# Observation of the Multimeric Forms of Concanavalin A by Electrospray Ionization Mass Spectrometry

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Mass spectrometry (MS) is becoming a powerful tool for the analysis of biomolecules due in large part to the capabilities produced by newer ionization techniques such as electrospray ionization (ESI). Arguably the most important contribution of electrospray ionization mass spectrometry (ESI/MS) to biomolecule characterization is the accurate molecular weight determination for biopolymers from the multiple charge-state distribution generally obtained with ESI/MS.[1] Recent results have demonstrated that relatively weak noncovalent associations can be preserved upon transfer into the gas phase with ESI, providing a new approach to the determination of both structurally specific[2] and nonspecific[3] noncovalent associations present in solution. At a minimum, such noncovalent associations require mild ionization and atmosphere–vacuum interface conditions for mass spectrometric detection while still providing sufficient desolvation.[1c,2,3] It has been well established that there is a relationship between solution conformation and charge-state distribution observed by ESI/MS,[4] where the more compact tightly folded solution structures are observed at lower charge states. The changes in charge-state distribution can be attributed to factors including Coulombic contributions and the inaccessibility of some of the charge sites compared to the open (unfolded) solution conformation. More recently, direct evidence of maintaining some portion of this solution conformation in the gas phase has been presented with gas-phase H/D exchange experiments.[5] Most noncovalent solution associations studied by ESI/MS have been observed at lower charge state (higher $m/z$) than the individual subunits,[2] as expected for compact and labile structures. Therefore, an extended $m/z$ range (>3000 ) mass spectrometer may be necessary for studying many noncovalent associations in near physiological pH solutions (where less extensive charging is typically observed) by ESI/MS. In this communication, we report the successful ionization and detection of the dimeric and tetrameric forms of concanavalin A (Con A) by ESI/MS.

Concanavalin A, the jack bean lectin, has been the subject of many biochemical studies, and it was one of the first lectins for which a three-dimensional structure was determined.[6] Con A has been shown to crystallize as the tetramer of nearly identical 237 amino acid ($M_r$ 25 500) protomers.[7] In solution, there exists a dimer–tetramer equilibrium that is temperature and pH dependent.[8] It has been reported that Con A is predominantly a tetramer at pH > 7 and a dimer at pH 5.5.[9] Molecular weight distribution analysis of Con A obtained by sedimentation equilibrium did not indicate the presence of any monomer, trimer, or oligomer species greater than the tetramer.[8] Therefore, only the dimer and tetramer of Con A should be observed by ESI/MS if interface conditions are sufficiently gentle to maintain the associations present in solution.

Positive ion ESI mass spectra of Con A solutions[10] were obtained using an extended $m/z$ range (~45 000) quadrupole mass spectrometer developed in our laboratory. The mass spectrometer employed a heated metal capillary interface.[11] The mass spectra were obtained under conditions intended to minimize heating of molecular ions (i.e., low capillary–skimmer interface voltage, low capillary temperature) but still providing sufficient desolvation.[12] Figure 1a shows a low-resolution mass spectrum of Con A in 10 mM $NH_4OAc$ (pH ~ 6.7) with 23 W applied to the inlet capillary and a capillary–skimmer voltage difference ($\Delta$CS) of 47 V. Three multiply charged molecular ion peaks indicative of the intact tetramer (Q) are observed near $m/z$ 5000 and labeled as $Q^{22+}$ to $Q^{20+}$, along with three peaks indicative of the dimeric form (D) near $m/z$ 3500 labeled $D^{16+}$ to $D^{14+}$.[13] The absence of trimer or pentamer species is convincing evidence that the tetramer (and likely dimer) species arise due to specific association in solution.[14]

Increasing the extent of heating or collisional activation of Con A molecular ions in the ESI/MS interface leads to disruption of these self-associated tetramers. Figure 1b shows the mass spectrum of Con A for the same solution conditions as Figure 1a, with the capillary heating increased to 26 W and the $\Delta$CS increased to 77 V. The ions due to the dimer species have increased

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(10) Concanavalin A was obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purifcation. Senear and Teller[8] have estimated that in commercial preparations of Con A, approximately 50% of the subunits are hydrolyzed between residues 118 and 119 (termed "fragmented"). However, the hydrolyzed subunits maintain their normal folded structure and are no smaller than dimers in solution. This heterogeneity in the sample would most likely be undetectable in these experiments due to the low resolution of the mass spectrometer employed.

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(12) Electrospray conditions utilized a 0.4 $\mu$L/min sample flow rate with no coaxial sheath flow, a coaxial flow of $SF_6$ (~100 mL/min) to help stabilize the electrospray and reduce corona discharge, and a countercurrent flow of heated $N_2$ for desolvation.

