Coordinatively Polymerized Bilayer Membranes Prepared with an Amphiphilic Derivative of 2-Thiazolylazophenol in Water

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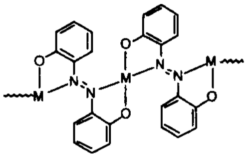
In search of new amphiphiles that form coordinatively polymerized bilayer membranes (CPBMs) in water, N-dodecyl-3-[2-hydroxy-3-(4-carboxymethyl-2-thiazolylazo)-5-methylphenyl]propanamide (**5**) was prepared. The bilayer membranes of **5** prepared in the presence of 2 equivalents of NaOH were further sonicated in the presence of transition metal ions such as Ni(II), Cu(II), Co(II), or Co(III) at >80 °C to obtain CPBMs. The CPBMs of **5** were characterized by transmission electron microscopy, gel filtration, resistivity against disruption in aqueous ethanol, visible spectra, and release of entrapped 5(6)-carboxyfluorescein. Remarkable stabilization of the bilayer membranes through coordinative polymerization was evidenced by the resistivity of the Ni(II)-CPBM of **5** against disruption in 40%(v/v) ethanol upon incubation for 20 hr.

Introduction

Bilayer membranes formed by synthetic surfactants are receiving intense interest as biomembrane models and for their ability of drug delivery, solar energy conversion, and other biomimetic functions.[1~10] Polymerization of bilayer membranes can eliminate the basic instability element of the membranes while retaining the structure and the physical characteristics of the parent membranes.[9,11~13] Coordination polymers derived from bilayer membranes could provide additional advantages originating from electronic, magnetic, or catalytic[14] properties of metal ions. Moreover, degree of polymerization of the membranes may be controlled more easily in coordinative polymerization compared with organic polymerization through variation of the content of metal ions involved in polymerization, thus allowing better balancing of fluidity and rigidity of polymerized bilayer membranes.

Previously, we have reported formation of coordination polymers (**1** or **2**; a molecular mechanics calculation indicated that **1** is more stable than **2**) from *o,o*'-dihydroxyazobenzene (DHA).[15,16] Synthetic amphiphiles consisting of polar heads and hydrophobic tails as well as rigid segments such as azobenzene moieties form bilayer membranes upon sonication in water.[17] If DHA is exploited as a rigid segment of amphiphiles as exemplified by **3**, the resulting bilayer membranes may produce coordination polymers in the presence of various metal ions.



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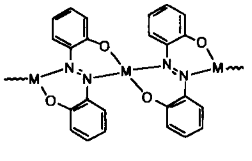

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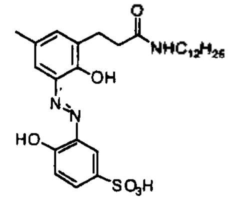
In a previous study, we have designed the first coordinatively polymerized bilayer membranes (CPBMs) with **3** by sonication in the presence of various transition metal ions in water.[18] The CPBMs of **3** may be schematically illustrated as **4**, in which circles, rectangles, and dark ellipses represent polar heads, rigid segments, and metal ions, respectively. Some of the metal ions of **4** may be missing, leading to defects in polymerization.



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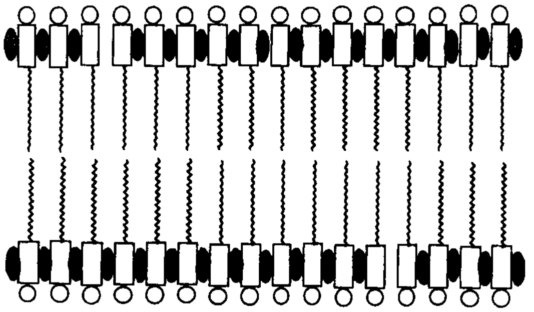

