Selective Precipitation of Salts on the Surface of a Gel State Phosphatidylcholine Membrane

Kazutoshi Iijima, Teruhiko Matsubara, and Toshinori Sato*
Department of Biosciences and Informatics, Keio University,
3-14-1 Hiyoshi, Kouhoku-ku, Yokohama 223-8522

(Received April 3, 2007; CL-070358; E-mail: sato@bio.keio.ac.jp)

We identified precipitates of salts on phospholipid bilayers in solution with an atomic force microscope and X-ray fluorescence analyzer. The precipitates on the surface of a dipalmitoyl phosphatidylcholine membrane in a gel state were found in Tris-buffered saline and grew with time. The salts precipitated were considered to be NaCl in buffer.

In physiological conditions, plasma membranes of animal cells are exposed to various ions. The interactions of phospholipid bilayers with monovalent and divalent ions have been investigated using ^{2}H NMR spectroscopy,[1] and the dependency of electrostatic potential on the variety of ion was evaluated according to the Gouy–Chapman theory of a diffuse double layer.[2] Recently, the interaction of sodium chloride (NaCl) with a 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC) and dipalmitoyl phosphatidylcholine (DPPC) bilayer was investigated by using molecular dynamic simulations, and it was revealed that NaCl in solution altered the conformation of phosphatidylcholine head groups and slightly changed the ordering of hydrocarbon tails.[3,4] However, the influence of the state of the phosphatidylcholine membrane on the binding of NaCl has not been investigated.

In the present study, we employed an atomic force microscope (AFM) and an X-ray fluorescence analyzer to observe the precipitation of salt on phospholipid bilayers in solution. The procedures for the preparation of lipid bilayer on mica were shown in Figure 1. An air–water interface lipid monolayer of POPC was prepared on a Langmuir–Blodgett trough (FSD-220, USI System Co., Ltd., Japan) at 25 °C with a subphase of Milli-Q water. The POPC monolayer was transferred to freshly cleaved mica by horizontal deposition at a surface pressure of 35 mN·m^{-1} and dried overnight in a desiccator. A second lipid monolayer of either DPPC or POPC was transferred to the POPC-coated mica by horizontal deposition at a surface pressure of 30 mN·m^{-1}. AFM measurements of the lipid bilayers cumulated on mica were carried out on a SPA-300 (Seiko Instruments Inc., Japan) in Milli-Q water or buffers at 25 °C. A 200-μm-long soft cantilever (SN-AF01, Olympus Optical Co., Ltd.) with integrated pyramidal silicon nitride tips having a spring constant of 0.02 N·m^{-1} was used for all measurements. A typical scan rate was 1 Hz.

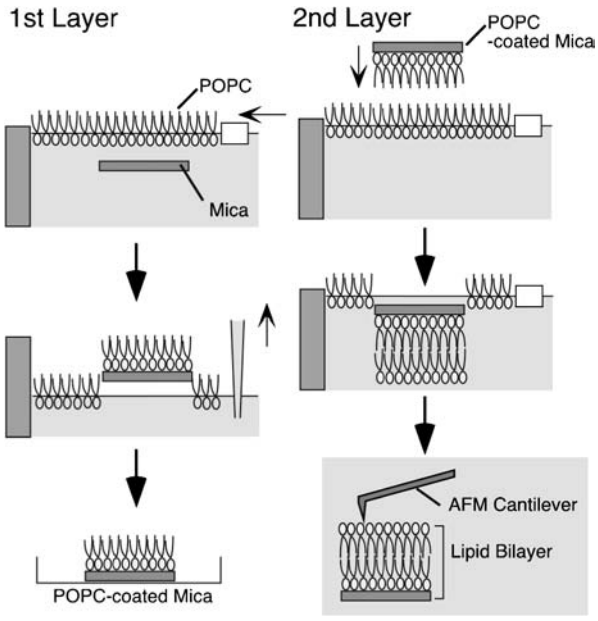

Figure 1. The procedures for the preparation of lipid bilayer on mica.

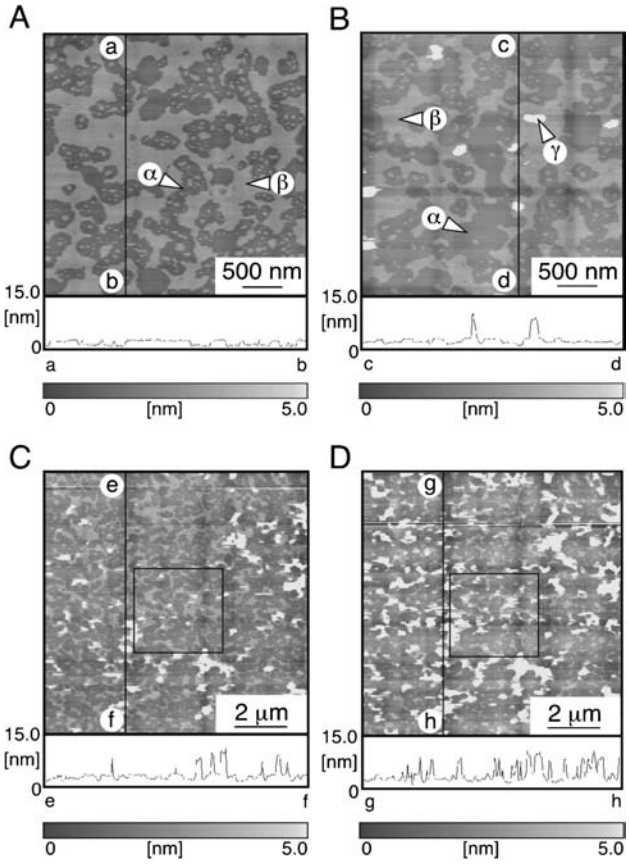

Figure 2. AFM images of the surface of the DPPC membrane in (A) water and (B–D) TBS. Incubation with the TBS lasted (B) 5 min, (C) 10 min, and (D) 20 min. Cross sections are shown at the bottom of each AFM image. Squares in (B) and (C) mean identical areas with (B).

