Transition between Higher-Level Self-Assemblies of Ligand−Lipid Vesicles Induced by Cu^{2+} Ion

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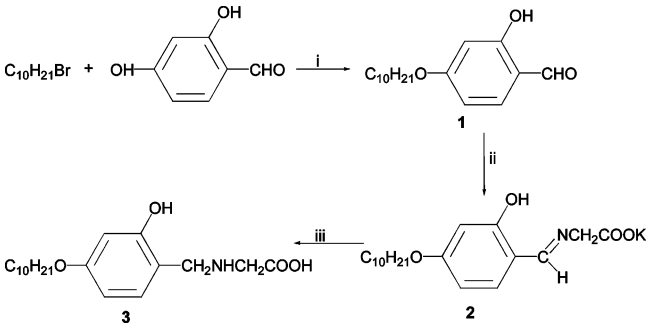
Introduction

Self-assemblies have attracted much attention because they allow the efficient formation of complex multidimensional structures from simple building blocks. Surfactant or polymeric molecules in solution can spontaneously assemble into large, stable structures with well-defined geometries, which are closely related to living systems.[1,2] Among these biomimetic structures, the simplest and most studied system is the vesicle. Proteins and other biologically active molecules can be incorporated into the vesicle bilayer or interior; therefore a vesicle can serve as a model of a biological cell.[2] However, even in the simplest cells or tissues there are invariably several levels of self-assemblies. The vesicle seems too simple to mimic a natural living system. The higher level of self-assemblies can be achieved through the specific and reversible association of vesicles into stable multivesicle aggregates by means of ligand−receptor coupling. These structures would be better models for biological tissues than a simple vesicle, and their spontaneous formation in the laboratory could lead to new methods for processing artificial tissues and "soft" composite materials.

The formation of higher-level self-assemblies from small aggregates can be brought about by a number of attractive or repulsive interactions, such as van der Waals, ion-binding, hydrophobic, polymeric bridging, and depletion forces, as well as ligand−receptor interaction.[3] Among these interactions, only the last one is specific and reversible. It was reported that the higher-level self-assemblies of lipid vesicles were obtained through bilayer-DNA[4,5] or bilayer-actin complexation,[6] metal-ion complexation,[7−9] and biotin-streptavidin molecular recognition.[10] However, little work was performed on the transition between these structures. Herein we report an observation of two higher-level self-assembled structures composed of lipid bilayers: one is an aggregate of lipid vesicles, and the other is a stacked lipid vesicle strip. Both of them are formed in a solution of ligand−lipid vesicles and induced by Cu^{2+} ion. The transition between them can be controlled artificially and reversibly by adjusting the concentration of Cu^{2+} ion.

Scheme 1[a]



[a] Reagents and Conditions: (i) KOH/CH_3OH, reflux 20 h under N_2, 80% yield; (ii) NH_2CH_2COOH, KOH/C_2H_5OH, room temperature, 2 h, 65% yield; (iii) $NaBH_4$, ice bath, 12 h, 46% yield.

Experimental Section

Materials. Dimyristoylphosphatidylcholine, 1-bromodecane, 2,4-dihydroxybenzaldehyde, glycine, copper chloride, and calcium chloride were obtained commercially. Water was distilled twice from an aqueous solution of $KMnO_4$, which was prepared more than 24 h in advance.

The ligand, (4-decyloxy-2-hydroxy-benzyl)amino-acetic acid, was synthesized in our laboratory (Scheme 1). 4-Decyloxy-2-hydroxy-benzaldehyde (**1**) was prepared according to a published procedure.[11] After being stirred with equimolar glycine in an ethanol solution of potassium hydroxide for 2 h, potassium [(4-decyloxy-2-hydroxy-benzylidene)-amino]-acetate (**2**) was precipitated. Reduction of **2** resulted in the formation of the desired compound **3**.

*Potassium[(4-decyloxy-2-hydroxy-benzylidene)-amino]acetate (**2**).* ^1H NMR (200 MHz, DMSO-d_6): δ 0.853 (s, 2H, CH_2), 1.241 (m, 21H, $C_{10}H_{21}$), 1.970 (s, 1H, CH), 6.961 (m, 3H, phenyl H). FT-IR (KBr, cm^{-1}): 3423 (OH), 2956, 2926, 2855 ($CH_3(CH_2)_9$), 1725 (C═N), 1626 (C═O). UV−vis (nm, pH = 12.0): 365, 260.

