18 HP TLC plates (Merck) in acetone.

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

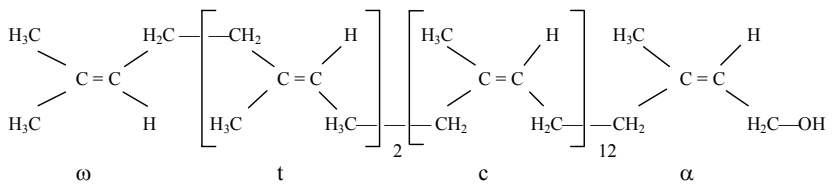

Fig. 1. Structure of hexadecaprenol; ω, an isoprene residue furthest from the hydroxyl group, t a trans-isoprene residue, c a cis-isoprene residue, α an "OH-terminal" isoprene residue.

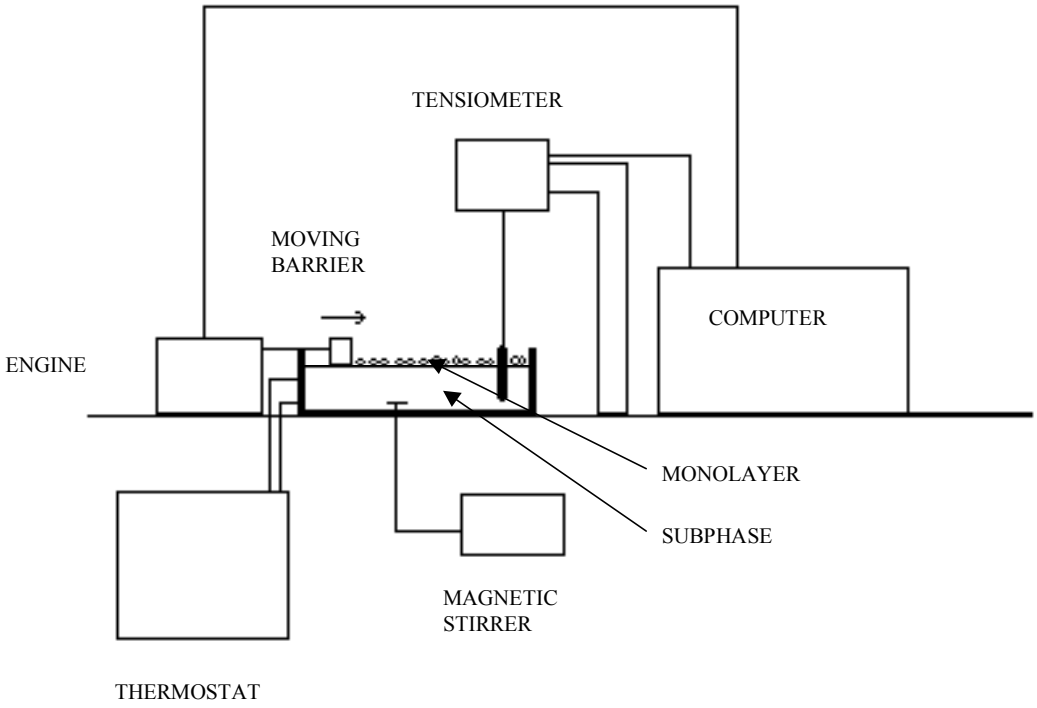

Fig. 2. The experimental set-up used for investigations of lipid monolayers.

Wilhelmy method (Gruszecki, Sujak, Strzałka, Radunz & Schmid, 1999a; Gruszecki, Grudziński, Banaszek-Glos, Matula, Kernen, Krupa & Sielewiesiuk, 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 minutes 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. Dionised 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 studies of lipid monolayers we measured the difference in surface tension between a clean or pure liquid surface one covered with a film. The surface pressure, Π, is generally considered to be equal to the reduction of the pure liquid-surface tension by the film, i.e.,

$$\Pi = \gamma_0 - \gamma \qquad (1)$$

where γ_0 is the surface tension of the pure liquid and γ is the tension of the film-covered surface. An entirely analogous expression is used for the surface pressure at a liquid-liquid interfacial tension with the film present. For a system, which is in either absolutely stable or metastable equilibrium, it is easy to show that equation 1 applies to the force measured by a Langmuir film balance (Gaines, 1966).

Bilayer formation and electrical measurements.

