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ORIGINAL PAPER

Influence of vitamin C on alcohol binding to phospholipid monolayers

M. Weis · M. Kopáni

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Abstract The simple model of the biological membrane is provided by well-controlled lipid monolayers at the air–water interface. The Maxwell displacement current technique (MDC) provides novel approach to conformation study of the membrane models. The effect of alcohols is interaction with membrane molecules, mainly with the lipid head group and consequent changes in physical–chemical properties of the membrane. The aim of study is to detect changes in structural, electrical and mechanical properties of dipalmitoyl-phosphatidylcholine (DPPC) monolayer on the subphase of methanol–water and ethanol–water mixtures before and after addition of antioxidant agent, vitamin C. Monolayers properties are investigated by a surface pressure analysis (including mechanical properties evaluation) and the Maxwell displacement current measurement, the dipole moment projection calculation. Surface pressure–area isotherms show similar behaviour of the DPPC monolayer on alcohol–water mixtures independently on presence of vitamin C. Binding/adsorption process induces change of electron density distribution across monolayer and thus the molecular dipole moment. We observe small or negligible binding of methanol molecules on oxygen bonds of DPPC. Thus the antioxidant, vitamin C, has no significant effect. For ethanol–water mixtures is observed recovery of electrical properties in presence of antioxidant agent. We suppose that vitamin C regulates DPPC–ethanol molecules interaction.

Keywords Langmuir film · Dipalmitoyl-phosphatidylcholine · Maxwell displacement current technique · Alcohols · Vitamin C

M. Weis
Department of Physics, Faculty of Electrical Engineering and Information Technology, Slovak University of Technology, Ilkovičova 3, 81219 Bratislava, Slovakia

M. Kopáni (✉)
Institute of Pathology, School of Medicine, Comenius University, Sasinkova 4, 81108 Bratislava, Slovakia
e-mail: martin.kopani@fmed.uniba.sk

Introduction

Much attention has been paid to the dynamic behaviour of the cell membrane. Well-controlled lipid monolayers that assemble spontaneously from solvated molecules at the air–water interface can provide simple and conventional models of biological membranes which can be considered as two superimposed lipid monolayers (Sackmann 1996; Lipowsky 1991). Moreover, they provide promising applications as models of biological membranes for studying lipid–protein interactions as well as characterization of molecular arrangement in presence of external stimulus. Particularly chemical stimulus is fundamental for biophysics for various applications (e.g. gene therapy). Monolayers at the air–liquid interface are a suitable model system for studying the ordering in two dimensions as well as interfacial reaction. The monolayer–subphase interaction can be easily varied by changing the subphase composition (Maltseva et al. 2005).

The Maxwell displacement current technique (MDC) provides for a new possibility of molecular ordering and conformation study of the membrane models (Weis et al. 2005). This novel approach in molecular biophysics in combination with standard methods can clarify membrane chemical and physical properties form new point of view.

Electrical properties of the membrane play important role in transmembrane diffusion and membrane properties. However, up to now, the membrane investigation was not focused on electrical properties evaluation because of measurement difficulty.

The membranes, bilayers of the amphiphilic molecules, represent the basic structural component of all biological systems. They participate in substance exchanges as well as have influence at the metabolism processes. Any small change in cell membrane environmental composition can affect the electrical charge of the membrane surface, mechanical properties, permeability and function of the membrane proteins. Any changes in physical–chemical properties can be evoked by chemical substances such as alcohols (Klemm and Williams 1996; Vierl et al. 1994).

The cell membrane is one of the main targets for alcohols. The influence of alcohol (particularly ethanol) on phospholipids monolayer has been often investigated. The main point of such studies can be reality that ethanol acts as an anaesthetic on biological membrane (Alakoskela et al. 2004; Goodman et al. 1996). General anaesthesia is a multidimensional phenomenon. In the mammalian central nervous system, most general anaesthetics act at multiple molecular sites (Antkowiak 2001). Goodman et al. (1996) examined the effect of surface pressure induced by different anaesthetics (halotane, isoflurane) on the rate of dipalmitoyl-phosphatidylcholine (DPPC) hydrolysis by phospolipase-C. They found the direct proportion between the surface pressure and the rate of DPPC hydrolysis. The rate of DPPC hydrolysis was affected mainly by monolayer surface pressure.

