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Gas phase hydration of electrospray ions from small peptides

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Abstract

Electrospray ions of several small peptides were dispersed at atmospheric pressure into nitrogen containing precisely controlled concentrations of water vapor. The resulting gas–ion mixture was passed through a supersonic free jet expansion into a vacuum chamber containing a quadruple mass analyzer. Mass spectra of the product ions revealed sequences of peaks due to ions of the parent peptide with varying numbers of adduct water molecules. The extent of hydration seemed to relate to the "hydrophobicity" of the parent peptide. In their hydration behavior the ions of the complex comprising a couplet of cyclosporin A (CSA) and gramicidin S (GRS) resembled ions of the former more than those of the latter. The simplest explanation for this similarity is that in bonding with the GRS molecule the CSA molecule blocks the subsequent hydration of hydrophilic sites on that GRS molecule. Thus, the overall hydrophilicity is less for the complex than for the sum of its parts. This result suggests that the solvation behavior of ions of non-covalent complexes may provide clues to their conformations. (Int J Mass Spectrom 219 (2002) 1–10)

Keywords: Cyclosporin A; Gramicidin S; Electrospray ions; Electrospray ion hydration; Peptide ions

1. Introduction

The solvation properties of gas phase ions have been the subject of extensive studies for many decades. Most of those studies have been carried out with small ions of inorganic atoms and molecules. The advent of electrospray ionization (ESI) has made possible the study of the gas phase solvation behavior of large polyatomic ions having complex structures and a multiplicity of charges [1]. Valuable information on the solvation of such ions under near-equilibrium conditions has recently been obtained in studies by Klassen et al. [2] and Woenckhaus et al. [3] among others using mass spectrometric methods pioneered by Kebarle. Mass spectrometry (MS) can in principle provide solvation information that is much more accurate, sensitive and complete than can be obtained by other techniques. Unfortunately, MS analysis can only be carried out in vacuo where it is difficult to insure that the measured distribution of solvation states of an ion species is an accurate representation of the distribution in some well defined equilibrium state. For example, consider a system comprising ions in equilibrium with vapor in gas at a pressure appreciably above that in the mass analyzer. It is difficult, if not impossible, to transfer a sample of vapor from that system into vacuum without substantial changes in the extent of ion solvation. Consequently, many if not most mass spectrometric studies of ES ion hydration have been limited

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to characterizing hydration states of ions after free jet expansion of a gas–ion mixture from an appreciable pressure into the vacuum environment of the mass analyzer. During such expansion, the temperature and pressure decrease precipitously from their initial values. It thus seems almost certain, except under unusual circumstances, that those terminal hydration states are unlikely to constitute an accurate picture of the initial solvation states of those ions, i.e., before expansion. Indeed, it seems unlikely that those terminal solvation states can be meaningfully related to what should be expected in *any* particular thermodynamic state under well defined equilibrium conditions, at least for any system in which pressure and temperature are influential variables. Moreover, in some studies, e.g., those by RodriguesCruz et al. [4] and Nguyen et al. [5], the mixture of ions and gas that was expanded into vacuum came directly from the electrospray chamber and was not at equilibrium to begin with. Consequently, in neither of those studies was it possible to determine how much of the observed hydration occurred during formation of the ions from charged droplets and how much occurred during the free jet expansion. This problem has haunted many if not most studies on the solvation of electrospray ions. However, Williams and coworkers [6], as well as Beauchamp and coworkers [7], have recently been able to measure equilibrium distributions of solvation states by FTICRMS under conditions for which the effective "vapor pressure" of the solvent from the ions at the ambient temperature of the cell walls is of the same order as the background pressure of the solvent molecules in the cell. The dependence of that solvation on the wall temperature thus provides the basis for thermodynamic calculations of the enthalpies and free energies of solvation. Unfortunately, this approach is limited to temperatures and pressures that may be quite remote from those encountered in many more commonplace situations.

In a previous paper, we reported a method for eliminating contributions from the ion formation process so that all the measured solvations were only due to the free jet expansion process [8]. The method consisted in removing all solvent molecules from the ES ions *before* dispersing them in a carrier or bath gas of precisely known composition that transported those ions into the vacuum system by way of a free jet expansion. Thus, all the solvation characterized by the mass analyzer becomes entirely attributable to what goes on during that expansion. Interpretations of results from this approach do not enjoy the elegance consequent to rigorous thermodynamic analysis of equilibrium states. However, they do allow one to obtain insight rooted in reproducible quantitative comparisons among various species of solvation states that have all been reached by the same accurately specified path.

