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Non-covalent Complexes of Nucleosides and Nucleobases with β-Cyclodextrin: a Study by Fast Atom Bombardment Mass Spectrometry and Collision-induced Dissociation†

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Naturally occurring nucleosides and nucleobases form inclusion complexes with β-cyclodextrin. These host–guest complexes could be detected by fast atom bombardment mass spectrometry. The collision-induced dissociation spectra of the protonated complexes showed mainly ions related to the guest molecules. However, deoxyribonucleoside complexes also exhibited facile elimination of the sugar moiety, whereas this fragmentation was absent in ribonucleoside complexes. The deprotonated inclusion complexes underwent collision-induced dissociation to give mainly the deprotonated host molecule. It appears, therefore, that the protonated complexes have protonated guests inside the cavity of the neutral host and the deprotonated complexes have neutral guests in the cavity of the deprotonated host. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: cyclodextrin; inclusion complexes; nucleosides; nucleobases; fast atom bombardment mass spectrometry; collision-induced dissociation

INTRODUCTION

The non-polar cavity of the cyclic oligosaccharide β-cyclodextrin (CD) is able to accommodate guest molecules, mainly by hydrophobic interactions. The resulting CD inclusion complexes with drugs are finding widespread application in the pharmaceutical industry as these complexes can improve the solubility, stability and bioavailability of the guest molecules.[1] Nuclear magnetic resonance (NMR) and diffraction methods give information on the structure, stoichiometry and stability of CD complexes in solution or in the solid phase.[2–4] Recently, there have been several investigations of such supramolecular non-covalent inclusion complexes by mass spectrometry using techniques such as fast atom bombardment (FAB),[5] plasma desorption (PD)[6] and electrospray ionization (ESI).[7] These soft ionization techniques have indicated that the CD inclusion complexes are also stable in the gas phase.

Nucleoside analogues constitute an important class of compounds with a wide range of biological activity. More importantly, all of the drugs currently licenced for the treatment of acquired immune deficiency syndrome (AIDS), viz. 3′-azidothymidine (AZT), dideoxyinosine (ddI), dideoxycytidine (ddC) and lamivudine (3TC), are nucleoside analogues.[8] The commonly encountered problem with nucleoside drug candidates is their poor stability and bioavailability.[9] As already stated, one possible approach to circumvent this difficulty is to prepare inclusion complexes with CD. As a first step towards this objective, a few CD inclusion complexes with naturally occurring nucleosides (**1**–**8**) and nucleobases (**9**, **10**) were prepared. In order to assess the gas-phase behaviour of these complexes, it was decided to examine their mass spectra under FAB conditions. The observed host–guest complexes were also subjected to collision-induced dissociation (CID).

EXPERIMENTAL

The cyclodextrin–nucleoside/nucleobase complexes were prepared by mixing 0.05 mmol of the nucleoside/nucleobase dissolved in 2 ml of methanol–propan-2-ol–water (depending on their solubility) with 0.05 mmol of CD dissolved in 2 ml of water. The mixture was stirred for 2 h and washed with 2 × 1 ml of chloroform. The aqueous layer was separated and concentrated in a Speedvac concentrator. The solid nucleoside/nucleobase–CD complex (1:1) so formed was used for mass spectral studies. Because of poor solubility the guanine–CD complex could not be prepared.

The FAB and CID mass analysed ion kinetic energy (MIKE) spectra were recorded on a JEOL (Tokyo, Japan) SX-102/DA-6000 mass spectrometer equipped with a FAB ion gun producing a beam of neutral xenon atoms (6 kV, 10 mA). Glycerol, thioglycerol and *m*-

