ESI-MS Analysis of Tetrapyridinium Macrocycle Complexation with Carboxylic Anions

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ESI-MS Analysis of Tetrapyridinium Macrocycle Complexation with Carboxylic Anions

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Complex formations of a quaternary tetrapyridinium macrocycle ($Cpy4Br_4$) with anionic species, mono-, di-, tri-carboxylic acids, adenocine 5′-triphosphate (ATP), diphosphate (ADP), and monophosphate (AMP) were observed in a neutral pH region. Electrospray ionization mass spectrometry (ESI-MS) was used to study reactivity and selectivity of the complex formations between the Cpy4 cation and the guest anions.

1. Introduction

Highly selective host–guest complex formations of anions play an important role in a biological process.[1] A full understanding of molecular mechanism of the complex formations in the biological process is of fundamental importance, and energetic efforts have been made in order to mimic the complex formations between synthetic organic molecules.[2] Synthetic macrocyclic or macropolycyclic polyammonium molecules[3),4] have been used to form complexes by electrostatic interaction with guest anions, such as carboxylic acids and phosphoric acids.[5)–7] Furthermore, studies for designing the molecular structures of macrocycles have been carried out to increase stability and structural selectivity toward the anions. An NMR or acid-base titration method has been used to study the complex formations between host molecules and organic anion species.[7)–10]

Electrospray ionization mass spectrometry (ESI-MS) has become known as an effective technique for the characterization of status of ion species in solution. ESI-MS is characteristics for its being a soft-ionization to produce multiply charged ions. The technique can extract weakly bound complex species directly from the solution into the gas phase without destroying the complex and perform a mass spectrometric analysis. It is an excellent technique for the analysis of ionic composition in solution. Many studies have been carried out on host–guest complex formation with cations as guest ion,[11)–13] but little has been reported on a host–guest reaction with anions as guest ion except a report by Collette *et al.*[14] They studied the host–guest complex formations between neutral receptors, a macrocycle and macropolycyclic polyamine, and dicarboxylic acids (HO_2C–$(CH_2)_n$–CO_2H) by ESI-MS in both a positive and negative-ion mode. The complex formations in an acidic region were detected and the stability of the complex in the gas phase was studied by MS/MS measurement.

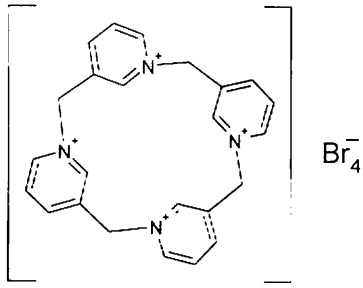

Fig. 1. $Cpy4Br_4$

$Cpy4Br_4$ (structure shown in Fig. 1) is a unique quaternary tetrapyridinium macrocycle, which differs from an ordinary macrocycle or macropolycyclic polyamine by possessing the positive charge even in a neutral or weak alkaline region. We have carried out the ESI-MS study on the complex formation of Cpy4 cation with various anion species, mono-, di-, tricarboxylic acid, adenocine 5′-triphosphate (ATP), diphosphate (ADP), and monophosphate (AMP) (structures in Table 1).

2. Experimental

ESI mass spectra were obtained with a sector-type mass spectrometer (JEOL-D300) connected with a laboratory-made ESI interface. The ESI ion source is described in detail elsewhere.[15] Briefly, a sample solution was sprayed at the tip of a needle by applied voltage of 3.5 kV. A distance between the needle and the counter electrode was *ca.* 1 cm. The counter electrode was made from a 12-cm long capillary tube (i.d. 0.5 mm) of stainless steel. The solvent in charged droplets was evaporated by a counter-current N_2 gas at approximately 50℃. Ions passed a vacuum system through the first, the second skimmer and a lens system, and entered the mass analyzer. The electrospray was performed with a sample flow-rate 2.5 μL/min. The voltage ΔV between the first and second skimmer was set at 50 V. Collision-induced dissociation (CID) occurs between the two skimmers in the ESI interface.

