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Comparison of the extraction behavior of metal ions by metal affinity ligands in an organic-aqueous two-phase system and in a liposome system

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The interaction of various metal ions with simple metal affinity ligands, 3-(hexadecylthio)propionic acid (HTP) and 2-(hexadecylthio)benzoic acid (HTB), was investigated in an organic/aqueous two-phase system and in a liposome system. Both ligands exhibited a high silver(I) affinity at low pH in the organic/aqueous two phase system but low selectivity in the extraction of other transition metal ions such as Cu(II), Ni(II), Co(II), Zn(II), Cd(II), and Hg(II) above pH 6. On the other hand, remarkably different behavior for metal adsorption was observed in the liposome system with HTP and HTB. HTB lost the ability to interact with metal ions when modified on the liposome surface. This may be attributed to the formation of a hydrogen bond between the carboxyl group of the HTB and the phosphate group of the phospholipid. The HTP-modified liposome effectively adsorbed metal ions of the zinc family, such as Zn(II) and Cd(II), which could interact with the phosphate group of phospholipid, and also adsorbed those transition metal ions, which could be extracted in the organic/aqueous two-phase system. This may be caused by two functions of the liposome, i.e., a gate function for metal ions at the surface of the HTP-modified liposome and additional stabilization by interaction between Cu(II) and the phosphate group of the phospholipid.

1. Introduction

The extraction of metal ions with metal affinity ligands has been used for hydrometallurgical processes and for wastewater treatment. Well-designed ligands such as the LIX series [1] have been utilized to separate copper(II) from copper ore leach liquors. In general, ligands that interact with copper(II) extract not only copper(II) but also other metal ions such as nickel(II) and cobalt(II) at high pH. A ligand with a higher affinity for copper(II) is required in the case of ammonia leaching solutions [2]. However, it is very difficult to design a highly selective and effective ligand for a specific metal ion from the properties of a single ligand.

Liposome is a highly-organized self-asssembly, which consists of a closed phospholipid bilayer membrane and has a hydrophobic interface of 4~5 nm inside the membrane. In addition, the liposome can undergo changes in response to environmental stresses, such as temperature [3], pH, and oxidation [4]. It is well known that hydrophobic compounds can be inserted in the liposome membrane and such membrane-inserted molecules have different characteristics compared to those in homogeneous solution. The difference is related to the orientation of the inserted molecules in the liposome as well as interaction with the phospholipid composing the liposome. It has been reported that the orientations and distributions of the membrane-inserted molecules are directly correlated with their structure [5]. The metal affinity ligands are, thus, expected to exhibit different abilities for metal interaction in the liposome system modified with the ligands as compared with to those in homogeneous solution.

The purpose of the present study is to compare the extraction behavior of metal ions by metal affinity ligands in both an organic/aqueous two-phase system and in a liposome system. The complex formation of the metal ion with our synthetic ligands (HTP and HTB), which differ in the bulkiness of their structure, was first investigated in an organic/water two-phase system. The effect of the modification of the ligands in the liposome on their metal interaction ability was then examined.

2. Experimental

2.1. Materials

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids Inc (Alabaster, AL). 2-Morpholinoethanesulfonic acid (MES) and 3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid (EPPS) were purchased from Dojindo (Kumamoto, Japan). Calcein was purchased from Sigma (St. Louis, MO). Phen Green was purchased from Molecular Probes (Eugene, OR). The other chemicals were of commercially guaranteed reagent grade and used without further purification. 3-(hexadecylthio)propionic acid (HTP) and 2-(hexadecylthio)benzoic acid (HTB), which were used as the functional ligands were synthesized according to a previously described method [6] with slight modification. 3-Mercaptopropionic acid or thiosaligenine and potassium carbonate were dissolved in chloroform. Under reflux conditions, hexadecylbromide was added in a dropwise manner. The mixture was refluxed at 60°C for 24 h. After cooling to room temperature, the mixture was evaporated and was washed with 4N HCl solution several times. The organic solution recovered was concentrated and was purified by column chromatography over silica gel to obtain a pure liquid. The identification of products were carried out by ^{1}H-NMR and ^{13}C-NMR using a Bruker AL400 (400MHz) NMR spectrometer.

2.2. Metal extraction in the aqueous/organic two phase system

The aqueous phase was prepared by dissolving metal nitrate into 1 M aqueous ammonium nitrate solution. The pH was adjusted by adding a small amount of nitric acid or

ammonia. The organic phase was prepared by diluting ligands with chloroform. Selectivity for metal ions was measured batchwise at 303 K for 24 h. The initial concentration of each metal was 1 mM.