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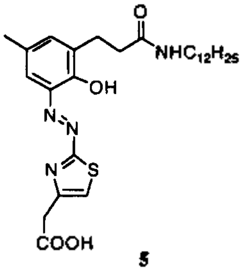
Coordinative polymerization of the bilayer membranes of **3** resulted in remarkable stabilization of the membranes as demonstrated by enhanced resistivity to disruption in aqueous ethanol. In addition, it has been shown that fluidity and rigidity of the coordinatively polymerized bilayer membranes can be balanced by changing the metal content.[18] Another unprecedented molecular function manifested by the CPBMs of **3** is fast hydrolytic cleavage of proteins. For example, chymotrypsin was multiply cleaved within a few minutes at 4 °C and pH 7.[19]

In order to expand the area of CPBMs, it is necessary to prepare additional CPBM-forming amphiphiles and characterize the resulting CPBMs. As a result of our continuing efforts to discover new CPBM-forming amphiphiles, we have prepared thiazolylazophenol derivative **5**. In this paper, the CPBMs prepared by coordinative polymerization of **5** with various transition metal ions are reported.



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Experimental Section

Preparation of *N*-dodecyl-3-[2-hydroxy-3-(4-carboxymethyl-2-thiazolylazo)-5-methylphenyl]propanamide (5). To a solution of 2-amino-4-thiazoleacetic acid (1 g; 6.3 mmol) dissolved in cold 9 M sulfuric acid (20 mL), $NaNO_2$ (0.44 g; 6.3 mmol) dissolved in 5 mL water was added slowly at 4 °C. The resulting solution was slowly added at 4 °C to a mixture obtained by mixing a solution of *N*-dodecyl-3-(2-hydrolxy-5-methylphenyl)propanamide (2.11 g; 6.3 mmol) dissolved in 100 mL ethanol with 50 mL 6 N NaOH. To the resulting deep violet mixture, 6 N HCl was added slowly until the mixture turned deep brown. The resulting precipitates were collected by filtration and dissolved in hot ethyl acetate. After the solution was dried with $MgSO_4$, the product was separated on silica gel by eluting with a 10 : 5 : 1 mixture of methylene chloride, ethyl acetate, and acetic acid, and was further purified by recrystallization from ethyl acetate-hexane, mp 178-180 °C. ^1H NMR (DMSO-d_6): 0.63-1.47 (m, 23H); 2.25 (s, 3H); 2.35-2.62 (t, 2H); 2.68-2.90 (t, 2H); 2.95-3.26 (q, 2H); 6.87-6.97 (m, 3H). Anal. Calcd. for $C_{27}H_{40}N_4O_4S$: C, 62.70; H, 7.80; N, 10.87; S, 6.21. Found: C, 62.25; H, 8.10; N, 10.84; S, 6.18.

Membrane Formation. Bilayer membranes of **5** were prepared by sonication at >80 °C of a solution obtained by mixing **5** (2.6 mg; 5 μmol), 200 μL water, and 200 μL of 0.050 N NaOH (10 μmol) solution. To the resulting dark brown solution of the bilayer membrane of **5**, 100 μL of 0.050 M solution of $CoCl_2 \cdot 6H_2O$, $[Co(NH_3)_5Cl]Cl_2$, $NiCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, or $ZnCl_2$ was added. Upon sonication for more than 15 min at >80 °C, CPBM of **5** was produced. When 250 μL solution of 0.30 M *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (pH 7.50) was added, pH of the resulting solution became 7.50.

Physical Measurements. Transmission electron microscopic measurement of bilayer membranes was carried out with a Jeol-200cx electron microscope operating at 200 kV. The bilayer membranes prepared in the absence of transition metal ions were negatively stained with a 2% (by weight) solution of uranyl acetate. Bilayer membranes prepared in the presence of transition metal ions were subjected to transmission electron microscopic measurements without treatment with uranyl acetate. Sonication was carried out with a Sae Han SH-50 sonicator. Differential scanning calorimetric measurements of bilayer membranes prepared with 10 mM **5** in the presence or absence of transition metal ions were performed with a Stanton Redcroft DSC calorimeter. UV-visible spectra were obtained with a Beckman DU-70 spectrophotometer. Fluorescence spectra were obtained with a Japan Spectroscopic Co. Fp-777 spectrofluorometer.

