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International Journal of Mass Spectrometry 193 (1999) L1–L6



Letter

Ionspray ionization and tandem mass spectrometry of Li^+ or NH_4^+ coordinated 1:1 γ -cyclodextrin/12-crown-4-ether double macrocyclic inclusion complex

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Received 7 June 1999; accepted 3 August 1999

Abstract

Ionspray mass spectrometry (MS) of a water-acetonitrile solution of 1:1 γ -cyclodextrin (γ CD)/12-crown-4 ether (12-C-4) double macrocyclic inclusion complex added with LiBr and ammonium acetate allowed the detection of 1:1:1 γ CD/12-C-4/ Li^+ or NH_4^+ gaseous associations, which were characterized by tandem-MS experiments performed with a triple quadrupole analyser. The low energy collision-induced dissociation (CID) products were both γ CD and 12-C-4 cationated with Li^+ or NH_4^+ , respectively. The CID of 1:1:1 γ CD/12-C-4/ NH_4^+ also yielded protonated γ CD and 12-C-4. Li^+ or NH_4^+ cationated γ CD were more abundant than the corresponding cationated 12-C-4. These results suggested preferential cationization of γ CD at the external hydrophilic surface with respect to 12-C-4 cation inclusion within the 1:1 double macrocyclic complex for entropy and steric hindrance reasons. Nevertheless, the 12-C-4 cationization did yet even occur to a significant extent, thus providing support for the surviving capability of a cationated gaseous CD host-guest complex with the charge located within the host cavity. (Int J Mass Spectrom 193 (1999) L1–L6) © 1999 Elsevier Science B.V.

Keywords: Cyclodextrin; Crown-ether; Double inclusion; Host-guest complex; Ionspray; Tandem mass spectrometry

1. Introduction

Noncovalent inclusion or host-guest (h-g) complexes are a class of supramolecular associations of general scientific and technological interest; they represent the molecular recognition expression of a number of concave macrocycles, which are able to

host into their internal cavity suitably sized guests such as bare ions or organic neutrals [1, 2]. Among the many types of synthetic or natural macrocycles used as host ligands, crown ethers are specifically able to include bare cations [3], leading to “charged” complexes, while the natural cyclooligosaccharides (cyclodextrins, CDs) [4–7] and some commercially available synthetic derivatives, having a torus-shaped structure with a rather rigid hydrophobic cavity, can

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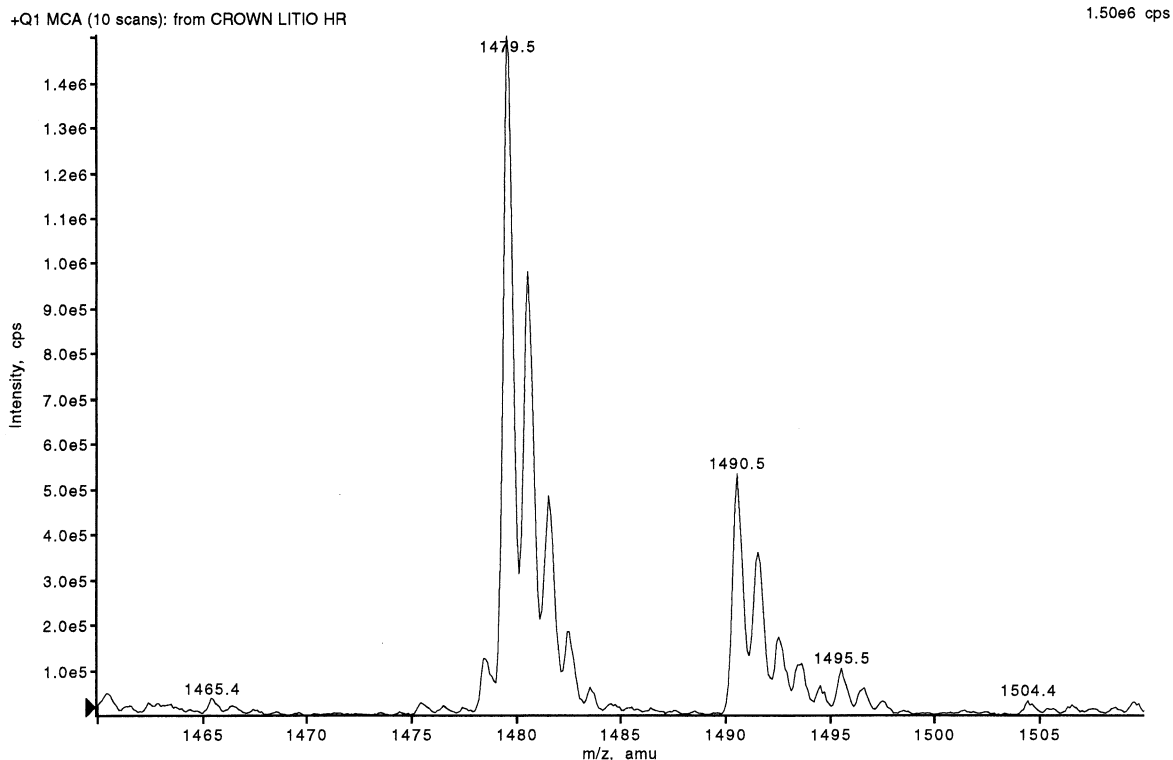


