Interaction between Model Membranes and a New Class of Surfactants with Antioxidant Function

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ABSTRACT The effect of two series of amphiphilic quaternary ammonium salts on some properties of phospholipid membranes was studied. The compounds of one series, *N*-benzyl-*N,N*-dimethyl-*N*-alkyl ammonium bromides, exert a destructive effect on membranes and are treated as reference compounds. The compounds of the other series, *N*-(3,5-di-*t*-butyl-4-hydroxy)benzyl-*N,N*-dimethyl-*N*-alkyl ammonium bromides, are derivatives of the former ones, exhibit antioxidant properties, and do only relatively slight damage to the membranes. The aim of the work was to explain the difference in molecular interaction with membranes between the two kinds of hydrophobic compounds. Thermodynamic methods, a new mixing technique, and monolayer and quantum calculation methods were used. It has been shown that the antioxidant molecules are less hydrophobic than those of the reference compounds and disturb the membrane organization to a lesser extent. On the basis of monolayer data, we suggest that the studied antioxidant behaves like a substitutional impurity, whereas the reference behaves like an interstitial one.

INTRODUCTION

In our earlier studies (Gabrielska et al., 1993a,b; Witek et al., 1994) we described a new type (Witek et al., 1995) of amphiphilic compound (DA-n) characterized by the following properties: 1) They are membrane-active, which means that they interact with membranes (in this case, because they are able to incorporate into biological and model membranes due to their hydrophobic alkyl chains). 2) They act as antioxidants thanks to their ability to scavenge free radicals, thereby protecting membrane components (particularly membrane lipids) against oxidation. 3) They are possibly nondestructive or only slightly destructive (in a certain concentration range) to membranes. The antioxidant compounds DA-n are amphiphilic quaternary ammonium salts, being functionalized permeatoxins with hindered phenolic substituent and being derivatives of a group of unsubstituted benzylammonium analogs BB-n (known as germicidal, fungicidal, and algicidal agents, and pronouncedly membrane-perturbing factors; Gabrielska et al., 1979; Isomaa, 1979; Kuczera et al., 1985; Ancelin and Vial, 1986; Gallova et al., 1990; Lindstedt et al., 1990; Przestalski et al., 1991; Devinsky et al., 1991; Sarapuk et al., 1992; Balgavy and Devinsky, 1994), which are used as the reference compounds. We studied (Gabrielska et al., 1993a; Witek et al., 1994, 1995) the interactions of DA-n and BB-n (n = 2, 3, 4, 5, 6, 9, 12, 14, and 16) with planar (BLM), liposome, and erythrocyte membranes using electrical, nuclear magnetic resonance, ESR, isotopic, and hemolytic methods. It was found that the stability of lipid and erythrocyte membranes decreases with increasing concentration of both compounds in the bath solution and with the number of carbon atoms in their alkyl chains. But in all experiments performed we noticed that the substituted benzylammonium salts have lower efficiency for membrane modification than those unsubstituted.

The comparison of both series of compounds shows that they are membrane active, but the activities differ significantly. The aim of this paper is to attempt to explain the difference on the molecular level in interactions between the related compounds and phospholipid membranes.

MATERIALS AND METHODS

The new type of amphiphilic quaternary ammonium salts, which are functionalized permeatoxins with a hindered phenolic substituent as a free radical scavenger, have been synthesized in our laboratory (Witek et al., 1995). The general formula of these compounds (DA-n) is presented in Fig. 1 *a*. A series of unsubstituted benzylammonium analogs (BB-n), treated as a reference, is shown in Fig. 1 *b*.

The transition of surfactant molecules (DA-n and BB-n) from the surface phase to the aqueous phase was studied by following the time course (relaxation time) of surface pressure after putting a certain amount of the surfactant on the water surface. The surfactant was placed on the water surface in the form of a chloroform-methanol solution of 8:2 volume ratio, containing 4×10^{17} surfactant molecules in 100 μl of solution. That amount of solution was spread on the surface of water contained in a Teflon vessel 80 mm in diameter, equipped with a Wilhelmy plate, an electrobalance, and an A/D transducer (model PCL 812 PG; Advantech Co.). The analog signal of the electrobalance was converted to a digital one and registered by data acquisition software PCLS-702 (Advantech Co.) every 2 s on Lotus 1-2-3 worksheets, where the data were recalculated to surface pressure-vs.-time plots. The accuracy of surface tension measurements was better than 1 mN/m. In our opinion it is a new approach to the determination of the hydrophobicity of amphiphilic compounds. The idea is based on the assumption that the rate of mixing of surface amphiphiles

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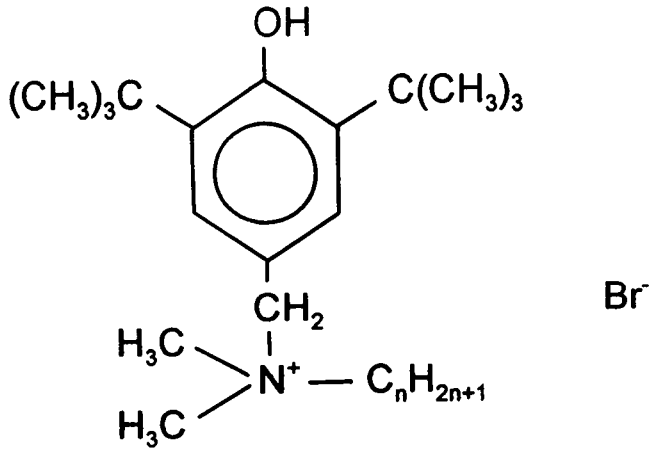

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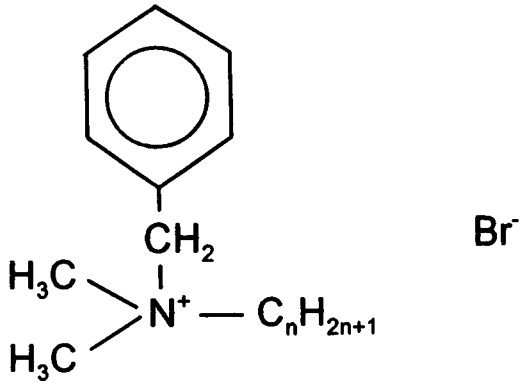

B

FIGURE 1 General formulae of the antioxidant compounds DA-n (*a*) and their analogs BB-n (*b*).

with a bulk water subphase depends on hydrophobicity (the rate of mixing increases with declining hydrophobicity).

