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Gas-phase memory of solution-phase protein conformation: H/D exchange and Fourier transform ion cyclotron resonance mass spectrometry of the N-terminal domain of cardiac troponin C

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Abstract

Electrospray ionization, followed by gas-phase hydrogen/deuterium exchange and Fourier transform ion cyclotron resonance mass analysis provide a means for correlating the gas-phase and solution-phase conformations of a protein. Multiple gas-phase conformations (based on different rate and extent of deuterium incorporation) are found for each of the 6+, 7+, and 8+ charge states of the N-terminal domain of cardiac muscle troponin C (cNTnC). We analyze the effects of variations in ion accumulation period and capillary temperature on protein ions of the *same* charge state following electrospray from either aqueous or methanolic solvent. The deuterium exchange-resolved conformations of the 8+ charge state are essentially the same for cNTnC electrosprayed from either solvent. However, the 6+ and 7+ charge states of cNTnC exhibit different gas-phase conformation (reflected in different deuterium uptake profiles) when electrosprayed from aqueous solution and highly organic solutions. The present experiments constitute some of the most direct evidence that gas-phase protein ions can retain some "memory" of their solution-phase conformation. (Int J Mass Spectrom 192 (1999) 319–325) © 1999 Elsevier Science B.V.

Keywords: Hydrogen/deuterium exchange; FTICR; Protein conformation in vacuo; Solvent

1. Introduction

Hydrogen bonding [1], hydrophobic forces [2] and van der Waals/steric forces [3] are important factors that determine protein folding in solution. Theorists have studied proteins *in vacuo* for some time [4]. During the 1990's, matrix-assisted laser desorption/ionization (MALDI) [5] and electrospray ionization (ESI) [6] have made possible mass analysis of large involatile biomolecules. Mass analysis approaches for examining gas-phase protein ion conformation include gas-phase hydrogen/deuterium (H/D) exchange [7,8], gas-phase proton transfer reactions [9–11], gas-phase ion mobility [11–17], gas-phase basicity [10], and energetic surface imprinting [18,19]. Gas-phase H/D exchange with FTICR mass analysis can resolve multiple conformations of gas-phase protein ions [7,8]. Ion mobility yields a spectrum of ion

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cross-sectional areas and thus provides a gross but direct measure of gaseous protein ion shape/size. Thus, the latter two techniques offer complementary probes of conformational heterogeneity and conformational stability of gas-phase protein ions [8]. Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) is especially well suited for gas-phase H/D exchange and other ion–molecule reaction experiments, by virtue of its ultrahigh mass resolving power [20–25] and the ability to trap and react gaseous ions for extended periods [26].

ESI enables the determination of gas-phase protein mass and charge. However, it is unclear how proteins react to desolvation during the process of electrospray ionization. The source conditions controlling ion formation (e.g. needle voltage, source pressure, capillary temperature, solvent composition, configuration, and dimensions) affect the final conformational distribution of gas-phase ions. A correlation between gas-phase and solution-phase of bovine cytochrome *c* and ubiquitin ion conformations has been inferred from gas-phase ion mobility measurements [13,17], whereas no measurable difference in gas-phase basicity of cytochrome *c* ions electrosprayed from different solvents was found [10]. A significant problem is that changing the solvent or electrospray conditions can affect the gas-phase protein charge state distribution, and different charge states may exhibit radically different conformational distributions [8,17]. Thus, the optimal comparison will be based on a protein from which ions of the *same* charge state and *essentially the same abundance* can be obtained following electrospray from solutions in which the protein conformation is known to differ.

In this investigation, we examine the conformations of gas-phase protein ions electrosprayed from different solvents, by use of gas-phase H/D exchange monitored by FTICR MS. We chose cardiac N-terminal domain troponin C (cNTnC) as a system to investigate because (unlike many other proteins), the *same* charge states of gas-phase protein ions are obtained on electrospray from different solvents. We are thus able to compare the deuterium uptake distributions of gas-phase cNTnC ions electrosprayed from water and highly organic solutions in which the protein conformation is native and denatured, respectively.

2. Materials and methods

2.1. Materials

D_2O (99.9 at.% D) was purchased from Aldrich Chemical Co. (Milwaukee, WI). 99.99% ^{14}N ammonium sulfate and 99.95% ^{12}C glucose were obtained from Isotech (Miamisberg, OH). All other chemicals and reagents were of the highest commercially available grade.