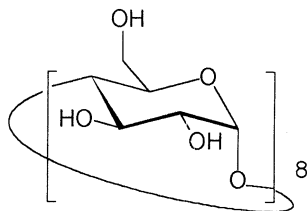
Figure 1. Ionspray mass spectrum of a water/acetonitrile 50/50 (v/v) solution of 1:1 γ CD/12-C-4 h-g complex added with LiBr and ammonium acetate.

host appropriately sized lipophilic organic molecules or part of them, leading to “neutral” h-g complexes. The CD complexes are currently applied to the pharmaceutical cosmetic, and food industries, where they can: (i) enhance the water solubility of lipophilic guests, (ii) control the release of volatile guests, and (iii) protect labile guests from degradation [4–8].

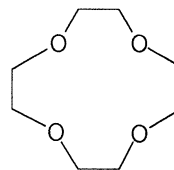
Structure and properties of h-g associations, which

can have just a 1:1 h/g molar ratio or a different stoichiometry, e.g. a 2:1 h/g molar ratio for the so-called “sandwich” structures, have been studied extensively in the solid state or in solution by exploiting most of the presently available diffractometric, spectroscopic, or other physicochemical methods and molecular modelling.

Mass spectrometry (MS) was exploited for gas-phase studies and proved to be mostly appropriate for



γ -cyclodextrin (γ -CD)



12-crown-4 (12-C-4)