Alcohols change the membrane structure and force the membrane proteins into unfavourable conformations. Other effect of alcohols is interaction with membrane molecules, mainly with lipid head group and consequent changes in physical–chemical properties of membrane (Patra et al. 2006; Ickenstein et al. 2006). Ethanol stimulates the transport of some compounds that cross the membrane by simple diffusion. This alteration in membrane permeability may be deleterious to the cells promoting the leakage of intracellular constituents or the entry of toxic extracellular substances disturbing the composition of the cytoplasm. The interaction between amphiphilic molecules of monolayer and subphase molecules are essential for understanding the structure (Wagner and Brezesinski 2007; Cavalcanti et al. 2006), mechanical (Whitehouse et al. 2004; Marrink and Mark 2003) and electrical properties (Lopez Cascales et al. 2006) of the monolayer.

The material properties of the cellular membrane are crucial to many biological processes. Elastic modulus expresses mechanical properties of the monolayer and interaction between DPPC molecules and subphase molecules. Elastic modulus minimum gives information about the transition between different phases of monolayer. Lipid membrane is a physical interface between the intracellular and extracellular nature. The cell membrane also regulates the permeation of molecules in and out of the cell, mechanical response to environmental stresses (Shoemaker and Vanderlick 2003; Fosnaric et al. 2006).

The mechanism by which the anaesthetics act is still lacking. Several theories exist, which explain how the anaesthetics works. The anaesthetics influenced the function of membrane proteins, lipids or ion channels (Miller 1985; Franks and Lieb 1978; Kukita and Mitaku 1993). Nowadays, ion channels are proposed to be the most important anaesthetics targets. Recently for the biophysical research of biomembranes lipid monolayers at the air–water interface are used. The experimental studies on simple model lipid membrane showing various sites of anaesthetics action in the lipid membrane such as acyl chain domain (Eckenhoff 1996), headgroup region (Garcaa et al. 2000; Gallova et al. 1992; Seelig et al. 1988), water/lipid interface (Pickholz et al. 2005; Cafiso 1998). On the other hand, it is assumed different distribution of some anaesthetics and alcohol molecules (Pohorille and Wilson 1996).

The aim of presented work is to detect changes in structural, electrical and mechanical properties of DPPC monolayer situated on the subphase of methanol–water and ethanol–water mixture after addition of vitamin C. Consequent comparison of subphases influence on this membrane model is analyzed and discussed. Monolayer properties are investigated by surface pressure analysis (including mechanical properties evaluation), the Maxwell displacement current measurement and subsequent dipole moment projection calculation.

Materials and experimental methods

Chemicals

The material used in this study as model phospholipid was 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine monohydrate (DPPC) purchased from Sigma-Aldrich. Lipid was dissolved in chloroform at the stock concentration 0.5 mg/ml and spread on the subphase using microsyringer (Hamilton, USA). As subphases were used pure water (bidistilled deionized water, 15 MΩ cm) and solutions of ethanol (spectrophotometric grade purity, Sigma-Aldrich) and vitamin C (Galvex, Banska Bystrica, Slovakia). For alcohol solutions, concentration of 20% and 100 mM concentration of vitamin C was used. All subphases were thermostated to the temperature 17°C. Monolayers were allowed to equilibrate and solvent to evaporate for 15 min. This time was sufficient for chloroform to evaporate and monolayer to stabilize.

Experimental methods

The methods of surface pressure measurements versus molecular area were chosen for the investigations of mechanical properties. The isotherms were measured during continuous compression of the monolayer, using a computer-controlled Langmuir trough (model 611, Nima Technology, UK). The total working area of the trough was 600 cm^2 and the compression rate was 50 cm^2/min, which corresponds to 6.8 $\times$ 10^{-3} nm^2/s per one molecule. The surface pressure–area isotherms were measured by the Wilhelmy plate method, surface pressure sensor PS4 (Nima Technology, UK), with accuracy of 0.05 mN/m.

Membrane curvation due to thermal fluctuations is essential for the shape and/or conformations of membranes as well as for cracks and defects generation. The elastic modulus characterizes the elasticity of the monolayer and is in analogy with bulk materials defined as

$$|E| = -A\left(\frac{\partial \pi}{\partial A}\right)_T \tag{1}$$

where π is surface pressure, A area per molecule and T is temperature. Elastic modulus (also called reciprocal compressibility) expresses the degree of rigidity of the Langmuir film under compression force influence.