Surface pressure vs. mean monolayer area isotherms were measured for monolayers of pure lecithin, pure antioxidant (or reference compound), or a mixture of both. The isotherms were taken using a rectangular trough with effective dimensions of 280 mm × 82 mm × 10 mm, equipped with the same Wilhelmy plate measuring setup as in previous studies (of transition of surfactants into the water subphase). Doubly distilled water was used as the subphase. The monolayer was allowed to stabilize for 20 min before compression. In all monolayer experiments the initial molecular area was 1.0 nm^2/molecule, and the rate of compression was 0.108 nm^2/molecule per min (3.60×10^{-5} m^2/s). The experiments were performed at room temperature. The data were sampled every 1–2 s and were stored by the data acquisition software.

The enthalpies of dilution were measured with a flow microcalorimeter LKB 2107 and a Thermometric TAM (flow mixing cell). Injection of the reactants into the calorimeter was done by means of a Gilson peristaltic pump (Minipuls 2), and the flows were determined by weight. Densities of the solutions were measured with a vibrating tube densimeter (Paar DMA 602) controlled by computer and calibrated with water and air. The temperature was kept constant (± 0.002°C) with a Heto proportional temperature controller. The osmotic measurements were made by means of a vapor pressure osmometer (Osmomat 070-SA; Gonotec) in which the molal decrease of vapor pressure of the used solvent was measured indirectly.

Quantum chemical calculations for the systems under consideration have been performed with the MO LCAO method, starting from their optimized geometries. In all cases the molecular geometries were optimized by using the PM3 all-valence approach (Stewart, 1989). The Gamess package of computer programs (Schmidt et al., 1993) has been used in the ab initio calculation of the electronic structure for standard choice of the coordinate origin.

RESULTS AND DISCUSSION

Relaxation experiments

First of all, we decided to compare the possible differences in hydrophobicity of both classes of compounds DA-*n* and BB-*n*. Therefore we performed relaxation experiments. Pure surface phases made of DA-12 and BB-12 compounds were studied by continuous-time surface pressure detection. The results of the experiment are shown in Fig. 2.

The plots show the relaxation (due to hydrophobic mismatch) of surface phases made of DA-12 and BB-12 in time. For the relaxation time range greater than 100 s, surface pressure of BB-12 decreases much slower than that of DA-12 in identical physical conditions (the same surface area for the same number of molecules and the same subphase and temperature). Although the surface area and the number of molecules of both compounds are the same, the number of layers they form in the surface phase are different. In Fig. 3 apparent molar volumes of DA-12 and BB-12 are shown as a function of molarity. The apparent molar volumes were calculated from the following formula (Różycka-Roszak and Fisicaro, 1993):

$$V_\phi = \frac{M}{d} - \frac{(d - d_o)}{m d d_o}, \quad (1)$$

where M is the molecular weight of the studied compound, m is molarity, and d and d_o are densities of the surfactant solution and pure water, respectively.

The intercept of the plot of the apparent molar volumes gives the partial molar volume at infinite dilution. The obtained values are 450 cm^3 mol^{-1} (0.75 nm^3/molecule) for DA-12 and 330 cm^3 mol^{-1} (0.55 nm^3/molecule) for BB-12. These values were used as a measure of the volumes of the studied compounds. As a consequence, the number of layers of the surface phase in the case of DA-12 is greater than that in the case of BB-12. Therefore, for relaxation times shorter than 100 s, DA-12 decreases the surface tension more than BB-12, and the surface pressure is greater for DA-12 than for BB-12. However, after this short relaxation time, the solubility of DA-12 in the bulk water phase becomes higher than the solubility of BB-12. It indicates, in our opinion, that the compound DA-12 is more hydrophilic than the compound BB-12.

Thermodynamic experiments

To verify the above conclusion, we used a thermodynamic method. For this we compared partial molar quantities of both kinds of compounds.