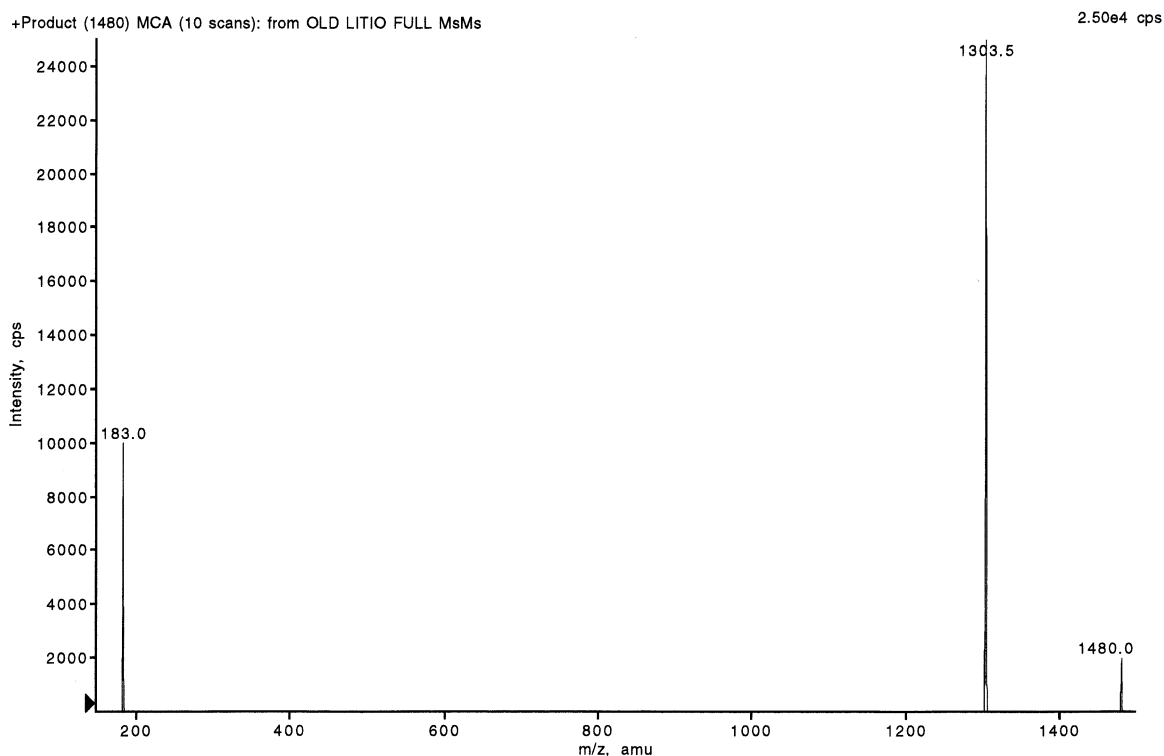


Figure 2. CID tandem mass spectrum of ${}^7\text{Li}^+$ coordinated 1:1 $\gamma\text{CD}/12\text{-C-4}$ ternary association (m/z 1479.5) generated by Ionspray ionization.

the charged h-g complexes, such as cationized crown ethers, because the charge is indispensable to this methodology [9]. MS was also applied to the study of neutral h-g complexes [9–22]; using soft ionization methods like fast atom bombardment (FAB) and electrospray (ESI) or ionspray (IS) (PE-Sciex, Concord, Ontario, Canada) protonated, deprotonated, cationated, or otherwise charged species of neutral h-g complexes, mainly CD complexes, actually existing in condensed phase, were detected as intact gaseous entities. These MS results, however, while able to provide molecular and/or chiral recognition evidence [10–13,21], as well as a possibly fast and easy analytical approach, were regarded prudently for the possibility that “false positives” due to the formation of gaseous ion-molecule h-g adducts as MS artifacts, e.g. proton-bound heterodimers, rather than the intact charged species of true “neutral” h-g complexes, could be detected [15,16]. In particular, it was

argued that with ESI or IS MS the charging process of the neutral h-g systems could perturb dramatically the intrinsically weak h-g binding interactions and that the following desolvating step would be too hard a process for the survival of the gaseous inclusion complexes [15]. Notwithstanding a few pieces of evidence, indirect support of the possibility of detecting true intact charged CD h-g complexes by MS was obtained from comparative experiments carried out on mixtures of the guest molecule with noncyclic CD analogues [16,17], and also by investigating a few multicomponent systems of current pharmaceutical interest for their free water solubility, in which a βCD inclusion complex of a basic or an acidic guest drug was associated to an organic acidic or basic counterion, respectively [18–21]. For instance, the protonated or deprotonated gaseous 1:1:1 $\beta\text{CD}/\text{drug}/\text{counterion}$ associations were detected by IS ionization whose tandem-MS dissociation allowed one to draw