For detection of changes in charge states of the molecules as well as relation with structural and conformational changes a contactless method based on analysis of Maxwell's displacement currents (MDC) was developed. This method was originally introduced by Iwamoto and Majima (1989, 1991) and improved in further works of various authors (Zakharov and Iwamoto 2002; Sulaiman et al. 2006; Vajda et al. 2004). Proposal of MDC experiment application for biological membrane phantom measurement was presented in our previous work for the first time (Weis et al. 2005). The top electrode was suspended in air, parallel to the interface, without a direct mechanical or electrical contact with a floating monolayer on the water surface (Fig. 1). The air gap between the top electrode and the water surface was regulated to a certain spacing (approx. 1 mm) by measuring the capacitance of the electrode system. The displacement current was detected with a Keithley 617 electrometer (Keithley Instruments, Cleveland, OH, USA). The sensitivity of measuring the current was 0.1 fA, the background noise was suppressed by a multiple electrical shielding of the electrode as well as whole measuring system to 2 fA. The area of top electrode was $A_E = 20$ cm^2.

Due to dynamic processes in the monolayer associated with the change in charge distribution caused by its compression, the induced charge in the top electrode

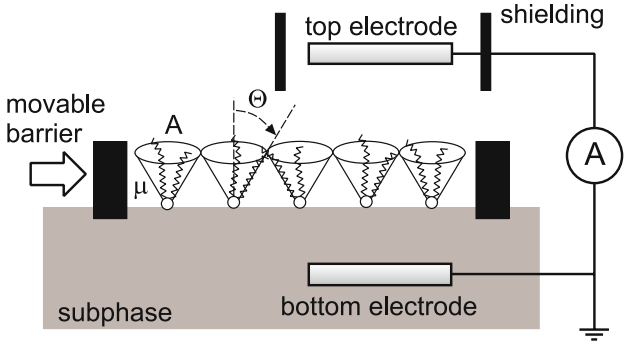

Fig. 1 Sketch of the MDC measurement apparatus. Rod-like chains of DPPC molecules execute precessional motion on the air/water interface with tilt angle Θ to the normal. A and μ represents the area per molecule and the dipole moment of molecule, respectively. As the movable barrier compress the monolayer, time-dependent charge is induced on top electrode, which results in a displacement current detected by an electrometer

varies with time and this generates a current flowing through the outer circuit via the electrometer. In this experimental setup, the observed dynamic charge processes are caused by lateral compression of the monolayer by a moveable barrier. Therefore any time-independent charge (particularly structured water layer and additional substances in subphase) distributed near/at the interface has no effect.

The major advantages of this method are high sensitivity and explicitness of measurement evaluation. As we showed in our previous studies (Weis et al. 2005; Vajda et al. 2004) the current flowing in the outer circuit can be expressed as a time change of the induced charge

$$I = \frac{\partial Q_i}{\partial t} = \mu N G \frac{\partial \langle \cos\, \Theta \rangle}{\partial t} + \mu \langle \cos\, \Theta \rangle \Gamma \frac{\partial N}{\partial t} \tag{2}$$

where μ is the dipole moment of one molecule (μ_Z is projection of μ to the normal), N is the number of molecules under the top electrode and Γ is the geometrical factor depending only on the distance between the top electrode and the top plane of the monolayer and on the shape and area of the upper electrode. The $\langle \cos\, \Theta \rangle$ stands for the statistical mean value cos Θ where Θ is the angle between the vector of dipole moment and the normal. By integrating the displacement current with respect to time, the induced charge Q_i can be obtained and in this way we also evaluated the vertical component of the molecular dipole moment. Thus, the dipole moment projection to the normal μ_z should be calculated as

$$\mu_z = \mu \langle \cos\, \Theta \rangle = \frac{1}{\Gamma} \int \frac{I}{N} \mathrm{d}t. \tag{3}$$

In this way the maximal dipole moment μ (i.e. molecular dipole moment) and molecular arrangement represented by $\langle \cos\, \Theta \rangle$ can be estimated.

Results

Pure water subphase

In contrast with the surface pressure–area isotherm analysis in MDC measurement is extremely sensitive also in the low surface pressure area, where surface pressure methods are useless. Comparison of surface pressure–area and dipole moment projection–area isotherms is shown in Fig. 2. By analysis of records of the Maxwell displacement current projection, we can calculate the dependence of the dipole moment on the area per molecule of the monolayer. DPPC monolayer at the water surface shows a rapid change of the dipole moment projection at value around 1.1 nm^2.

This change represents a phase transition of the Langmuir film from gaseous phase to liquid phase (Kaganer et al. 1999). From results of the measurement of the surface potential by Kelvin probe (Vogel and Möbius 1988), the value of the dipole moment of DPPC molecule was determined as 820 mD. Our recordings show the value around 815–825 mD, which is in accordance with the values obtained by independent measurements.