2.2. Protein preparation

The N-terminal domain of cardiac troponin C (cNTnC) was expressed from *E. coli* cells, strain BL21(DE3)pLysS, from a culture in which the sole nitrogen and carbon sources were 99.99% ^{14}N ammonium sulfate and 99.95% ^{12}C glucose, as previously described [27]. The resulting ^{13}C, ^{15}N doubly depleted cNTnC protein exhibits a much narrower isotopic distribution than that of cNTnC having natural isotopic abundance [28], and thus makes it easier to resolve different gas-phase protein ion conformations based on different rate and extent of deuterium incorporation during gas-phase H/D exchange. Of course, because not every protein ion has exchanged an identical number of deuteriums after a particular incubation period, the isotopic distribution will always widen following H/D exchange.

2.3. Gas-phase H/D exchange

Gas-phase H/D exchange of cNTnC ions was performed as previously described for bovine ubiquitin [8]. Experiments were performed on a previously described 9.4 T ESI FTICR mass spectrometer configured for external accumulation [29,30]. Samples (10–20 μM) were infused into a tapered 50 μm i.d. fused silica micro-ESI needle at a rate of 300 nL/min at a needle voltage of 2.5 kV and typical heated capillary current of 2.5 A. Ions were accumu-

lated in a linear octopole ion trap (operated at 1.5 MHz, 100 $V_{p\text{-}p}$) for typically 3 s and then transferred to the ICR cell through a second octopole ion guide (operated at 1.5 MHz, 100 $V_{p\text{-}p}$). The isotopic distributions for ^{13}C, ^{15}N-depleted cNTnC ions of a given charge state were isolated by stored waveform inverse Fourier transform (SWIFT) dipolar excitation [31–33]. Immediately following isolation, the ions were cooled by trapping in the cell for ~10 s. The parent ions were then allowed to react with D_2O pulsed into the vacuum system via a previously described three-way pulse valve/leak valve combination [34,35]. The partial pressure of D_2O during the pulse rose to 2×10^{-7} Torr within ~2 s and remained stable throughout the course of the H/D exchange period. Ions were allowed to react with the neutral exchange reagent for different time periods. The exchange reagent pulse was followed by a 5 min pumpdown where the pressure rapidly dropped to 5×10^{-8} Torr (~10 s) and achieved a final pressure of 2×10^{-8} Torr. Neutral pressure was measured with a Granville Phillips (Boulder, CO) Model 274 ion gauge. Typical base pressure for the instrument was 2×10^{-9} Torr. The ions were then subjected to broadband frequency sweep excitation (50–300 kHz) and detection (300 kHz Nyquist bandwidth and 256 KWord data). An Odyssey™ data station (Finnigan Corp., Madison, WI) controlled all experiments.

2.4. Circular dichroism spectroscopy

All circular dichroism (CD) spectra were recorded with an AVIV Model 62A DS spectropolarimeter (Lakewood, NJ), at a protein concentration of 5 μM, in a quartz cell with a 1 mm path length. The measured ellipticity data were averaged over three runs and converted into mean residue ellipticity, $[\theta]$, defined as $[\theta] = \theta/10\ n\ C\ l$, in which θ is the measured ellipticity in millidegrees, n is the number of amino residues in the protein, C is protein concentration (mol/L), and l is the path length of the cell (cm). The CD data were plotted as mean residue ellipticity $[\theta]$ versus wavelength in 1.0 nm steps.

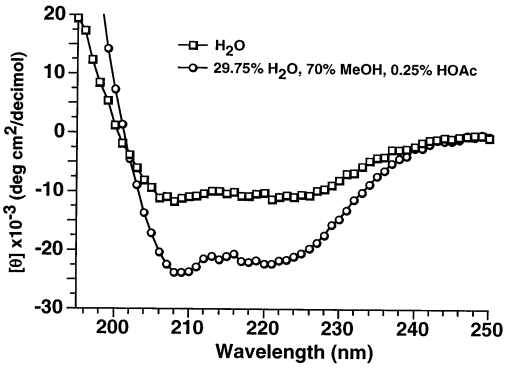

Fig. 1. Circular dichroism spectra of the N-terminal fragment of 5 μM cardiac muscle troponin C (cNTnC) in H_2O and 29.75% H_2O/70% MeOH/0.25% HOAc solutions.