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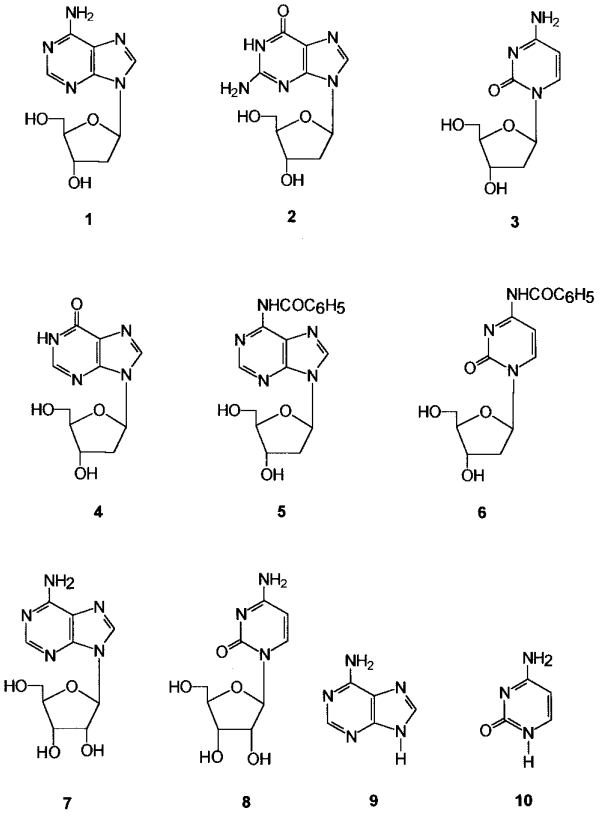

nitrobenzyl alcohol (NBA), obtained from Tokyo Kasei Kogyo (Tokyo, Japan) were tried as the matrices. Thioglycerol gave the best results and hence all subsequent analyses were carried out using thioglycerol as the matrix. The samples were dissolved in thioglycerol (0.01 M). The FAB-desorbed ions were accelerated to 10 keV. The mass spectra were recorded by scanning the magnetic field over the mass range 1–2500 with a scan speed of 10 s and a cycle time of 12 s at a resolution of 3000. Helium was used as the collision gas in the second field-free region collision cell for CID MIKE measurements. The pressure of helium in the cell was adjusted so that the parent beam intensity was reduced by 50%. The CID MIKE spectra were recorded using a scan speed of 20 s and a cycle time of 25 s. Each spectrum reported here is an average of 4–5 scans.

Thymine and thymidine–CD complexes did not yield any appreciable peaks corresponding to the inclusion complexes. It is presumed that their complexes are unstable and do not survive in the gas phase under the conditions we employed.

RESULTS AND DISCUSSION

The ion abundances in the FAB mass spectra of the CD complexes of **1–10** are given in Table 1 and the partial FAB mass spectra of a representative example is given in Fig. 1. In all the spectra protonated guest molecules gave rise to the most abundant ion. However, the lower mass region is excluded in Table 1 and Fig. 1, which focus only on the host and the adduct ion region. The samples **1–10** gave abundant protonated ions corresponding to the 1:1 host–guest adducts with CD. For example, in **1** this peak appears at *m/z* 1386. The next lower mass peak is at *m/z* 1270, corresponding to loss of the deoxysugar moiety. This fragmentation is absent in the ribonucleoside, **7**. The host molecule is present mostly as sodiated species, $[Hs + Na]^+$ at *m/z* 1157. Peaks corresponding to $[Hs + H]^+$ (*m/z* 1135) and $[Hs + K]^+$ (*m/z* 1173) are also observed. However, there are no peaks corresponding to $[Hs + Na + G]^+$ which could represent an ion–molecule complex. The FAB mass spectra, therefore, suggest the existence of a host–guest inclusion complex.

The CID spectra of these complexes were measured by the MIKE technique. As representative examples, the CID MIKE spectra of **1** and **7** are given in Fig. 2 and the ion abundances in the CID MIKE spectra of the CD inclusion complexes of the other compounds are listed in Table 2. Non-covalent complexes, such as CD inclusion complexes, are expected to fragment into their constituents under CID.[10] Previous reports of tandem mass spectra of CD inclusion complexes (whether 1:1[5e,7b] or 1:1:1[7c,7e] complexes) show only the loss of the components and not fragmentation of the guest molecule. However, these nucleoside–CD complexes also show fragment ions corresponding to the protonated guest molecules and nucleobases. It is clear from Fig. 2 and Table 2 that when the sample contains