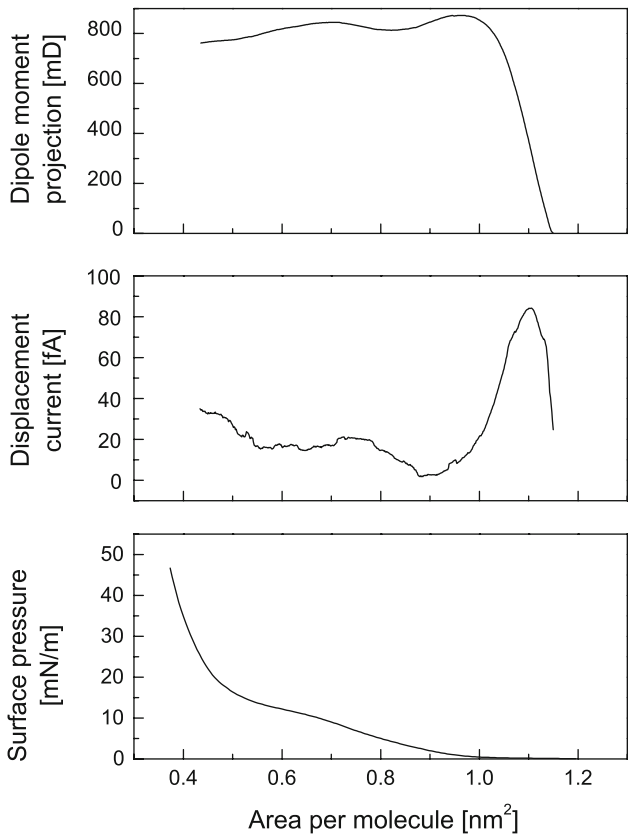

Fig. 2 Surface pressure–area (*bottom*), displacement current–area and dipole moment projection–area (*top*) isotherms of DPPC monolayer situated on pure water subphase

Water–alcohol subphase

On the DPPC monolayer on the water–methanol subphase (Fig. 3a) we can observe a time shift of surface pressure–area isotherm to bigger areas per molecule. This shift of isotherm also corresponds to the shift of dipole moment projection, without any noticeable change of phase transition shape.

At water–ethanol subphase DPPC surface pressure–area isotherms exhibits a very sharp phase transition from gaseous to liquid phase at area about 0.8 nm^2 (observable only for first compression) and from liquid phase to solid phase at 0.5 nm^2. Also a very slight time shift is observable. However, this effect is not as obvious as it was at DPPC monolayer on the water–methanol subphase. Moreover, a significant change in the dipole moment projection of DPPC molecules forming the monolayer appeared (Fig. 3b). A time shift to lower values of the dipole moment projection is noticeable and achieves negative values. Due to adsorption of ethanol molecules to the PC polar head in the polar heads of monolayer consisting of DPPC molecules, reversion of the dipole moment projection of the DPPC molecule is observed.

Water–alcohol–antioxidant subphase

Dependence of surface pressure an area per molecule for DPPC monolayer on methanol–water–vitamin C subphase and ethanol–water–vitamin C subphase is shown in Fig. 4a, b. The sharp phase transition from gaseous to liquid expanded state ($G–L_E$) and a slight change for liquid-expanded–liquid condensed ($L_E–L_C$) and liquid condensed–solid state ($L_C–S$) phase transition is noticeable for both alcohols. The increase of molecular area, which is growing depending on time, is also observable in both cases. Limiting area per one molecule is conserved and its value is approximately in agreement with DPPC known dimensions as well as surface pressure–area isotherm.

Displacement current–area isotherms recorded for DPPC monolayers on water–methanol–vitamin C subphase for various times (after monolayer creation) are shown in Fig. 4a (middle). Sharp maximum at area about 1.7 nm^2 is noticeable. In addition, with increasing time it is possible to observe peak shift to the larger area per molecule. In opposite, the measurement of the Maxwell displacement current of DPPC monolayer situated on ethanol–water–vitamin C (Fig. 4b) as a function of area per molecule exhibit time-stable strong current maximum about 0.8 nm^2, for which a shift is hardly observable.

DPPC monolayers under compression on the methanol–water–vitamin C subphase are rapidly changing the dipole moment projection at molecular area about 1.6 nm^2 (Fig. 4a) and reach maximal dipole moment 660 mD.