2. Apparatus and procedures

All mass analyses were carried out on a triple quadruple instrument (Delsi-Nermag 30-10) equipped with an electrospray ion source (Analytica of Branford, CT). Peptide-containing sample solution was infused through the spray needle at 0.5–1.0 μL/min by a syringe pump (Harvard Apparatus, Model 11). Water was injected into a metered stream of carrier gas (nitrogen) by another syringe pump (kds Model 100) to produce bath gas having a precisely known water content. Peptides angiotensin I–III, gramicidin S (GRS) were obtained from Sigma (St. Louis). Cyclosporin A (CSA) was a gift from Prof. R.E. Handschumacher of the Pharmacology Department and the Yale Medical School. Glacial acetic acid (ACS reagent grade) and methanol (HPLC grade) were from J.T. Backer (Phillipsburg, NJ). Deionized water was from a Nanopure Deionization System fitted with an organic-free filter (Barnstead, Dubuque, IA)

The apparatus is shown schematically in Fig. 1 and is fully described in [8]. Carrier gas from a high pressure source, throttled by valve A to a pressure indicated by gauge B, flows via sonic orifice C through heated vaporizing chamber D into which solvating species is injected in liquid form at a desired rate by syringe pump E. (The mass flow through a sonic orifice for a gas at a particular temperature is linear in the upstream pressure and independent of the downstream pressure as long as the ratio of the former to

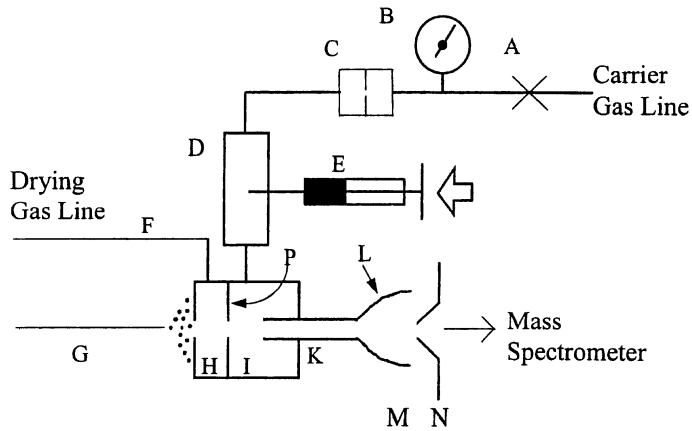

Fig. 1. Schematic representation of the apparatus. Needle valve A throttles high pressure gas to pressure shown by gauge B from which it flows through sonic orifice C through heated vaporizer D into which liquid solvating species is injected at a desired rate by syringe pump E. The resulting "bath gas" enters chamber I at a rate to maintain the pressure in I only slightly above that in drying chamber H. A potential difference between injection needle G and drying chamber H electrosprays sample solution into charged droplets that are driven by the field into chamber H counter current to a flow of drying gas through line F into chamber H from which it exits counter current to the entering flow of charged droplets and ions from electrospray needle G. Partition P is at a potential intermediate between chamber H and "soaking tube" K so that ions from evaporating droplets are driven through the entrance of H and thence through the partition orifice to the entrance of "soaking tube" K to be entrained in entering bath gas, emerging as a supersonic free jet M of ion-bearing gas into the first stage of the vacuum system. A core portion of that free jet passes through skimmer N into a separately pumped chamber containing the mass analyzer. This apparatus gave highly reproducible results in both this investigation and the earlier studies of [8].

the latter is greater than a critical value which is about 2.5 for most simple gases and was always 4.0 or more in our experiments.) The resulting "bath gas" (carrier gas plus solvent vapor) enters chamber I at a rate such that the pressure in I is only very slightly above that in H. All parts of the system downstream of D are heated to well above the dew point of the "bath gas" (carrier gas plus adduct vapor). A potential difference between injection needle G and drying chamber H disperses sample solution into charged droplets that are driven by the field to the entrance of chamber H, counter current to drying gas emerging from H. Partition P is at a voltage intermediate between chamber H and soaking tube K (an 8 cm length of brass capillary tubing with a bore of 0.5 mm in most of our experiments) so that ions from evaporating droplets are driven through the entrance of H and the partition orifice to the entrance of K to be entrained in entering bath gas, emerging as a supersonic free jet M of ion-bearing gas in the first stage of the vacuum system. A core portion of the ion-bearing jet gas flows through skimmer N into the next stage of the vacuum system and thence to the mass analyzer. This system is very effective in mixing ES ions with a bath gas of known composition and temperature, both of which are readily variable over a wide range. Moreover, the compositions of bath gas, drying gas and sample solution could be varied independently.

3. Results and discussion

First to be discussed will be experiments with three small peptides, angiotensins I–III, whose compositions and ESI mass spectra are shown in Fig. 2. The most abundant ions for each of these species are doubly charged monomers, but their abundance is some three times as great for III as for II, which in turn is 30% greater than for I. Moreover, the abundance of the singly charged monomer follows the same trend, highest for III and lowest for I, which is the only one of the three that produces a triply charged ion.

