Proof of Inclusion Complex Formation between Cyclophane and Aromatic Guests by Fast Atom Bombardment Mass Spectrometry

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Some obstacles for the analysis of host–guest complexes by proton nuclear magnetic resonance (1H NMR) are the problem of requiring for a large excess of host, high concentration and high solubility of host and guest in order to get a good resolution. By 1H NMR spectrometry it is very difficult to detect the complex which the peaks of guest become overlapped by host peaks. Our primary purpose in this report is to judge if fast atom bombardment mass spectrometry (FAB-MS) is a suitable measurement tool for the determination of the host–guest complex stoichiometry in stead of 1H NMR spectrometry.

1. Introduction

A number of synthetic supramolecular complex such as cyclophanes, catenanes, cyclodextrins, crown ethers, and rotaxanes have been identified by fast atom bombardment mass spectrometry (FAB-MS).1−6 Phosphoryl transfer reactions are involved in the regulation of biological molecular recognition. Phosphorylation and dephosphorylation of proteins play an important role in the signal transduction.7 For the point of view, the host–guest complex formation between cyclophane (TGMI) as a host and 4-nitrophenyl phosphate (NPP), 4-nitrophenol (NP), and O-phosphorylethanolamine (PEA) as guests have been previously investigated by proton nuclear magnetic resonance (1H NMR).8

However, in the case of using the L-phosphotyrosine (PPT) as a guest, the peaks of PPT exhibited upfield shifts with broadening and became overlapped by the host peaks. Therefore, it was impossible to confirm a complex formation between PPT and TGMI. To see if TGMI forms a complex with PPT, the FAB-MS was measured using glycerol as a matrix.

2. Experimental

2.1 Materials

TGMI8) and 4-nitrophenyl phosphate (NPP)9) were synthetic compounds, which had been already reported elsewhere. PPT, 4-nitrophenol (NP), and O-phosphorylethanolamine (PEA) were purchased from Tokyo Kasei Kogyo Co., Ltd and were used without further purification (Fig. 1).

2.2 FAB mass spectral measurements

FAB-MS was performed using a JEOL JMS-GCmate instrument. Measurement conditions were as follows: For the FAB-MS experiments TGMI (0.02 M in 2 µL DMSO), the guest compounds (0.02 M in 2 µL H2O), and glycerol (2 µL) as matrix in DMSO (4 µL) were mixed. Then 2 µL of the mixture was loaded. Xe was employed as a fast atom bombardment gas. Scanning was performed from m/z 100 to 1,500 in 10 s and several scans were summed to obtain the final spectrum. In the case of NP as a guest, the negative-ion mode was employed and all of others were employed positive-

Fig. 1. Structures of cyclophane host and guests.

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2.3 Job's plots

Equimolar solutions (8 × 10^{-3} M) of host in DMSO and guest in H₂O were prepared and mixed in various amounts ([H]:[G]=1:3, 1:2.3, 1:1.5, 1:1, 1.5:1, 2.3:1, and 3:1). Glycerol was used as matrix. FAB-MS spectra of the mixture were recorded, and the absolute intensities of the host-guest complex were analyzed by Job's method¹⁰ for FAB-MS results.

3. Results and Discussion

Ion peaks for [TGMI+H]⁺, [TGMI+PPT+H]⁺ were observed at m/z 863 and 1,124, respectively (Fig. 2). Molecular ion peaks of higher mass could not be detected. The FAB-MS measurement suggests that TGMI forms the 1:1 complex with PPT.

The 1:1 complex stoichiometry for the complex between TGMI and PPT was also confirmed by using Job's method of continuous variations using FAB-MS. The Job's method gave a maximum at 0.5, indicative of a 1:1 stoichiometry (Fig. 3). In the case of using NPP and NP as guests, the same results were obtained and ion peaks for [TGMI+NPP+H]⁺, [TGMI+NP−H]⁻ were observed at m/z 1,082 and 1,000, respectively (Figs. 4 and 5). On the other hand, in the case of using alkyl phosphate such as PEA as a guest, the 1:1 complex ion peak for TGMI-PEA was not observed. It was suggested that TGMI forms complex with aromatic guests but not with alkyl phosphate such as PEA. The
phenomenon is identical to a result of previously reported $^1$H NMR measurement method.\(^8\)

In conclusion, $^1$H NMR measurement is widely used for determination of host–guest complex formation, however, in case either the peaks of guest and host overlap each other or these signals show broad, the method cannot be used for measuring confirmation of host–guest complex formation. In these cases, the FAB-MS measurement is useful for confirmation of host–guest complex formation. In particular, Job’s plot using FAB-MS was not known. In this paper, it was concluded that FAB-MS can be use to perform Job’s plot which is useful method for determination of host–guest stoichiometry.

References


**Keywords:** FAB-MS, Host–guest complex formation, Cyclophane

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