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International Journal of Mass Spectrometry 266 (2007) 180–184
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Short communication

Formation of stoichiometric complexes between dibenzo-30-crown-10 and guanidinium moiety containing compounds

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Received 14 June 2007; received in revised form 10 July 2007; accepted 17 July 2007
Available online 21 July 2007

Abstract

Inclusion complexes between protonated guanidinium moiety and a large crown ether, dibenzo-30-crown-10, have been studied by electrospray ionization mass spectrometry (ESI-MS). In order to determine which solvent is the most suitable for generation of these complexes, methanol, water, acetonitrile and their mixtures (most common solvents for ESI) have been tested. Acetonitrile/water (1/1) has been found the best choice. In acetonitrile/water solution, the formation of stoichiometric complexes has been established (stoichiometric means that a peptide attaches the number of crown ether molecules equal to the number of guanidinium moieties it contains). For example, for the peptide containing three arginine residues the peak of the respective complex with three crown ether molecules is clearly seen.

Keywords: Guanidine; Arginine; Crown ether; Electrospray ionization

1. Introduction

Protonated guanidine is strongly complexed by large crown ethers (e.g., 27-crown-9 [27C9], 30-crown-10 [30C10], dibenzo-30-crown-10 [DB30C10]) due to the formation of hydrogen bonds (Scheme 1) [1].

As a consequence, a protonated alkyl-guanidinium side chain of arginine also forms inclusion complexes with large crown ethers. Therefore, arginine-containing peptides form complexes with these crown ethers and such complexes have been successfully analyzed by electrospray ionization mass spectrometry (ESI-MS) [2,3]. Those complexes are stable in the gas phase, however, are characterized by rather low solution stabilities [3]. ESI-MS analysis of such complexes may have some interesting applications in experiments where the presence of arginine in small peptides is of interest. For example, addition of respective crown ether (e.g., [DB30C10]) would be an excellent method for the selective recognition of the arginine fragments from a tryptic digest. However, some problems appear for the peptides containing multiple arginine residues since respective stoichiometric complexes (stoichiometric means that a peptide attaches the number of crown ether molecules equal to the number of guanidinium moieties it contains) have not been observed or have been characterized by a low signal to noise ratio [2,3]. Lysine-containing peptides easily form stoichiometric complexes with 18-crown-6 [4,5]. Thus, the problems with generating stoichiometric complexes between arginine-containing peptides and DB30C10 result from the steric hindrance. The inclusion complex between protonated alkyl-guanidinium side chain and large crown ether is quite a bulky moiety. In this communication, we show that signals of the title complexes can be detected in the ESI mass spectra by using a properly selected solvent.

2. Experimental

Guanidine hydrochloride, arcaine sulphate and dibenzo-30-crown-10 were obtained from Sigma (Poznań, Poland), Cystapep 1 was synthesized as described elsewhere [6,7], peptide AQKRRR was synthesized manually by a solid-phase method using the Fmoc/Bu^t strategy (fluorenylmethyloxycarbony/tert-butyl) [8].

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doi:10.1016/j.ijms.2007.07.005

Scheme 1. Inclusion complex between protonated guanidine and dibenzo-30-crown-10 (DB30C10).

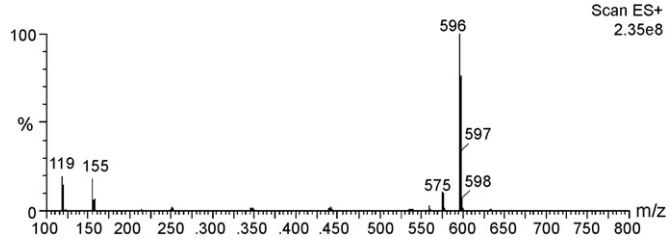


Fig. 1. ESI mass spectrum obtained for a methanol solution containing guanidine (G) hydrochloride and dibenzo-30-crown-10 (DB30C10). $[G+H+DB30C10]^+$ *m/z* 596, $[DB30C10+K]^+$ *m/z* 575, $[2G+2H+Cl]^+$ *m/z* 155, $[2G+H]^+$ *m/z* 119.

The ESI mass spectra were obtained on a Waters/Micromass (Manchester, UK) ZQ2000 mass spectrometer (single quadrupole type instrument, Z-spray, software MassLynx V3.5, Manchester, UK). The sample solutions were prepared in methanol, water, acetonitrile (most common solvents for ESI) and their 1/1 mixtures at a crown ether and guest concentration 5×10^{-5} mol/dm^3, and mixed by using ultrasonic bath. The sample solutions were infused into the ESI source using a Harvard pump, the flow rate of 80 μl/min. The ESI source potentials were capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 30 V. The source temperature was 120 °C and the dessolvation temperature was 150 °C. Nitrogen was used as the nebulizing and dessolvating gas at flow-rates of 100 and 300 l h^{-1}, respectively.