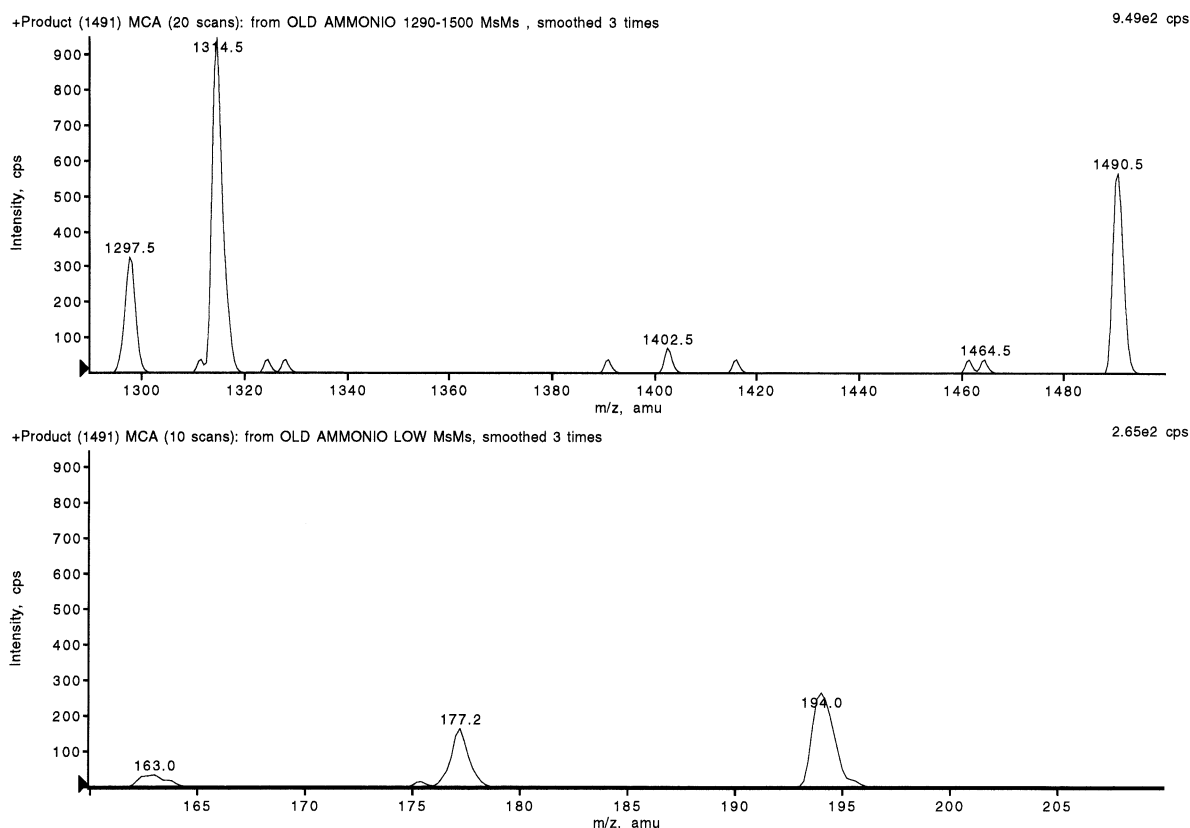


Figure 3. CID tandem mass spectrum of NH_4^+ coordinated 1:1 $\gamma\text{CD}/12\text{-C-4}$ ternary association (m/z 1490.5) generated by Ionspray ionization: (A) expanded upper and lower mass sections; (B) whole spectrum with nominal mass values.

the rank order of the intermolecular noncovalent binding interactions within the gaseous ternary ions [20]. Besides, the strategic role of the strongest ionic bond between drug and its counterion within the neutral multicomponent association was outlined, whereas while this bond vanished upon protonation or deprotonation, it was able to address the charge of the resulting ternary ion well outside the CD cavity, thus avoiding any perturbation effects of the charge to the weak h-g interactions [20].

γCD cavity is capable to host lipophilic or amphiphilic and suitably sized macrocycles such as 12-crown-4-ether (12-C-4) or cryptands as guests, leading to double macrocyclic h-g complexes, which can include a cation [23]. For instance, the structure of a lithium-coordinated 1:1 $\gamma\text{CD}/12\text{-C-4}$ double macrocyclic inclusion complex in solid phase was determined by x-ray diffraction [24] and we considered

this complex an interesting substrate for an MS study inasmuch as: (i) it is a charged h-g association, which should provide a straightforward detectability as for cation-coordinated crown ethers and (ii) the charge is placed just inside the γCD cavity by the cationated 12-C-4 guest; this could allow one to check the possible perturbation effects of an internal charge on the $\gamma\text{CD}/12\text{-C-4}$ h-g interaction.

