ELSEVIER

International Journal of Mass Spectrometry 204 (2001) 77–85

International Journal of Mass Spectrometry

Differences between positive and negative ion stabilities of metal–sulfur cluster proteins: an electrospray ionization Fourier transform ion cyclotron resonance study

Keith A. Johnson[a], Marc F.J.M. Verhagen[b], Michael W.W. Adams[b], I. Jonathan Amster[a]*

[a]*Department of Chemistry, University of Georgia, Athens, GA 30602, USA*
[b]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, USA*

Received 9 March 2000; accepted 1 June 2000

Abstract

The stability of iron–sulfur proteins during electrospray ionization in both positive ion and negative ion modes was investigated using Fourier transform ion cyclotron resonance mass spectrometry. Positive ion and negative ion mode mass spectra of iron and zinc rubredoxin from Clostridium pasteurianum and ferredoxins from Pyrococcus furiosus containing [3Fe–4S], [4Fe–4S], [3FeNi–4S], and [3FeMn–4S] clusters are compared. The results demonstrate that all clusters are stable as negative ions, whereas only the [4M–4S], (M = Fe, Mn, Ni) clusters are stable as positive ions. This is the first direct evidence of the existence of the [3FeMn–4S]-containing cluster from Pyrococcus furiosus. The formal oxidation state of each metal–sulfur cluster is determined by the mass-to-charge measurement, and is found to be the same for both positive and negative ions. (Int J Mass Spectrom 204 (2001) 77–85) © 2001 Elsevier Science B.V.

Keywords: Electrospray; Metalloproteins; Ferredoxin; FTICR

1. Introduction

Electrospray ionization (ESI) has become a valuable tool in the investigation of protein interactions by mass spectrometry. It is capable of preserving the noncovalent interactions that exist in solution when the complex is transferred to the gas phase. This nondestructive transformation is especially important when analyzing metal–protein interactions. Binding stoichiometry, binding cooperativity, and conformational changes of protein in the presence of metal ions are targets for analysis by ESI mass spectrometry. The use of ESI mass spectrometry for the analysis of noncovalent interactions has recently been reviewed [1–3]. ESI has been used in both positive and negative modes of ionization, generating positive protein ions by the addition of protons during electrospray and generating negative ions through the extraction of protons. The choice of ionization modes is generally based on the isoelectric point (pI) of a given protein. Normally, positive ion mode is chosen for basic or neutral proteins, whereas negative ion mode is used for the ionization of acidic proteins, however, it has

* Corresponding author. E-mail: jamster@uga.edu

PII S1387-3806(00)00337-7

been shown that ESI can generate positive ions from acidic amino acids and negative ions from basic amino acids [4,5]. Questions concerning the differences between these modes of ionization and the information that can be extracted from data collected in each mode have recently been investigated by Konermann and Douglas [6]. One result of the investigation shows that the tertiary structure of proteins can be monitored using denaturing and nondenaturing conditions for positive ions, but the same data are not always available from the mass spectra generated under the same solvent conditions in negative ion mode.

The higher order structure of metalloproteins has also been monitored in both modes of ionization. There have been numerous studies of metal-containing proteins in positive mode ESI, and range from measurements of metal atom stoichiometry of nonheme iron containing rubrerythrin [7] and calcium binding parvalbumin [8] to the investigation of conformational changes associated with the unfolding of cytochrome *c* and myoglobin [9]. Several studies of metal-protein interaction using negative mode electrospray ionization have been published. These include calcium induced noncovalent interactions between proteins as well as measurements of the stoichiometry and cooperativity of metal atom binding to proteins [7,10,11]. A comparison of the two modes of ionization for a single metalloprotein has been published [12]. The iron and zinc forms of Clostridium pasteurianum (Cp) rubredoxin and its recombinant counterpart purified from Escherichia coli (E. coli) have been investigated using electrospray ionization mass spectrometry to isolate conditions that prevent the loss of the metal from the two forms of the protein [13]. Iron–sulfur cluster containing ferredoxins have been investigated using both positive and negative modes of electrospray ionization and the clusters are more stable in negative mode than in positive mode [14,15]. The stoichiometry and identification of iron–sulfur clusters from Sulfolobus acidocaldarius [16] and Rhodobacter capsulatus [17] ferredoxins have been determined using electrospray mass spectrometry. More recently, an intermediate cluster structure from the high potential iron–sulfur protein (HiPIP) from Chromatium vinosum (Cv) has been investigated by mass spectrometry as a possible model for characterizing the assembly of metal–sulfur clusters in ferredoxin [18]. The effect of the oxidation state of iron in heme-containing proteins was resolved by Henion and co-workers, who investigated ferric heme associated with myoglobin and ferrous heme associated with cytochrome *c*, in which only a one mass unit difference distinguished the two oxidation states of heme [19]. The influence of the oxidation state of the metal centers on ions in electrospray have also been described for rubrerythrin and hemerythrin [7], and for Cd^{2+} and Zn^{2+} metallothioneins [20].