Bilayer lipid were formed according to the technique described previously (Janas, Kuczera, Chojnacki & Krajewska-Rychlik, 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

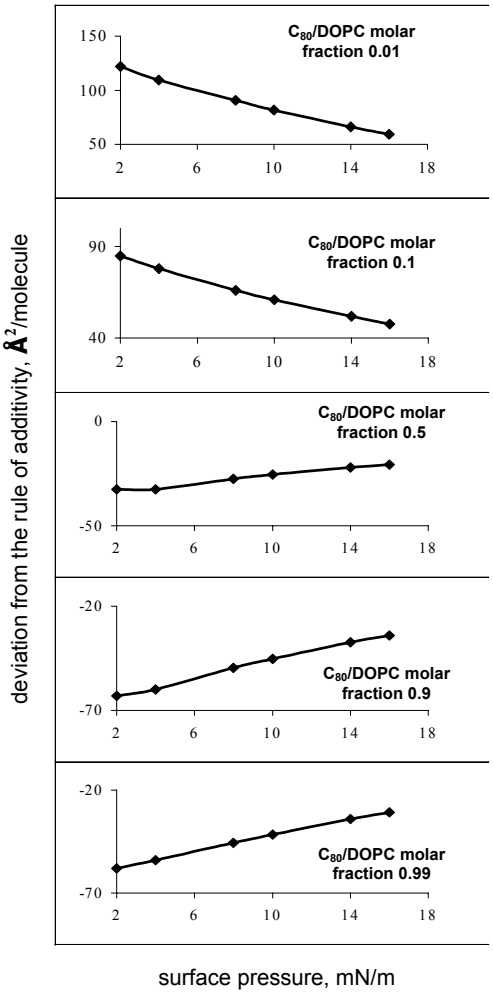

Fig. 4. Deviations from the rule of additivity of mixed monolayers at indicated molar fractions shown as a function of surface pressure.

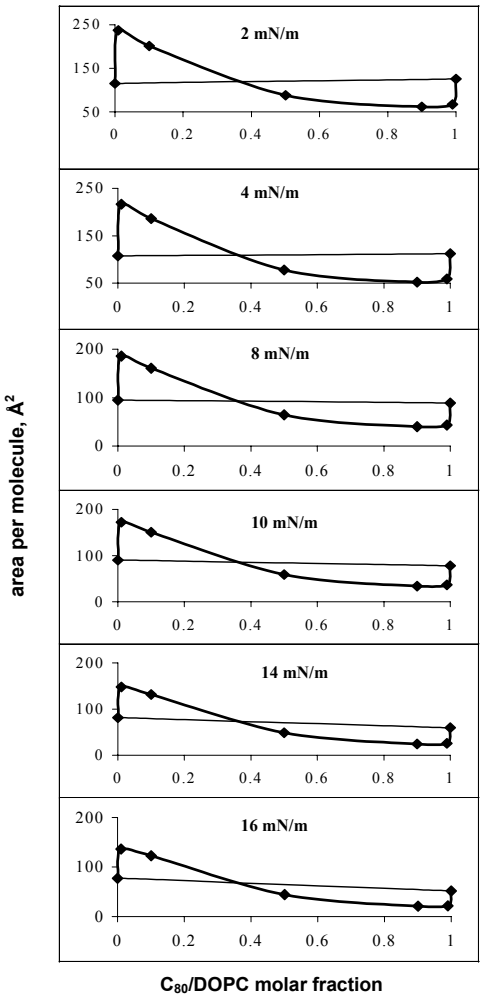

Fig. 3. The dependence of area per molecule, A^{π}, of the C_{80}/DOPC monolayer mixture on the molar fraction of ODA/DOPC at constant indicated surface pressures.

conductance of the membrane, G, was calculated from current-voltage characteristics.

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

$$P_{Na+} = (E_{Na+} \times t_{Na+} \times G)/F(c_2 - c_1) \qquad (2)$$

where c_1 and c_2 are the concentrations of NaCl inside and outside the spherical bilayer, respectively; F is the Faraday constant; E_{Na+} is the equilibrium potential for Na^+ 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, Robello, Usai & Marchetti, 1982; Janas, Walińska, Chojnacki, Świeżewska & Janas, 2000a; Janas, Nowotarski, Gruszecki & Janas, 2000b):

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

which correlates the potential difference, $\Delta V_{m.}$, 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. Electrical conductance, G, was calculated