*5-Decyloxy-2-[(2-hydroperoxy-ethylimino)-methyl]-phenol (**3**).* ^1H NMR (200 MHz, DMSO-d_6): δ 0.857 (s, 3H, CH_3), 1.245−1.770 (m, 16H, $-(CH_2)_8$), 3.201 (s, 2H, $-CH_2-CO-$), 3.557 (t, 1H, $-OCH_2-$), 6.896 (m, 3H, phenyl H). FT-IR (KBr, cm^{-1}): 3343 (OH), 2927, 2855 ($CH_3(CH_2)_9$), 1724 (C═N), 1616 (C═O). UV−vis (nm, pH = 12.0): 220, 270.

Method. The ligand−lipid vesicles were prepared by mixing compound **3** with dimyristoylphosphatidylcholine (DMPC) (**3**/DMPC = 1:20 (molar ratio)) in chloroform solution and then removing the solvent thoroughly under a vacuum. The mixed lipids were hydrated in an aqueous solution of 0.01 M KOH and sonicated for 20 min at 50 °C. The concentration of DMPC was 5×10^{-3} M. The resulting ligand−lipid vesicle solution was translucent. Then it was incubated in the dark for 12 h before use. Transmission electron microscopy (TEM) was carried out on a JEM-100CX II electron microscope. The sample was prepared by freeze-fracture replication according to standard techniques with a high-vacuum freeze-etching system (Balzers BAF-400D). Turbidity was recorded on a Shimadzu UV-250 spectrophotometer. The monitoring wavenumber was set at 700.0 nm because no absorbance was found there for our system.

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(1) Israelachvili, J. *Surfactants in Solution*; Mittal, K. L., Bothorel, P., Eds.; Plenum: New York, 1986; Vol. 4, pp 3−33.
(2) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.
(3) Israelachvili, J. *Intermolecular and Surface Forces*; Academic Press: London, 1992.
(4) Rädler, J. O.; Koltover, I.; Salditt, T.; Safinya, C. R. *Science* **1997**, *275*, 810.
(5) Artzner, F.; Zantl, R.; Rapp, G.; Rädler, J. O. *Phys. Rev. Lett.* **1998**, *81*, 5015.
(6) Wong, G. C. L.; Tang, J. X.; Lin, A.; Li, Y.; Janmey, P. A.; Safinya, C. R. *Science* **2000**, *288*, 2035.
(7) Constable, E. C.; Meier, W.; Nardin, C.; Mundwiler, S. *Chem. Commun.* **1999**, 1483.
(8) Waggoner, T. A.; Last, J. A.; Kotula, P. G.; Sasaki, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 496.
(9) Li, C.; Lu, X. C.; Luo, X. Z.; Liang, Y. Q. *Chem. Commun.* **2001**, 1440.
(10) Chiruvolu, S.; Walker, S.; Israelachvili, J.; Schmitt, F. J.; Leckband, D.; Zasadzinski, J. A. *Science* **1994**, *264*, 1753.
(11) Claramunt, R. M.; Forfar, I.; Cabildo, P.; Lafuente, J.; Barberá, J.; Giménez, R.; Elguero, J. *Heterocycles* **1999**, *51*, 751.