The present letter shows the results of a study by IS MS and tandem MS of lithium or ammonium cationated $\gamma\text{CD}/12\text{-C-4}$ double macrocyclic inclusion complexes.

2. Experimental and materials

2.1. Complex preparation

γCD and 12-C-4 were purchased from Aldrich (Milan, Italy) and used without any further purifica-

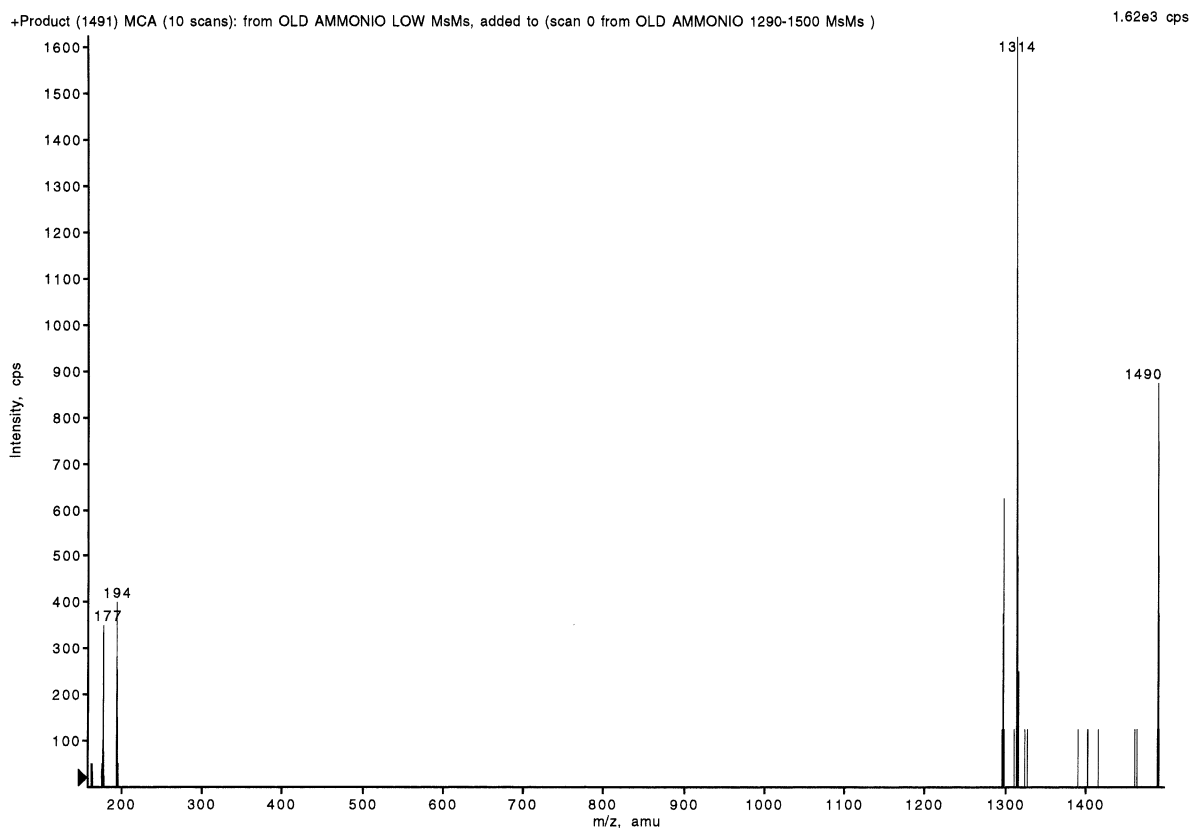


Fig. 3. (continued)

tion. The 1:1 complex between γ CD and 12-C-4 was obtained as follows: 40 mg of γ CD (3.1×10^{-5} mol, 1 eq.) were mixed with 5.4 mg ($5 \mu\text{L}$, 1 eq.) of 12-C-4. The mixture was suspended in 1 mL of deionized water stirred at 40°C until complete dissolution. The solution was then freeze-dried.