3. Results and discussion

Methanol, acetonitrile, water and their mixtures are the most common solvents used for ESI-MS. Very high signal of inclusion complexes (1:1 stoichiometry, Scheme 1) between protonated guanidine and DB30C10 was observed in each solvent (and in solvent mixtures) as shown in Fig. 1 for a methanol solution.

Arcaine contains two guanidinium moieties (Scheme 2), thus it is expected to form a 2:1 complex with DB30C10. It is

Scheme 2. Structure of arcaine.

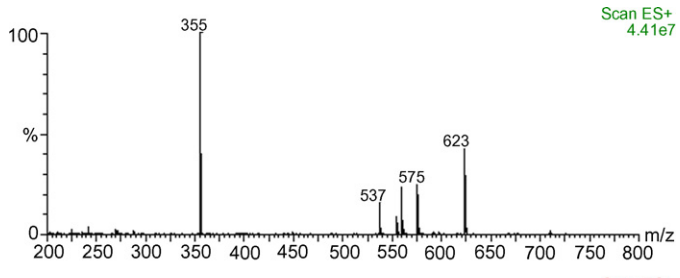


Fig. 2. ESI mass spectra obtained for acetonitrile/water (top) and methanol/water (bottom) solution containing arcaine sulphate and DB30C10. $[Arcaine+2H+DB30C10]^{2+}$ *m/z* 355, $[arcaine+2H+2DB30C10]^{2+}$ *m/z* 623, $[DB30C10+K]^+$ *m/z* 575, $[DB30C10+Na]^+$ *m/z* 559 and $[DB30C10+H]^+$ *m/z* 537.

worth mentioning that arcaine is an effective agent for reducing *N*-methyl-D-aspartate receptors [9,10]. When acetonitrile/water (1/1) was used as a solvent, high signals of the 1:1 and 2:1 complexes were obtained (Fig. 2, top). For other solvents, e.g., for methanol, the signals of complexes were characterized by low abundances in comparison to that of the signal of cationized DB30C10 (Fig. 2, bottom).

Another compound taken for the study, shown in Scheme 3, is an effective antimicrobial agent called Cystapep 1 [6,7]. Cystapep 1 contains one arginine residue, thus it is expected to form the 1:1 complex with DB30C10. This complex was observed and, similarly to arcaine, the respective signal was the most intense when using acetonitrile/water mixture as a solvent (Fig. 3).

Finally, the peptide containing three arginine residues (Scheme 4) was studied. This compound we called AQKRRR. It is worth noting that anthraquinone peptides show interesting biological activities [11,12]. For clarity, the mass spectra are shown in Figs. 4 and 5 in the range 200–600 and 600–1000 *m/z*, respectively. As shown in Fig. 4, in the range 200–600 *m/z* there is not much difference between the results obtained for acetonitrile/water and methanol/water, although the ion $[AQKRRR+3H+DB30C10]^{3+}$ at *m/z* 453

Scheme 3. Structure of Cystapep 1.

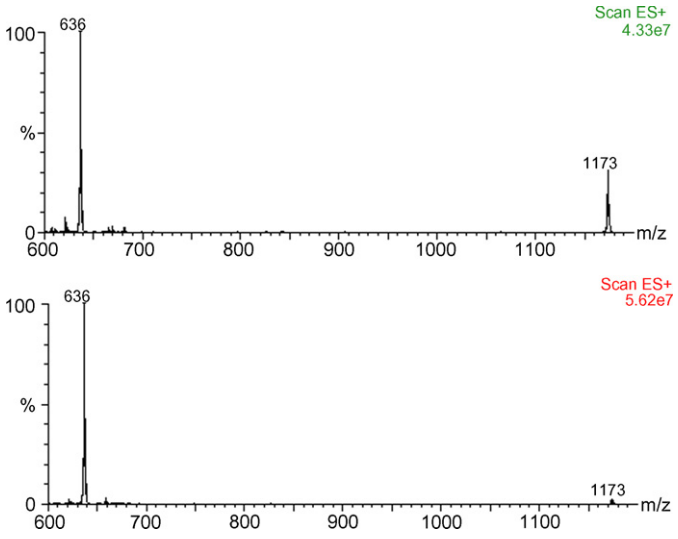
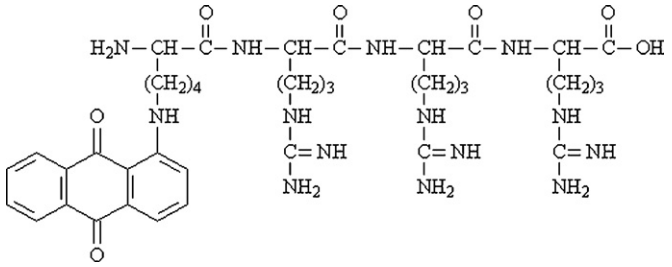