3. Results and discussion

3.1. CD spectra of cNTnC at different solvent composition

Methanol-induced conformational transitions in proteins have been studied by, e.g. near- and far-UV CD, tryptophan fluorescence, microcalorimetry, diffusion measurements, and nuclear magnetic resonance (NMR) [36–40]. At a high alcohol concentration (usually more than 65% of alcohol) a protein is typically highly helical, based on its more pronounced far-UV CD relative to the native protein conformation. NMR also shows that β-structural or irregular chain regions of a native protein become helical upon alcohol-induced denaturation [37,38]. Troponin C (TnC) is an EF-hand calcium binding protein of the thin filament of muscle and plays a regulatory role in skeletal and cardiac muscle contraction. TnC consists of two similar globular domains (N-terminal domain and C-terminal domain). The cardiac N-terminal domain of troponin C (cNTnC) consists of a single polypeptide chain (10 063.4 Da) with 89 amino residues, including 23 acidic and 8 basic residues. NMR of native cNTnC reveals 5 α-helices (N, A, B, C, D helices), two short twisted β-sheets and three loops [41]. Fig. 1 shows CD spectra of cNTnC in H_2O and 29.75% H_2O/70% MeOH/0.25% HOAc, respectively. [A CD spectrum can be analyzed to identify and quantitate secondary structural elements (α-helix, β-sheet,

and random coil) of a protein in solution. In particular, the more negative the mean residue ellipticity at 222 nm, the greater the degree of α-helicity in the protein.] As for other proteins, the CD-determined helicity of cNTnC at high alcohol concentration increases two-fold compared to that of the native state. The question is, can that difference in solution structure carry over into the gas-phase after electrospray ionization.

3.2. ESI measurement of charge state distributions of cNTnC electrosprayed from different solvent compositions

Gas-phase protein ions with different charge states may exhibit different conformations, and different reactivity toward (gaseous) D_2O [7]; Freitas, 1998 #5]. Therefore, in order to compare the H/D exchange rates of gas-phase protein ions electrosprayed from different solvents, it is important to chose a protein that has essentially the *same* charge state distribution when electrosprayed from different solvents. Incidentally, it is not easy to compare solution-phase and gas-phase H/D exchange patterns, because it has been suggested that the mechanism for H/D exchange is very different in solution versus gas phase [8,34,42–44].

It has long been known that solvent composition can greatly affect the distribution of charge states produced by electrospray [45]. That is unfortunate, because different charge states can have different conformations. It is thus essential to compare the *same* charge state of a protein from two *different* solutions. The effect is more pronounced in proteins with many basic sites and thus a wide distribution of charge states. In aqueous solution at neutral pH, low charge states are observed in the ESI FTICR mass spectrum, whereas a very wide distribution of charge states is observed on electrospray from highly organic and/or low-pH solution of a denatured protein. By choosing a protein such as cNTnC, with just a few basic amino acid residues, we are able to generate nearly the same charge state distribution on electrospray from H_2O and 70% MeOH/0.25% HOAc, respectively, as shown in Fig. 2. (Because cNTnC has 2 arginines and 6 lysines, the 6+, 7+, and 8+ charge states of gas-phase cNTnC ions are readily generated from either aqueous or highly organic solutions.) Thus cNTnC offers the best available system to test for correlation between solution-phase and gas-phase protein structures.

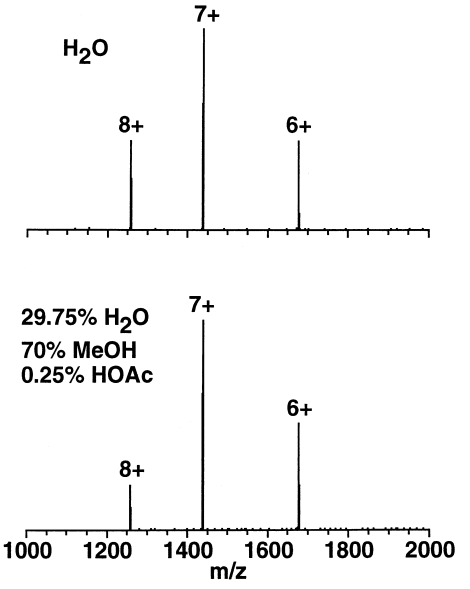

Fig. 2. FTICR mass spectra of cNTnC when electrosprayed from H_2O (top) and 29.75% H_2O/70% MeOH/0.25% HOAc solution (bottom). Note that the *same* charge states are obtained following electrospray ionization of cNTnC from both solutions.

