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Cation-specific Transition from Vesicle to Lamella for an Aromatic Diamine Lipid in Aqueous Solutions

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Addition of Cu^{2+} or Pd^{2+} into a novel aromatic diamine lipid in water induced vesicle–lamella transition. Combining scattering and calorimetry revealed that the metals first coordinated on the vesicle surface and then induced flattening and stacking of the bilayer to form highly ordered lamellae. This is the first report of the Pd^{2+}-induced structural transition of micelles.

Molecular design of nano-ordered structures from metal containing building blocks has become a topic of increasing interest in recent years.[1–3] Surfactants with metal binding sites have been developed and successfully applied to not only nanotechnology and material science but also biochemistry and pharmaceutical fields. In the present study, we show that a cationic lipid containing an ethylenediamine group (DA, Figure 1) assembles into a lamellar structure induced by Cu^{2+} or Pd^{2+} ions in dilute solutions by use of X-ray scattering, microscopy, and calorimetry.

As reported already, DA forms a stable bilayer vesicle in aqueous solutions with a diameter of 50–100 nm and a bilayer thickness (D_{bi}) of 3.0 nm.[4] The SAXS (small-angle X-ray scattering) profile of the DA solution shows the characteristic form factor of bilayers. It can be well fitted with a simple triple layer model consisting of two outer-layers and one inner-layer (solid line and see the Supporting Information[12]). Only when Cu^{2+} or Pd^{2+} was added to this solution (the lipid and metal concentrations are $[DA] = 6.4$ and $[Metal] = 3.2$ mM, respectively), the scattering was significantly changed as presented in Figure 2b. Multiple diffraction peaks appeared after the Cu^{2+} or Pd^{2+} addition and the relation of the peak position indicated formation of lamellar structures. By use of the Bragg relation, the lamellar spacing (L) of the DA/Cu^{2+} and DA/Pd^{2+} complex were calculated to be 4.2 and 3.6 nm, respectively. This structural change can be interpreted to mean that preceding vesicles are flattening and stacked with each other by adding the cations. In this model, the cations adhere and connect vesicles with each other (see the Fourier transformation analysis of the SAXS profile[12]). Some divalent cations such Ca^{2+} display a similar function in biological vesicles.[5]

The examined metals in Figure 2a did not induce any changes in the DA vesicle except for Fe^{2+}. Transmission electron microscopy (TEM) indicated that Fe^{2+} induced random aggregation (Figure S2[12]) thus its increasing SAXS intensity at low q can be attributed to the fractal scattering from the aggregate of the vesicles. It is believed that Fe^{2+} does not have sufficient affinity to induce the stacking of the DA bilayers.

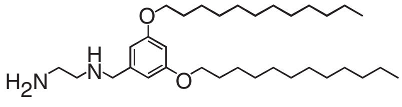

Figure 1. Chemical structure of DA.

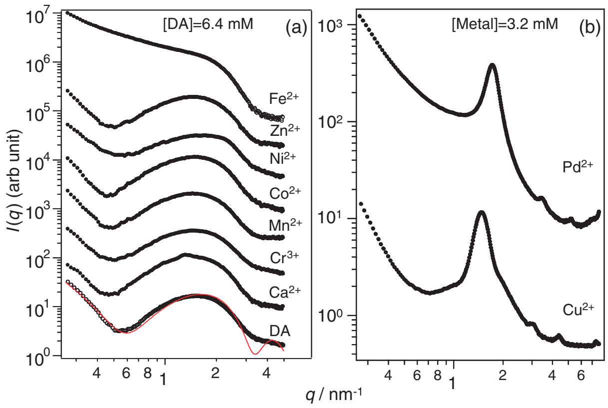

Figure 2. Comparison of the SAXS profiles upon addition of various metal ions. Before the addition, the profiles were fitted with a bilayer model (red solid line). Only addition of Pd^{2+} and Cu^{2+} induced the lamellar formation (b).

The ion selectivity for the DA may be correlated to the coordination geometry at metal ion. Cu^{2+} and Pd^{2+} complexes of ethylenediamine derivatives can have a square-planar geometry, this coordination may bind two DA lipids to form lamella. The other metals can have tetrahedral, octahedral as well as square-planar and thus be unfavorable for ordered structures. The phenomenon is now under investigation.

TEM for DA/Cu^{2+} complex confirmed self-assembly of the bilayer into lamellar structure (Figure 3). The grain size of the stacked lamellae was about 50–60 nm and L was evaluated to be 4–5 nm from the TEM image, consistent with that of SAXS.

Figure 4a shows the [Cu^{2+}] dependence of SAXS profiles. At higher [Cu^{2+}], well-developed lamellar profiles were observed, however at lower concentrations the profiles can be attributed to mixtures of lamellae and vesicles. Figure 4b plots the relative scattering intensities at $q = 1.5\,\mathrm{nm}^{-1}$ (lamellar peak) against the molar concentration ratio. The peak was not appreciable at $[Cu^{2+}]/[DA] < 0.05$ and then its intensity showed a sigmoidal increase with [Cu^{2+}], and finally the increase seemed to finish at $[Cu^{2+}]/[DA] > 0.5$. This indicates cooperative transition from vesicle to lamella, similar to the ganglioside–cholesterol/Ca^{2+} system.[5] We observed that the DA/Pd^{2+} system exhibited a similar sigmoidal growth with DA/Cu^{2+}, except for the stoichiometry: the growth of the lamella seemed to finished at $[Pd^{2+}]/[DA] = 1$ (Figure S1[12]). This difference can be related to the greater affinity of Cu^{2+}.