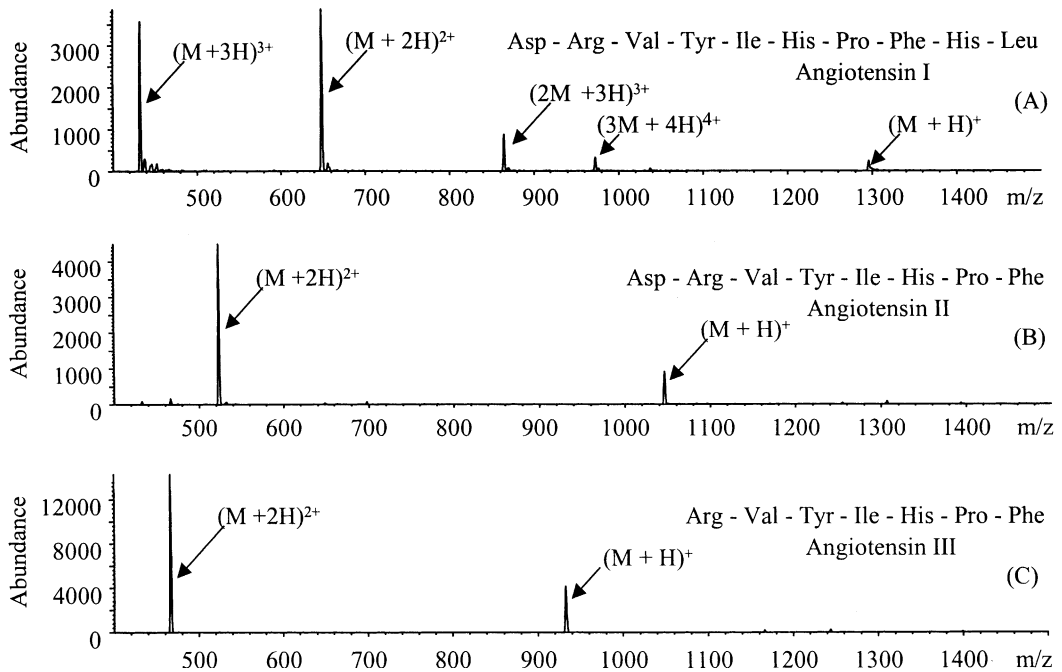

Fig. 2. ESI mass spectra for (A) angiotensin I (Mr = 1296); (B) angiotensin II (Mr = 1046); (C) angiotensin III (Mr = 931). In each case the peptides were at concentrations of 400 μmol/L in 50–50 methanol–water containing 0.1% acetic acid. The ions were completely desolvated before transport into the MS vacuum system by way of a free jet expansion of dry nitrogen.

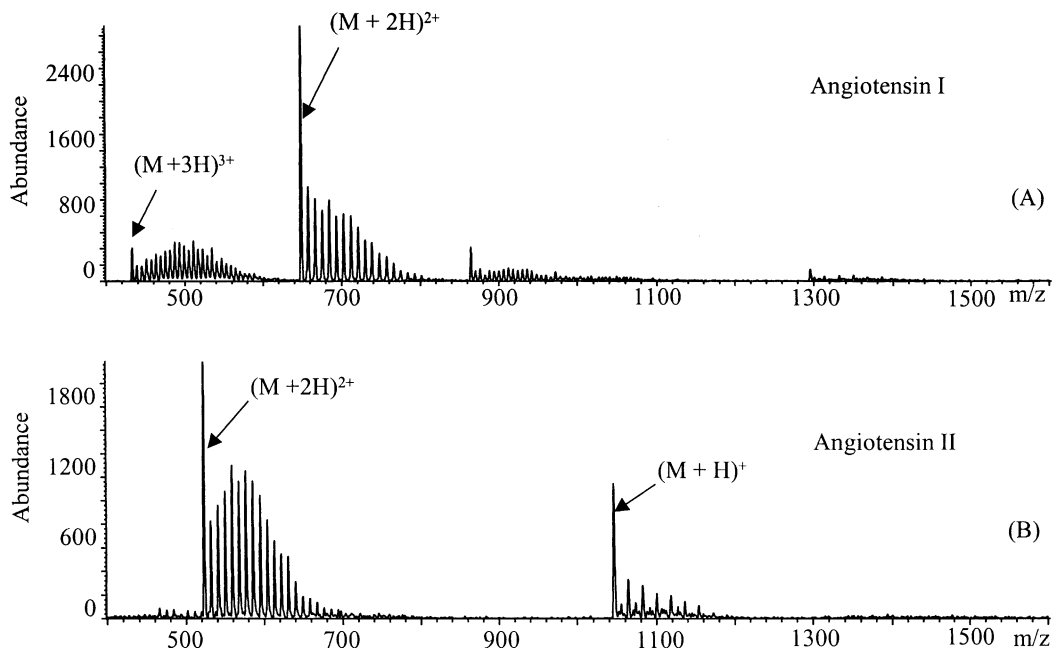

Fig. 3. ESI mass spectra for (A) angiotensin I; (B) angiotensin II. Everything was the same as for the spectra in Fig. 2 except that the bath gas contained 0.08% water vapor.