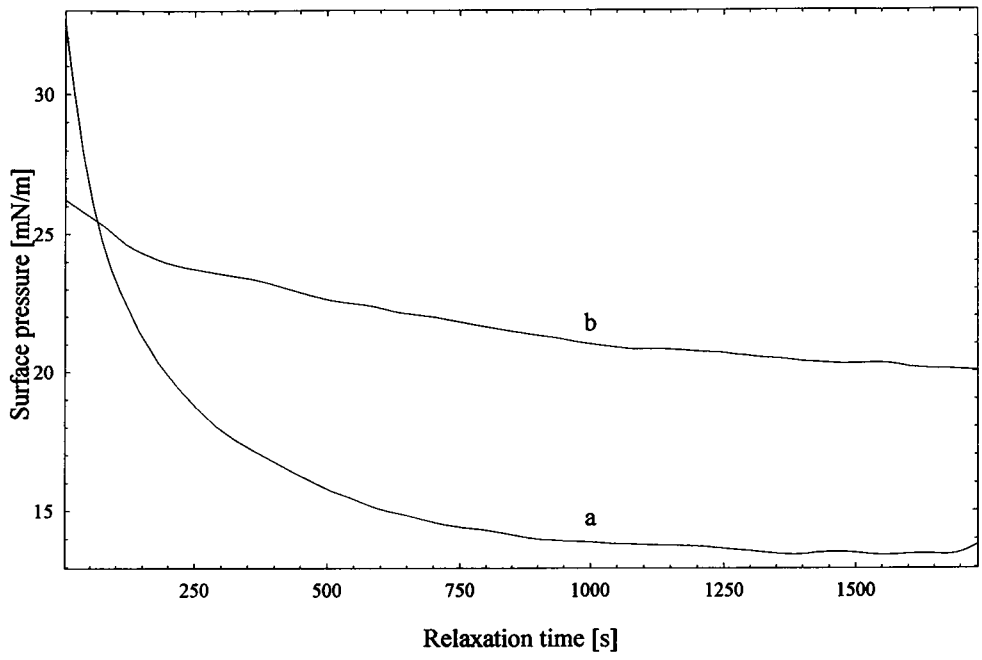

FIGURE 2 Comparison of surface pressure relaxation for DA-12 (*a*) and BB-12 (*b*). The transition of surfactant molecules (DA-n and BB-n) from the surface phase to the aqueous phase was studied by following the time course (relaxation time) of surface pressure after putting a certain amount of the surfactant on the water surface. The surfactant was placed on the water surface in the form of a chloroform-methanol solution of 8:2 (v/v), containing 4×10^{17} surfactant molecules in 100 μl of solution. That amount of solution was spread on the surface of water contained in a Teflon vessel 80 mm in diameter and equipped with a Wilhelmy plate, an electrobalance, and an A/D transducer (model PCL 812 PG; Advantech Co.). The analog signal of electrobalance was converted to a digital one and registered by data acquisition software PCLS-702 (Advantech Co.) every 2 s on Lotus 123 worksheets, where the data were recalculated to surface pressure versus time plots. The accuracy of surface tension measurements was better than 1 mN/m. In our opinion the this is a new approach to the determination of the hydrophobicity of amphiphilic compounds. The idea is based on the assumption that the rate of mixing of surface amphiphiles with the bulk water subphase depends on hydrophobicity (the rate of mixing increases with declining hydrophobicity).

Assuming infinite dilution as the reference state, the integral molar enthalpies of dilution ΔH_d are given by

$$\Delta H_d = \phi_{L,f} - \phi_{L,i}, \tag{2}$$

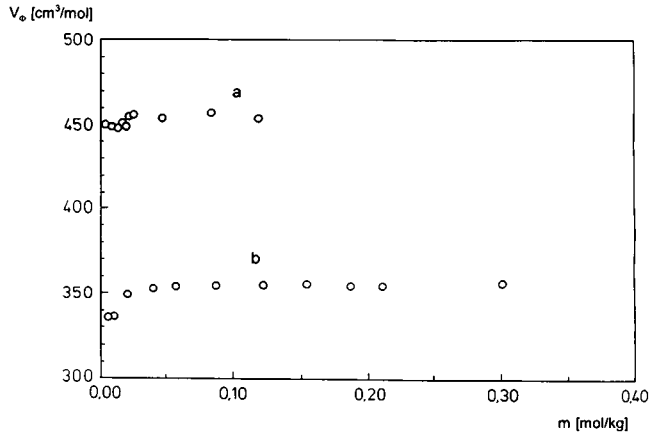

FIGURE 3 Molar volumes V_ϕ of DA-12 (*a*) and BB-12 (*b*) as a function of concentration (m). Apparent molar volumes were obtained from density measurements. Densities of the solutions were measured with a vibrating tube densimeter (Paar DMA 602) controlled by computer and calibrated with water and air. The temperature was kept constant (±0.002°C) by a HETO proportional temperature controller.

where $\phi_{L,f}$ and $\phi_{L,i}$ are the apparent molar relative enthalpies for the final and initial concentrations, relatively.

For an ionic surfactant in the premicellar region the relative apparent molar enthalpy can be expressed (De Lisi et al., 1979, 1990; Desnoyers and Perron, 1987; Fisicaro et al., 1990; Różycka-Roszak and Fisicaro, 1992) by means of a polynomial of $m^{1/2}$ (m, concentration). Stopping the serial expression at the third term, we obtain

$$\phi_L = Am^{1/2} + Bm + Cm^{3/2}, \tag{3}$$

where ϕ_L is the apparent molar relative enthalpy, and A ($= 2390$ J mol$^{-1/2}$ kg$^{1/2}$ at 40°C) (De Lisi et al., 1979) is the limiting Debye-Hückel slope for relative enthalpies accounting for long range solute-solute interactions. Parameters B and C are averaged on the experimental points in the premicellar region by least-squares curve fitting:

$$\Delta H_d - A(m_f^{1/2} - m_i^{1/2}) = B(m_f - m_i) + C(m_f^{3/2} - m_i^{3/2}), \tag{4}$$

where m_f and m_i are final and initial concentrations, respectively.

Experiments and calculations performed on this basis lead to the results shown in Fig. 4.

