Effects of Water-Soluble Alcohols on the Surface Conductance of Lipid Monolayers: Bimodal Action

Tadayoshi Yoshida,* Yasushi Yamamoto, and Keijiro Taga

Department of Applied Chemistry, Nagoya Institute of Technology, Showa-Ku, Nagoya 466-8555, Japan

Hiroshi Kamaya and Issaku Ueda*

Anesthesia 112A, DVA Medical Center, Salt Lake City, Utah 84148

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We (Yoshida et al. *J. Phys. Chem. B* **2000**, *104*, 1249) devised a submersible horizontal electrode for estimation of the conductance of surface monolayers. The effects of 1-propanol on the electrical conductance of surface monolayers of stearic, elaidic, and oleic acids were estimated. The effects depend on the structures of the hydrophobic part of the monolayers. The surface electrical conductance of a stearic acid monolayer increased 18-fold by the addition of 1-propanol. However, the response of an oleic acid monolayer was almost negligible. The result suggests that alcohol effects on the surface electrical conductance depend strongly on the packing of the monolayers, therefore it is affected by changes in the alkyl tails. Because alcohol molecules in water anchor to the monolayer surface, sandwiched water molecules between the alcohol and monolayer form two-dimensional structures. The solid condensed form of the stearic acid membrane tends to form two-dimensional structures of sandwiched water. The magnitude of their structures depends on the condensability of the monolayer. We contend that the proton flow through the hydrogen-bond network of water (electrical conductance) increases.

Introduction

We reported that alcohols (ethanol, 1-propanol, and 1-butanol), added to the aqueous phase, affect the electrical conductance of the proton at the dipalmitoyl-L-α-phosphatidic acid (DPPA) surface monolayer bimodally.[1] At low alcohol concentrations, surface conductances increased, while at high alcohol concentrations, conductances decreased. From Fourier transform infrared (FTIR) spectra, it was found that alcohol molecules with short hydrocarbon chains distribute to the membrane surface. They interact with the polar P=O group, rather than penetrate into the hydrophobic core.[2,3] At the hydrophilic surface of lipid membranes, hydrogen-bond networks are formed. Alcohols[4] and anesthetics[5,6] destroy these networks. Destruction of these hydrogen bonds induces fluctuation of the membrane structure.[6,7]

Accordingly, we considered that the change in the surface conductance by alcohols (ethanol, 1-propanol, and 1-butanol) is in part caused by the partial destruction of the hydrogen-bond networks which are present at the membrane surface due to the interaction between the polar group of the membrane and the OH of the alcohols.[1]

Similarly, polar groups are present at the surface of proteins as a result of the dissociation of amino acid side chains. The surface electrical conductance on proteins is likely to be affected by alcohol molecules.[1] The proteins of E. coli contain Glu and Asp with COOH radicals. These carboxylic radicals are capable of creating hydrogen-bond networks with hydration water molecules of these proteins.[8] These hydrogen-bond networks create higher-order structures and affect their functions.

Since the OH radicals of added alcohols can form hydrogen bonds with water molecules, these molecules can compete with the hydrogen-bonded networks among carboxylic radicals. As a result, these added alcohol molecules may cause fluctuations among the hydrogen-bonded molecular assemblies.

In the present study, we used hydrophobic carboxylic acid with different three-dimensional structures (stearic, elaidic, and oleic acids) to form surface monolayers. From the differences in the effects of the added alcohols on these monolayers, we found that there are differences in the action modes of alcohols on the surface of these monolayers. When the monolayers are more condensed (more solid), we assume that alcohol molecules form a two-dimensional structure. Further, the proton flow on the surface of the most condensed stearic acid monolayer tends to be influenced most by these alcohols. Depending on the concentration of alcohol, the velocity becomes eighteen times faster.