2.3. Liposome Preparation

Liposome solutions were prepared by the following procedure. Ligand and POPC were dissolved in chloroform/methanol; the solvent was evaporated and the resulting thin film was dried for 2 h under vacuum. The lipid film was hydrated by dispersing in 1M ammonium nitrate and 50 mM MES or EPPS buffer to form multilamellar vesicles. The multilamellar vesicle suspension was frozen in dry ice-ethanol (-80°C) and then was dispersed at above the phase transition temperature for five cycles. The solution so obtained was then passed through two stacked polycarbonate filters of 100-nm pore size by using an extrusion device.

2.4. Metal adsorption in the ligand-modified liposome system

The metal adsorption experiment was carried out by shaking the liposome suspension in the presence of metal ion at 25°C for 24 h. After the above operation, the liposome and metal ion were separated by ultralfiltration (Ultra free MC; Millipore) at the specific centrifugal condition of 5000 G for 10 min. The concentration of metal ion in the filtered solution was measured by using metal ion fluorescent indicators, such as calcein and Phen Green.

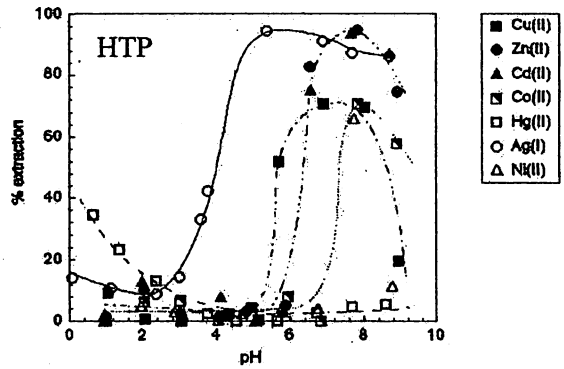

Fig.1 Effect of pH on the extraction of various metal ions in the chloroform/1 M ammonium nitrate system with HTP. Concentration of HTP is 10 mM. Initial metal concentration is 1 mM.

3. Results and Discussion

3.1. Metal extraction with ligands from the aqueous to the organic phase

Figures 1 and **2** show the effect of pH on the extraction of metal ions with HTP and HTB in the chloroform/1M ammonium nitrate system. In general, both ligands exhibit a similar extraction behavior for each metal ion. HTP and HTB possess thioether-type sulfur and carboxyl-type oxygen as the coordination sites for metal ions. It is

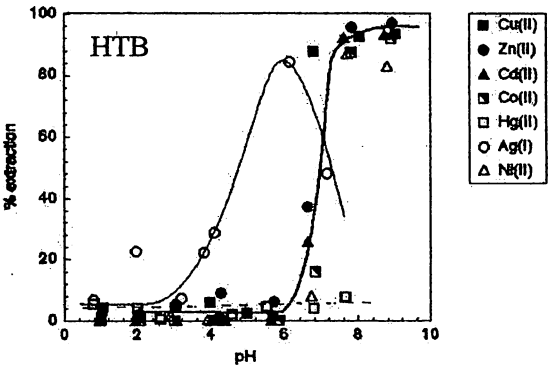

Fig. 2 Effect of pH on the extraction of various metal ions with HTB in the chloroform/1 M ammonium nitrate solution system. Concentration of HTB is 10 mM. Initial metal concentration is 1 mM.

considered that the affinities of metals for both sulfur and oxygen will be important for metal extraction. In the pH range 0~3 which the carboxyl group proton can not dissociate, "soft" metal ions such as silver(I) and mercury(II) were extracted with HTP but not HTB. This difference in the extraction behavior between HTP and HTB is attributable to the electron density on the sulfur atom of the ligand. The sulfur atom of HTB may have a relatively lower electron density than that of HTP because of the resonance effect with the benzene ring of HTB. At a higher pH range (pH3~6), in which the carboxyl group proton dissociates to give a negative charge, silver(I) ion was predominantly extracted with both ligands, although the other transition metal ions were not extracted. This selectivity for silver(I) extraction may suggest that silver(I) has an affinity for both the thioether-type sulfur and carboxyl-type oxygen of the ligands. The extraction of transition metal ions such as copper(II), nickel(II), cobalt(II), zinc(II), and cadmium(II) took place at a relatively high pH(5~10). Rather different behavior was observed between the two ligands; in the case of HTP, metal ions were extracted with increasing pH in the following order: Cu(II), Zn(II), Cd(II), Co(II), Ni(II), while there was no difference between any of the metal ions in the case of HTB. It is shown that the extraction of metal ions with HTB may be related to the contribution of the carboxyl oxygen only and is independent of that of sulfur. This difference between the coordination ability of the two ligands for metal ions may cause the difference in their extraction behavior at high pH (pH>8) in which metal ions form ammine complexes with ammonia. In the case of "soft" silver(I), the silver(I) complex with HTP, which has a high stability due to the high electron density on the sulfur atom, is extracted at high pH. On the other hand, the percent extraction of silver(I) with HTB is reduced by the formation of the silver(I)-ammine complex. In the case of "intermediate~hard" metal ions such as Cu(II), Ni(II), Co(II), Zn(II), and Cd(II), the opposite phenomena occurred.