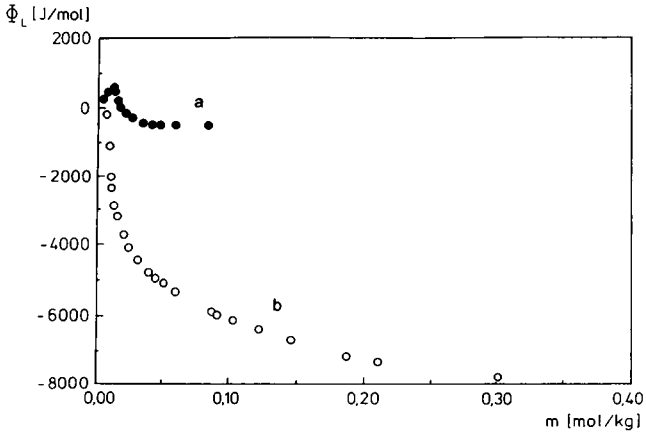

FIGURE 4 Apparent molar relative enthalpies ϕ_L of DA-12 (*a*) and BB-12 (*b*) as a function of concentration (*m*). Apparent molar relative enthalpies were obtained from enthalpies of dilution. The enthalpies of dilution were measured with a flow microcalorimeter LKB 2107 and a Thermometric TAM (flow mixing cell). Injection of the reactants into the calorimeter was done by means of a Gilson peristaltic pump (Minipuls 2), and the flows were determined by weight.

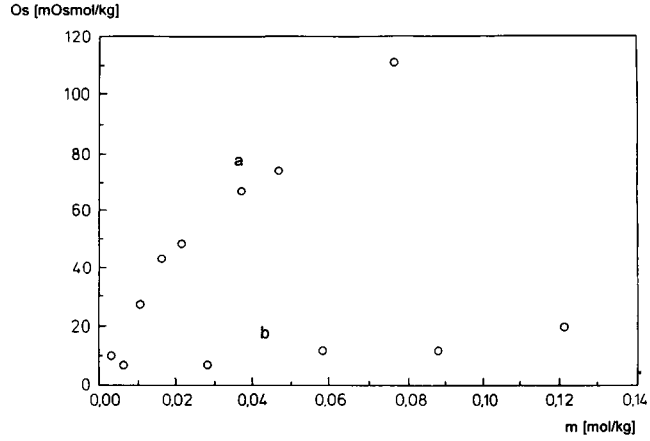

FIGURE 5 Osmolarity (Os) versus concentration (*m*) for DA-12 (*a*) and BB-12 (*b*). The osmotic measurements were made by means of a vapor pressure osmometer (Osmomat 070-SA; Gonotec), in which the molal decrease of vapor pressure of the used solvent was measured indirectly.

Fig. 4 shows that in the region of higher concentration the values of ϕ_L for BB-12 are lower than in the case of DA-12. The decrease is typical of the increase in hydrophobicity of a compound (De Lisi et al., 1990; Fisicaro et al., 1990; Różycka-Roszak and Fisicaro, 1993). So the much higher value of the apparent molar relative enthalpy of DA-12 than that of BB-12 indicates that the introduction of one hydroxy group into the benzene ring reduces its hydrophobicity.

A similar conclusion can be drawn from osmolarity measurements presented in Fig. 5. For the same concentration the number of osmoles is much greater in the case of DA-12 than in BB-12. Thus, aggregation of DA-12 molecules occurs to a much lower extent than in the case of BB-12 molecules, and BB-12 has a much greater tendency to form aggregates. This confirms that compound BB-12 is more hydrophobic than DA-12.

Quantum chemical methods

To understand such differences in the properties of these two compounds, the quantum chemical methods were used to study the electronic structure of DA-*n* and BB-*n* molecules. The calculations for the system under consideration (an isolated molecule of the surfactant) have been performed with the MO LCAO method, starting from their optimized geometries. The results of the calculations are shown in Figs. 6 and 7, where the atomic net charges are presented for both molecules under study. To simplify calculations, the alkyl chains of the molecules studied were limited to 2-carbon residues. The justifica-

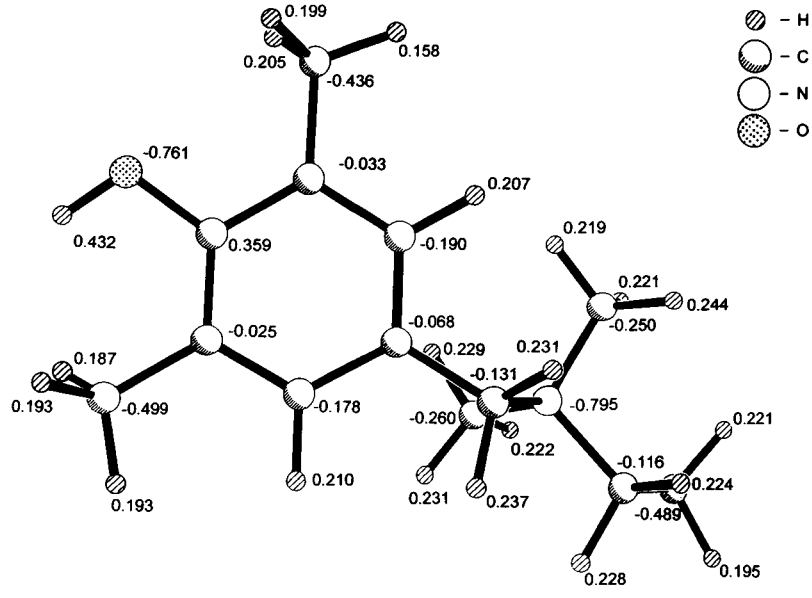

FIGURE 6 Optimized structure and respective atomic net charges for the DA-n ($n = 2$) system. Quantum chemical calculations for an isolated molecule have been performed with the MO LCAO method, starting from the molecular geometry optimized by the PM3 all-valence approach (Stewart, 1989). The Gamess package of computer programs (Schmidt et al., 1993) has been used in the ab initio calculation of the electronic structure for standard choice of the coordinate origin.