Experimental Method

The lateral electrical conductance on the lipid monolayer surface was measured with a novel horizontal electrode. The gas phase is filled with N_2 under completely CO_2-free conditions.[1] The horizontal electrodes are formed by depositing a pair of rectangular gold films (15 × 60 mm) with 4 mm distance on a Pyrex plate (50 × 60 × 5 mm) by vacuum evaporation.[1] The plate was immersed 1 mm below the water surface. The horizontal electrode eliminates the meniscus formation around vertical electrodes partially immersed in water. Shapovalov et al.[9] and Cavalli[10] also used electrodes submerged in water to avoid meniscus effects. The present electrode has about 20- to 100-fold higher sensitivity than their electrodes.[11]

The lipids are stearic acid (octadecanoic acid), elaidic acid (*trans*-9-octadecanoic acid), and oleic acid (*cis*-9-octadecenoic acid), purity better than 99% (Sigma, St. Louis, MO). Water

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* Author to whom correspondence should be addressed.

Alcohols and Surface Conductance of Lipid Monolayers

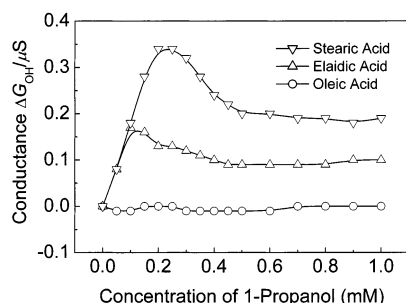*J. Phys. Chem. B, Vol. 107, No. 14, 2003* **3197**

Figure 1. Surface Electrical Conductance of Stearic Acid, Elaidic Acid, and Oleic Acid in 1-Propanol.

was purified by a super water-purifying system (Pureline WL21-P, Yamato, Tokyo, Japan), with purity better than 0.07 μS cm^{-1}, then boiled in a greaseless Pyrex glass flask for 15 min under N_2 gas to eliminate CO_2, and then cooled. The CO_2-free water was transferred to a Pyrex glass bottle, placed in a constant-temperature water bath maintained at 25 °C, and then transferred into the monolayer-forming trough. To avoid contamination by air during these transfers, Teflon tubes with Teflon stopcocks were used under N_2 gas. The electrical conductance between the two thin gold electrodes was measured by a digital conductance meter (CM-11P, Toa, Tokyo) at a frequency of 80 Hz. The lipids are dissolved in benzene. The surfactant molecules spread spontaneously on the water surface until a saturated monolayer is produced.

We used 1-propanol, purity better than 99.8% (Wako, Osaka, Japan), which was previously[1] found to show the strongest effect on the DPPA monolayer. 1-Propanol was added to the water to the final concentration of 1.0 mM, in eleven divided doses. During this procedure, shifts of the surface conductance from the original value, ΔG_{OH}, were measured in comparison to the original value.

The surface potential of the monolayer was measured by the ionization method. For the air electrode we used a stainless sheet (diameter 25 mm and 1 mm thick) containing ^{241}AM 2.4 MBq (Japan Isotope Inst., Tokyo, Japan) for the α-emitter. For estimation of the surface potential, one of the terminals of the horizontal electrode is used as the ground and the other terminal is used for the water electrode. The surface potential was measured by an electrometer with high internal resistance (200 TΩ) (Keithley, model 6512).

The molecular surface area was obtained by the surface pressure–area curves, obtained by the Wilhelmy method. All experiments were performed at 25 °C.

Results and Discussion

The conductance effect, ΔG_{OH}, of added alcohol, showed a bimodal pattern with a peak. This is similar to the alcohol effect on the DPPA monolayer. The magnitude of the alcohol effect was in the following order: stearic acid > elaidic acid > oleic acid.

The alcohol effect on the oleic acid monolayer was negligibly small. The differences of the alcohol effects among these monolayers are apparently caused by the discrepancy in the hydrocarbon parts of these fatty acids.

The surface conductance of stearic acid monolayers did not show appreciable surface conductance (0.02 μS). In the presence of 1-propanol, the surface conductance increased up to 18-fold (Figure 1). There are two possible mechanisms to increase the surface conductance. (1) The number of movable protons is increased by the increase of the dissociation of stearic acid. (2) The travel speed of the movable protons is increased.