Table 1. Ion abundances in the FAB mass spectra of 1–10 (*m/z* with relative abundances (%) in parentheses)

Compound	$[Hs + H]^+$	$[Hs + Na]^+$	$[Hs + K]^+$	$[Hs + G + H]^+$	$[Hs + G + H - S]^+$ [a]	Others
1	1135 (15)	1157 (80)	1173 (27)	1386 (100)	1270 (18)	
2	1135 (12)	1157 (63)	1173 (18)	1402 (100)	1286 (25)	1670 (10)[b]
3	1135 (20)	1157 (55)	1173 (24)	1362 (100)	1246 (58)	
4	1135 (35)	1157 (100)	1173 (23)	1387 (57)	1271 (22)	
5	1135 (20)	1157 (100)	1173 (28)	1472 (53)	1356 (36)	
6	1135 (30)	1157 (59)	1173 (20)	1466 (100)	1350 (28)	
7	1135 (74)	1157 (100)	1173 (33)	1402 (77)	—	
8	1135 (70)	1157 (100)	1173 (21)	1378 (32)	—	
9	1135 (50)	1157 (100)	1173 (36)	1270 (60)	—	
10	1135 (16)	1157 (32)	1173 (10)	1246 (100)	—	

[a] S = $C_5H_8O_3$ (deoxysugar).
[b] $[Hs + 2G + H]^+$.

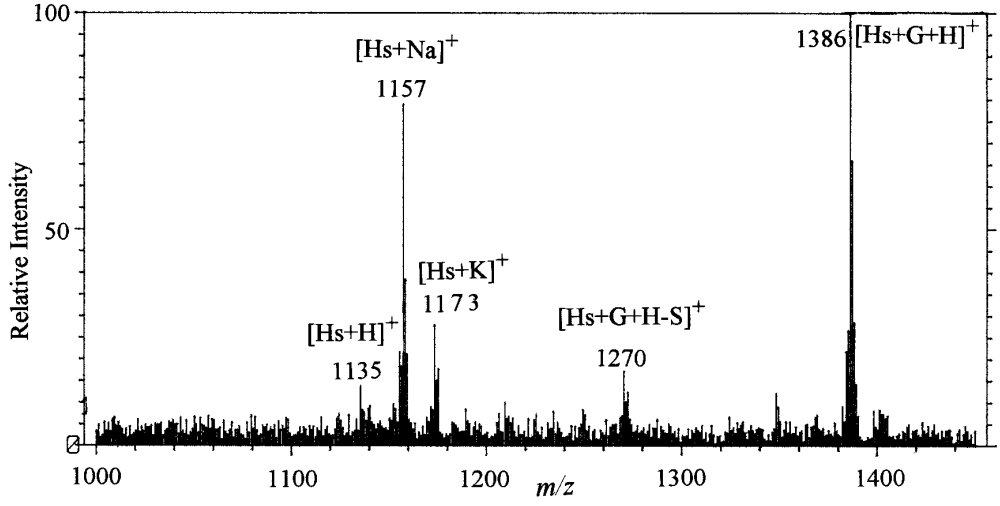

Figure 1. FAB mass spectrum of CD inclusion complex of **1**.

a deoxysugar, the complex easily undergoes decomposition by loss of the sugar moiety, whereas this does not happen when it is a ribosugar. This is understandable in view of the high susceptibility of the *N*-glycosidic bond to cleavage in deoxysugars in an acidic environment.[11] Protonation of the base will induce this cleavage much faster in a deoxysugar than in a ribosugar. CD is known to facilitate ester hydrolysis.[12] It is possible, therefore,