Results and Discussion

Amphiphile **5** formed bilayer membranes upon sonication at >80 °C in water when 2 equivalents of NaOH was added. The resulting bilayer membranes turned into precipitates when kept at room temperature within few days.

The bilayer membranes of **5** prepared in the presence of 2 equivalents of NaOH were further sonicated in the presence of transition metal ions such as Ni(II), Cu(II), Co(II), Co (III), or Zn(II) at >80 °C. Intense colors (deep blue for Ni(II) and deep green for Co(II), Co(III), or Cu(II) ion) were developed upon sonication of the bilayer membranes in the presence of the transition metal ions. The bilayer membranes prepared in the presence of Cu(II) or Zn(II) turned into precipitates when pH was lowered to 7.5, whereas those prepared with Ni(II), Co(II), or Co(III) were stable even at pH 7.5. In the presence of Fe(II) or Fe(III) ions, the bilayer membranes of **5** formed precipitation immediately. Transmission electron micrographs of the self-assemblies of **5** prepared in the absence or presence of Ni(II), Co(II), Co (III), or Cu(II) are illustrated in Figure 1. The bilayer membranes prepared in the absence of transition metal ions were rodlike. For those prepared in the presence of Ni(II) or Cu (II) ion, spherical shapes were dominant. Those prepared in the presence of Co(II) or Co(III) ion were mostly rodlike with minor amounts of spherical shapes.

When the bilayer membranes prepared in the presence of transition metal ions were subjected to gel filtration on Sepharose 4B, the bilayer membranes were eluted prior to

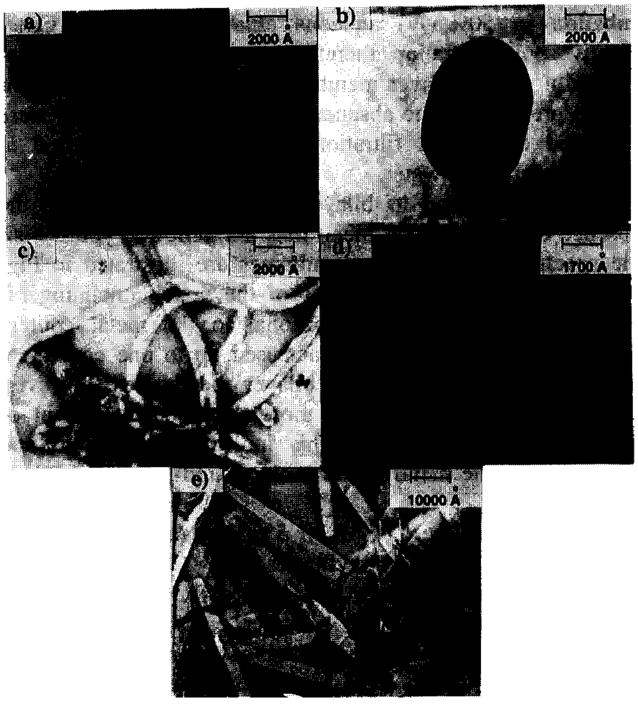


Figure 1. Transmission electron micrographs of assemblies of various bilayer membranes of **5** prepared in the presence of an equivalent of Ni(II) (a), Co(III) (b), Co(II) (c), or Cu(II) ion (d) or in the absence of any transition metal ion (e).