Electrospray Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectrometry has become a valuable tool in the arsenal of techniques used to probe the structure of proteins [3]. The soft ionization of electrospray ionization along with the high resolution and high mass accuracy that are possible with the technique facilitate the investigation of the stoichiometry and oxidation states of metalloproteins. Iron–sulfur cluster containing proteins that range from mononuclear metal centers to [4Fe–4S] clusters have been investigated to determine the effect of the oxidation state and the presence or absence of disulfide bonds on the measured mass [15]. The high resolution and high mass accuracy of FTICR mass spectrometry enable the detection of small changes in mass of metalloproteins that accompany the formation or removal of disulfide bonds and mass differences associated with oxidized versus reduced forms of metal centers [15,21]. In this article, we compare the stability of various iron–sulfur cluster proteins by ESI-FTICR mass spectrometry using both positive ion and negative ion modes of ionization.

2. Experimental

All proteins examined here were produced by over-expression in E. coli. The preparation and purification of recombinant wild-type Pyrococcus furiosus (Pf) ferredoxin has been described previously [22]. The [4Fe–4S] cluster was converted to the [3Fe–4S] form by treating the ferredoxin with a five fold excess

of ferricyanide for 15 min. The oxidant was subsequently removed by gel filtration [23]. Prior to analysis, protein solutions were desalted by ultrafiltration in a microcentrifuge tube with 1 mL of 15 mM ammonium acetate solution with a 5 kDa cutoff membrane (Millipore Corporation, Bedford, MA). The protein was then resuspended in water to yield a final protein concentration of 15 μM.

All mass spectra were acquired with a Bruker BioApex 7 tesla FTICR mass spectrometer equipped with an Analytica electrospray ionization source. The source was modified by replacing the glass capillary with a heated metal capillary which provides gentler desolvation of ions produced during ESI without using higher voltages that can cause loss of the metal cluster. Capillary tips for electrospray ionization were produced in our laboratory from 100 μm inner diameter fused silica capillary (Polymicro Technologies, Phoenix, AZ). The capillary was pulled to a fine tip by heating it with a micro-torch (Microflame, Inc., Minnetonka, MN) while applying a constant force. The flow rates for sample introduction to the mass spectrometer ranged between 6 and 10 μL/h. A voltage of 1.2 kV (-1.2 kV for negative ions) was applied to the syringe needle that infused the sample into the spray capillary, forming a liquid electrical junction [24]. The ion source parameters varied between positive mode and negative mode electrospray. For negative mode ionization, the ion source parameters (average values) were as follows: skimmer, -4.80 V; hexapole dc offset, 2.35 V; hexapole extract plate, -1.89 V; hexapole trap, -6.74 V For positive mode: skimmer, 5.44 V; hexapole dc offset, -4.74 V; hexapole extract plate, 1.22 V; hexapole trap, 6.78 V. The heated metal capillary was maintained at a temperature between 110 and 130 °C for the experiments. Although the signal intensity was adjusted through the variation in temperature of the metal capillary, no significant improvement or deterioration of the signal was observed for slightly higher temperatures. At temperatures lower than 100 °C, the signal became unstable. Ions were stored in the source region in a hexapole ion guide for 0.5–0.75 s and were pulsed through a series of electrostatic lenses into the detection cell where trapping potentials of 0.90 and 0.95 V (-0.90 and -0.95 V for negative ions) were used on the front and back trapping plates of the cell to isolate ions for excitation and detection. The time domain signal (transient) was collected by averaging approximately 50 scans. The summed transient was apodized prior to application of the Fourier transform, followed by calibration to yield a mass spectrum.