Fig. 3. ESI mass spectra obtained for acetonitrile/water (top) and methanol/water (bottom) solution containing Cystapep 1 and DB30C10. $[Cystapep\ 1 + H]^+$ *m/z* 636 and $[Cystapep\ 1 + H + DB30C10]^+$ *m/z* 1173.



Scheme 4. Structure of AQKRRR.

is more abundant in the former solvent. In the mass range 600–1000, there is an essential difference (Fig. 5). Namely with the acetonitrile/water solvent, the stoichiometric 3:1 ion $[AQKRRR + 3H + 3DB30C10]^{3+}$ at *m/z* 811 is clearly seen,

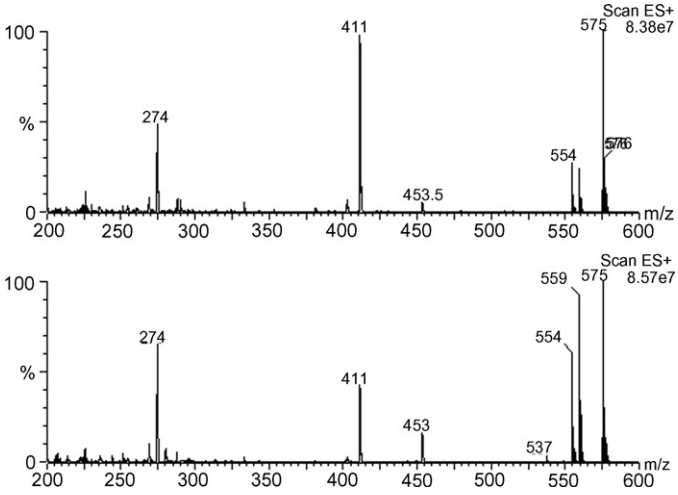


Fig. 4. ESI mass spectra at range 200–600 *m/z* obtained for methanol/water (top) and acetonitrile/water (bottom) solution containing AQKRRR and DB30C10. $[AQKRRR + 3H]^{3+}$ *m/z* 274, $[AQKRRR + 2H]^{2+}$ *m/z* 411, $[AQKRRR + 3H + DB30C10]^{3+}$ *m/z* 453, $[DB30C10 + K]^+$ *m/z* 575, $[DB30C10 + Na]^+$ *m/z* 559, $[DB30C10 + NH_4]^+$ *m/z* 554 and $[DB30C10 + H]^+$ *m/z* 537.

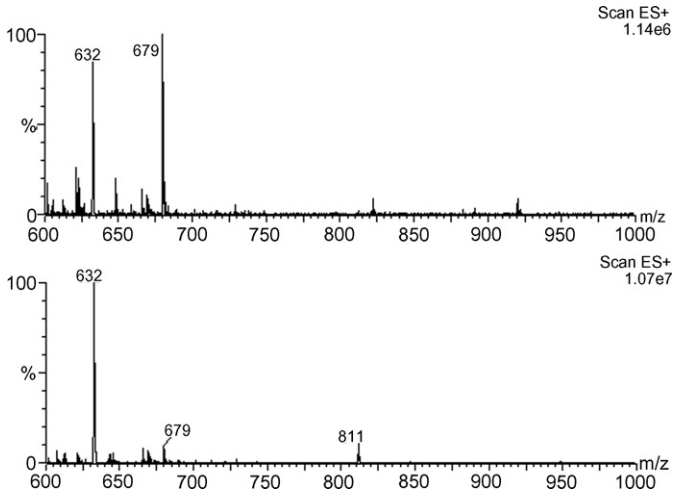


Fig. 5. ESI mass spectra at range 600–1000 *m/z* obtained for methanol/water (top) and acetonitrile/water (bottom) solution containing AQKRRR and DB30C10. $[AQKRRR + 3H + 2DB30C10]^{3+}$ *m/z* 632, $[AQKRRR + 2H + DB30C10]^{2+}$ *m/z* 679 and $[AQKRRR + 3H + 3DB30C10]^{3+}$ *m/z* 811.

indicating the presence of three arginine residues in the peptide analyzed. To be honest, we must add that the peak of ion $[AQKRRR + 3H + 3DB30C10]^{3+}$ was much lower than the base peak in the spectrum namely $[DB30C10 + K]^+$ at *m/z* 575. Their abundance ratio was about 1/70, thus, in the full range mass spectrum the peak of ion $[AQKRRR + 3H + 3DB30C10]^{3+}$ would not be clearly seen.