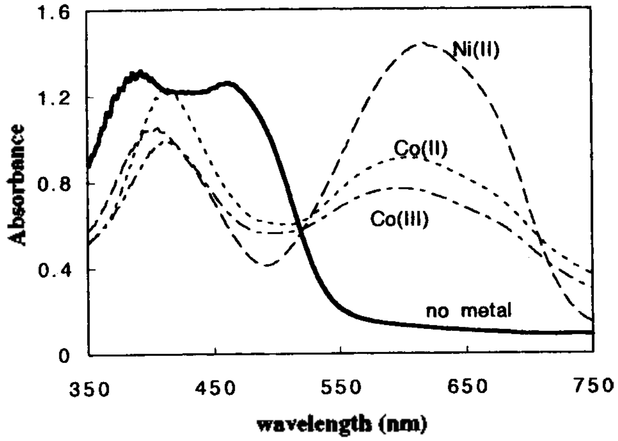


Figure 2. Visible spectra of bilayer membranes of **5** prepared in the presence of Ni(II), Co(II), or Co(III) or in the absence of any transition metal ion. The bilayer membranes were prepared with 0.12 mM **5**. Visible spectra of bilayer membranes of **5** prepared in the presence of Cu(II) or Zn(II) were similar to those prepared in the presence of Co(II) or Co(III).

the blue dextran marker (MW 2×10^6) with a minor amount of unassembled amphiphile being eluted slowly. Sepharose 4B is generally employed in separation of globular proteins with molecular weights of 6×10^4-2×10^7 or dextrans with molecular weights of 3×10^4-5×10^6. When the bilayer membranes prepared with Ni(II) was tested right after and one week after preparation, almost identical separation patterns were obtained. The Ni(II)-bilayer membranes were not disrupted even after storage in 40%(v/v) ethanol solution for 24 hr at room temperature as checked by the gel filtration. The Ni(II)-bilayer membrane was totally disrupted during incubation in 50%(v/v) aqueous ethanol for 3 hr. Coordinative polymerization, therefore, results in remarkable stabilization of the bilayer membranes of **5**. The bilayer membranes prepared in the absence of transition metal ions were disrupted during gel filtration even without incubation in ethanol-containing water.

Amphiphile **5** and its bilayer membranes obtained in the absence of the transition metal ions are yellow. Typical visible spectra of the bilayer membranes are illustrated in Figure 2. The absorbance changes of the metal-containing bilayer membranes were proportional to the metal content when the metal content was increased up to one equivalent of **5**. The absorbance of the bilayer membranes, however, did not change further when more than one equivalent of the metal ion was added. This indicates that up to one equivalent of the metal ion is incorporated into the CPBMs of **5**.

The fluidity of the CPBMs of **5** was examined by release of entrapped 5(6)-carboxyfluorescein (CF). When the CPBM was formed in the presence of 0.2 M CF and purified by gel filtration, CF becomes entrapped within the CPBM. Due to the self-quenching of fluorescence at high concentrations of CF, CF is weakly fluorescent when entrapped within the CPBM but becomes strongly fluorescent upon release from the CPBM. By following the increase in the fluorescence (λ_{ex}=494 nm, λ_{em}=514 nm) of CPBM with CF entrapped within, the amount of CF released was measured and the results are summarized in Table 1. The results indicate that the fluidity increases noticeably between 35 °C and 45 °C.

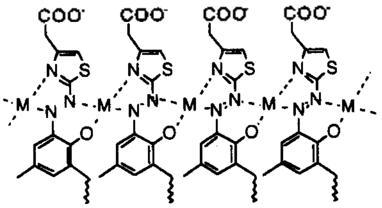
Table 1. Degree of release over the period of 30 min of CF entrapped in the Co(III)-CPBM or Ni(II)-CPBM of **5**

	25 °C	35 °C	45 °C	55 °C
Ni(II)-CPBM	6%	45%	86%	95%
Co(III)-	28%	33%	62%	96%

[a] The amount of released CF was measured by fluorescence increase during the period of incubation and the total amount of CF was measured by fluorescence increase over 5 days.