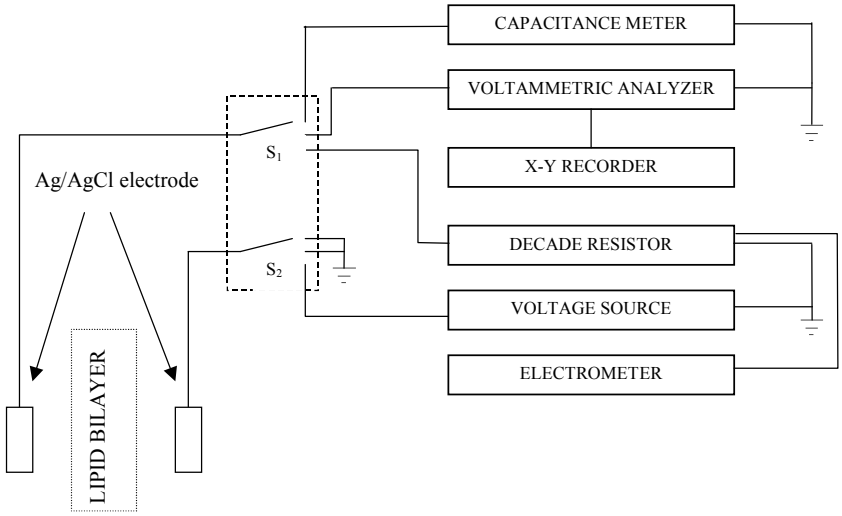

Fig. 5. Experimental set-up used for electrical measurements of bilayer lipid membranes.

from current-voltage characteristics (the precision of voltage and current measurements: 0.1 mV and 0.01 nA, respectively).

Results and discussion

The Langmuir monolayer technique was used to investigate the interaction of hexadecaprenol with lipid films. Fig. 3 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, 10, 14 and 16 mN/m. One can see the family of maximal values of A^{π} in the range (136÷237) Å^2 for C_{80}/DOPC molar fraction equal to 0.01. The family of minimal values in the range (20÷61) Å^2 corresponds to C_{80}/DOPC molar fraction equal to 0.9. The isotherms of pure C_{80} and pure DOPC monolayers make it possible to evaluate the mean area per molecule in the mixed monolayer at a different surface pressure by assuming that the molecules of both components occupy the same area in the mixed monolayer as in a one component system (N'soukpoé-Kossi, Sielewiesiuk, Leblanc, Bone & Landrum, 1988; Gruszecki, Zelent, Heidar-Ali, Tajmir-Riahi Wang, Taleb, Veeranjaneyulu & Leblanc, 2000):

$$A_{\pi 12} = x_1 A_{\pi 1} + x_2 A_{\pi 2} \qquad (3)$$

where $A_{\pi 12}$ is the average molecular area at a surface pressure π in the monolayer of the two components with molar fractions x_1 and x_2; $A_{\pi 1}$ and $A_{\pi 2}$ are the molecular areas in the single component monolayers at the same surface pressure π. The subscripts 1 and 2 correspond to C_{80} and DOPC, respectively. Eq. 3 (known as the additivity rule) has often been used to analyse some molecular interactions. The deviations (both negative and positive) from the additivity rule for monolayers of molar fraction C_{80}/DOPC equal to 0.01, 0.1, 0.9 and 0.99 are presented in Fig. 4. One can see the family of maximal positive values of these deviations for the molar fractions equal to 0.01 and 0.1 (for surface pressure equal to 2 mN/m). The family of minimal negative values corresponds to the molar fraction equal to 0.5, 0.9 and 0.99 (for surface pressure equal to 2 mN/m). The changes in isothermic isobars and deviations from the rule of additivity shows that hexadecaprenol can modify molecular organisation of lipid monolayer. Similar conclusions were presented for xanthophyll – lecithin monolayers (Gruszecki *et al.*, 1999a, b). As it can be noticed in Fig. 3, there are two extremes of the isothermic isobars. The increase of the area per molecule seems to be the cause of the existence (in the mixture) of the areas at the border of phases not occupied by molecules. The negative deviation in the two component system, which indicates the decrease of the area occupied by molecules in the mixture, can be seen in Fig. 4. Negative deviation from the rule of additivity can be explained as due to formation of the complexes in which the molecules occupy a smaller area than in the monolayer without any interaction (N'soukpoé-Kossi *et al.*, 1988; Gruszecki *et al.*, 2000).