3.3. Gas-phase H/D exchange of cNTnC

Fig. 3 shows the deuterium incorporation for the 6+, 7+, and 8+ charge states following each of several different periods of exchange with gaseous D_2O. For example, of the 154 exchangeable hydrogens in cNTnC (65 from side chains, 86 from amide backbone, and 3 from the C- and N-termini) only ~70 deuteriums (or ~45% of deuterium incorporation) are incorporated into the fast exchanging form of the 7+ charge state after 15 minutes of exchange with D_2O. Each charge state gives rise to a wide variance in rate and extent of gas-phase H/D exchange. The differences in H/D exchange rate and extent are likely due to different non- or slowly interconverting conformers, each with a different overall accessibility to solvent.

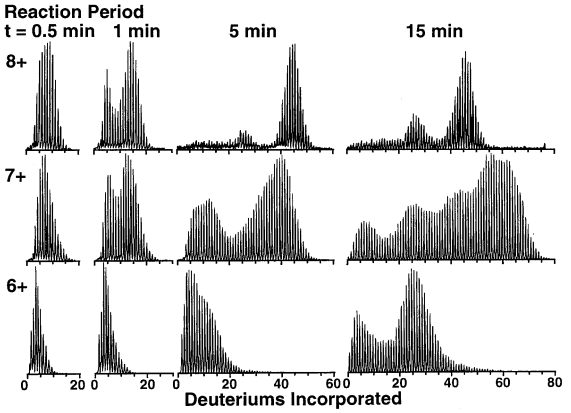

Fig. 3. Deuterium incorporation by gas-phase cNTnC ions for the 6+ (bottom), 7+ (middle), and 8+ (top) charge states electrosprayed from 29.75% H_2O/70% MeOH/0.25% HOAc solution, with 3 s of external ion accumulation, obtained following gas-phase H/D exchange for each of several indicated reaction periods.

Ion mobility measurements of gas-phase bovine ubiquitin ions have resolved conformers whose relative populations depend strongly on solvent composition and the capillary temperature used for electrospray ionization [17]. In a related study, the differences in measured ion drift time distributions for cytochrome *c* ions electrosprayed from aqueous and highly organic solutions suggested gas phase conformations that correlate to those present in solution [13]. Ion mobility experiments can determine the conformations that are elongated or compact. In general, as the charge on a protein ion increases, the ion-mobility measured collision cross section also increases, suggesting that coulombic repulsion drives the "unfolding" of the highly charged gas phase ion [46,47]. However, more extended structures do not necessarily result in faster or more extensive gas phase H/D exchange [7,8,48,49]. Thus conformations with similar collision cross sections can often be resolved according to their different chemical reactivity to H/D exchange.

Gas-phase H/D exchange rates point to gas-phase protein conformer distributions that are sensitive to ionization and ion transport conditions [7,8]. Fig. 4 shows the very high reproducibility of deuterium incorporation profiles for the 6+, 7+, and 8+ charge states of cNTnC ions. Thus, we can be confident that

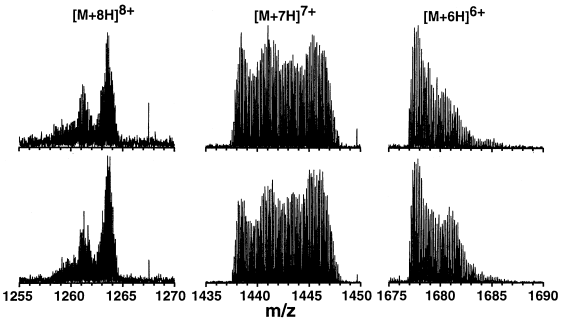

Fig. 4. FTICR mass spectra of cNTnC electrosprayed from 29.75% H_2O/70% MeOH/0.25% HOAc solution, with 1 s of external ion accumulation, for 8+, 7+, and 6+ charge states in a first run (top) and the second run (bottom).

even small differences in those profiles are real, and not due to fluctuation in experimental conditions or instrument response.