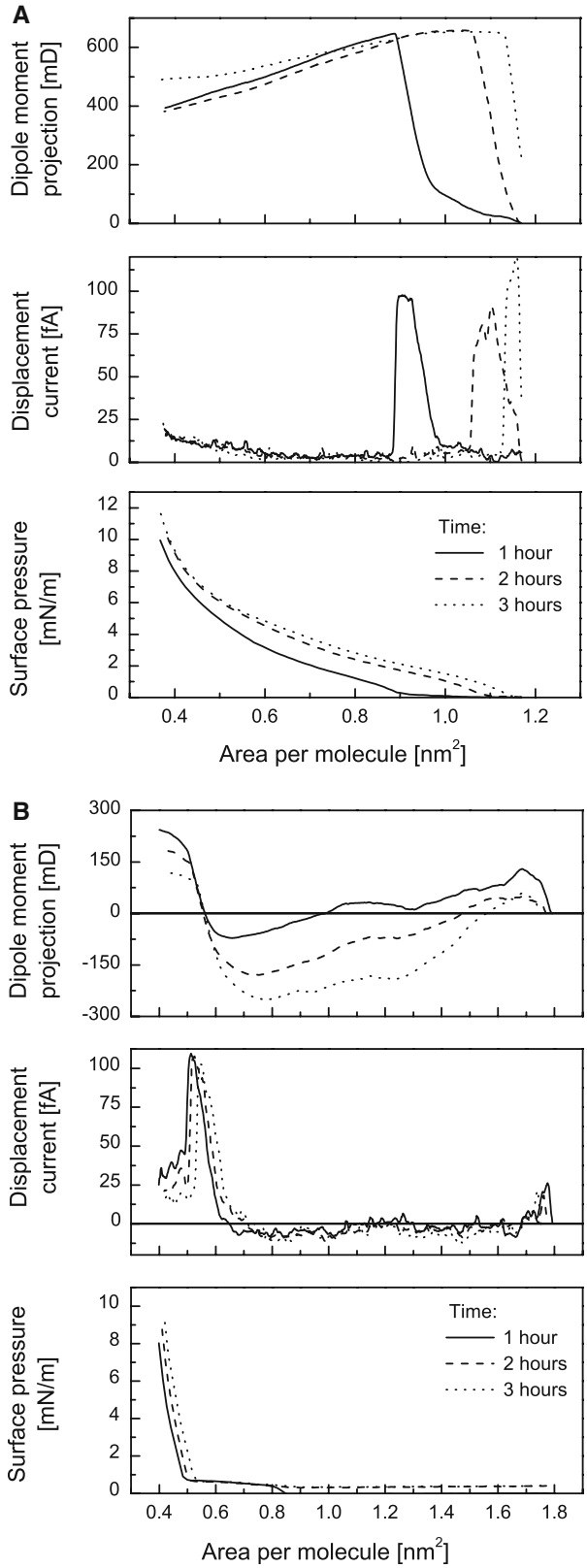

Fig. 3 Surface pressure–area (*bottom*), displacement current–area and dipole moment projection–area (*top*) isotherms of DPPC monolayer. Monolayers were situated on water–methanol (**a**) and water–ethanol (**b**) subphase