Both the macrocycle $Cpy4Br_4$ and the guest anions were dissolved in water–acetonitrile (1 : 1, v/v) at 0.2

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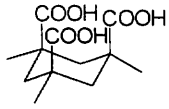
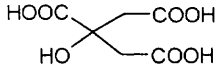
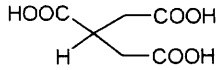
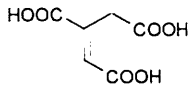
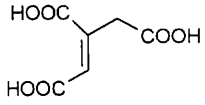
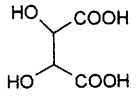
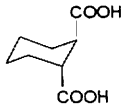
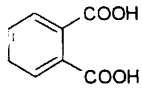
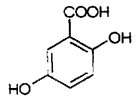
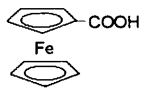
Table 1. Complexation of Cpy4 Cation with Carboxylic Acid Anions in ESI Mass Spectra

	Guest anions		Mass	Ion (m/z)	Ratio*
	Tricarboxylic acids				
1	Kemp's acid		258	$[M-4X+C-2H]^{2+}$ (312) $[M-3X+C-2H]^{+}$ (703)	0.34
2	*cis,cis*-1,3,5-Cyclohexane tricarboxylic acid		216	None	—
3	Citric acid		189	None	—
4	Tricarboxylic acid		176	None	—
5	*trans*-Aconic acid		174	$[M-4X-C-3H]^{+}$ (539) $[M-3X+C-2H]^{+}$ (618)	0.09
6	*cis*-Aconic acid		174	$[M-4X+C-3H]^{+}$ (539) $[M-3X+C-2H]^{+}$ (618)	0.08
	Dicarboxylic acids				
7	L-Tartalic acid		150	None	—
8	1,2-*trans*-Cyclohexane dicarboxylic acid		172	$[M-3X+C-H]^{2-}$ (309) $[M-3X+C-2H]^{+}$ (618) $[M-4X+C-2H]^{2+}$ (269)	0.26
9	Phthalic acid		166	$[M-4X+C-2H]^{2+}$ (266) $[M-3X+C-H]^{2+}$ (306)	0.09
	Monocarboxylic acids				
10	2,5-Dihydroxybenzoic acid		155	$[M-3X+C-H]^{2+}$ (301) $[M-4X+2(C-H)]^{2-}$ (338)	0.12
11	Ferrocenecarboxylic acid		230	$[M-3X+C-H]^{2-}$ (338) $[M-4X+2(C-H)]^{2+}$ (413)	0.38
12	Acetic acid		60	None	—
	Adenosine-5′-tri-, di-, mono-phosphate				
13	ATP·2Na		551	$[M-4X+A-2Na]^{2+}$ (437)	0.07
14	ADP·2Na		471	$[M-4X+A-2Na]^{2+}$ (397)	0.06
15	AMP·2Na		391	$[M-4X+A-2Na]^{2+}$ (357) $[M-3X+A-2Na+H]^{2+}$ (397)	0.07

* The complex ion intensity relative to sum of ionic Cpy4 species.
M, $Cpy4Br_4$; C, guest's carboxylic acids; X, Br^- anion; A, adenosine phosphates.

mM concentration. Since $Cpy4Br_4$ tends to decompose in an acidic pH, the solution was neutralized to pH 7 by the addition of $NaHCO_3$. $Cpy4Br_4$ was synthesized by the published method.[16] The anions **1**, **2**, and **8** (see Table 1) were obtained from Aldrich, and all the other anions were from Nacalai Tesque, Inc.

3. Results and Discussion

The complex formation between Cpy4 ion and Kemp's acid **1** of the tricarboxylic acid in the pH 7 solution is shown in Fig. 2a. Two complexes were formed between the positive Cpy4 and the negative Kemp's acid ion. They were detected as $[M-4X+C-2H]^{2+}$ ($m/z=312$) and $[M-3X+C-2H]^{+}$ ($m/z=703$), where M, C, and X represent $Cpy4Br_4$, the carboxylic acid, and the counter ion Br^-, respectively. The non-complexed ions, $[M-2X]^{2+}$ and $[M-3X]^{3+}$, were also found. It was clear from the spectrum that the doubly charged ion of