With respect to hydration, the results in Fig. 3 show that under the same conditions (0.08% water vapor in the bath gas) the fraction of doubly charged ions that remain unhydrated is larger for I than for II. The monotonic decrease in abundance with extent of solvation in I, as opposed to the rise and then fall in abundance as solvation increases shown by II is another symptom of the greater hydrophilicity of II relative to I. That latter characteristic is also evident in the solvation pattern of the triply charged ions of I and suggests that the amount of charge per se can have an enhancing effect on apparent hydrophilicity. That enhancement by charge is also evidenced by the increased hydrophilicity of the doubly charged ion of II relative to its singly charged ion.

The results in Fig. 4 were obtained some months after the spectra in Fig. 2 and are limited to a comparison of the hydration behavior of angiotensins II and III with twice the concentration of water vapor in the bath gas, i.e., 0.16%. It is noteworthy that in Figs. 3 and 4, the spectra for singly charged ions of II show a series of intermediate minor peaks that correspond to doubly charged dimers of II. This dimerization is not exhibited by I or III, but was displayed by the results with leucine enkephalin reported in [8]. Nor is it apparent in the spectrum for unsolvated ions of II in Fig. 2. It is an example of information that solvation of ions can readily reveal which might otherwise go unnoticed except with instruments having enough resolution to resolve isotope peaks. Taken together these spectra indicate that II and III are quite similar in their hydration behavior and therefore are both more hydrophilic than I. This conclusion is consistent with both retention times of these species in reverse phase liquid chromatography (LC) [9] and calculated hydrophobicities [10]. Also interesting is the relative absence of “magic number” solvation for these species.

The results obtained with CSA and GRS are somewhat more provocative. The sequences of the component amino acids for these two cyclic oligopeptides are given in Fig. 5. Fig. 6 shows the spectra obtained by ESIMS analysis of solutions containing these

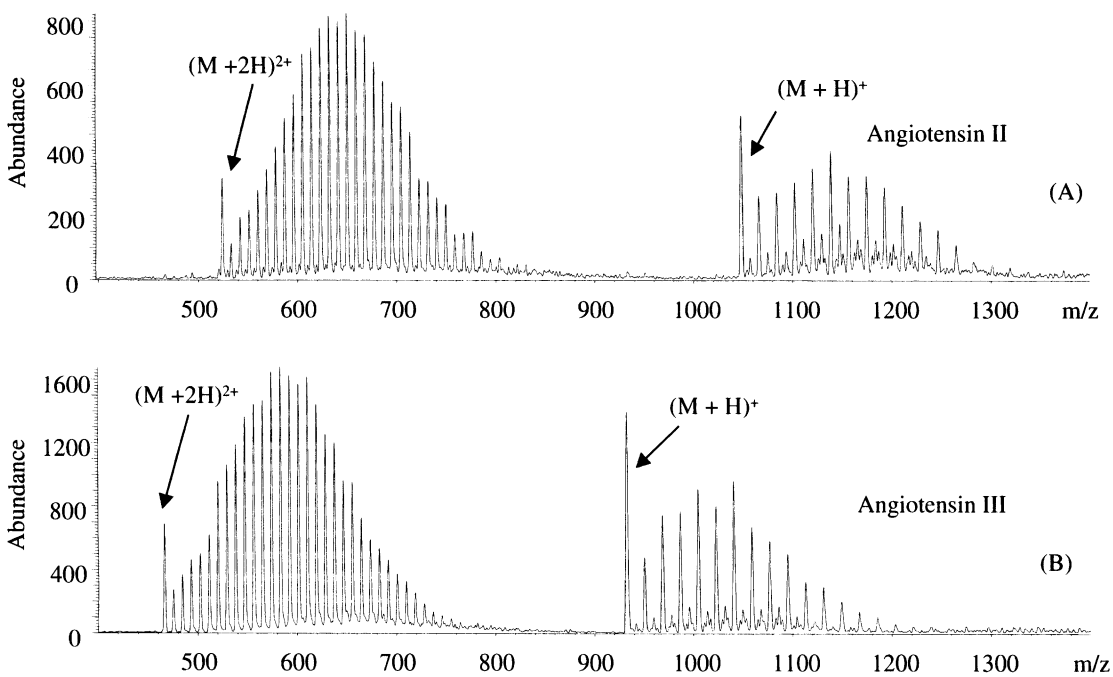

Fig. 4. ESI mass spectra for (A) angiotensin II; (B) angiotensin III. Everything was the same as for the spectra in Fig. 2 except that the bath gas contained 0.16% water vapor.