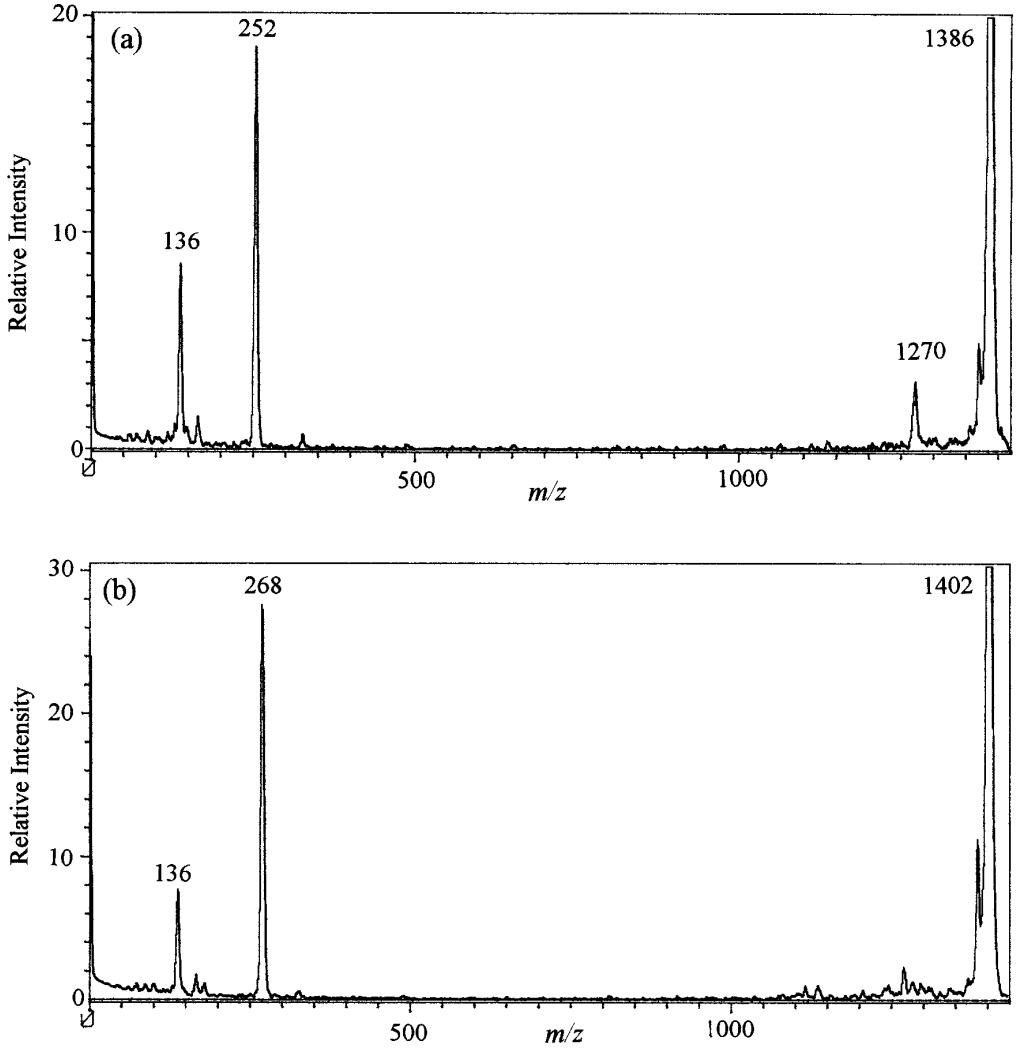

Figure 2. CID MIKE spectra of $[Hs + G + H]^+$ of **1** (*m/z* 1386) and **7** (*m/z* 1402).

Table 2. Ion abundances in the CID MIKE spectra of $[Hs + G + H]^+$ ions of 1–10 (m/z with relative abundances (%) in parentheses)

Compound	$[Hs + G + H - S]^+$ [a]	$[G + H]^+$	$[Base + H]^+$	Others
1	1270 (16)	252 (100)	136 (43)	1135 (2), 163 (7)
2	1287 (100)	268 (30)	152 (65)	1135 (2), 163 (3)
3	1246 (100)	228 (10)	112 (56)	1135 (2)
4	1271 (100)	253 (5)	137 (21)	1135 (20)
5	1356 (100)	338 (22)	222 (43)	1135 (4)
6	1350 (100)	322 (24)	216 (70)	1135 (8)
7	—	268 (100)	136 (29)	1135 (3)
8	—	244 (100)	112 (22)	1135 (4)
9	—	136 (100)[b]	136 (100)[b]	1135 (2)
10	—	112 (100)[b]	112 (100)[b]	1135 (5)