However, there is a little difference in the metal extraction behavior between HTP and HTB in the organic/aqueous two-phase system. It was thus found that HTP and HTB could have a similar metal affinity in the organic/aqueous two-phase system, in which the ligand can move without sterical and physicochemical restriction.

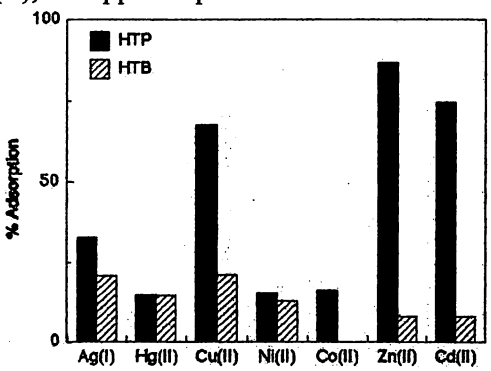

Fig. 3 Percent adsorption of metal ions on metal affinity ligand-modified liposome at pH 8. Concentration of HTB and phospholipid are 10 mM and 50mM, respectively. Initial metal concentration is 1 mM. Buffer: 1 M ammonium nitrate, 50 mM EPPS , pH 8. After metal adsorption, the liposome was separated from the solution by ultrafiltration. Non adsorbed metal concentration was measured by using a fluorescence probe, calsein or phen green.

3.2. Metal adsorption on ligand-modified liposome

Figure 3 shows the adsorbed amount of metal on HTP- and HTB-modified liposome at pH 8 in which there is no

selectivity for metal ions with HTP and HTB in the organic/aqueous two-phase system(**Fig. 1** and **2**). In the case of HTP-modified liposome, the metal ions of the zinc family, such as zinc(II) and cadmium(II), were adsorbed on the liposome surface to a greater degree compared to that of silver(I) and copper(II). It has been reported that a zinc(II) ion and the phospholipid membrane can interact with each other on the liposome surface. The above results suggest that the liposome phosphate group was involved in the zinc family adsorption on the HTP-modified liposome, as described by Binder et al. [7]. On the other hand, the adsorption of the zinc family was not detected in the case of the HTB-modified liposome. This behavior can be qualitatively explained by the difference in the chemical structure as schematically shown in **Fig.4**. HTP is a compact linear structure. Thus, HTP has a high affinity for the hydrophobic part of the phospholipid and locates in the depths of the liposome membrane. On the other hand, because of its benzene ring HTB has a bulky structure and the coordination group is located on the liposome surface and forms a hydrogen bond with the phosphate group of the phospholipid. The liposome modified with HTB could not adsorb the zinc family due to the hydrogen bonding network between the phospholipid and HTB. In the HTB-modified liposome, the amounts of other metal ions adsorbed as well as those of the metal ions of the zinc family were also significantly low, as compared to those of the HTP-modified liposome (**Fig.3**). The formation of the hydrogen bond network in the HTB-phospholipid system can also explain the low amount of adsorption of other metal ions on HTB.

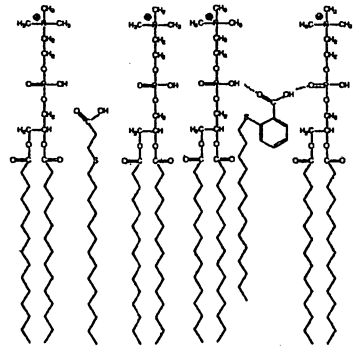

Fig. 4 Schematic illustration for the interaction between (A) HTP or (B) HTB and phospholipid in the HTP- and HTB-modified liposome. HTP locates in the depths of the phospholipid membrane. HTB locates at the membrane interface and forms a hydrogen bond with the phospholipid. Thus, HTB-modified liposome can hardly adsorb metal ions of the zinc family, which are capable of interacting with the phosphate group of the phospholipid, and other metal ions, which are capable of interacting with HTB.