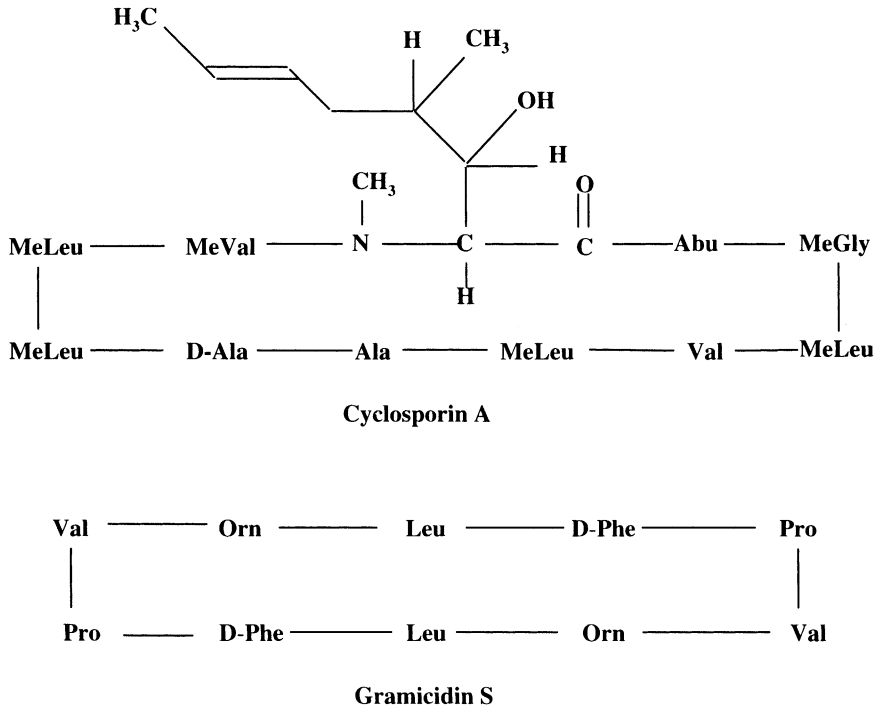

Fig. 5. Schematic diagram showing the identities and sequences of amino acids in cyclosporin A (CSA) and gramicidin S (GRS).

compounds at 500 μmol/L concentrations in 50–50 methanol–water containing 0.025% of acetic acid. In spectrum A for CSA the two major peaks are for singly and doubly charged ions, the former being roughly 30% more abundant than the latter. These two major peaks have small satellites that might at first seem due to one adduct water molecule on each charge, evidence of incomplete desolvation. In fact, they are due to unidentified impurities. In spectrum B for GRS, the single major peak is due to doubly charged ions of that peptide. There is also a very small peak at an *m*/*z* value of 773 corresponding to a triply charged ion of a dimer having an adduct HCl, an acid with which GRS readily forms a salt. At this concentration the abundance of singly charged ions of GRS is very small but becomes comparable to that for doubly charged ions at higher initial concentrations, i.e., millimolar.

The hydration behavior of these CSA and GRS ions is revealed in Fig. 7 for the case when the desolvated ions were dispersed in nitrogen containing 0.08 mol% water vapor. Spectrum A, for CSA, shows that for both the singly and doubly charged ions, the extent of solvation is so limited that most of the ions remain dry. Spectrum B, for GRS under the same conditions, shows that the majority of the ions are solvated. Doubling the water vapor content in the bath gas, to the still relatively low value of 0.16%, substantially increased the extent of solvation for both species, as shown in Fig. 8. Most of the singly charged CSA ions still remain dry, but a majority of the doubly charged ions are solvated to a substantial extent. For GRS, in contrast, both the singly charged ions, present in only trace amounts, and the doubly charged ions comprising the vast majority, are so extensively solvated that completely dry ions are barely detectable. These observations constitute strong evidence that GRS is far more hydrophilic than CSA, a result that is entirely consonant with the chromatographic behavior of these peptides. Column retention times in

