NOTE

Determination of Association Constant of Inclusion Complex between Cyclophane and Biological Relevant Phosphates by Fast Atom Bombardment Mass Spectrometry

Koichi METORI,^{*a)} Yumiko KIMURA,^{a)} and Muneharu MIYAKE^{a)}

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By ^1H NMR spectrometry it is impossible to confirm the association constant (K_s) of the host-guest complex which peaks of guest become broad and overlapped by host peaks. In order to solve the obstacles and raise the usefulness of FAB mass spectrometry in host-guest chemistry, we examined whether FAB mass spectrometry would be a suitable measurement tool for K_s value of the host-guest complex. As the result, the K_s value calculated by FAB mass spectrometry was mostly in agreement with the K_s value calculated by ^1H NMR spectrometry. It was concluded that FAB mass spectrometry is available for the determination of K_s value.

1. Introduction

The design and synthesis of macrocyclic ring systems play an important role in host-guest chemistry.¹⁾ Cyclophane, which form inclusion complexes with nucleotides, have been subjected of intensive synthetic studies²⁾ since nucleotides play a central role in the cellular process of metabolism and replication³⁾ and also phosphoryl transfer reactions are involved in the regulation of biological molecular recognition. Phosphorylation and dephosphorylation of proteins play an important roles on the signal transduction.⁴⁾ Previously we reported that FAB mass spectrometry can be used to perform Job's plot which is useful for determination of host-guest stoichiometry⁵⁾ and also a novel water-soluble cyclophane (TGDMAP) (Fig. 1) has been designed and synthesized as a host to trap biologically relevant phosphates by hydrophobicity of their aromatic ring groups and electrostatic interactions of 4-dimethylaminopyridinium groups in neutral aqueous solution.⁶⁾ Our primary purpose in this report is to judge if FAB mass spectrometry is a suitable measurement tool for the determination of the association constant (K_s) of the host-guest complex. In the previous report,⁶⁾ examinations on the complex formation of TGDMAP with 4-nitrophenyl phosphate (NPP), *O*-phospho-L-tyrosine (PPT), guanosine 5'-monophosphate disodium salt (GMP), cytidine 5'-monophosphate disodium salt (CMP), and uridine 5'-monophosphate disodium salt (UMP) were made by ^1H NMR spectrometry in 0.1 M phosphate buffer solution at pD 6.8. These K_s values are summarized in Table 1. However, in the case of using of adenosine 5'-monophosphate disodium salt (AMP) as a guest, the signal of proton at C-1' of AMP exhibited upfield shifts with broadening and the signals of aromatic protons were not detected in the presence of TGDMAP. Therefore, it was impossible to confirm a complex formation between AMP and

Fig. 1. Structure of cyclophane host.

Table 1. The K_s (M^{-1}) Values of Guests in the Presence of TGDMAP by MS and NMR Methods

Method	Guest					
	PPT	NPP	GMP	AMP	UMP	CMP
MS	630	120	330	150	120	100
NMR ^{a)}	600	130	410	N.D.	120	110

^{a)} In 0.1 M phosphate buffer (pD 6.8) at 303 K. [Guest]: 2.5×10^{-3} M. [Host]: 6.25×10^{-4} – 7.5×10^{-3} M.

N.D.: not detected.

TGDMAP. To see if TGMAP forms a complex with AMP and also FAB mass spectrometry is a suitable measurement tool for the determination of K_s of host-guest complex in stead of ^1H NMR spectrometry.

2. Experimental

2.1 Materials

TGDMAP⁶⁾ and NPP⁷⁾ were synthetic compounds, which had been already reported elsewhere. Disodium salts of PPT, GMP, CMP, and UMP were purchased from Tokyo Kasei Kogyo Co., Ltd., and AMP from Wako Pure Chemical Industries, Ltd. were used without further purification.

^{*a)} College of Pharmacy, Nihon University (7-7-1, Narashino-dai, Funabashi, Chiba 274-8555, Japan)

Fig. 2. Structures of guests.

2.2 FAB mass spectral measurements

FAB mass spectrometry was performed using a JEOL JMS-GCmate instrument. Measurement conditions were as follows: equimolar solutions (5×10^{-3} M) of host and guest were prepared in 0.02 M triethylammonium acetate (TEAA) solution containing 50% glycerol (pH 7.0). An equal volume ($5 \mu\text{L}$) of the host and guest solution were mixed and then $1 \mu\text{L}$ of the mixture was loaded. Xe was employed as a fast atom bombardment gas. Scanning was performed from m/z 100 to 2,000 in 10 s and several scans were summed to obtain the final spectrum. All of FAB mass spectra of the mixture were employed positive-ion mode.

2.3 Job's plots

Equimolar solutions (5×10^{-3} M) of host and guest were prepared in TEAA solution containing 50% glycerol (pH 7.0) and mixed in various amounts ($[H]:[G] = 1:3, 1:2, 1:1.5, 1:1, 1.5:1, 2:1$, and $3:1$). FAB mass spectra of the mixture were recorded from m/z 700 to 1,300 in 10 s (positive-ion mode), and the absolute intensities of the host–guest complex were analyzed by Job's method^{(5), (8)} for FAB mass spectra results.