We have also checked if the value of the acetonitrile/water ratio affects the signals of the complexes studied. For the ratios of 2/1, 1/1 and 1/2, the obtained signal intensities were very similar.

The question is why using acetonitrile/water as solvent increases the signals of the complexes. Water enables protonation of each of the guanidinium moieties and their presence in the protonated form is necessary to attach a stoichiometric number of the crown ether molecules. For example, in pure methanol the triply charged ion $[AQKRRR + 3H]^{3+}$ at *m/z* 274 was not observed; there was only the signal of the doubly charged ion $[AQKRRR + 2H]^{2+}$ at *m/z* 411. This phenomenon was also observed for lysine-containing peptides [5]. Attachment of crown ethers to lysine-containing peptides increases their hydrophobicity [4]. It can be taken for granted that in the case of arginine-containing peptides hydrophobicity increase also takes place. Acetonitrile is less polar than methanol and water, so more hydrophobic ions are prone to exist in such solvent. Thus, it seems reasonable that a mixture of acetonitrile/water is a "good compromise" enabling the formation of multiply protonated ions and the formation of hydrophobic complex. In order to confirm above explanation, another (less polar than methanol) solvent, namely acetone, was used. Fig. 6 shows the ESI mass spectrum obtained for acetone/water solution containing Cystapep 1 and 30DBC10. The signal of the respective complex ion is clearly seen. As follows from a comparison of the mass spectra shown in Fig. 3, the acetone/water mixture is not as good as the acetonitrile/water one, however, it is better than the methanol/water one.

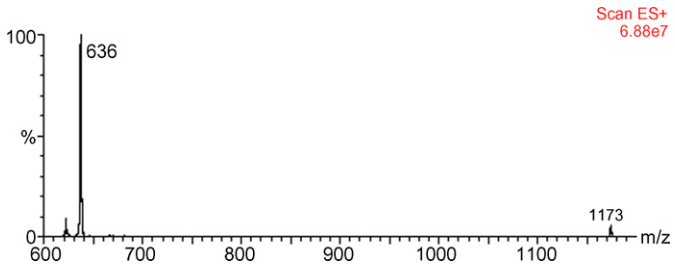


Fig. 6. ESI mass spectrum obtained for acetone/water solution containing Cystapep 1 and DB30C10. [Cystapep 1 + H]$^+$ *m/z* 636 and [Cystapep 1 + H + DB30C10]$^+$ *m/z* 1173.

Another non-polar solvent, chloroform, was also considered. However, this solvent is not miscible with water. ESI mass spectra taken for the methanol/chloroform mixture as a solvent yielded the peaks of the complexes characterized by low signal to noise ratios.

The effect of acidification of the organic solvent on the signals of the respective complexes has also been checked. Fig. 7 shows the ESI mass spectra taken for acetonitrile (bottom) and methanol (top) containing Cystapep 1, DB30C10 and 0.1% of formic acid. It is clear that in acetonitrile the signal of the complex is higher.

It is well known that crown ethers form very stable complexes with alkali metal cations [13–15]. These cations, Na^+ and K^+, are present as impurities and the signals of cationized organic molecules are very often observed in the ESI mass spectra. Without adding any inorganic salt, DB30C10 yielded high peak of [DB30C10 + K]$^+$ ion at *m/z* 575 and a lower peak of [DB30C10 + Na]$^+$ ion at *m/z* 559. Concentration of Na^+ ion is surely higher than that of K^+, however, large crown ethers prefer a larger cation. Therefore, protonated guanidinium moiety competes with alkali metal cations to occupy the crown ether cavity. Acetonitrile tends to coordinate alkali metal cations, and respective peaks were observed in the ESI mass spectra [16,17].