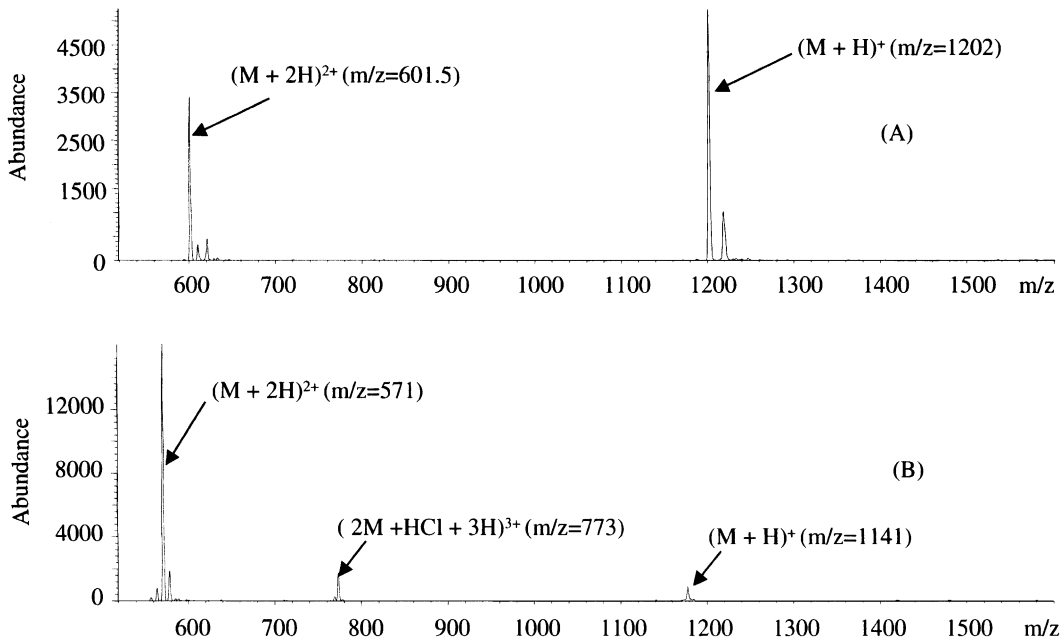

Fig. 6. ESI mass spectra for (A) cyclosporin A (Mr = 1201) and gramicidin S (Mr = 1140). In each case the electrosprayed solution contained the peptide at a concentration of 500 μmol/L in 50–50 methanol–water containing 0.025% acetic acid. The ions were (almost) completely desolvated before transport into the vacuum system of the mass spectrometer in a free jet expansion of dry nitrogen.

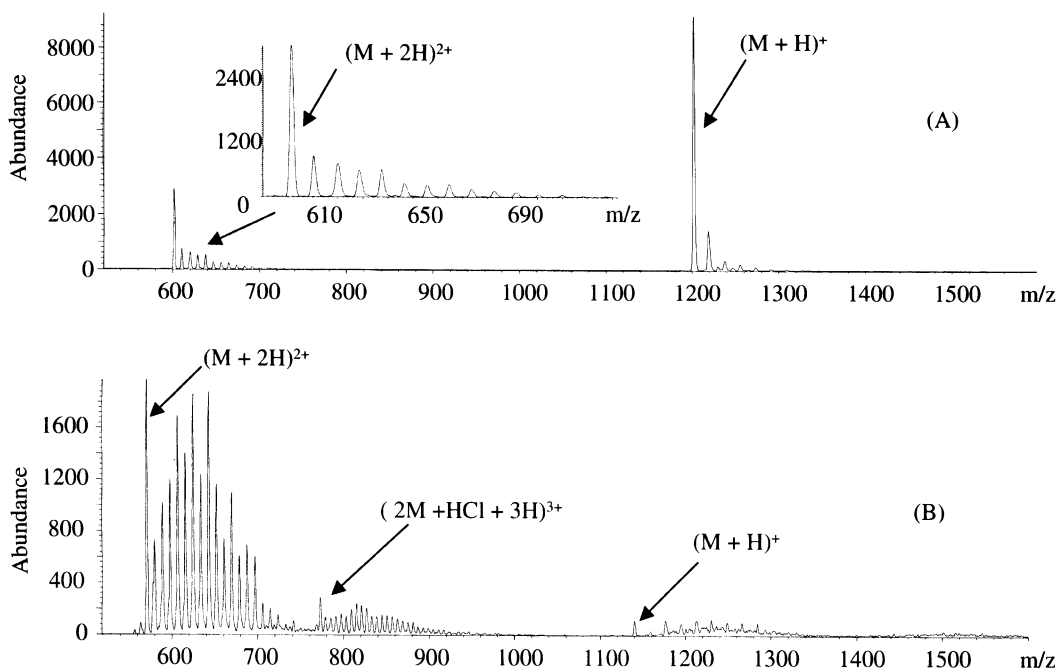

Fig. 7. ESI mass spectra for (A) cyclosporin A; (B) gramicidin S. Everything was the same as for Fig. 6 except that the bath gas contained 0.08% water vapor.