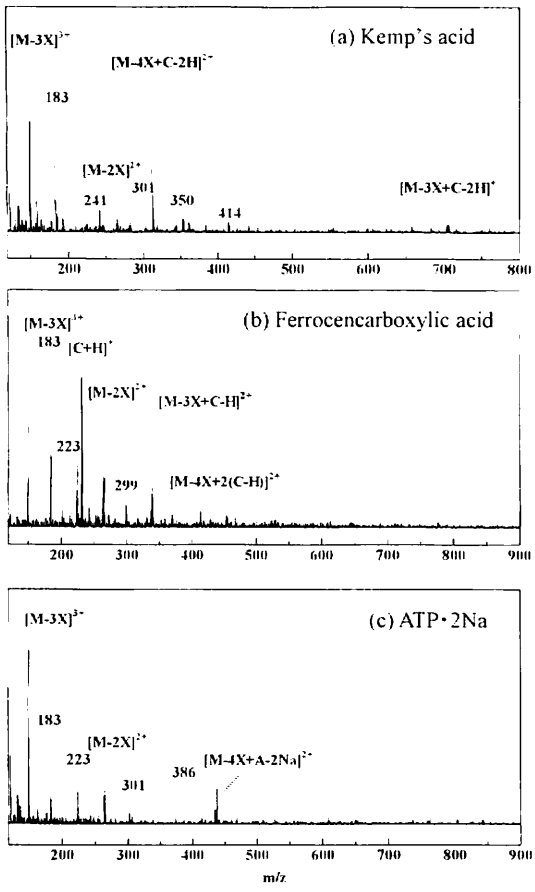


Fig. 2. Positive ion ESI mass spectra of mixture solutions of $Cpy4Br_4$ with (a) Kemp's acid **1**, (b) ferrocenecarboxylic acid **11** and ATP·2Na **13**.

Kemp's acid was involved in the complex formation. When an isomer *cis,cis*-1,3,5-cyclohexane tricarboxylic acid **2** was used in the same experimental condition, no complex formation was indicated. The results obtained with the other tricarboxylic acids are shown in Table 1.

The complex formation of Cpy4 ion with the dicarboxylic acid 1,2-cyclohexane dicarboxylic acid **8** was showed by the three ions, $[M-4X+C-2H]^{2-}$, $[M-3X+C-H]^{-}$, and $[M-3X+C-2H]^{+}$. Similarly, the formation was studied with phthalic acid **9** and L-tartaric acid **7**. In the combination of Cpy4 ion and phthalic acid, the ions $[M-4X+C-2H]^{2+}$ and $[M-3X+C-H]^{+}$ were observed, but no complex formation was found between Cpy4 ion and L-tartaric acid. The monocarboxylic acids, 2,5-dihydroxybezoic acid **10** and ferrocenecarboxylic acid **11** (Fig. 2b) formed two types of the complex ions, $[M-3X+C-H]^{2+}$ and the ion with two carboxylic acid molecules, $[M-4X+2(C-H)]^{2+}$; however, no complex formation with acetic acid was indicated. The ESI spectrum of an equal molar mixture of $Cpy4Br_4$ and ATP is shown in Fig. 2c. The spectrum indicates the formation of the complex ion $[M-4X+A-2Na]^{2+}$ ($m/z=437$), where A represents ATP molecule. The same complex ion formations were observed with both ADP and AMP.

In Table 1, we summarize the complex formations of the Cpy4 cation with the anion species. Intensity of the complex ions was expressed by the ratio of the complex ion intensity to sum of ionic Cpy4 species detected in the spectrum. Table 1 indicates that the Cpy4 cation forms complex with the carboxylic acids regardless of the number of carboxylic function in the acids. The complex formation appears to have certain selectivity toward the structure of carboxylic acid, but no relationship can be deduced at this time. Cpy4 forms complexes with ATP, ADP, and AMP, which indicate that the length of the phosphate chains does not affect the complex formation.

In an aqueous solution, binding constants K between the Cpy4 cation and the guest anions are **1** ($\log K=4.1$) $<$ **4** (4.4) $\cong$ **5** (4.5) $<$ **6** (5.1).[16] Although the K values were obtained in a solvent system different from ours, for example, $\log K=4.1$ for Kemp's acid **1** corresponds approximately to the 50% complex formation of the host Cpy4 ion under the concentration of 0.2 mM. Therefore, the intensity ratio of 0.34 in Table 1 is reasonable from the binding constant. On the other hand, we were unable to observe the complex formation of Cpy4 cation with the isomer of guest anion **2**. Since the binding constants obtained by the ^{1}H-NMR titration[16] was based on the assumption that the complex formed the 1 : 1 stoichiometric compound, which would not differentiate the formation of $[M-4X+C-2H]^{2+}$ or $[M-3X+C-2H]^{+}$, or the mixture of the both ions. Furthermore, a change in the NMR titration curve of guest anion **2** was too small to match the theoretical curve of the 1 : 1 stoichiometry, which would suggest the presence of multiple complex species.