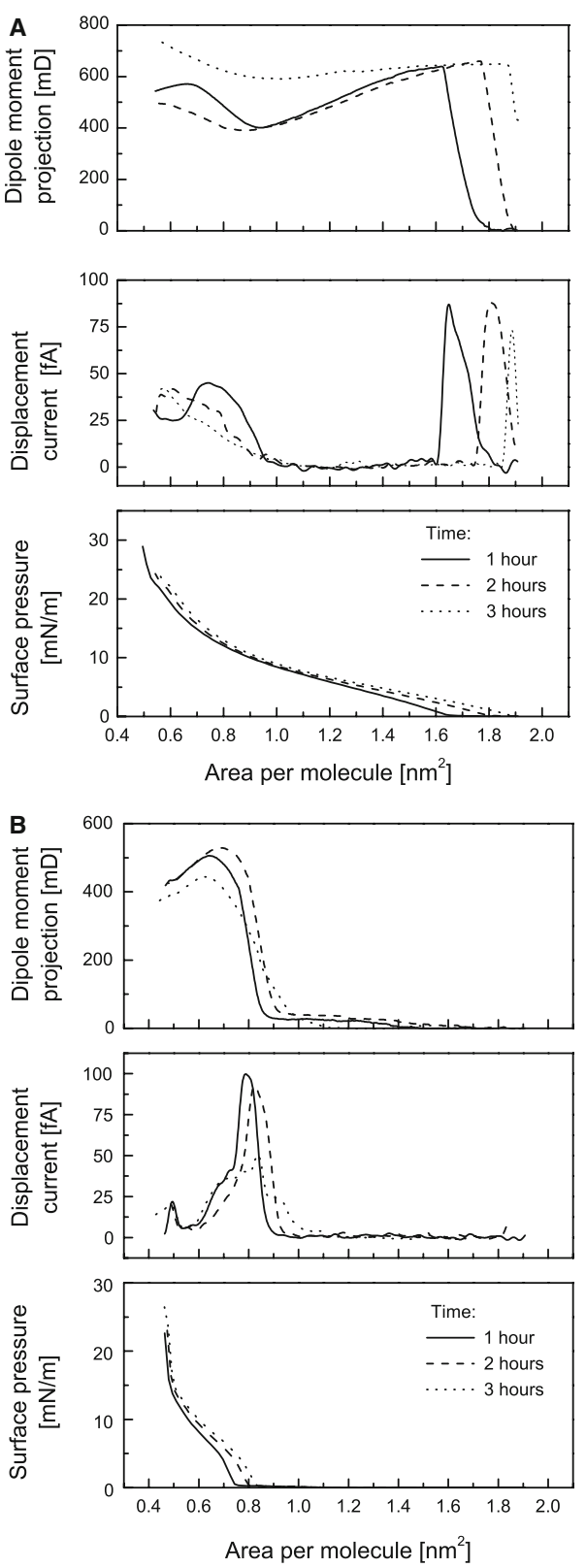

Fig. 4 Surface pressure–area (*bottom*), displacement current–area and dipole moment projection–area (*top*) isotherms of DPPC monolayer. Monolayers were situated on water–methanol–vitamin C (**a**) and water–ethanol–vitamin C (**b**) subphase

Calculations of dipole moment projection–area isotherms for various times of DPPC monolayer on ethanol–water–vitamin C subphase show phase transition from gaseous phase to liquid phase at area about 0.8 nm^2 per molecule. Moreover, evaluations illustrate slight shift in time domain to larger areas per DPPC molecule, which corresponds to the previous experiment (Fig. 2).

Additional information about mechanical properties can be obtained from the elastic modulus–area per molecule isotherms. For the elastic modulus dependence on water–alcohol (i.e. water–methanol and water–ethanol) in comparison with pure water subphase, a significant fall of elastic modulus was detected (Fig. 5). Partial recovery was observed in presence of vitamin C in the subphase (water–methanol–vitamin C and water–ethanol–vitamin C) of mechanical properties of the DPPC monolayer. From the obtained results we can assume that membrane fluidity increases (rigidity decreases) with alcohol binding to the monolayer. This effect can be partially inhibited by presence of vitamin C in the subphase. Moreover, rapid changes of elastic modulus are sensitive and reliable evidence of phase transition in the monolayer. Thus, they can be exploiting for assignation of specific phases to changes observed in dipole moment projections.

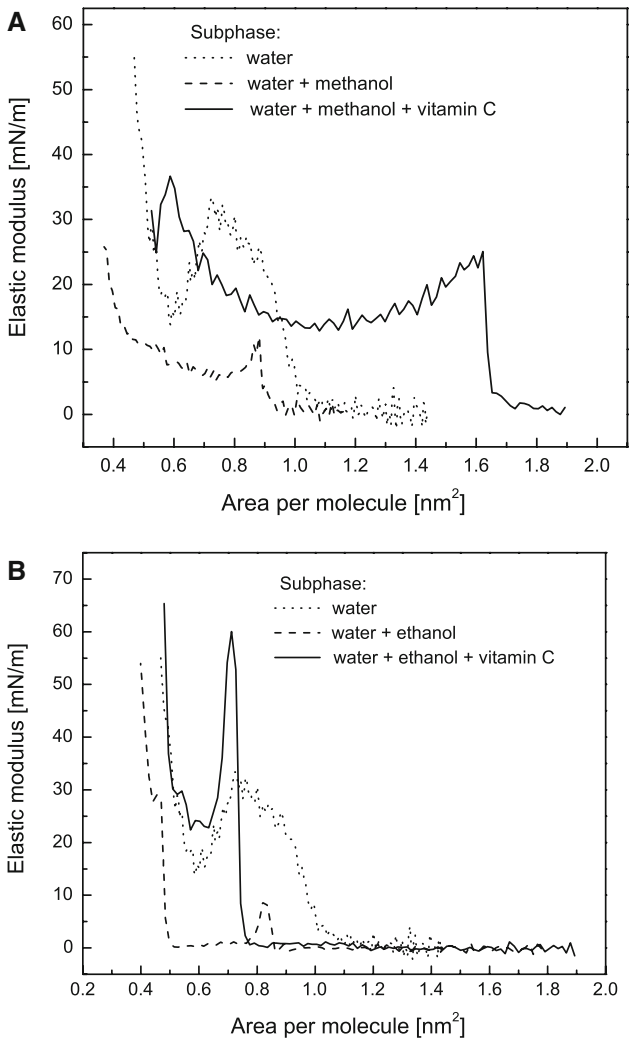

Fig. 5 Isothermal elastic modulus curves of DPPC monolayer on tree different subphase: **a** water, water–methanol, water–methanol–vitamin C or **b** water, water–ethanol, water–ethanol–vitamin C. *Curves* depict analysis of surface pressure–area isotherm after 1 h