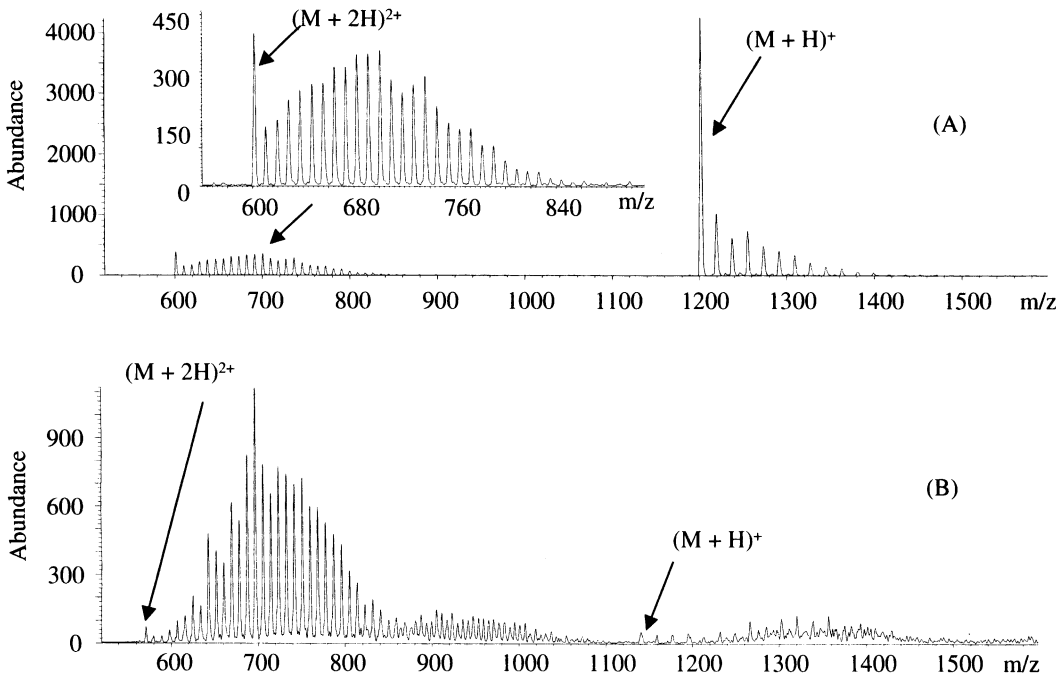

Fig. 8. ESI mass spectra for cyclosporin A and gramicidin S. Everything was the same as for Fig. 7 except that the bath gas contained 0.16% water vapor.

reverse phase HPLC, are far longer for CSA than for GRS [11].

4. Conformation implications of ion solvation

Fig. 9 shows the ESIMS spectrum obtained for a solution comprising CSA and GRS in 50–50 methanol–water containing 0.025% of acetic acid. Each peptide was at a concentration of 250 μmol/L. As we discovered some years ago, a major peak in the spectrum for such a mixture corresponds to a dimer containing one molecule of each peptide. The hydration behavior of this complex is shown in Fig. 10. Spectrum A was obtained when the water concentration was 0.08%, spectrum B when it was 0.16%.

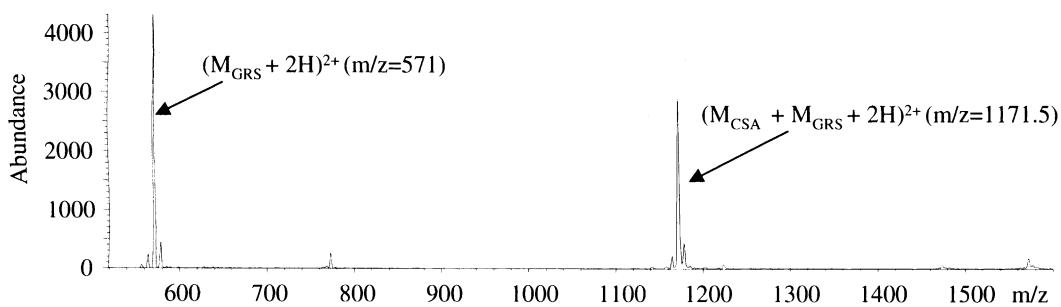

Fig. 9. ESI mass spectrum for a solution containing both cyclosporin A and gramicidin S, each at a concentration of 250 μmol/L in 50–50 methanol–water containing 0.025% acetic acid. The ions were completely desolvated before transport into the vacuum system by way of a free jet expansion of dry nitrogen.