Calculations of isotope distributions for the proteins were made by using IsoPro software (available from http://www.members.aol/msmssoft). Experimentally obtained isotope distributions and calculated distributions were matched using the chi-squared test as described by McLafferty and co-workers [25]. The estimation of the monoisotopic mass was made after assigning the isotope composition of each of the peaks in the experimentally observed distribution as described previously [15].

3. Results and discussion

ESI-FTICR mass spectrometry is a valuable tool in identifying the small changes in mass associated with a metalloprotein's oxidation state or in counting disulfide bonds within a protein. The isotopically resolved mass spectra that are obtained from the data yield the monoisotopic molecular weight of the protein. Monoisotopic molecular weight is defined as the sum of the lowest atomic weight isotopes in the molecular formula, and for most proteins, this corresponds to ^{12}C, ^{1}H, ^{14}N, ^{16}O, and ^{32}S, the lowest mass and most abundant isotopes of these elements. For metals, the lowest mass isotope is often not the most abundant. We define the monoisotopic mass of a metalloprotein as the sum of the monoisotopic molecular weight of the apoprotein plus the atomic mass of the most abundant isotope of the metal or metals that make up the metal center. For example, we used ^{56}Fe instead of ^{54}Fe because their natural abundances are 91.8% and 5.82%, respectively. Since the mass difference between peaks containing different numbers of iron correspond to the mass of ^{56}Fe rather than ^{54}Fe, this arbitrary convention is more practical than

the strict definition of monoisotopic molecular weight [15].

The molecular weight for a protein is normally calculated assuming that all of the ionizable sites in the amino acid sequence for the protein are neutral. However, the metals in metalloproteins exist in oxidation states other than zero, which make this rule impractical. Using this rule for metalloproteins would result in molecular weights that vary according to the oxidation state of the metal center, because metalloproteins with metal centers having different oxidation states would require different numbers of protons to achieve charge neutrality. To avoid this problem, we define the "apparent mass" as the mass that is calculated from the data making the assumption that all the charge present on an ion is due to excess protons (or a deficit of protons, for negative ions). By using this convention, we make no assumptions about the oxidation state of the metalloprotein prior to calculating a molecular weight. We also define the "calculated mass" as the monoisotopic mass that is calculated from the sum of all of the atomic weights of the lowest mass isotopes of the amino acids in the sequence of the protein plus the atomic weight of the most abundant isotope of the metal or the components of the metal–sulfur cluster. Calculation of the molecular weight by this method requires no assumptions about internal disulfide bonds. This method allows the derivation of the oxidation state or the number of disulfide bonds in the metalloprotein by calculating the difference between the apparent mass and the calculated mass for the protein. A more complete analysis of this procedure has been discussed recently [15].

Positive and negative ion ESI-FTICR mass spectra of iron Cp rubredoxin are shown in Fig. 1. The 3+ and 4+ charge states are detected for positive ions and charge states 3− through 7− are detected for negative ions for the iron form of rubredoxin. The apparent mass derived from the mass spectral data of iron rubredoxin is 6096.58 Da and the calculated mass is 6099.55 Da. The difference between the masses indicates that the metal is present in the 3+ oxidation state. This result is the same for both the positive and negative mode data. This is the biologically relevant

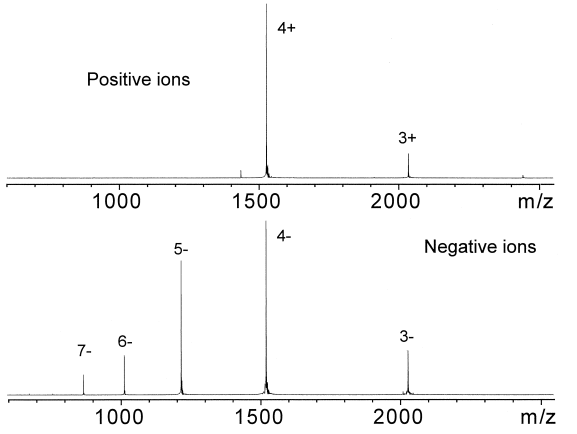

Fig. 1. Positive mode (top) and negative mode (bottom) ESI-FTICR mass spectra of iron rubredoxin from C. pasteurianum. The positive mode spectrum exhibits the 4+ and 3+ charge states, whereas the negative mode shows a wider range of charge states from 3− to 7−. For both ionization modes, only holoprotein is detected.