The behaviour of hexadecaprenol/DOPC membranes as a function of applied potential was studied by performing current-voltage experiments. As presented in Fig. 6, the curves are symmetric and linear for values in the potential range –20 to +20 mV. The value of the slope increases with the

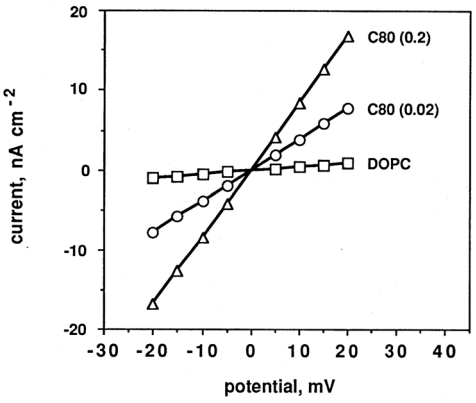

Fig. 6. Current-voltage, I/V, steady-state characteristics of bilayer lipid membranes made from DOPC (□); C_{80}/DOPC mole ratio 0.02 (○); C_{80}/DOPC mole ratio 0.2 (Δ). Experiments were performed at 25±0.1°C. Each point represents the mean value ±S.D. obtained for 6-8 different macrovesicular bilayer lipid membranes.

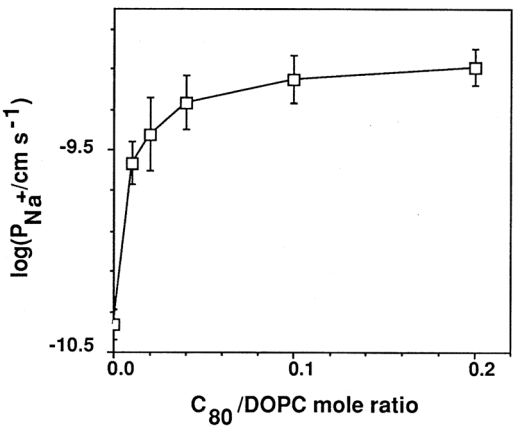

Fig. 7. Membrane permeability coefficient for sodium ions, P_{Na+}, vs. C_{80}/DOPC mole ratio. Each point represents the mean value (±S.D.) obtained from six to eight different macrovesicular bilayer lipid membranes. Experiments were performed at 25±1°C.

increased percentage of hexadecaprenol in the membrane. The dependence of P_{Na+} ions on the percentage of hexadecaprenol in the bilayer is shown on a semilogaritmic scale in Fig. 7. The value of P_{Na+}, calculated according to equation (2), increases with the increasing percentage of C_{80} in the bilayer. The value of P_{Na+} equal to $(4.2\pm0.7)\times10^{-11}$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 C_{80}/DOPC mole ratio equal to 0.2. For lower concentrations of the polyprenol in the membrane, the rate of increase of P_{Na+} is considerable. For higher concentrations of C_{80} in the membrane, a slight increase in the value of P_{Na+} is observed in comparison with DOPC bilayers. Bilayer lipid membranes prepared from C_{80}/DOPC mixtures exhibited much higher permeability for sodium ions in comparison with DOPC bilayers. The perfect linearity of current-voltage characteristics for hexadecaprenol containing DOPC bilayers indicates that the increase in membrane permeability is incompatible with the formation of carriers by hexadecaprenol molecules. It was demonstrated (Lai & Schutzbach, 1984) 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. 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. Valterson, van Duyn, Verkleij, Chojnacki, de Kruiff and Dallner (1985) demonstrated, that in the absence of transmembrane potential, α-saturated polyprenol (dolichol). destabilise phosphatidylethanolamine bilayers but not the phosphatidylcholine ones. We report an increase of sodium ions permeability in the presence of transmembrane potential, which was observed for C_{80}/DOPC bilayers containing no phosphatidylethanolamine. The permeability of liposomes to water, glucose, Ca^{2+} and alkaline cations was monitored (Boscoboinik, Feliz, Disalvo & Belocopitow, 1985) using a rapid reaction stopped-flow spectrophotometer. Net permeability of phosphatidylcholine bilayers to alkaline cations was induced by the incorporation of dolichol. This effect was not observed in the case of non-charged solutes like glucose or in that of alkaline earth cations such as calcium. Permeation of K^+ was significantly increased above the phase transition temperature. It was demonstrated (McCloskey & Troy, 1980) that polyisopenols form clusters in phospholipid bilayers. We suggest the existence in hexadecaprenol/phosphatidylcholine bilayers of polyprenol microdomains, which can form transmembrane pores. The hydrogen bonds between hydroxyl group of hexadecaprenol and the ester oxygen of phosphatidylcholine can stabilise these microdomains. These microdomains can modulate the permeability of hexadecaprenol-phospholipid membranes.

In conclusion, the results of model lipid membranes investigations show that electrical and packing properties change under the influence of hexadecaprenol. The results indicate that transmembrane potential can facilitate the formation of pores in polyprenol-phosphatidylcholine bilayers. The results also indicate that polyprenyl molecules can modulate the molecular organisation of lipid bilayers and can modify lipid membranes by the formation of fluid microdomains.

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