Supramolecular Complexation of Conjugated Schiff-Base Macrocycles with Amino Acids and Small Peptides by ESI-MS and ESI-MS/MS

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Objectives

➤ Probe the formation and stabilities of supramolecular complexes of novel conjugated Schiff-base macrocycles with amino acids and small peptides in the gas phase using ESI-MS and ESI-MS/MS.

R=OC$_n$H$_{2n+1}$, n=3,4,5,6
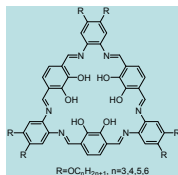
Scheme 1: Structure of conjugated Schiff-base macrocycles

Methods

➤ ESI-MS experiments were carried out on Bruker Esquire~LC and Micromass LCT.

➤ Stock solutions of the synthesized macrocycle samples were prepared in CH$_2$Cl$_2$ or CHCl$_3$. The working solutions were made by dilution of stock solutions with methanol and mixed with the amino acid or peptide solutions.

➤ MS/MS spectra of the supramolecular complexes of macrocycle with amino acids or peptides were measured on Bruker Esquire~LC. LCT was used to measure the accurate masses of fragment ions formed with in-source CID.

Results and Discussion

Amino Acids

➤ Only lysine and arginine were found to form supramolecular complexes with conjugated Schiff-base macrocycles (Fig. 1).

➤ MS/MS of macrocycle-amino acid complexes showed only the formation of [M+H]$^+$, a result of simple breakage of the hydrogen bond (Fig. 2). Fragmentation of [M+H]$^+$ formed fragment ions a, b, c and d.
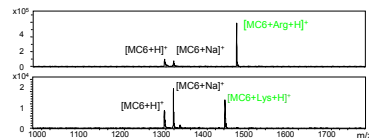

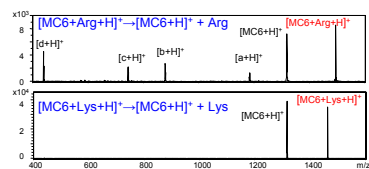Fig. 1: ESI-MS of macrocycles (MC6) with lysine and arginine

Fig. 2: MS/MS spectra of 1:1 complexes, [MC6+Lys+H]$^+$ and [MC6+Arg+H]$^+$

Peptides containing one lysine residue

➤ For di- or tripeptides, supramolecular complexes are formed with only lysine and arginine containing peptides (Fig. 3).

➤ For di- or tripeptides containing only one lysine residue, macrocycle-peptide complexes dissociate exclusively by loss of peptides (Fig. 4). This suggests that the Schiff-base macrocycle has a higher proton affinity (PA) than small peptides.
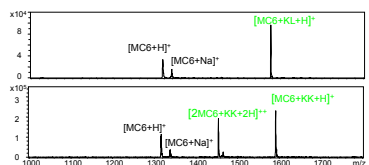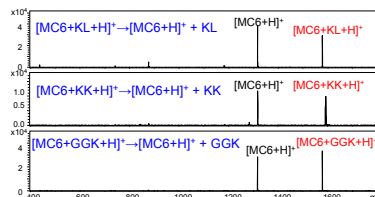

Fig. 3: ESI-MS of macrocycle (MC6) with KL and KK

Fig. 4: MS/MS spectra of 1:1 macrocycle-peptide complex with KL, KK and GGK

Peptides containing multiple lysine residues

➤ For tri- or tetrapeptides containing multiple lysine residues, doubly-charged 2:1 and even 3:1 adducts were also observed along with the 1:1 complexes (Fig. 5).

➤ The cleavage of macrocycle covalent bond was found to be competitive with the hydrogen bond cleavage for the complexes [M+KYK+H]$^+$ and [M+KKK+H]$^+$ (Fig. 6). The MS/MS data for MC6 and MC5 are summarized and compared in Table 1. Same neutral losses were found for macrocycle-KKK complexes with different peripheral alkoxy chains (Table 1). Three fragment ions are named as x, y and z. The complexes were strongly bound by multiple hydrogen bonds.