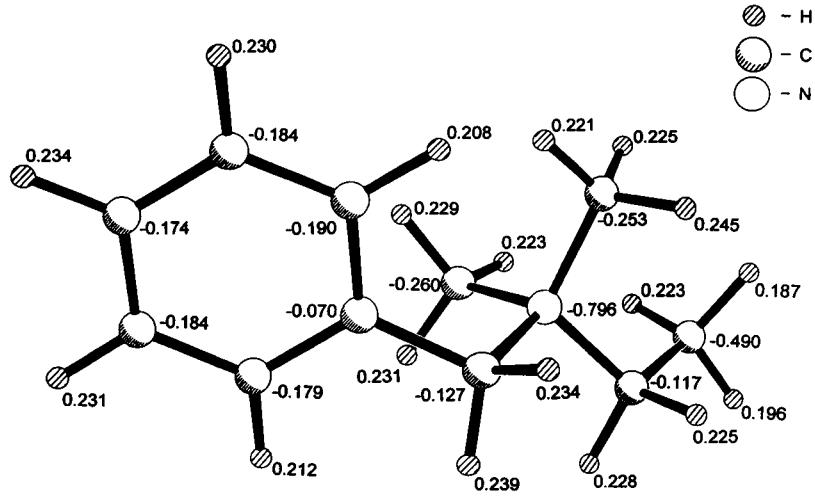

FIGURE 7 Optimized structure and respective atomic net charges for the BB-n (n = 2) system. Quantum chemical calculations for an isolated molecule have been performed with the MO LCAO method, starting from the molecular geometry optimized by the PM3 all-valence approach (Stewart, 1989). The Gamess package of computer programs (Schmidt et al., 1993) has been used in the ab initio calculation of the electronic structure for standard choice of the coordinate origin.

tion for such "truncation" of chains follows from zero dipole moments of all alkanes. Truncation of chains was necessary to limit computation time. The origin of coordinate systems was taken at the mass center in both cases. Dipole moments, calculated in such systems of coordinates, are essentially different for these cationic surfactant molecules. The dipole moment value of the DA-n molecule is 12.47 D, and that for BB-n is more than two times smaller (5.54 D). This result suggests that changes in chemical structure affect the electrical properties of the molecules studied in a very strong manner and therefore may influence their hydrophobic and hydrophilic properties. The results of calculations show also that the greatest (in modulus) values of atomic net charges are at nitrogen in both molecules; in the case of DA-n molecules a nearly equal net charge is located at oxygen.

The theoretical results are obtained for truncated chains of the molecules and do not allow for any solvent presence, i.e., isolated molecules were considered. That is why the experiments shown in Fig. 2 were carried out. The relaxation of surface phases on water, under the same physical conditions, was experimentally compared, and we have shown that the surface pressure of the DA-12 surface phase initially decays more rapidly and, finally, to an essentially lower value than for BB-12. We have obtained similar indications concerning hydrophobicity from thermodynamic considerations. Thus the computed electric properties (the greater dipole moment of DA-n than of BB-n) correlate well with the greater hydrophilicity of DA-n in comparison with the hydrophilicity of BB-n.

This conclusion may serve as the basis for an explanation of the fact that compounds DA-n disorganize membranes less than compounds BB-n. It seems that the higher the hydrophilicity of the polar head of an amphiphile, the smaller the immersion of the molecule into the hydrophobic core of the bilayer and the smaller the disorganization of the whole membrane.

Results of monolayer experiments

To verify this notion by other means, we carried out monolayer experiments using DA-16 and BB-16 compounds (considering these compounds as most hydrophobic in both series).

Fig. 8 shows a three-dimensional plot: the surface pressure of a mixed monolayer made of lecithin and DA-16 (a modifier) as a function of area per molecule in the monolayer and the molar fraction of the modifier. Thus the plot shows compression isotherms of the mixed monolayer systems in the full range of molar fraction of DA-16.

In Fig. 9 the horizontal cuts of Fig. 8 are shown. The cuts show the dependence of area per molecule of the lecithin DA-16 monolayer on the molar fraction of DA-16 at constant surface pressures of 4, 6, 8, and 10 mN/m.

The three-dimensional plot of the surface pressure of the lecithin–BB-16 mixed monolayer system versus the mean molecular area and molar fraction of the modifier (BB-16) is shown in Fig. 10, and the horizontal cuts of this plot (the isothermic isobars for surface pressures 4, 6, 8, and 10 mN/m) are shown in Fig. 11.

A comparison of the presented relationships shows pronounced differences. The system shown in Fig. 8 exhibits slightly nonlinear dependence of surface pressure at any constant area per molecule, which increases with the molar fraction of DA-16. The dependence shown in Fig. 10 is also nonlinear, but the surface pressure of a mixed monolayer exhibits mainly a decreasing tendency with respect to the molar fraction of BB-16 at constant values of mean molecular area. The surface pressure difference between the lecithin–DA-16 system and the lecithin–BB-16 system is shown in Fig. 12 as a function of mean molecular area and molar fraction of modifier. The only structural difference between DA-16 and BB-16 molecules, which is two t-butyl groups (instead of two hydrogen atoms) plus one hydroxyl group (instead of one hydrogen atom), affects the surface pressure of the monolayer in a very strong manner. The