The possibility of the first reason depends on a magnitude of the change in the monolayer surface potential. The surface potential $V(w)$ of the stearic acid monolayer on the super-purified water (purity better than 0.07 μS cm^{-1} and pH 6.8) was 260 mV. The surface potential $V(HCl)$ of the stearic acid monolayer on 10 mM HCl solution was 346 mV. Therefore, the electric potential close to the polar radical (electrical double layer) is -86 mV (= 260 $-$ 346 mV).

On the basis of the surface potential, and according to Graham's equation,[12] the dissociation constant of the carboxylic radical of the stearic acid monolayer was estimated to be 9 $\times$ 10^{-4}. This value is extremely small when compared with the dissociation constant of acetic acid (10^{-1}), estimated from the depression of freezing point.[13]

On the other hand, the dissociation constant of carbonic acid in the aqueous phase tends to decrease as the carbon chain length increases.[14] The tendency is attributed to the increase of hydrogen-bond formation in the next-neighbor molecule when the carbon chain length increases. Stearic acid monolayers can form hydrogen bonds in the one-dimensional form in a zigzag chain.[8] Therefore, the dissociation of molecules in the stearic acid monolayers is suppressed.

To form a monolayer, a benzene solution of stearic acid was added to the water surface successively by droplets. When it stopped to spread on the water surface, the surface pressure became maximum (18.5 mN/m) and a saturated monolayer was formed. The corresponding molecular area was 22.5 Å^2. Simultaneously, the surface electrical conductance became maximum (0.02 μS). This conductance value is close to our lowest experimental limit, 0.01 μS, and shows that the proton transport along the stearic acid monolayer is almost null.

The Unwin group[15] used a submarine ultramicroelectrode (UME) and measured lateral proton fluxes and the lateral proton diffusion coefficient. They concluded that the lateral diffusion coefficient of protons is smaller than those in the bulk aqueous phase, or they could not detect the lateral proton diffusion. This means that the proton diffusion along the membrane surface is suppressed in comparison to the bulk. Their conclusion is compatible with our small electrical conductance along the stearic acid monolayer.

The surface electrical conductance of dipalmitoyl-L-α-phosphatidylcholine (DPPC) monolayer, which the Unwin group used, was too small to obtain meaningful data for the stearic acid monolayer. The surface conductance of the DPPA monolayer was 18-times larger than that of the stearic acid monolayer and was 0.37 μS.[1]

Stearic acid, DPPC, and DPPA molecules contain dissociable radicals. In the stearic acid monolayer, the one-dimensional chain forms by hydrogen bonding between carboxylic radicals.[8] In the DPPC monolayer, a zwitterion structure forms by electrostatic force between the negative charge of phosphate and the positive charge of choline.[16]

These interactions among neighboring molecules suppress dissociation of the charged radicals. Therefore, the surface electrical conductance of stearic acid and DPPC monolayers became very small. Suppression of dissociation indicates strong interactions between the proton and the polar group of the monolayer membrane, and small mobility of the proton along the monolayer surface. In the DPPA monolayer, however, these molecular pairs do not form. Phosphate groups and their hydrations form proton pathways which increase the surface electrical conductance.[1]

On the other hand, the addition of 1-propanol increased the surface electric conductance 18-fold (1800% increase) (Figure

1). However, addition of 1-propanol did not affect the surface potential of the stearic acid monolayer more than 10%. When the electric potential changes 10%, the dissociation constant changes 20% according to Graham's equation.[12] Therefore, the large increase of the surface electric conductance cannot be explained by the increase of the dissociation.

To use Graham's equation, one has to assume that the multivalent ion concentration is below 1 μM. In our experiment, the materials which come in contact with water are Teflon and a gold-plated Pyrex glass. There is little chance that multivalent metal ions solubilize into the water. When we reused the super purified water (<0.07 μS/cm) to form a monolayer again, the electrical conductance and surface potential varied less than 10% from the initial experimental result. Considering that monolayers tend to adsorb multivalent ions, the above experimental result indicates nonexistence of multilvalent ions that affect the membrane characteristics.