Discussion

In the presented results, the influence of alcohol on the model of lipid membrane as well as protective effect of vitamin C was measured. Even though the used concentrations of alcohol and antioxidant agent are out of range of physiological limits, these conditions are recently studied (Patra et al. 2006; Pinisetty et al. 2006) for the possibility to obtain a thorough understanding of the phenomena.

Measurements of influence of water–methanol subphase on DPPC monolayer did not exhibit significant effect (degradation) on mechanical and electrical properties. In this case, monolayer properties are rather conserved. Experiments prove trend in rising of molecular area with time. Hence, methanol molecules adsorption to DPPC monolayer is assumed, however without strong influence on the monolayer.

Evaluations of dipole moment projection–area per molecule of DPPC monolayer exhibit strong rising at area of 1.7 nm^2 and then small decrease down to area about 0.9 nm^2. This local minimum does not represent a change in the order of DPPC monolayer (L_C–S phase transition is observed at area $\sim$0.65 nm^2). Therefore, the change in dipole moment corresponds to the changes in DPPC molecule (bonds orientation or intermolecular interaction energy). In this way, we expect small or negligible binding of methanol molecules on oxygen bonds of DPPC molecule. Vitamin C, as an antioxidant agent, has no significant effect on DPPC monolayer (Figs. 3a, 4a).

The elastic modulus (Fig. 5) is characterized by sharp transition from gaseous (G) phase state to liquid expanded phase (L_E) at area of 1.6 nm^2. Following continual decrease of the elastic modulus means the presence of intermediate phase (liquid expanded and liquid condensed phase). For area about 0.6–0.65 nm^2 DPPC monolayer on water–methanol subphase is system going to solid phase (Fig. 5a). Adsorption effect results in 'shift' of gaseous state–liquid expanded state phase transition to larger molecular areas, however mechanical properties are conserved. This result is in accordance with increase of area per DPPC molecule predicted by molecular dynamics simulations (Patra et al. 2006; Pinisetty et al. 2006). Moreover, the position of liquid condensed–solid state phase transition (around area of 0.8 nm^2) is untouched.

Comparison of the DPPC monolayer elastic modulus on water–methanol–vitamin C subphase with monolayer on pure water and water–methanol subphase is possible to observe an improvement of mechanical properties (decrease of membrane fluidity). However, the monolayer is on water–methanol subphase and hence an influence of methanol on surface tension is still noticeable. Its impact is mainly noticeable as expansion of intermediate phase area (in case of DPPC on pure water it is about 0.6 nm^2) and therefore elastic modulus dependence can appear like new state of the monolayer.

The DPPC monolayer on water–ethanol subphase exhibits significant decrease of dipole moment projection in range of area per molecule 0.6–1 nm^2 (Weis et al. 2006). In addition our measurements show that ethanol has no preferable order in near-to-surface layer. With increasing of the surface pressure one can occur the change in reorientation of ethanol molecules, as well as change in DPPC–ethanol interaction (change in binding between DPPC molecules, and between DPPC and ethanol molecules). Important part of molecular dipole moment of DPPC is created by phosphatocholine (PC polar head) group of hydrophilic head. From this point of view in experiments of the DPPC monolayer on water–ethanol subphase are not measured only a DPPC molecules, however dipole moment of the DPPC–ethanol molecular system. The adsorption (binding) of ethanol molecules on PC group can essentially change bond property. This interaction can lead to change of electric charge on the membrane surface, mechanical properties, and permeability as well as to change of ion diffusion across the membrane.

Klemm and Williams (1996) investigate that ethanol changes the order of water molecules around PC group. By ethanol binding to PC head of DPPC water molecules are shifted and are accumulated around alkyl group of alcohol (Nishikawa and Iijima 1993; Sato et al. 2000). Also Patra et al. (2006) assume that alcohol changes the structure of the membrane. Alcohol induces creation of unsuitable protein structure, which prevent from their normal physiological function.