Figs. 5–7 show the effect of solvent composition and ion accumulation period on the conformations of the 8+, 7+, and 6+ charge states of cNTnC ions, as manifested after 15 min of gas-phase H/D exchange. In our instrument, the current supplied to the heated capillary and the ion accumulation period for the octopole ion trap have the greatest influence on the appearance of the final FTICR mass spectrum after gas-phase H/D exchange [8]. Here, we chose the current supplied to the heated capillary to be as low as

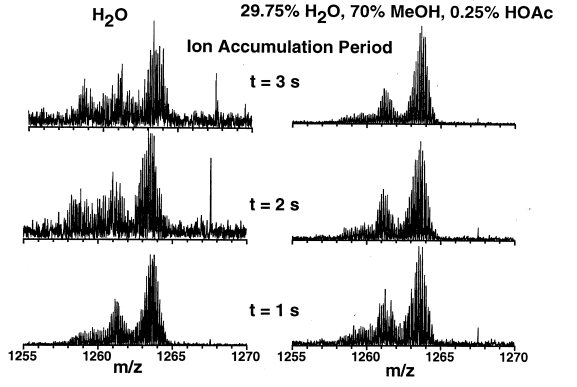

Fig. 5. FTICR mass spectra (8+ charge state) of cNTnC when electrosprayed from H_2O (left) and 29.75% H_2O/70% MeOH/0.25% HOAc solution (right) following gas-phase H/D exchange for 15 min after 1 s (bottom), 2 s (middle), and 3 s (top) external ion accumulation.

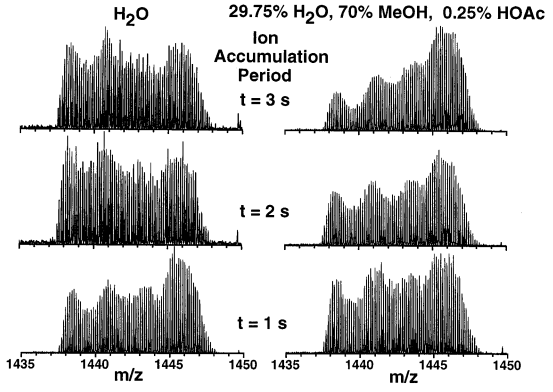

Fig. 6. As in Fig. 5, but for the 7+ charge state of cNTnC.

possible, 2.5 A. Higher capillary current (not shown) shifts the conformational distribution of cNTnC toward fast-exchanging conformations, as observed previously for ubiquitin [8]. Fig. 5 shows no significant difference in deuterium incorporation distribution for 8+ charge state for cNTnC electrosprayed from either H_2O or 70% MeOH/0.25% HOAc, at any of several external ion accumulation periods. Moreover, the deuterium incorporation patterns for the 6+ and 7+ charge states at short accumulation period (1 s), are essentially the same when electrosprayed from different solvents (Figs. 6 and 7). In contrast, the conformational distribution for the 6+ or 7+ charge states differs significantly when cNTnC is electrosprayed from aqueous vs. highly organic solution after longer external ion accumulation period (e.g. 3 s).

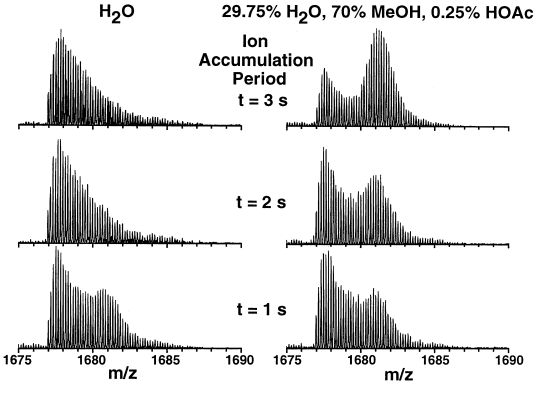

Fig. 7. As in Fig. 5, but for the 6+ charge state of cNTnC.