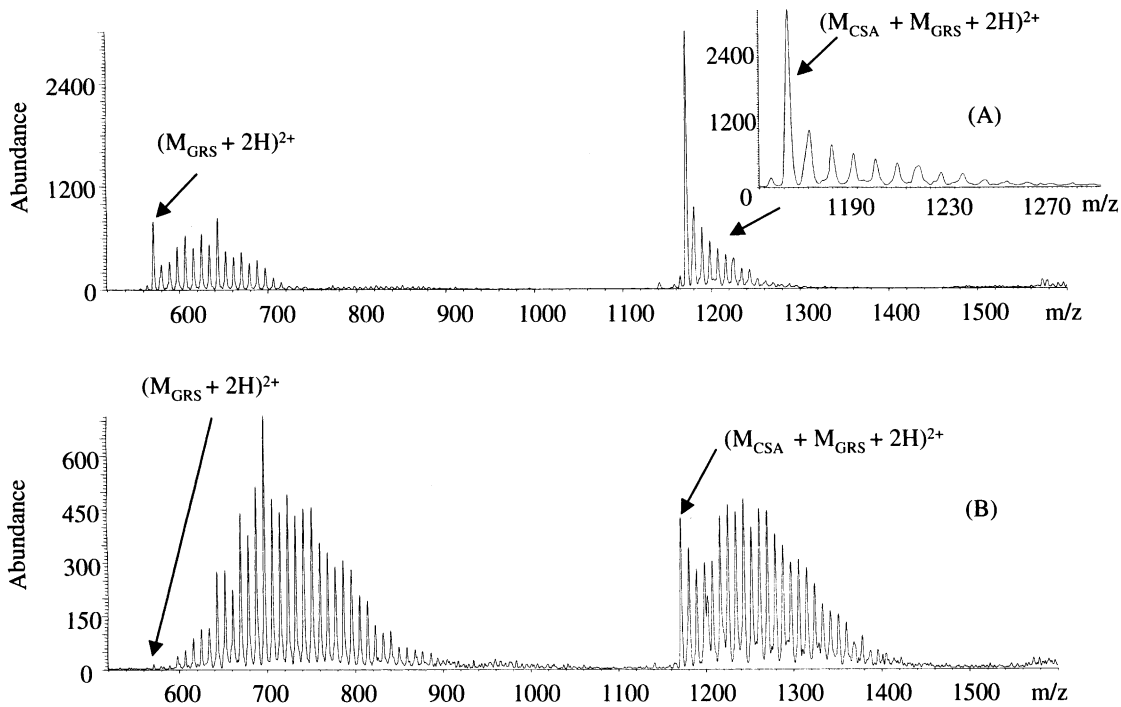

Fig. 10. Everything the same as in Fig. 9 except that the bath gas contained water vapor at concentration of 0.08% for (A) and 0.16% for (B).

Comparison of Fig. 10 with Figs. 7 and 8 shows very clearly that the complex is much more like CSA than GRS in its hydration behavior. That is, the extent of hydration for the complex is about the same as for CSA, i.e., much less than for GRS.

The inescapable conclusion from this result is that the structure of the complex is such that the hydration sites in its GRS component are somehow prevented from hydrating, e.g., by being shielded from access by water molecules. Moreover, because both peptide molecules are about the same size, such shielding cannot be due to an envelopment of an entire GRS molecule by a CSA molecule. In other words, the locus of attachment of GRS to CSA is selective in the sense that it affects those parts of the GRS molecule that constitute hydration sites. As already noted, both their relative retention times on a reverse phase LC column and their relative hydration behavior shown in Figs. 7 and 8, indicate that CSA is overall much more hydrophobic than GRS. This conclusion is to be expected in view of the fact that all of CSAs component amino acids are considered to be hydrophobic whereas 4 of the 10 amino acids on GRS are strongly hydrophilic. Moreover, the literature indicates that a betasheet model with two-fold molecular symmetry is generally accepted as the most likely structure for GRS [11–13]. “This model has the hydrophobic surface directed to one side of the average backbone plane and the charged side chains of ornithine directed to the other side.” It is thus tempting to conclude that the structure of the complex is such that the CSA molecule attaches to the side of the GRS molecule that contains the hydrophilic ornithines, thereby insulating them as prospective hydration sites from access and/or occupation by the gas phase water molecules. The main “rationale” for this fairly crude conjecture now rests solely on the basis that “like sticks to like” but it is consistent with the experimental observations. A more rigorous explanation for those observations awaits detailed molecular modeling studies.

5. Conclusions

Solvation studies with water and other solvents, similar to those reported here and in [7], along with those of other investigators, can provide a lot of information on the nature of complex molecules of biological importance. Of most value would be measurements on the distribution of solvation states under well defined equilibrium conditions. Unfortunately, such data are extremely difficult to obtain. More easily obtainable measurements, short of that ideal, can also be rich sources of insight but it is important that they relate to conditions that are reproducible and well defined even though they are not at equilibrium. In particular, one must be careful in drawing conclusions from results in which more than one process can contribute to the observed result. In view of the rapidly growing awareness of the variety and intricacy of the reactions and processes in which the peptides and proteins of living systems engage, there will be a growing need for methods of elucidating the mechanisms of these processes. Of special impact is the emerging realization that two or more different species may “collaborate” in a single process by way of non-covalently bound complexes. In this perspective, it seems likely that the most important result in this communication is that solvation studies may provide valuable insight on the conformation and structure of such complexes.

Acknowledgements

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