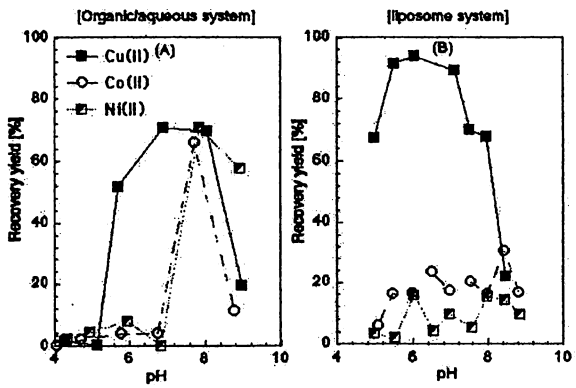

Fig.5 Comparison of the recovery yield of Cu(II), Ni(II), and Co(II) from the diluted aqueous solution between the organic/aqueous two-phase and liposome system with HTP.

There are many factors affecting the metal adsorption of the liposome system modified with

a metal affinity ligand; adsorption of metal ion on the phospholipid composed of liposome, adsorption on the metal ligand, and interaction between the phospholipid and the metal ligand. These characteristics suggest the possibility that metal adsorption could be controlled by designing the type of phospholipid and by changing the membrane properties due to environmental stress such as temperature and pH.

3.3. Comparison of metal interaction between the organic/ aqueous two phase and the liposome system

Figure 5 compares the recovery yield of Cu(II), Ni(II), and Co(II) with HTP in the organic/aqueous two phase and in the liposome systems. These metal ions have similar electronic properties, resulting in difficulties in their separation. **Figure 5(A)** indicates an incomplete separation ability of HTP in the organic/aqueous two phase system, especially above pH 8. On the other hand, the HTP-modified liposome exhibited high selectivity for Cu(II) over the wide pH range measured here, as shown in **Fig.5(B)**. The appearance of the selectivity for Cu(II) in the HTP-modified liposome could be attributed to the distribution state of HTP on the liposome surface. As shown in **Fig.4**, HTP was considered to be localized at depth in the liposome membrane, resulting in the formation of a barrier to the metal ion interaction. This barrier contributed to the appearance of the selectivity for Cu(II) due to steric hindrance and electrostatic repulsion of the positive charged coline group. Cu(II), which can bypass the above barrier, is thought to form a complex with HTP. Further, the interaction between Cu(II) and the phosphate group may provide an additional stabilization for the Cu(II)-HTP complex. These factors (Cu(II) affinities for HTP and the phosphate group, steric hindrance, and electrostatic repulsion) may be the reason for the Cu(II) selectivity on the HTP-modified liposome.

As compared with the results in **Fig.5 (A)** and **(B)**, the recovery yield of Cu(II) in the liposome system was found to be significantly higher than that of the organic/aqueous two-phase system. The interaction between Cu(II) and HTP can be calculated as 1: 1 coordination in the liposome system. In this situation, there are two or four vacant coordination sites for Cu(II). Thus, Cu(II) adsorbed on HTP can acquire an additional stability by interacting with the phosphate group in the empty coordination sites.

In general, it is very difficult to provide both a high stability constant between a metal ion and a ligand and a high selectivity for a specific metal ion in the organic/aqueous two-phase system. The liposome system, which possesses a hydrophobic interface, 4~5 nm in depth, may possibly provide the effective properties such as selectivity and the ability for metal adsorption; a ligand having a general affinity for metal ions and liposome having selectivity and additional stability.

4. Conclusion

It was found that metal affinity ligands, HTP and HTB, had similar properties in relation

to metal interaction in the organic/aqueous two-phase system; silver(I) was extracted above pH 4, and other transition metal ions were extracted above pH 6. In the liposome modified with the ligands, a difference of ligand type on metal adsorption was observed. The adsorption ability of the HTB-modified liposome was significantly low. This inhibition for metal adsorption was caused by the formation of a hydrogen bond between HTB and the phosphate of the phospholipid composed of the liposome. On the other hand, the HTP-modified liposome exhibited a high adsorption ability, especially for the metal ions of the zinc family, Zn(II) and Cd(II), by interacting with the phosphate group of the phospholipid. Further, high selectivity and high extractability of Cu(II) was observed in the liposome system compared with that for the organic/aqueous two-phase system. This could be due to two functions of the liposome, i.e., a gate function for metal ions at the surface of the HTP modified liposome and additional stabilization by the interaction between Cu(II) and the phosphate group of the phospholipid.

The liposome system may be able to selectively adsorb metal ions by controlling the membrane properties.

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