3.4. Solution-phase versus gas-phase cNTnC conformation

Figs. 6 and 7 indicate that the gas-phase conformations of cNTnC produced by electrospray ionization from aqueous and highly organic solvents are clearly different. Moreover, the CD spectra show that the protein exhibits greater helicity in the highly organic solution than in aqueous solution. Of course, the electrospray process itself may change the protein conformation because of, e.g. the huge change in dielectric constant of the medium (~80 for water versus 1 for vacuum). Moreover, in our instrument, ions are accumulated and stored for several seconds in an external octopole ion trap before injection into the Penning trap for FTICR excitation/detection. Whereas in the external trap (at ~1 mTorr), the ions undergo thousands of ion–neutral collisions and are in addition heated (probably by a few tens of C) due to the applied rf electric field. For example, after prolonged external accumulation, protons may be stripped from the protein ions (lowering their charge state) and/or unfolded. That is why our deuterium incorporation profiles are different for cNTnC ions stored for different lengths of time in our external octopole ion trap (Figs. 5–7). One possible explanation is that the slowest-exchanging gas-phase cNTnC conformers generated from aqueous solution are more stable than those from highly organic solution. Whatever the detailed nature of those effects, we nevertheless observe a measurable difference between the gas-phase deuterium incorporation patterns of cNTnC electrosprayed from two solutions in which the solution-phase cNTnC conformations are significantly different. [Although we cannot rule out the possibility that some proton transfer reactions of the stored ions with solvent may take place in our external ion trap (at ~10% H_2O, 100 000 collisions/s at the Langevin ion–molecule limit at 10 mTorr), we do not think that the collisions are reactive, for the same reason that we don't observe charge stripping in the external ion trap (on a 3 s time scale).] In summary, it seems reasonable to infer that some memory of solution-phase protein structure is carried through the electrospray/ion accumulation process to the gas phase.