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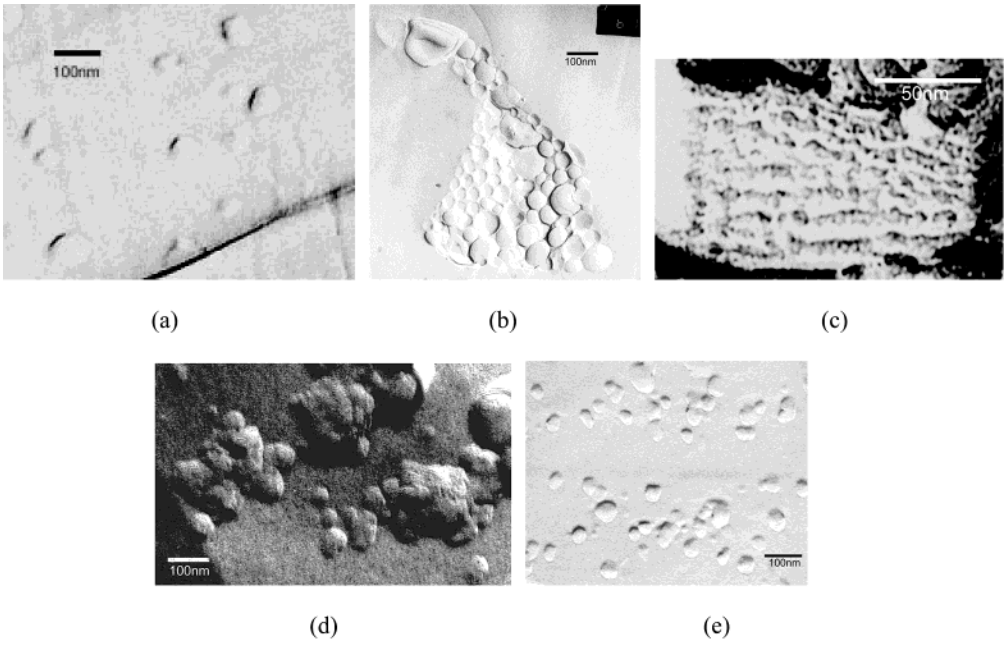


Figure 1. Freeze-fracture electron micrograph of self-assemblies formed by ligand (2.5×10^{-4} M) and lipid ([DMPC] = 5×10^{-3} M): (a) $[Cu^{2+}] = 0$; (b) $[Cu^{2+}] = 2 \times 10^{-4}$ M; (c) $[Cu^{2+}] = 10^{-3}$ M; (d) $[Cu^{2+}] = 10^{-3}$ M and [EDTA] = 10^{-3} M; (e) $[Cu^{2+}] = 10^{-3}$ M and [EDTA] = 10^{-2} M.

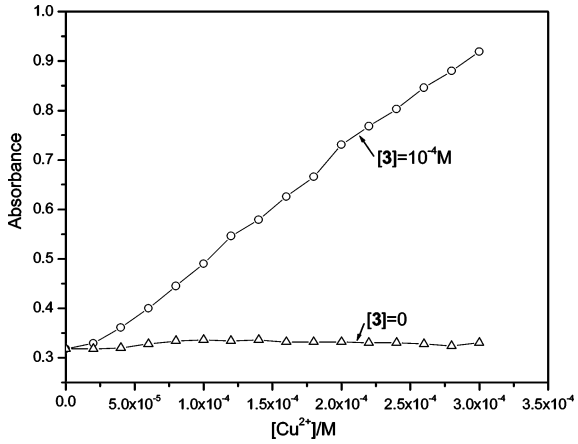
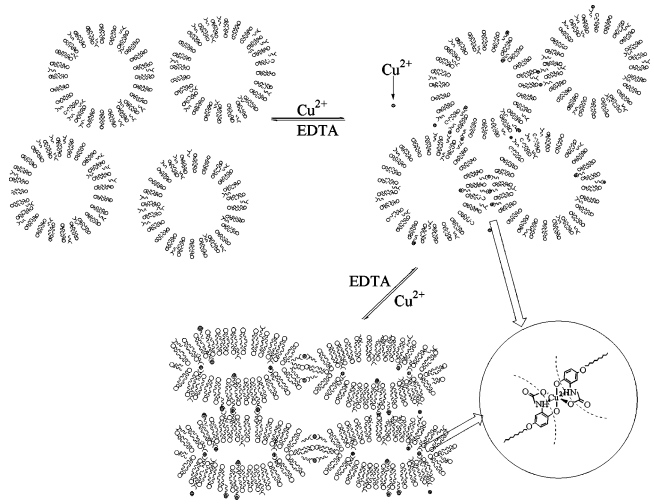


Figure 2. Relation between turbidity and $[Cu^{2+}]$ for DMPC vesicles at 30.0 °C. [DMPC] = 5×10^{-3} M, λ = 700.0 nm.