As expressed in an earlier paper[11] our novel horizontal electrode has 100-fold-plus sensitivity, and is designed to suppress experimental errors. Additionally, we confirmed by a blank test that the addition of alcohol shows no effect on the surface electric conductance in the absence of a monolayer.[1] The large increases in conductance are not caused by experimental artifacts.

Considering that alcohol did not increase the dissociation constant strongly enough to explain the large increase in the surface electrical conductance, we proposed a model where the added 1-propanol forms a two-dimensional cluster of water molecules (sandwich-type). In this model, a proton can move inside of the newly formed two-dimensional water cluster. The new proton pathway is the planar hydrogen-bonded network, constructed between the polar groups of the monolayer membrane and the OH radicals of the hydrating alcohol.

The order of alcohol effect on the surface electrical conductance was stearic acid > elaidic acid > oleic acid. The molecular areas occupied in the spread monolayers were stearic acid 22.5 Å^2, elaidic acid 26.9 Å^2, and oleic acid 33.8 Å^2 at 25 °C. The reason for these differences in occupied areas is the interactions among alkyl chains. The interaction among oleic acids is weak due to the steric hindrance between double bonds. Both elaidic acid and oleic acid contain double bonds. However, the steric hindrance is less in elaidic acid because it is in the trans form. As a result of the presence of a saturated hydrocarbon chain, the stacking tendency of stearic acid is stronger than that of elaidic acid. The two-dimensional water clusters formed by alcohols are easier to build on the membrane surface when membrane structure is stable. As shown in Figure 1, the effect is strongest with a stearic acid monolayer.

The alcohol molecules in the aqueous phase tend to anchor under the monolayer by the attractive forces between the alcohol OH and the carboxyl radical of the monolayer.[14] It is generally considered that since alcohol molecules contain only one OH, which is capable of forming hydrogen bonds, the assembly may be limited to the linear form. However, the information on the radial distribution function obtained by X-ray diffraction and mass spectra shows that alcohols can maintain two-dimensional assembly in water, due to the stacking tendency.[17,18] A model has been proposed where several two-dimensional assemblies can stack together. The water molecules form two-dimensional clusters (sandwich-type structure).

On analogy to the water cluster model, the water molecules sandwiched between carboxyl radicals of the monolayer and the anchoring alcohols tend to form two-dimensional water clusters. These water structures are not rigid. They maintain a flexible fluidlike form. Nevertheless, their position and movement are restricted to a sheetlike two-dimensional structure. Therefore, proton translocation may occur with high efficiency inside the two-dimensional water clusters among hydrogen bonds. The proton movement at the planar lipid membrane surface is small, as Unwin proposed. However, the presence of alcohol molecules leads to constructing a new planar hydrogen-bonded network within the electrical double layer. This leads to the increase of the total electrical conductance along the surface of the monolayer. As a result, the added alcohols increase the surface electrical conductance.

When the alcohol concentration is increased, a maximum appeared in the surface electrical conductance. We presume that the maximum is produced by distortion and partial destruction of the new planar hydrogen-bonded network.

Recently we found that the surface electrical conductance along a cetyl alcohol monolayer increased from 0.01 μS to 0.2 μS or above by the addition of water-soluble alcohol (1-propanol). Though cetyl alcohol contains an undissociated polar group (−OH), it forms a condensed and solid monolayer on the water surface similar to stearic acid. The alcohol effect on the surface electrical conductance is not under direct influence of the structure of membrane polar groups. This non site-specific effect of alcohol is consistent with anesthesia, and might mean that anesthesia by water-soluble alcohol may be associated with disturbances in the proton conductance.

As discussed above, larger conductance occurs for charged monolayers. However, the large increase in conductance due to alcohol was not accompanied by a large increase in the number of protons, according to the surface potential measurements.

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