Since the complexes in this study contain the unstable guest anions which undergo easily protonation and deprotonation, the stability of complex in the solution may be different from that of complex in the gas phase. That may be a reason why we could not observe a systematic relationship on the selectivity of the complex formation among the guest anions. Figure 3a shows the spectrum of the mixture solution of $Cpy4Br_4$ and Kemp's acid **1** without addition of $NaHCO_3$. The pH of the solution was 4.8. Moreover, in the spectrum of $Cpy4Br_4$ itself without Kemp's acid, the peak intensities of $[M-nX]^{n+}$ were not greatly influenced by a change in pH. The spectrum of the mixture solution is quite different from that at pH 7 (Fig. 2a). This is because the dissociation equilibrium of the tricarboxylic acid changes largely with a small change in pH.

If the complex is unstable in the gas phase, the CID may occur at the ESI interface with a residual collision gas, and the desired complex ion can not be detected. Figure 3b shows the CID result that the spectrum was unchanged with an increase in the skimmer voltage ΔV from 50 V to 70 V. The complex between the Cpy4 cation and the Kemp 's acid anion by electrostatic interaction is considerably stable against the CID. The spectra of Cpy4 complex formation with Kemp's acid **1** and ferrocenecarboxylic acid **11** were unchanged against the CID with an increase in the skimmer voltage ΔV up to 120 V. But the signal-to-noise ratio for the complex ions became worse in the case of $\Delta V>100$ V, since ion transmission between the two skimmers decreased.

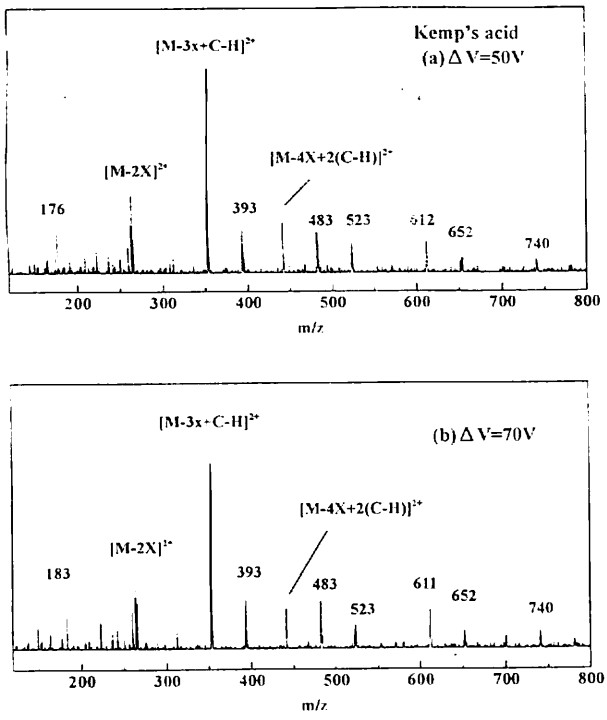


Fig. 3. Positive ion ESI mass spectra of a mixture solution of $Cpy4Br_4$ and Kemp's acid 1, without any addition of $NaHCO_3$ (pH 4.8); (a) the skimer voltage $\Delta V=50$ and (b) $\Delta V=70$.

For instance, it was reported the hydrogen-bonded tetramer complex of 1,1′-ferrocenedicarboxylic acid dissociated completely by the CID at $\Delta V=70$ V.[17] In addition, the $Ru(bpy)_3^{2+}$ ion with bidentate bpy (=2,2′-bipyridine) ligands dissociated at $\Delta V=190$ V and the $Ru(bpy)_2^{2-}$ and $Ru(bpy)^{2+}$ fragment ions appeared in the CID spectrum.[18] Finally, no definite systematic relationship on the complex formation could be observed. This is not due to the unstability of the complex in the gas phase but to that in the solution. The ESI-MS analysis of the complex formation may have an advantage in the characterization of the complex species which can not be achieved by the NMR method. On the other hand, the ESI may have the difficulties in detecting complex ion peaks when intensities of the spectral peaks are weaken by the formation of multiple complex ions.

In summary, we have succeeded in the observation of the complex formations between the quaternary tetrapyridinium macrocycle Cpy4 cation and various types of the anion species in the solutions at the neutral pH region. The Cpy4 cation formed complex with mono-, di-, and tricarboxylic acids regardless of the numbers of carboxylic groups in the guest anions; however, there has been observed some structural selectivity of the complex formation with guest anions. We also confirmed that the Cpy4 formed complex with the phosphate anion compounds, ATP, ADP, and AMP.

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