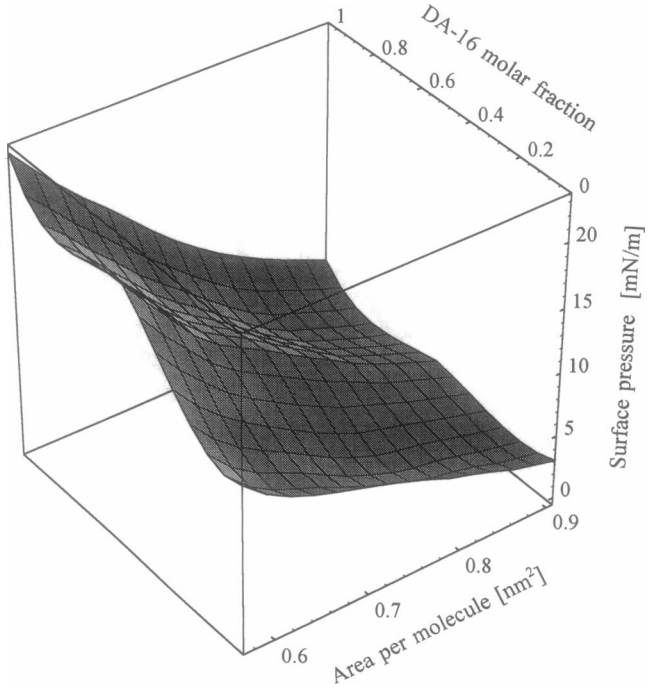

FIGURE 8 Relationship between surface pressure, area per molecule, and molar fraction of the DA-16 compound. The plot shows compression isotherms of mixed monolayer systems (lecithin–DA-16) in the full range of molar fraction of DA-16. Accuracy of surface tension measurements was better than 1 mN/m. The isotherms were taken using a rectangular trough with effective dimensions of 280 mm × 82 mm × 10 mm, equipped with a Wilhelmy plate, an electrobalance, and an A/D transducer (model PCL 812 PG; Advantech Co.). Doubly distilled water was used as the subphase. The monolayers were allowed to stabilize for 20 min before compression. In all monolayer experiments the initial molecular area was 1.0 nm^2/molecule, and the rate of compression was 0.108 nm^2/molecule per min (3.60×10^{-5} m^2/s). The experiments were performed at room temperature. The analog signal of electrobalance was converted to a digital one and registered by data acquisition software PCLS-702 (Advantech Co.) every 2 s on Lotus 123 worksheets, where the data were recalculated to surface pressure versus mean molecular area plots. Data from single experiments (for a specific value of the molar fraction of DA-16) were collected and analyzed further with the Mathematica (v. 2.2) software package (Wolfram Research).

greater the molar fraction of modifier and the smaller the mean molecular area, the greater the surface pressure difference is.

Analysis and consideration of monolayer experiments

The above experimental material enables us to present the following analysis and consideration. We may notice that the isothermic isobars shown in Fig. 9 exhibit a local maximum of mean area per molecule of the monolayer at equimolar concentrations of lecithin and DA-16. The curves for surface pressures 6, 8, and 10 mN/m exhibit two local minima; the first minimum exists at a DA-16 molar fraction of about 1/7, and the second one at a DA-16 molar fraction of about 4/5. Some of the isothermic isobars shown in Fig. 11 exhibit local minima of mean area per molecule of the monolayer at a molar fraction of BB-16 of about 1/4 and local maxima at about 1/2. The first family of local minima corresponding to a molar fraction of DA-16 equal to about 1/7 can be understood as the most packed state of the monolayer and thus the aggregation or association of its molecules. This aggregation corresponds to each molecule of DA-16 having six molecules of lecithin as its neighbors. This could lead to the conclusion that molecules of DA-16 during mixing enforce (over the pressure range considered) hexagonal headgroup packing of lecithin molecules in the monolayer and that this process results in lowering the mean molecular area of the monolayer. For DA-16 molar fractions greater than 1/7, not all DA-16 molecules can have six lecithin neighbors, and the mean molecular area must increase. If the molar fraction of DA-16 is about 4/5, the monolayer is mainly composed of DA-16 molecules; each molecule of lecithin aggregates with four molecules of DA-16, causing the existence of the second family of local minima on the isothermic isobars of the mixed monolayers at the studied surface pressures. Thus one can also expect a percolation-like transition (in a sense of domain connectivity transition; Vaz et al., 1989; Sankaram et al., 1992; Glaser, 1993) when increasing the molar fraction of DA-16 compound in mixed monolayer system. The aggregation preference of BB-16 for lecithin seems to be different, as there are local minima of mean area per molecule of the monolayer at a molar fraction of about 1/4 in this case. These minima can be interpreted as such an aggregation preference of BB-16 molecules for lecithin, which enforces (in the considered range of surface pressures) a trigonal arrangement of lecithin headgroups in the vicinity of a single molecule of BB-16; each molecule of this modifier is situated at an equal distance from its lecithin neighbors—i.e., in the interstitial position at this trigonal lattice. Nothing can be said about the existence of the second family of local minima on isothermic isobars of lecithin monolayers modified by BB-16 on the basis of the presented experimental material. Comparing the values of the mean molecular area at local minima in Fig. 9 and Fig. 11, we can see that the mean molecular area of the lecithin monolayer modified by DA-16 is higher than in the case of monolayers modified by BB-16, when considering curves for the same values of surface pressure.