oxidation state of the iron center under oxidizing conditions. Likewise, the zinc-containing form of Cp rubredoxin, Fig. 2, shows the 2+, 3+, and 4+ charge states in positive mode and charge states ranging from 3− to 7− in the negative mode. These data yield an apparent mass of 6105.58 Da and a calculated mass of

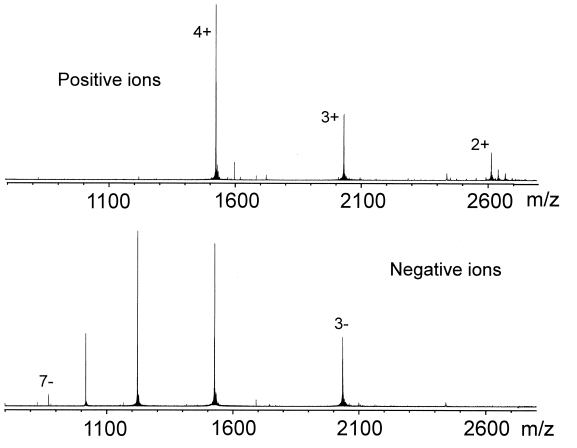

Fig. 2. Positive mode (top) and negative mode (bottom) ESI-FTICR mass spectra of zinc rubredoxin from C. pasteurianum. Charge states of 2+, 3+, and 4+ are found in the positive mode spectrum, whereas a wider range of charge states, 3− to 7− are observed in the negative mode mass spectrum. In both mass spectra, only holoprotein is detected.

6107.55 Da. The difference of 2 Da agrees with the known oxidation state for Zn in rubredoxin, 2+.

Positive and negative mode mass spectra of metalloproteins obtained under nondenaturing conditions show a consistent pattern in their charge state distributions. The negative ion mass spectra always exhibit a broader distribution of ions than the positive mode mass spectra, as can be seen in Figs. 1 and 2. In positive mode spectra one normally observes that denatured proteins have a broader distribution of charge states of lower mass-to-charge (i.e. with more charge) than are observed in the mass spectra of proteins electrosprayed in their native conformations. It has been proposed that this is the case because the unfolded protein exposes more ionizable sites than the folded protein, which results in the formation of higher charge states [26]. The positively and negatively charged protein ions observed for rubredoxin, Figs. 1 and 2, however, were obtained using nondentauring solutions and exist in their native conformations with the metal center intact. If the protein ions had unfolded, one would expect the metal to be lost, as is the case for mass spectra obtained under denaturing conditions. This behavior supports the argument that only positive mode ionization can be used to monitor tertiary structure (i.e. conformational changes) in a protein [6]. One might falsely conclude that the abundance of charge states that are formed for negative ions result from the denaturation and exposure of more ionizable sites along the peptides chain. The presence of the intact metal cluster in the negative ion mass spectrum shows that denaturation has not occurred.

Fig. 3 shows the positive mode mass spectrum of Pf ferredoxin, which contains a [4Fe–4S] cluster. The apparent mass, the mass derived assuming all of the excess charge is from protons, is 7509.83 Da. The calculated mass, which is the combined molecular weight of all of the amino acids in the sequence, making no assumptions about disulfide bonds, is 7513.87 Da. The 4 mass unit difference between the two values can be attributed to the 2+ oxidation state of the metal cluster and the presence of one disulfide bond that is known to exist distant from the active site of the protein [15,23]. The [4Fe–4S] protein, like the

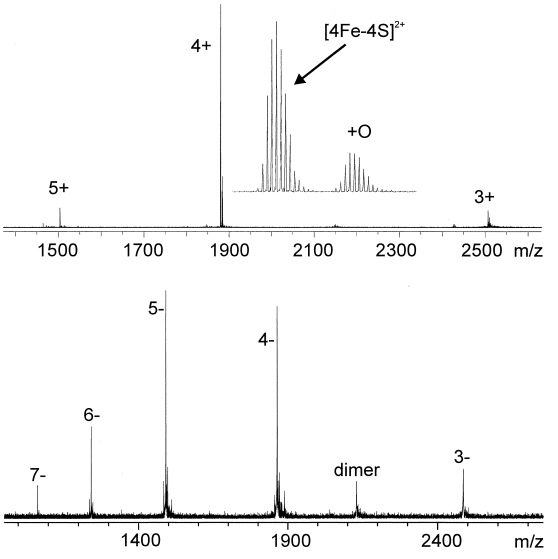

Fig. 3. ESI-FTICR mass spectra of ferredoxin from P. furiosus. An intact [4Fe–4S] cluster is observed in the ESI-FTICR mass spectrum in both the positive mode (top) and negative mode (bottom). Dimer is observed at *m/z* 2130 in the negative mode mass spectrum.