Thermal analysis by differential scanning calorimetry of the CPBMs did not indicate phase transition temperatures below 70 °C. Detection of phase transition temperatures provides strong evidence for regular structures of bilayer membranes. However, there are several preceding reports of failure to detect phase transition temperatures even if bilayer membranes were formed.[20]

In summary, treatment of amphiphile **5** with 2 equivalents of NaOH and sonication of the resulting anionic amphiphile at >80 °C in the presence of various transition metal ions produced CPBMs. When the metal ion used was Ni(II), Co(II), or Co(III), the CPBM was stable even when the medium was neutralized to pH 7.5. Enhancement in the bilayer membrane structure was demonstrated by resistivity to disruption in 40%(v/v) aqueous ethanol. Fluidity of the CPBMs was checked with release of CF entrapped within the bilayer membranes. The CPBMs prepared with **5** can be also schematically represented by **4**. Here, the polar heads are carboxylate anions and the rigid bodies are the thiazolylazobenzene moieties. In view of the previous report for the coordination of thiazoles to metal ion through the nitrogen atom instead of the sulfur atom,[21] the portion of the coordination polymers involved in the CPBMs of **5** can be illustrated by **6**.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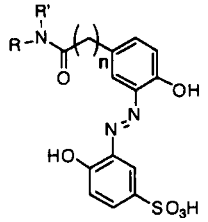
The first CPBM has been prepared with amphiphile **3** in the previous study.[18] In search of additional amphiphiles that form CPBMs, analogues of **3** such as **7-10**, have been prepared.[18] In spite of structural features closely related to **3**, amphiphiles **7-10** failed to produce CPBMs in the presence of various transition metal ions although they formed bilayer membranes in the absence of transition metal ions. This suggests that minute structural changes in the bilayer membranes can lead to failure in coordinative polymerization. This is not surprising since the bond angles and bond lengths of the coordination sphere of the CPBMs must match the geometry of the hydrophobic tails assembled in a large number in order to reduce the strains of the super structure of the CPBM.



7: $n = 1$, $R = C_{12}H_{25}$, $R' = H$
8: $n = 1$, $R = R' = C_{12}H_{25}$
9: $n = 2$, $R = C_{12}H_{25}$, $R' = H$
10: $n = 2$, $R = R' = C_{12}H_{25}$

Compound **5** is the second amphiphile ever discovered to form CPBMs in water. Apparently, the structure of the coordination sphere of the CPBMs of **5** matches the geometry of the assembly of the hydrophobic tails. Many molecular functions can be expected from the CPBMs as exemplified by the hydrolytic cleavage of chymotrypsin by the Co(III)-CPBM of **3** within a few minutes at pH 7 and 4 °C.[19] In this respect, characterization of additional functions of CPBMs can be undertaken with both **3** and **5**.

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Theoretical Studies on the Base-Catalyzed Deprotonation of 4-Phenacylpyridinium Cations

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Theoretical studies on the base-catalyzed deprotonation of 4-phenacylpyridimium cations, R^1-CO-CH_2-C_5H_4N-R^2, **I** (R^1=YC_6H_4- and R^2=CH_3), and **II** (R^1=C_6H_5 and R^2=$CH_2C_6H_4Y$) have been carried out with bases, NH_3 and $XC_6H_4NH_2$ using AM1 MO method. The Brϕnsted α values are 0.20 and 0.22 and the β_B values are 0.62 and 0.61, respectively for cations **I** and **II**. The negative I (=α-β_B) values obtained are in accord with the experimental results in aqueous solution, although the theoretical gas-phase α values for **I** are somewhat smaller than the experimental values in water due to neglect of solvation effect. It has been stressed that the Brϕnsted α is distorted not only by the lag in the resonance and solvation development in the carbanion, but also by the difference in the distance between the anionic center and substituents in the TS and in the product anion.

Introduction

In most chemical reactions, more than one process occurs concurrently: typically, bond formation or cleavage may be accompanied by solvation or desolvation, delocalization or localization of charge, hyperconjugative transfer of charge, etc. However, often these processes make unequal progress at the transiton state (TS) leading to an "imbalanced" TS.[1] In such situation, the progress of reaction at the TS depends on which process is adopted as the reaction coordinate.

Transition-state imbalances are a common phenomenon in many reactions including proton transfers involving most