A comparison to the influence of anesthetics and cholesterol on lipid membranes

An apparently similar problem was studied by Jørgensen et al. (1993), namely the influence of some anesthetics and cholesterol on lipid membranes, by the MC method of computer simulation. A cholesterol molecule was assumed to take up a site for an acyl chain on the triangular lattice, giving rise to interactions with six nearest neighbors on the triangular lattice, whereas the anesthetic molecules were considered as interstitial molecules occupying the centers of

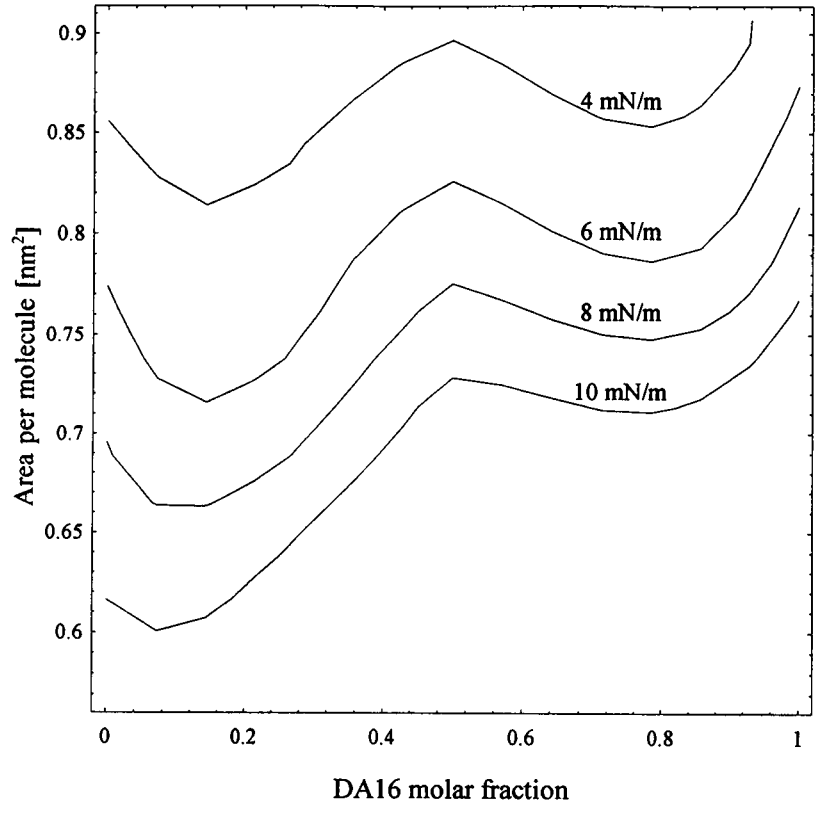

FIGURE 9 Dependence of area per molecule of the lecithin–DA-16 monolayer mixture on a molar fraction of DA-16 at constant surface pressures of 4, 6, 8, and 10 mN/m (from Fig. 8).

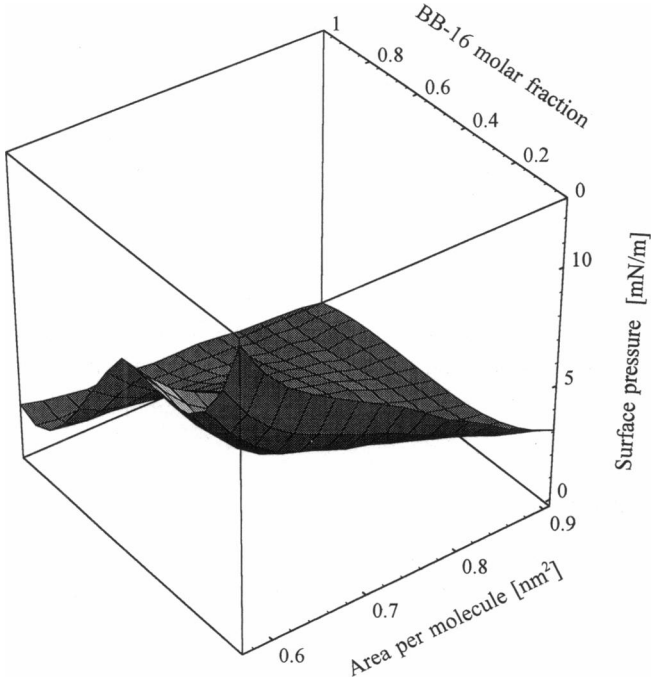

FIGURE 10 Relationship between surface pressure, area per molecule, and molar fraction of BB-16 compound. The plot shows compression isotherms of mixed monolayer systems (lecithin–BB-16) in the full range of molar fraction of BB-16. The accuracy of surface tension measurements was better than 1 mN/m. The experimental procedure for obtaining the plot, the same as that for the lecithin–DA-16 system, is described in detail in Fig. 8.

the triangles formed by three neighboring lipid-acyl chains. The membrane lipid association properties of BB-*n* resemble those assumed for anesthetics in the paper of Jørgensen et al. (1993), whereas the association properties of antioxidant series DA-*n* are similar to those assumed for cholesterol in that paper. The interstitial affinity of BB-*n* to membrane makes this series of compounds interact more strongly than the antioxidant series DA-*n*, which has an affinity for membrane in sites of its trigonal lattice and thus disorganizes the membrane to a smaller extent than the reference compounds series.

Hydrophobicity and molecular shape concepts

The affinity of the compounds studied for membrane seems to be measured by their hydrophobicity. The affinity of BB-*n* seems to be greater than that of DA-*n*, as the hydrophobicity of BB-*n* is greater than that of DA-*n*. Thus we may conclude (Schwarz, 1994) that the structural reorganization of membrane caused by BB-*n* is greater than that in the case of DA-*n*. In our previous studies (Hładyszowski and Przestalski, 1990) we considered the surface activity of different amphiphilic compounds in relation to their molecular shape. According to the steric considerations following from the molecular shape concept we expected a higher destructive ability of single-chain amphiphilic molecules with greater polar heads. As the polar head of DA-*n* is greater than that of BB-*n*, we expected a higher destructive ability for DA-*n*. This, as we have learned, is not the case.