Chiou et al. (1992) had observed by FTIR spectroscopy that after addition of small amount of ethanol to water-in-oil reversed micelles of DPPC molecules, the part of the water molecules (bounded by hydrogen bonds to DPPC molecules) was replaced by ethanol molecules. Ethanol molecules had strong interaction with phosphate moiety (PO_2 group) and water was replaced by this process. Authors discuss that interaction of alcohols with PC head of membrane lipids results in weakening of membrane–water interaction and destabilize the membrane. Investigation on the influence of alcohols with various chain lengths was observed direct connection between broken hydrogen bonds (water bounded to z PO_2 group of PC head) and number of carbon atoms in alcohol molecule. The efficiency of the anaesthesia was directly proportional to broken hydrogen bounds (Chiou et al. 1990). Some authors assume similar effect with opiates and intoxicants (Yurttas et al. 1992).

Dipole moment projection of DPPC monolayer on subphase water–ethanol–vitamin C reaches higher value as DPPC on water–ethanol subphase (Figs. 3b, 4b). Therefore, it is possible to assume that vitamin C has influence on DPPC–ethanol molecules interaction (or with PC group). Two effects are expected: (1) vitamin C molecules interact with DPPC molecules or (2) vitamin C molecules preferable bounded to ethanol molecules and in this way restrict their binding to DPPC molecules. Because water–ethanol–vitamin C subphase contain bigger amount of ethanol molecules in comparison with vitamin C, it is possible to predict that vitamin C has antioxidant effect on DPPC and has no significant influence on ethanol molecules. Vitamin C molecules are adsorbed to monolayer which consists of DPPC molecules and this process protects phospholipid monolayer. This concept is considerable if vitamin C binding to phospholipid is energetically more favourable as alcohol binding. Brazesinski et al. (2001) made assumption that ethanol molecules cause decrease of the probability of hydrogen bonds creation between PC head and water molecules. In this meaning, ethanol reduces the size of envelope created by water molecules around head of DPPC molecule.

Our results show slow recovery (increase) of area per molecule of phase transition of DPPC monolayer on water–ethanol–vitamin C subphase in comparison with subphase without antioxidant agent (Fig. 4b). Therefore, we can conclude that vitamin C molecules shield PC polar head from ethanol (Liu 1995; Blokhina et al. 2003) and on that account has impact on structured water around PC group. It is in accordance with our previous assumption about preferable binding of vitamin C to DPPC in comparison with DPPC–ethanol interaction (Cerda et al. 2002; Vázquez et al. 1995). In addition, adsorption of vitamin C molecules has no relevant impact on oxygen bonds and hence a dipole moment is comparable with measurements of DPPC situated on pure water.

Mechanical properties of the cell membrane play an important role in various biological processes (Cerda et al. 2002). For instance, the elastic modulus expresses the rigidity of the membrane. It is a function of many parameters as is presence of lipids, enzymes, cell membrane temperature and membrane environment. Obtained experimental results show similar behaviour of the DPPC monolayer on water–ethanol and water–ethanol–vitamin C subphases (Fig. 5b). Mechanical properties are in major influenced by ethanol molecules and this effect is observable also in case of inhibited binding to the monolayer

molecules (i.e. the environment is changed, for example surface tension of water–ethanol mixture was shifted from 72 mN/m, which represents pure water surface, to tension of 52 mN/m. This fact is in accordance with (Vázquez et al. 1995). In this case mechanical properties represent only a presence of alcohol near to the surface, but do not express the ethanol–phospholipid interaction. On water–ethanol and water–ethanol–vitamin C subphases elastic modulus of DPPC monolayer reaches higher values in comparison with pure water subphase. We have investigated that vitamin C have no significant effect on decrease of the elastic modulus (i.e. membrane rigidity). Hence, we do not assume change of mechanical properties of the monolayer due to the alcohol binding to the phospholipids. Monolayer created by DPPC molecules on pure water is more elastic and flexible towards external mechanical stimulus as the DPPC monolayer on subphase containing ethanol.

Electrical measurements provide essential information about molecular arrangement in the monolayer (so-called order parameter) as well as about orientation of bond polarizations. Binding/adsorption process induces change of electron density distribution across monolayer and thus value of molecular dipole moment. Binding of ethanol molecules in the subphase changes dipole moment of DPPC molecule from 820 mD to value about 200 mD (Pinisetty et al. 2006). The addition of vitamin C fully recovers dipole moment projection behaviour and partially restores the maximal dipole moment up to value of 520 mD (Fig. 4b). Therefore, we can conclude that the bond between DPPC and vitamin C is energetically favourable, PC group is shielded from ethanol molecules and in this way vitamin C molecules has essential influence on structured water around PC group. We suppose that vitamin C can affect interactions of DPPC–ethanol molecules and thus it may influence the anaesthetic effect of ethanol.

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