2.2. Mass spectrometry

A bench-top Perkin Elmer-Sciex API 2000 triple quadrupole mass spectrometer was operated in ion evaporation mode with a TurboIonSpray source set at 5600 V and declustering potential from 20 to 60 V. Resolution was set at 0.7 amu full width at half height (FWHM) on both the resolving quadrupoles. Resolution setting and mass calibration were performed by a 10^{-4} polypropylene glycol (PPG) solution.

The solid sample of the γ CD/12-C-4 h-g complex

was dissolved in water/acetonitrile 50/50 (v/v), added with $20 \mu\text{g}/\text{mL}$ of LiBr and with acetonitrile ammonium acetate 2 mM and then introduced into the ion source by an infusion pump with a flow rate of $5 \mu\text{L}/\text{min}$.

Collision induced dissociation (CID) tandem MS experiments were performed with a LINAC (Concorde, Ontario, Canada) collision cell, using nitrogen as collision gas at 8 mTorr (1 Torr = 133.3 Pa) and a collision energy (E_{lab}) of 35 eV.

3. Results and discussion

The formation of the 1:1 inclusion complex between γ CD and 12-C-4 was confirmed by high-resolution nuclear magnetic resonance (NMR) experiments in D_2O solution; these results will be published elsewhere [25].

As shown in Fig. 1, the IS MS spectrum of a

water/acetonitrile 50/50 (v/v) solution of the freeze-dried solid sample of 1:1 γ CD/12-C-4 h-g complex added to the LiBr and ammonium acetate allows one to detect the 1:1 double macrocyclic association, coordinated with ${}^7\text{Li}^+$ or NH_4^+ , at m/z 1479.5 and 1490.5, respectively. The weak peaks at m/z 1478.4 and 1495.5 are due to ${}^6\text{Li}^+$ isotope and to Na^+ contaminant, respectively.

Both the CID spectra of ${}^7\text{Li}^+$ or NH_4^+ coordinated ternary associations, shown in Figs. 2 and 3, appear absolutely simple, whereas the only noticeable charged products are ${}^7\text{Li}^+$ or NH_4^+ cationated γ CD (m/z 1303.5 or 1314.5) and ${}^7\text{Li}^+$ or NH_4^+ cationated 12-C-4 (m/z 183 or 194), respectively. In addition, protonated host (γ CD, m/z 1297.5) and guest (12-C-4, m/z 177.2) are also obtained from the NH_4^+ coordinated ternary parent ion (Fig. 3) by loss of NH_3 , as previously observed for CID of other ammonium cationated h-g complexes [13].

The CID spectra of ${}^7\text{Li}^+$ or NH_4^+ coordinated ternary associations (Figs. 2 and 3) also show that cationated or protonated γ CD are relatively more abundant products than the cationated or protonated 12-C-4, i.e. the dissociating parent assemblies eliminate the 12-C-4 guest more as a neutral than as a cationated species. The most reasonable and straightforward interpretation we can put forward for these CID results would be that the cation added to the solution of 1:1 γ CD/12-C-4 h-g complex should coordinate more easily with the hydroxyl groups of the external γ CD host surface than it could be fitted into the 12-C-4 guest cavity. The prevalence of the former process should be supported by favourable statistic/entropic factors, due to the number of possible cation coordination sites at the external γ CD host surface, and also by the practical absence of steric hindrance. Accordingly, chelation or multisite coordination can increase the ligand-metal ions complex stability [26,27]. Conditions for the latter process appear to be opposed to this, as there is only one cation coordination site, which is placed into the 12-C-4 cavity, access to which could be hindered by the inclusion into the γ CD host. However, in spite of such unfavourable conditions, cation coordination into the 12-C-4 cavity does actually occur to a significant extent.

Hence, we would like to conclude that these results can provide further support for the surviving capability of gaseous charged CD h-g complexes even if the charge is located just inside the host cavity.

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