Scheme 2



Results and Discussion

In our experiment, DMPC was used as the host. The ligand−lipid vesicles were prepared as described in the Experimental Section. Freeze-fracture transmission electron microscopy revealed the presence of dispersed vesicles (see Figure 1a). The average size of vesicles provided by electron microscopy is about 50 nm.

Adding a certain concentration of Cu^{2+} (2×10^{-4} M) to the ligand−lipid vesicle solution caused the vesicles to aggregate immediately (Figure 1b). Almost all of the vesicles were present in the large aggregates; few free vesicles were observed in the micrograph. As a comparison, when Cu^{2+} was added to a solution of pure DMPC vesicles, no such vesicle aggregates but free vesicles were observed. A turbidity experiment has also been carried out at 700.0 nm. Figure 2 shows the significant difference for DMPC vesicle solutions in the absence and presence of ligand **3**. There was no obvious alternation found for the vesicle solution free of ligand. However, this was not the case for the solution containing ligand. The obvious increase in turbidity with the addition of Cu^{2+} suggested that aggregates bigger than vesicles were formed in the solution. Combined with the observation of electron microscopy, the bigger aggregate can be assigned to be a higher-level aggregate based on lipid vesicles.

Furthermore, with the addition of more Cu^{2+} (10^{-3} M), the vesicle aggregate was transformed into a stacked vesicle strip (Figure 1c). The length of the whole stacked structure is about 160 nm, and the width is about 60 nm. Each strip appears to be composed of several compressed lipid vesicles, which are on average 60 nm long and 8 nm thick. According to the thickness, the strips are assumed to consist of the vesicular bilayers with little solution between them. This structure is quite similar to the one reported by Waggoner et al.[8] except that the latter is columnar in shape.

The transition between the vesicle aggregates and stacked vesicle strips has been proved to be reversible. The addition of equimolar EDTA (10^{-3} M) resulted in the disappearance of stacked structures (Figure 1d). And a 10-fold excess amount of EDTA (10^{-2} M) leads to the decrease of vesicle aggregates and the increase of free vesicles (Figure 1e).

Table 1. Effect of Ligand and Metal Ion on the Morphology of Higher-Level Aggregates

ligand and ion	vesicle aggregate	stacked vesicle strip
2 and Cu^{2+}	yes	no
3 and Ca^{2+}	yes	no
3 and Cu^{2+}	yes	yes

We infer that the formation of vesicle aggregates and the stacked structure could be attributed to the binding of the ligand **3** to a Cu^{2+} center. The low dilution of **3** within individual vesicles means that intervesicle coordination is favored and each coordination event will clip two vesicles together. With the increase of the concentration, more Cu^{2+} exists in the interior of the vesicles. The intravesicle coordination becomes more significant. This intravesicle interaction will promote deformation of the vesicles and thus lead to the formation of stacked self-assemblies (Scheme 2).

The addition of EDTA and then sonication will remove Cu^{2+} ion from the interior, surface, and exterior of vesicles and form the complex of Cu[EDTA]. A lesser amount of EDTA will just transform the stacked vesicle strips into aggregated vesicles. With the addition of more EDTA, $[Cu^{2+}]$ will be greatly reduced due to the formation of Cu[EDTA]. As a result, the vesicle aggregation will gradually disappear, and the amount of free vesicles will be obviously enhanced.

The high affinity of Cu^{2+} to ligand **3** should facilitate the process. Two controlled experiments were carried out in order to understand the mechanism (Table 1). In the first one, we replaced Cu^{2+} with Ca^{2+}, which is of less affinity and might not provide sufficient adhesion. It could not show similar results. The experimental results suggested that it is not electrostatic but coordination interaction that induces the formation of the higher-level structure. In another controlled experiment, ligand **2** was embedded in lipid vesicles instead of **3**, and no stacked vesicle strips but only vesicle aggregates were observed after the addition of Cu^{2+}. This result can be attributed to the weaker binding ability of **2** with Cu^{2+}.

In conclusion, we have shown that Cu^{2+} can be used to induce modified lipid vesicles to form higher-level self-assemblies, and these structures can be controlled artificially and reversibly by adjusting the concentration of Cu^{2+} ion in the solution. This research may open a new vista of self-assembly studies and shed light on biomimetic and material science. Further work is going on in our laboratory.

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