➤ The cleavage of macrocycle covalent bond is the only fragmentation pathway for [M+KKKK+H]$^+$ (Fig. 7 and Table 2), suggesting stronger binding of KKKK to macrocycle than KKK. Same neutral losses were found with [M+KKKK+H]$^+$ as [M+KKK+H]$^+$.

➤ The proposed fragmentation pattern was supported by accurate mass measurements on the fragments listed in Table 3.
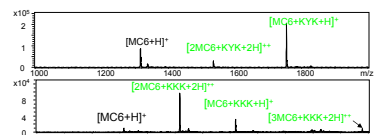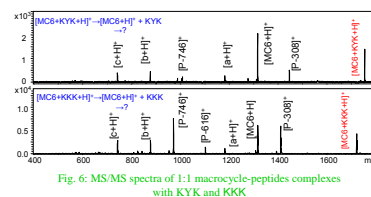

Fig. 5: ESI-MS of macrocycles (MC6) with KYK and KKK

Fig. 6: MS/MS spectra of 1:1 macrocycle-peptides complexes with KYK and KKK

Table 1: MS/MS data for 1:1 complexes, [M+KKK+H]$^+$

Ion	MC6 R=OC$_6$H$_{13}$	MC5 R=OC$_5$H$_{11}$	Neutral Loss
[M+KKK+H]$^+$	1718	1634	
x$^+$	1410	1354	R$_2$C$_6$H$_6$N$_2$
y$^+$	1102	1074	R$_4$C$_{12}$H$_{12}$N$_4$
z$^+$	972	944	R$_4$C$_{20}$H$_{14}$N$_4$O$_2$
[M+H]$^+$	1317	1234	KKK
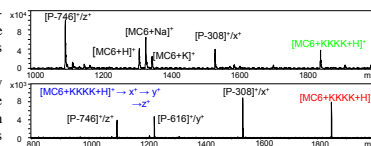
Fig. 7: ESI-MS of macrocycle (MC6) with KKKK and MS/MS of 1:1 macrocycle-KKKK complex, [MC6+KKKK+H]$^+$

Table 2: MS/MS data for 1:1 complexes, [M+KKKK+H]$^+$

Ion	MC6 R=OC$_6$H$_{13}$	MC5 R=OC$_5$H$_{11}$	Neutral Loss
[M+KKKK+H]$^+$	1846	1762	
X$^+$	1538	1482	R$_2$C$_6$H$_6$N$_2$
Y$^+$	1230	1202	R$_4$C$_{12}$H$_{12}$N$_4$
Z$^+$	1100	1072	R$_4$C$_{20}$H$_{14}$N$_4$O$_2$

Table 1: High-resolution accurate mass data for some ions of 1:1 macrocycle-KKKK complex, [M+KKKK+H]$^+$

Ions	Elemental Composition	Calculated Value	Observed Value	Error (ppm)
[M+KKKK+H]$^+$	C$_{102}$H$_{153}$N$_{14}$O$_{17}$	1846.1538		
X$^+$	C$_{84}$H$_{121}$N$_{12}$O$_{15}$	1537.9077	1537.9150	4.75
Y$^+$	C$_{66}$H$_{89}$N$_{10}$O$_{13}$	1229.6613	1229.6647	2.76
Z$^+$	C$_{58}$H$_{87}$N$_{10}$O$_{11}$	1099.6556	1099.6537	-1.73
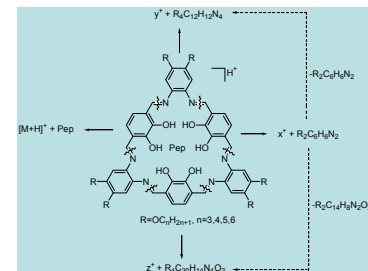
Scheme: Proposed fragmentation pattern for 1:1 macrocycle-peptide complex, [M+Peptide+H]$^+$

Conclusions

➤ Selective binding of Schiff-base macrocycles to lysine and arginine was found. Side chain of lysine or arginine is responsible for the specific hydrogen bonding.

➤ Stabilities of supramolecular complexes are related to lysine resides in the peptides.

Acknowledgement