A typical AFM image of the DPPC membrane was shown in Figure 2A. Domains 1.0 nm in height (β) from the lower region (α) were observed. This result suggested that two-phase state having different heights coexisted. The domain β and region α were considered to correspond to DPPC in a gel state and liquid-crystal state, respectively. Brewster angle microscopy showed that at 25 °C, DPPC separated into the two domains, a gel state and a liquid-crystal state.[5] The present AFM results were well consistent with the literature.

Then, the water phase covering the lipid membrane was replaced with Tris-buffered saline (TBS; 50 mM Tris-HCl, 150 mM NaCl, pH 7.5) in the AFM apparatus. After 5 min, a new domain γ having a height of 8–10 nm was observed by AFM (Figure 2B, bright dots). The occupied areas of domain γ were 1.6, 6.2, and 14.9% at 5, 10, and 20 min, respectively. Though the area of γ increased depending on the incubation time, the height showed no increase (Figures 2B, 2C, and 2D). Since domain γ was not observed in Tris-HCl buffer (50 mM Tris-HCl, pH 7.5), the precipitates were considered to be NaCl. Interestingly, domain γ was detected only on domain β corresponding to DPPC in a gel state (Figures 2B, 2C, and 2D). The precipitates disappeared on washing with Milli-Q water (data not shown). On DPPC membranes incubated with Tris-HCl buffer containing other salt such as KCl and $MgCl_2$, the precipitation was not observed (data not shown).

The atomic species of the precipitates observed on the membrane were identified by energy dispersive X-ray fluorescence (EDXRF) analysis. The lipid membranes cumulated on mica were incubated with TBS, then the solution was removed, and measurements were carried out at 25 °C with an X-ray analytical microscope (XGT-2700, Horiba, Ltd.). It was difficult to detect the spectrum from sodium because the energy of the spectrum is as low as the detection limit of the instrument. Existence of chlorine was estimated from Kα and Kβ peaks in EDXRF spectrum at an operating voltage of 15 kV with a standard less quantification procedure.[6] The EDXRF of each sample was done with a 30-s counting time. When the DPPC membrane was exposed to TBS for a couple of seconds, the chlorine peaks of Kα and Kβ from chlorine were slightly detected (intensity of chlorine, 0.35 ± 0.31 cps·mA^{-1}, Table 1). After 45 min, these peaks were clearly detected (2.07 ± 0.36 cps·mA^{-1}). When the DPPC membrane was exposed to Milli-Q water, chlorine was not detected. Increasing of the intensity of chlorine depending on the incubation time was observed. The existence of chlorine strongly supported that the precipitates on the DPPC monolayer corresponded to NaCl.

The formation of precipitates of NaCl only on solid DPPC may relate to the ordering of electric dipoles of zwitterionic phosphatidylcholine (PC). A molecular dynamics simulation has shown that Na^+ ions were closer to phosphate groups and Cl^- has some coordination with nitrogen in choline.[4] The interactions of Na^+ and Cl^- with PC would be due to the crystallization of NaCl on PC membrane.

To examine the influence of the kind of ion and pH of the buffer on the precipitation of salt, AFM measurements were performed using a phosphate-buffered saline (PBS). The precipitation of NaCl on DPPC was observed in PBS at pH 7.2, but not at pH 5.7 (data not shown). A quaternary amino group is fully positive at any reasonable pH value, and an ionizable phosphate group has an intrinsic pK of $\approx$1.5.[7] Although phosphatidylcholine is in a zwitterionic state at both pH 7.2 and 5.7, the protonation of the phosphate groups increases at pH 5.7.

Table 1. Intensities of chlorine on DPPC membrane surface by X-ray fluorescence analyses

Condition	Intensity of Chlorine /cps·mA^{-1}
Milli-Q	0.05 ± 0.07
PBS (few seconds)	0.35 ± 0.31
PBS (45 min)	2.07 ± 0.36

Next, POPC or mixed DPPC/cholesterol (60:40 by mol.) membranes were employed to examine the influence of membrane fluidity on the precipitation of salts. For the POPC membrane in a liquid-crystalline state, no precipitation was observed with the AFM, and X-ray fluorescence analysis showed no signal from chlorine (data not shown). Furthermore, for the DPPC/cholesterol (60:40) membrane, precipitation was not observed. Using differential scanning calorimetry, it has been found that cholesterol decreases the crystallinity of phospholipids in a gel state.[8] These results suggested that the ordered electric dipoles of the PC head group induced the precipitation of salts on DPPC membrane.

In conclusion, we observed the selective precipitation of salts on a gel state DPPC membrane. AFM observations of lipid membranes in solution brought the unexpected precipitation of salts on the surface of the DPPC membrane. The precipitated salts were considered to be NaCl from the composition of the buffer and results of X-ray fluorescence analysis. Further investigation will improve understanding of the interaction of inorganic ions with biomembranes and the preparation of organic–inorganic hybrid materials.

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Published on the web (Advance View) June 9, 2007; doi:10.1246/cl.2007.860