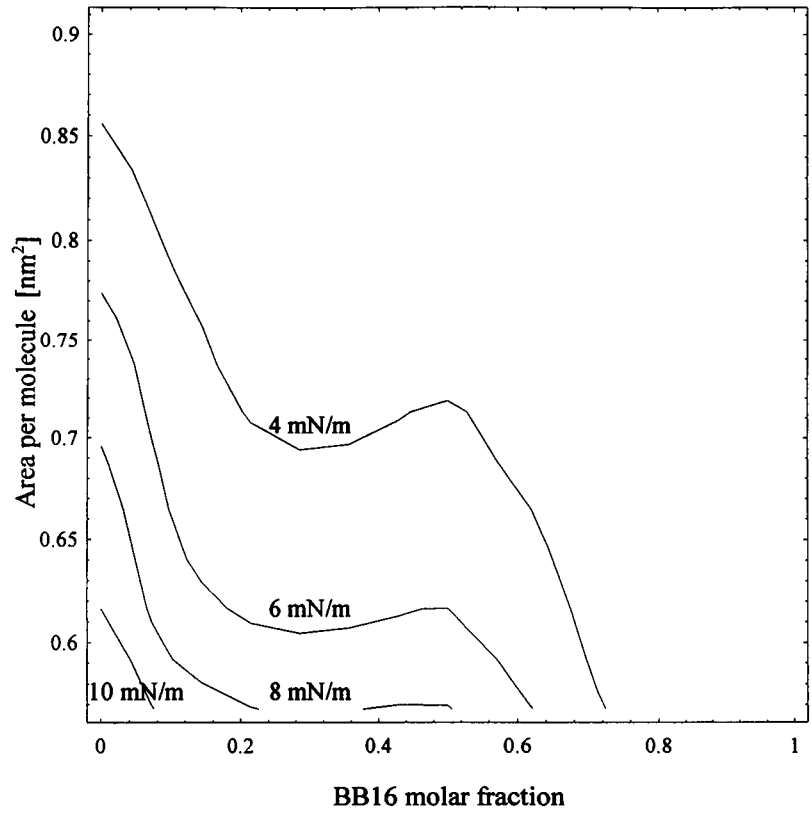

FIGURE 11 Dependence of area per molecule of the lecithin–BB-16 monolayer mixture on the molar fraction of BB-16 at constant surface pressures of 4, 6, 8, and 10 mN/m (from Fig. 10).

However, it does not contradict the molecular shape concept. If the molecules considered are not incorporated totally, the only shape to be considered in the concept should be the shape of the fragment introduced into the membrane.

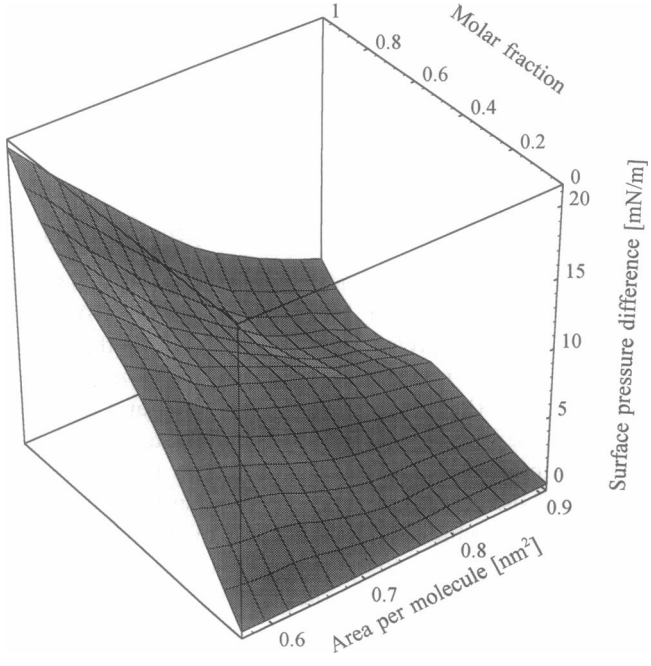

FIGURE 12 Difference in surface pressure between the lecithin–DA-16 and lecithin–BB-16 monolayer mixtures (from Figs. 8 and 10).

We know that DA-*n* is more hydrophilic than BB-*n*, and because of this fact, molecules of DA-*n* are much less immersed in the membrane. From this point of view, strict steric interpretation of molecular shape would be possible only for molecules of practically the same hydrophobicity. In our case the steric interpretation should concern only the shapes of the fragments of molecules, which are known to be incorporated into the membrane; the molecular shape concept has a less important role to play than hydrophobicity. Hydrophobicity plays a primary role in establishing the fragments of molecules to be incorporated into the membrane. However, both concepts should be taken into account in the consideration of amphiphile interaction with membranes.

CONCLUSIONS

Experimental and theoretical evidence shows that the compounds DA-*n* (antioxidants) and BB-*n* (the reference compounds), both being amphiphiles, have different hydrophobicities. The present results help to explain the differences in interactions of both series of compounds with biological and model membranes. The molecules with lower hydrophobicity (DA-*n*) are probably immersed in the hydrophobic core of the membrane to a shallower depth than those with higher hydrophobicity (BB-*n*), and that is why DA-*n* compounds disorganize the membrane structure to a lower degree than BB-*n* compounds.

The compounds DA-*n* and BB-*n* affect monolayers in different ways and have different affinities to membranes, because of their different aggregation preferences for lecithin. On the basis of our monolayer experiments and a comparison to the cited MC studies on the influence of anesthetics and cholesterol on lipid membranes, we suspect that the interstitial affinity of BB-*n* for membrane makes this series of compounds interact more strongly than the antioxidant series DA-*n*, which has an affinity for membrane in sites of its trigonal lattice and thus disorganizes the membrane to a smaller extent than the reference compound series.

We suggest that the aggregation preferences of the studied compounds for membrane lipids characterizes in an essential way the compounds' weak or strong interaction with membranes; however, the aggregation preferences strongly depend on the hydrophobicity of the compounds.

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