2.4 Determination of K_s values of the complexes by FAB mass spectrometry

The K_s values of the host–guest complexes were determined using the absolute intensities of host–guest complexes on the basis of the double reciprocal plots. The stock solutions of the host and all of the guests were prepared in TEAA solution (pH 7.0) containing 50% glycerol. The concentration of the host for nucleotides was 2.5×10^{-3} M, while those of the guest ranges from 8.3×10^{-4} M to 7.5×10^{-3} M (6 points). The host concentration for PPT and NPP was 1.25×10^{-3} M and the guest concentration ranges from 6.25×10^{-4} M to 2.5×10^{-3} M (5–6 points). The double reciprocal plots ($1/\text{absolute intensity } (I)$ vs. $1/[G]$) gave excellent linearity with a correlation coefficient $r \geq 0.997$.

Fig. 3. FAB mass spectrum for the inclusion complex formation between TGDMAP and AMP in a glycerol matrix. Experimental conditions: magnetic scan; accelerating voltage 1.7 kV, resolution 1,300.

Fig. 4. Job's plots for the inclusion complex formation between TGDMAP and AMP. The absolute intensity was measured at m/z 1,254.7 (host–guest complex). The total concentration (TGDMAP + AMP) was 5 mM.

3. Results and Discussion

Ion peaks for $[(\text{TGDMAP}-2\text{Cl})+(\text{AMP}-2\text{Na})+\text{H}]^+$ was observed at m/z 1,254.7 triethylammonium acetate (TEAA) solution (pH 7.0) and molecular ion peaks

Fig. 5. FAB mass spectrum for the inclusion complex formation between TGDMAP and a) GMP, b) UMP, c) CMP, d) PPT, or e) NPP in a glycerol matrix. Experimental conditions: see Fig. 3.

Fig. 6. The double reciprocal plots for the inclusion complex formation between TGDMAP and AMP. The absolute intensity was measured at m/z 1,254.7 (host-guest complex). The final concentration of TGDMAP was 2.5 mM and AMP concentration ranges from 0.83 mM to 7.5 mM.

of higher mass could not be detected (Fig. 3). The FAB mass spectrometry measurement suggests that TGDMAP forms the 1 : 1 complex with AMP. The 1 : 1 complex stoichiometry for the complex between TGDMAP and AMP was also confirmed by using Job's method of continuous variations using FAB mass spectrometry.⁶⁾ The Job's method gave a maximum at

0.5, indicative of a 1 : 1 stoichiometry (Fig. 4). In case of using other nucleotides as guests, the same results were obtained and the ion peaks of the 1 : 1 complexes of TGDMAP with GMP, UMP, and CMP showed at m/z 1,270.6 $[(\text{TGDMAP} - 2\text{Cl}) + (\text{GMP} - 2\text{Na}) + \text{H}]^+$, 1,231.6 $[(\text{TGDMAP} - 2\text{Cl}) + (\text{UMP} - 2\text{Na}) + \text{H}]^+$, and 1,230.7 $[(\text{TGDMAP} - 2\text{Cl}) + (\text{CMP} - 2\text{Na}) + \text{H}]^+$, respectively (Fig. 5). Examinations on the complex formation of TGDMAP with PPT and NPP were made by FAB mass spectrometry in TEAA solution (pH 7.0). Ion peaks for $[(\text{TGDMAP} - 2\text{Cl}) + (\text{PPT} - 2\text{H}) + \text{H}]^+$ and $[(\text{TGDMAP} - 2\text{Cl}) + (\text{NPP} - 2\text{H}) + \text{H}]^+$ were observed at m/z 1,168.8 and 1,126.7, respectively. On addition of PPT to the host solution under controlled conditions, the absolute intensity value was noted as a function of the concentration of the variable component. The K_s value (630 M^{-1}) of the result calculated by FAB mass spectrometry was mostly in agreement with the K_s value (600 M^{-1})⁶⁾ of the result calculated by ^1H NMR spectrometry and in case of using AMP as guest, the K_s value was 150 M^{-1} . The K_s values for TGDMAP with other guests are given in Table 1. The K_s values of the result obtained by MS measurement method were also mostly identical to the results of previously reported ^1H NMR measurement method⁶⁾ in spite of using different the temperature and the solvent in the two methods.

In conclusion, ^1H NMR measurement is widely used for determination of host-guest complex formation, however, the method can not be used for measuring confirmation of host-guest complex formation in case

either the peaks of guest and host overlap each other or these signals show broad. For the purpose to solve the obstacles, in this report, we investigated the measurement of K_s value by use of FAB mass spectrometry instead of ^1H NMR spectrometry. The K_s value calculated by FAB mass spectrometry was most similar to the K_s value calculated by ^1H NMR spectrometry. Therefore, we report here FAB mass measurement is useful method not only for confirmation of host-guest complex geometry but also for determination of K_s values of host-guest complexes.

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