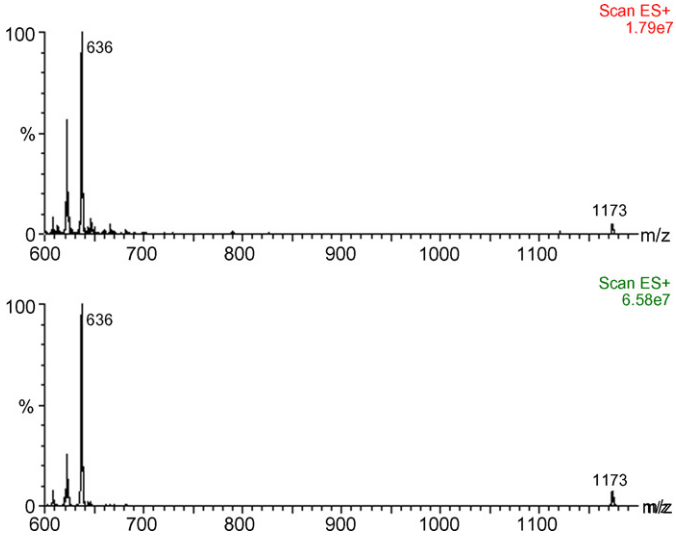


Fig. 7. ESI mass spectra obtained for acidified solution containing Cystapep 1 and DB30C10, top—methanol solution and bottom—acetonitrile solution. [Cystapep 1 + H]$^+$ *m/z* 636, [Cystapep 1 + H + DB30C10]$^+$ *m/z* 1173.

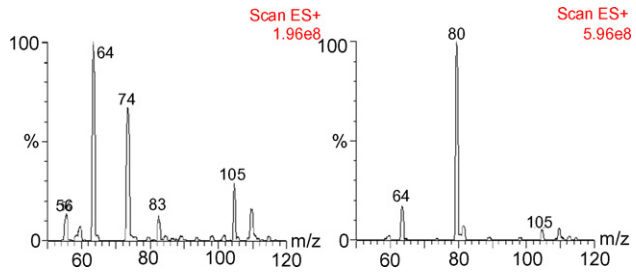


Fig. 8. ESI mass spectra obtained for acetonitrile/methanol solution, left—pure solution; right—after adding KCl. [methanol + Na]$^+$ *m/z* 56, [acetonitrile + Na]$^+$ *m/z* 64, [methanol + acetonitrile + H]$^+$ *m/z* 74, [(acetonitrile)$_2$ + H]$^+$ *m/z* 83, [(acetonitrile)$_2$ + Na]$^+$ *m/z* 105 and [acetonitrile + K]$^+$ *m/z* 80.

Coordination of K^+ and Na^+ by acetonitrile prevents formation of [DB30C10 + K]$^+$ and [DB30C10 + Na]$^+$ ion. Instead of [DB30C10 + K]$^+$ ion formation, formation of the complexes between crown ether and protonated guanidinium moiety can proceeds.

In order to confirm the hypothesis that acetonitrile competes with crown ethers for alkali metal cations, we have examined the mass spectra obtained for a mixture methanol/acetonitrile (1/1) in the low mass range. Fig. 8 (left) shows the ESI mass spectrum obtained for the pure mixture and Fig. 8 (right) shows the ESI MS spectrum obtained after adding KCl. It is clear that the attachment of acetonitrile molecule to the alkali metal cations is more favoured than that of methanol.

ESI mass spectra of the complexes studied were also obtained at higher cone voltage. Increase in this parameter led to the so-called fragmentation/dissociation "in source". For the complexes studied, we observed the loss of a neutral crown ether molecule. For the solution containing only AQKRRR the triply charged ion [AQKRRR + 3H]$^{3+}$ at *m/z* 274 was not observed at cone voltages higher than 40 V. For the solution containing AQKRRR and DB30C10 the ion [AQKRRR + 3H]$^{3+}$ was observed until the cone voltage of 70 V, since it was formed from [AQKRRR + 3H + DB30C10]$^{3+}$ *m/z* 453, [AQKRRR + 3H + 2DB30C10]$^{3+}$ *m/z* 632 and [AQKRRR + 3H + 3DB30C10]$^{3+}$ *m/z* 811. Analogous observations have been made for the complexes of lysine containing peptides with 18C6 [5].

4. Conclusions

Summing up, mixture of acetonitrile/water (1/1) is a good solvent for formation inclusion complexes between a protonated guanidinium moiety and a large crown ether, e.g., DB30C10, as demonstrated for three biologically important compounds namely arcaine, Cystapep 1 and AQKRRR. This result can be explained by taking into account the requirements of protonation of all guanidinium residues, hydrophobicity of formed complexes and competition between alkali metal cations and protonated guanidinium moiety for crown ether cavity (as described above). The complexes between DB30C10 and arginine-containing peptides are stable in gas phase; however, they are characterized by low-solution stabilities [3]. Thus, the use of respective solvents increases their concentration in solu-

tions, and as a consequence, the higher signal in ESI mass spectrum is obtained.

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