(13) It is noteworthy that while even charge-state ions can be given other assignments (as dimers or monomers), the odd charge-state ions have effectively unique assignments. For example, the ion labeled $Q^{20+}$ which is assigned to the 20+ tetramer has the same $m/z$ value as the 10+ dimer ion and the 5+ monomer ion of the individual subunit, whereas the $Q^{21+}$ ion is uniquely the 21+ tetramer ion ($M_r$ ~ 102 000). Due to the observation of these odd charge-state ions and the corresponding spacing of the three ion species, the given assignments are the most plausible for the ions observed.

(14) The aggregation of gas-phase multiply charged molecules to form these dimers and tetramers is very unlikely. Details have been addressed in: (a) Rockwood, A. L.; Busman, M.; Smith, R. D. *Int. J. Mass Spectrom. Ion Proc.* **1991**, *111*, 103. (b) Ogorzalek Loo, R. R.; Udseth, H. R.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 695.

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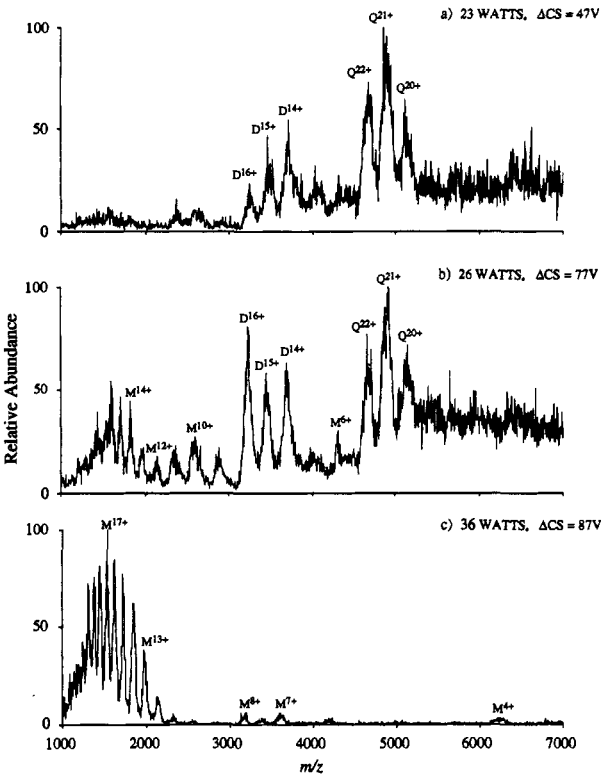


**Figure 1.** Positive ion ESI mass spectrum of concanavalin A in 10 mM $NH_4OAc$ obtained on the extended *m/z* range quadrupole mass spectrometer with differing interface conditions. Peaks labeled M are from the monomeric form, D are from the dimeric form, and Q are from the tetrameric form of Con A. (a) Mild interface conditions, capillary–skimmer voltage (ΔCS) = 46 V and capillary heating = 23 W (~180 °C at external surface). (b) Intermediate interface conditions, ΔCS = 77 V and capillary heating = 26 W (~185 °C). (c) Relatively harsh interface conditions, ΔCS = 87 V and capillary heating = 36 W (~250 °C).

in intensity relative to the tetramer ions, and a new charge-state series of ions has appeared (labeled $M^{16+}$ to $M^{6+}$) due to the breakup of the associated species into the monomeric subunits. Further dissociation of the Con A multimers can be observed in Figure 1c, where the capillary heating is increased to 36 W and the ΔCS = 87 V. Now only the monomeric form of Con A is observed, even though the solution conditions remain the same. Clearly, the interface conditions are crucial for observing in the gas phase the tetrameric and dimeric forms of Con A. Lowering the pH of the solution showed the expected decrease in tetramer species to be observed by ESI/MS. A more detailed study of the effects of solution pH and interface conditions on the observation of this association is in progress.

These results show that self-association of Con A subunits to form dimers and tetramers can be observed upon transfer to the gas phase with ESI/MS and that careful choice of interface conditions is crucial for preserving these noncovalent associations, consistent with earlier reports for the analysis of noncovalent complexes.[23] It is noteworthy that the average charge per monomer unit has the order monomer > dimer > tetramer, with a decrease of approximately two charges (per monomer unit) for the tetramer-to-dimer transition. An extended *m/z* range is useful for such studies since the tetrameric form of Con A is observed only beyond *m/z* 4000. Higher charge-state ions for a given species are collisionally activated to a greater extent in the interface than lower charge-state ions of the same species,[14a,15] are less stable due to repulsive Coulombic forces, and thus are more likely to dissociate due to the same processes required to desolvate the lower charge-state ions. The much different average *m/z* values for the monomer, dimer, and tetramer species indicates that dissociation of the multimers occurs *not* in the gas phase but earlier, during heating of the electrosprayed droplets, since dissociation of desolvated multimers would yield much lower charge-state monomers. These observations suggest that the rate of molecular ion desolvation (i.e., the effective heating profile through the ESI/MS interface) may be particularly important in preservation of such associations. Finally, observation of the relatively labile quaternary structure of this protein raises the possibility that the tertiary (native folded) structure of proteins can be retained on the ESI/MS time scale.

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