[a] S = $C_5H_8O_3$ (deoxysugar).
[b] G = base.

that the cleavage of the *N*-glycosidic bond is assisted by CD during CID of these protonated complexes. The CID mass spectra of the protonated CD inclusion complexes do not show any appreciable amount of protonated CD ($[Hs + H]^+$), unlike the FAB mass spectra, which show predominant ions corresponding to $[Hs + H]^+$ and $[Hs + Na]^+$, suggesting that the FAB mass spectra reflect the dissociation equilibrium of the CD complexes.

In order to obtain some insight into the strengths of the bindings in these complexes, we examined the CID MIKE spectra of the proton-bound dimeric species ($[2G + H]^+$) of the guest molecules. Although not prominent, a discernible peak corresponding to loss of

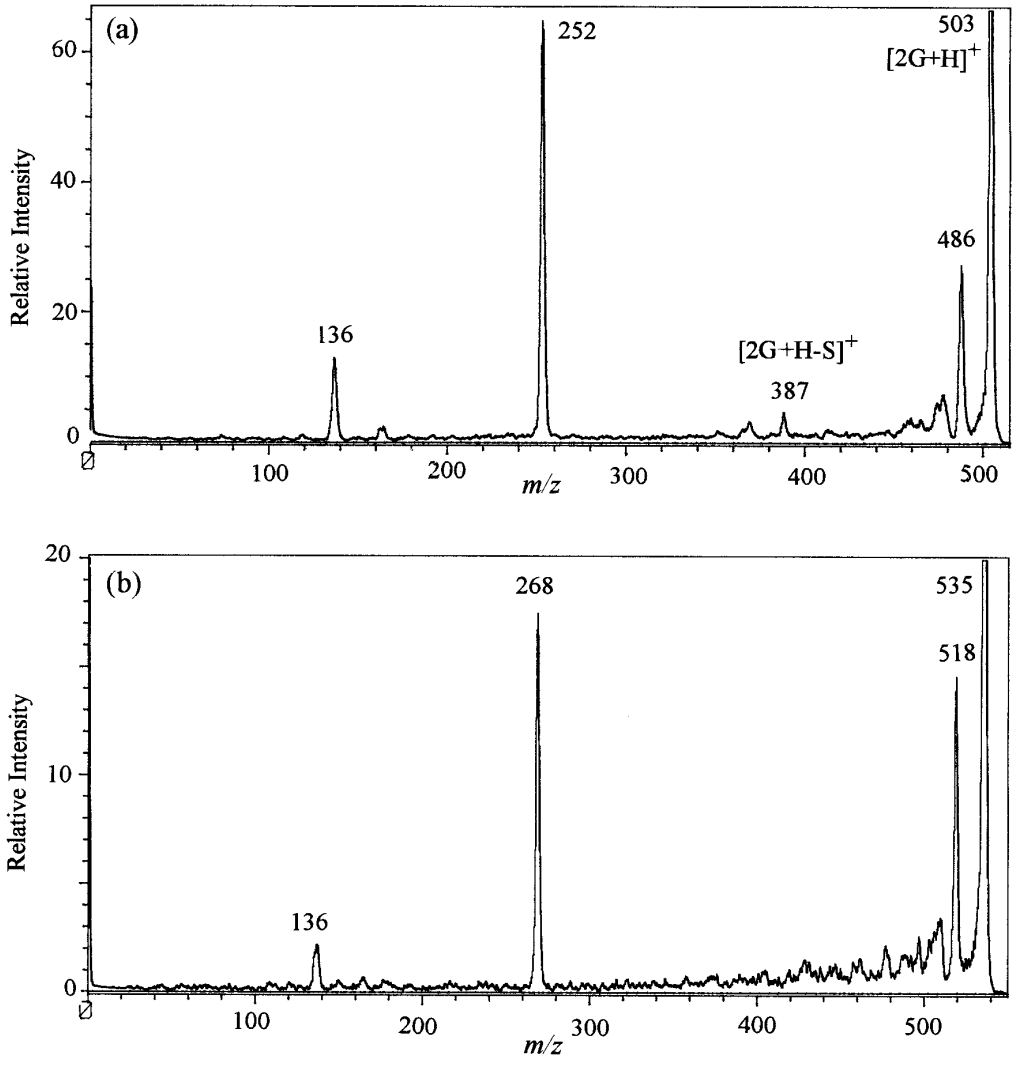

Figure 3. CID MIKE spectra of $[2G + H]^+$ of **1** (m/z 503) and **7** (m/z 535).

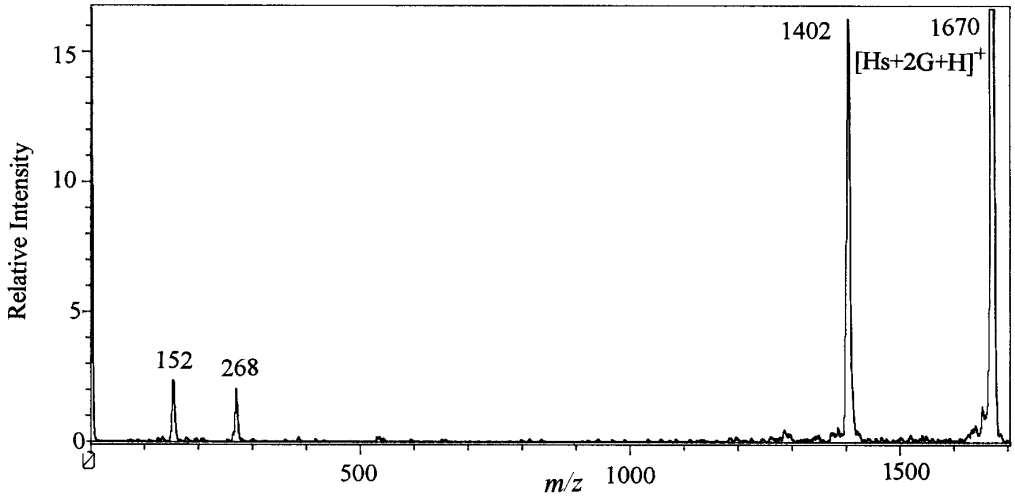

Figure 4. CID MIKE spectrum of the 2:1 complex of **2**, $[Hs + 2G + H]^+$ (*m/z* 1670).

the sugar unit is observed from the dimeric species when the sugar is a deoxysugar and not when it is a ribosugar (Fig. 3). Loss of the monomer to give the protonated nucleoside is the predominant fragmentation. One of the compounds (**2**) gave a fairly abundant 2:1 complex (having two molecules of **2** and one molecule of CD) at *m/z* 1670. Its CID MIKE spectrum is shown in Fig. 4. Comparing the CID MIKE spectra of