Acknowledgements

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References

[1] L. Pauling, R.B. Corey, H.R. Branson, Proc. Natl. Acad. Sci. USA 37 (1951) 205.
[2] W. Kauzmann, Adv. Protein Chem. 14 (1959) 1.
[3] F.M. Richards, J. Mol. Biol. 82 (1974) 1.
[4] J.A. McCammon, S.C. Harvey, Dynamics of Proteins and Nucleic Acids, Cambridge University Press, Cambridge, UK, 1987.
[5] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[6] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64.
[7] T.D. Wood, R.A. Chorush, F.M. Wampler III, D.P. Little, P.B. O'Connor, F.W. McLafferty, Proc. Nat. Acad. Sci. USA 92 (1995) 2451.
[8] M.A. Freitas, C.L. Hendrickson, M.R. Emmett, A.G. Marshall, Int. J. Mass Spectrom. 185/186/187 (1999) 565.
[9] R.R.O. Loo, R.D. Smith, J. Am. Soc. Mass Spectrom. 5 (1994) 207.
[10] P.D. Schnier, D.S. Gross, E.R. Williams, J. Am. Chem. Soc. 117 (1995) 6747.
[11] S.J. Valentine, A.E. Counterman, D.E. Clemmer, J. Am. Soc. Mass Spectrom. 8 (1997) 954.
[12] D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, J. Am. Chem. Soc. 117 (1995) 10141.
[13] R.R. Hudgins, J. Woenckhaus, M.F. Jarrold, Int. J. Mass Spectrom. 165/166 (1997) 497.
[14] Y. Liu, S.J. Valentine, A.E. Counterman, C.S. Hoaglund, D.E. Clemmer, Anal. Chem. 69 (1997) 728A.
[15] S.J. Valentine, J.G. Anderson, A.D. Ellington, D.E. Clemmer, J. Phys. Chem. 101 (1997) 3891.
[16] K.B. Shelimov, M.F. Jarrold, J. Am. Chem. Soc. 119 (1997) 2987.
[17] J. Li, J.A. Taraszka, A.E. Counterman, D.E. Clemmer, private communication.
[18] C.T. Reimann, P.A. Sullivan, J. Axelsson, A.P. Quist, S. Altmann, P. Ropestorff, I. Velazquez, O. Tapia, J. Am. Chem. Soc. 120 (1998) 7608.
[19] P.A. Sullivan, J. Axelsson, S. Altman, A.P. Quist, B.U.R. Sunqvist, C.T. Reimann, J. Am. Soc. Mass Spectrom. 7 (1995) 329.
[20] M.V. Buchanan, R.L. Hettich, Anal. Chem. 65 (1993) 245A.
[21] I.J. Amster, J. Mass Spectrom. 31 (1996) 1325.
[22] T. Dienes, S.J. Pastor, S. Schürch, J.R. Scott, J. Yao, S. Cui, C.L. Wilkins, Mass Spectrom. Rev. 15 (1996) 163.
[23] D.A. Laude, E. Stevenson, J.M. Robinson, in Electrospray Ionization Mass Spectrometry, R.B. Cole (Ed.), Wiley, New York, 1997, p. 291.
[24] M.K. Green, C.B. Lebrilla, Mass Spectrom. Rev. 16 (1997) 53.
[25] A.G. Marshall, C.L. Hendrickson, G.S. Jackson, Mass Spectrom. Rev. 17 (1998) 1.
[26] A.G. Marshall, S. Guan, Rapid Commun. Mass Spectrom. 10 (1996) 1819.
[27] F. Wang, W. Li, M.R. Emmett, A.G. Marshall, D. Corson, B.D. Sykes, J. Am. Soc. Mass Spectrom. 10 (1999) 711.
[28] A.G. Marshall, M.W. Senko, W. Li, M. Li, S. Dillon, S. Guan, T.M. Logan, J. Am. Chem. Soc. 119 (1997) 433.
[29] M.W. Senko, C.L. Hendrickson, L. Pasa-Tolic, J.A. Marto, F.M. White, S. Guan, A.G. Marshall, Rapid Commun. Mass Spectrom. 10 (1996) 1824.
[30] M.W. Senko, C.L. Hendrickson, M.R. Emmett, S.D.-H. Shi, A.G. Marshall, J. Am. Soc. Mass Spectrom. 8 (1997) 970.
[31] A.G. Marshall, T.-C.L. Wang, T.L. Ricca, J. Am. Chem. Soc. 107 (1985) 7893.
[32] A.G. Marshall, T.-C.L. Wang, L. Chen, T.L. Ricca, in American Chemistry Society Symposium Series, Vol. 359, M.V. Buchanan (Ed.), American Chemical Society, Washington, DC, 1987, p. 21.
[33] S. Guan, A.G. Marshall, Int. J. Mass Spectrom. Ion Processes 157/158 (1996) 5.
[34] M.A. Freitas, C.L. Hendrickson, M.R. Emmett, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 1012.
[35] C.Q. Jiao, D.R.A. Ranatunga, W.E. Vaughn, B.S. Freiser, J. Am. Soc. Mass Spectrom. 7 (1996) 118.
[36] C. Tanford, Adv. Protein Chem. 23 (1968) 121.
[37] A.T. Alexandrescu, Y.L. Ng, C.M. Dobson, J. Mol. Biol. 235 (1994) 587.
[38] M. Buck, S.E. Radford, C.M. Dobson, Biochemistry 32 (1993) 669.
[39] P. Fan, C. Bracken, J. Baum, Biochemistry 32 (1993) 1573.
[40] V.E. Bychkova, A.E. Dujsekina, S.I. Klenin, E.I. Tiktopulo, V.N. Uversky, O.B. Ptitsyn, Biochemistry 35 (1996) 6058.
[41] L. Spyracopoulos, M.X. Li, S.K. Sia, S.M. Gagne, M. Chandra, R.J. Solaro, B.D. Sykes, Biochemistry 36 (1997) 12138.
[42] S. Campbell, E.M. Marzluff, M.T. Rodgers, J.L. Beauchamp, M.E. Rempe, K.F. Schwinck, D.L. Lichtenberger, J. Am. Chem. Soc. 116 (1994) 5257.
[43] S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 117 (1995) 12840.
[44] T. Wyttenbach, M.T. Bowers, J. Amer. Soc. Mass Spectrom. 10 (1998) 9.
[45] S.K. Chowdhury, V. Katta, B.T. Chait, J. Am. Chem. Soc. 112 (1990) 9012.
[46] K.B. Shelimov, D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, J. Am. Chem. Soc. 119 (1997) 2240.
[47] A.L. Rockwood, M. Busman, R.D. Smith, Int. J. Mass Spectrom. Ion Processes 111 (1991) 103.
[48] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler III, F.W. McLafferty, Proc. Natl. Acad. Sci. USA 90 (1993) 790.
[49] F.W. McLafferty, Z. Guan, U. Haupts, T.D. Wood, N.L. Kelleher, J. Am. Chem. Soc. 120 (1998) 4734.