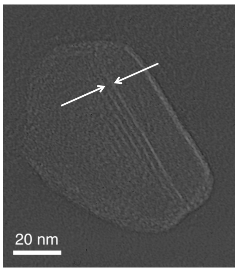

Figure 3. A typical TEM image for the DA/Cu^{2+} complex after freeze-drying, showing the bilayer stacked lamellae. The arrows indicate the adjacent layers with $L = 3$–4 nm.

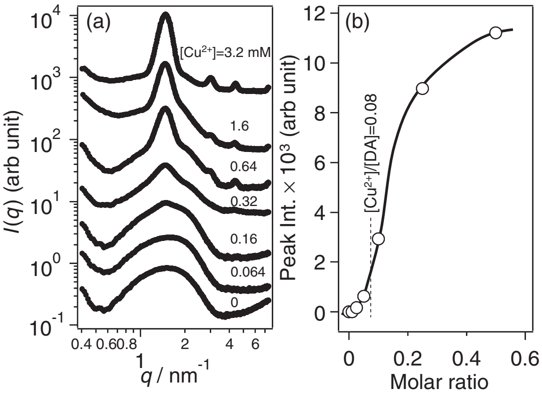

Figure 4. (a) Cu^{2+} ion concentration dependence of the SAXS profiles. [DA] = 6.4 mM for all. (b) The peak intensity plotted against Cu^{2+} ion concentration. The solid line was fitted with the data points with at the discretion of visual inspection. The dotted line indicate the transition point from I to II in ITC.

To our knowledge, there have been no reports of Pd^{2+}-induced vesicle–lamellar transition to date, although many lipids can bind various metals such as Cu^{2+},[3] Fe^{3+},[6,7] Co^{2+},[8] Hg^{2+},[9] Ru^{2+},[10] and Ag^{+}.[11] Since palladium is a rare metal that must be recovered from industrial waste, the present finding may lead to a new methods of palladium recycling.

Figure 5 shows the isothermal titration calorimetric (ITC) thermogram when $CuCl_2$ solution was added to DA solution at 25 °C. The thermogram can be divided into two regions; region I: the injection peak showed only a single sharp exothermic signal, and region II: combination of a sharp exothermic signal (a) and a broad exothermic signal (c) followed by a sharp endothermic peak (b) (for example the 9th injection is magnified in the inset). The dividing point between I and II is located at the fourth injection or $[Cu^{2+}]/[DA] = 0.08$. This point almost coincides with the ratio where the first lamellar peak becomes appreciable in SAXS (see the dotted line in Figure 4b). This coincidence suggests that the sharp exothermic peak in I can be related to coordinate interaction between Cu^{2+} and the diamine. In region II, the lamellae are formed and thus the three peaks are related to its formation. The exothermic peak (a) should be ion-pair formation similar to I. The peak (b) can be attributed to an entropy driven process and thus presumably related to rearrangement of the hydrophobic alkyl tails. In our case, its origin may be the flattening of the vesicles. Once the vesicles adhere to each other through coordination causing bilayer flattening, they can stack together to form lamellae. The driving force of the bilayer stacking is the attractive coordinate interaction between the bound Cu^{2+} on one vesicle and the amino group on other vesicle and this process is expected to be slow because of diffusion of the vesicles. This is the interpretation of the broad exothermic peak (c). In the later injections in the region II, the peak (b) was not observed since the lamellae formation was completed.

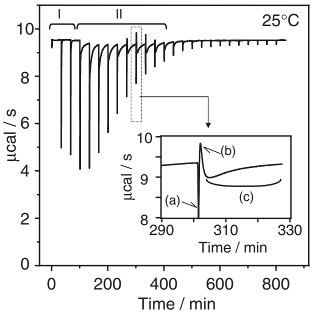

Figure 5. ITC thermograms for 5 mM $CuCl_2$ titrated into 1 mM DA at 25 °C. Inset: Magnified raw data showing three stepwise events upon addition of Cu^{2+} to DA.

In conclusion, we have found that addition of Cu^{2+} or Pd^{2+} into DA aqueous solutions induces flattening of preexisting vesicles and subsequent transformation into highly ordered lamellae by sandwiching or coordinating the cations between the ethylenediamine head groups. The lamellar peak showed a sigmoidal development with SAXS. When the lamellar peak emerged, ITC showed that three thermodynamic events occur sequentially; the first sharp exothermic signal (a) due to ion-pair formation, the second endothermic peak (b) due to the flattening of the vesicles, and the last broad exothermic signal (c) due to the bilayer stacking. This investigations into the mechanism of formation of these bilayer stacks may shed light on biomimetic and material science.

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12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.