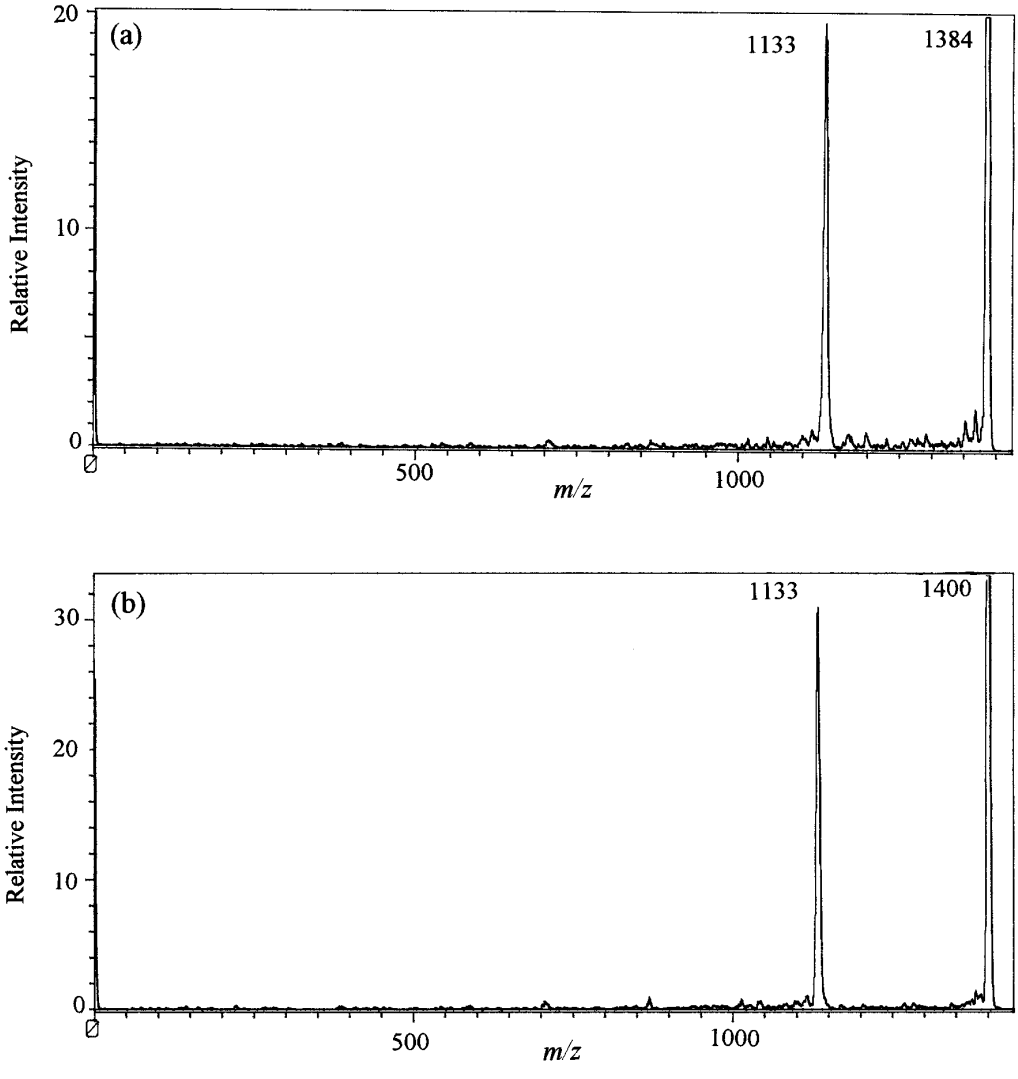

Figure 5. CID MIKE spectra of $[Hs - H + G]^+$ of **1** (*m/z* 1384) and **7** (*m/z* 1400).

the 1:1 and 2:1 complexes, it is seen that the most intense peak in the 1:1 complex corresponds to loss of the deoxysugar unit, whereas that in the CID MIKE spectrum of the 2:1 complex at m/z 1670 corresponds to the 1:1 complex. These observations suggest that the bonds between the guest and CD are stronger than the bonds between two guest molecules.

Some of these inclusion complexes also gave fairly abundant ions corresponding to $[Hs - H + G]^-$. The CID MIKE spectra of these complexes were also measured. For example, the CID MIKE spectra of $[Hs - H + G]^-$ of **1** and **7** are given in Fig. 5. The only prominent fragmentation is the elimination of the guest molecule leading to the formation of $[Hs - H]^-$ at m/z 1133. Unlike in the CID MIKE spectrum of the protonated complex, the loss of the sugar unit is not important in the CID MIKE spectrum of the deprotonated complex of **1**.

The CID mass spectrum of the protonated complex shows guest-related ions. In contrast, the CID mass spectrum of the deprotonated complex shows deprotonated host as the only product. It can therefore be concluded that the protonated complex consists of a protonated guest in the cavity of a neutral CD, whereas the deprotonated complex consists of a neutral guest in the cavity of a deprotonated CD. This is similar to the observations in a recent study on the CID spectra of protonated and deprotonated C_{60}–γ-CD complexes by Her and co-workers.[13]

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