iron and zinc-containing rubredoxins, is stable in positive ion mode and contains only the intact cluster, with no degradation products. The negative mode spectrum, also shown in Fig. 3, exhibits a broader distribution of charge states. An oxidation state of 2+ for the [4Fe–4S] cluster is derived from the negative ion data as well.

In solution, the [4Fe–4S] form of this ferredoxin can be converted to a [3Fe–4S] cluster by reaction with ferricyanide, Fig. 4 [23]. The [4Fe–4S] cluster exists in vitro with a formal oxidation state of 2+ in its most oxidized form, but the [3Fe–4S] cluster exists in a 1+ oxidation state in its oxidized form [23]. Fig.

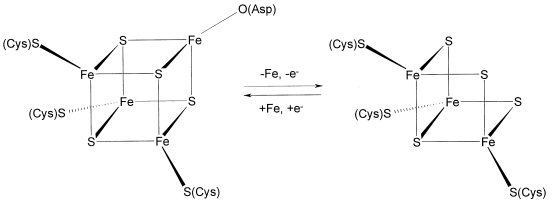

Fig. 4. Iron–sulfur cluster structures illustrating the reduction of the [4Fe–4S] form to the [3Fe–4S] form. Ligation of the cluster through the aspartic acid residue allows the facile conversion of the [4Fe–4S] form to the [3Fe–4S] form.

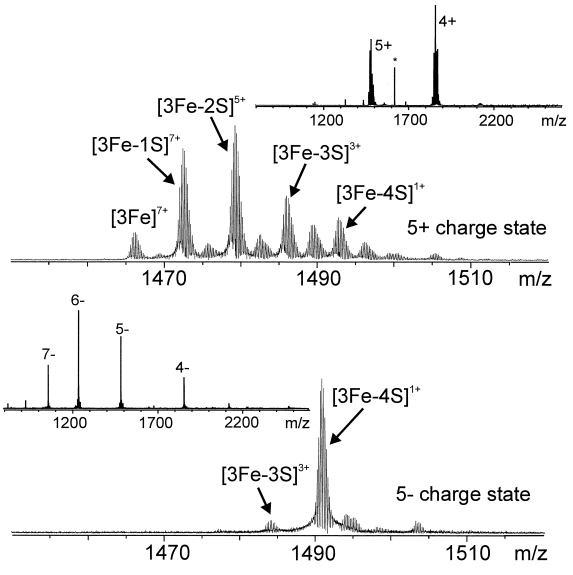

Fig. 5. ESI-FTICR mass spectra obtained for [3Fe–4S]-containing ferredoxin in positive ion mode (top) and negative ion mode (bottom). A stable cluster is observed only for the negative mode spectrum. The insets show an expansion of the 5+ and 5− charge states. The peaks corresponding to protein with an intact cluster are labeled. Major peaks represent degradation products that consist of [3Fe–3S], [3Fe–2S], [3Fe–1S], and [3Fe] are found only in the positive ion mass spectrum. Additional peaks corresponding to sodium and oxygen adducts are present at lower intensity.

5 shows positive and negative ion mass spectra of the [3Fe–4S] form of ferredoxin. Again, the negative mode mass spectrum contains a wider distribution of charge states than does the positive ion mass spectrum. Moreover, the positive ions show much more complexity for each charge state, corresponding to ions containing various degradation products of the [3Fe–4S] cluster. The apparent mass derived from the holoprotein data containing the intact [3Fe–4S] cluster is 7454.92 Da and the calculated mass is 7457.94 Da, indicating an oxidation state of 1+ as well as one disulfide bond, as expected between C21 and C48. An expansion of the 5+ and 5− charge states for these two mass spectra is also shown in Fig. 5. The positive mode mass spectrum is more complex than that of the [4Fe–4S] form, with many degradation products of the intact cluster detected. These products represent losses of inorganic sulfide from the iron–sulfur cluster to yield $[3Fe–4S]^{1+}$, $[3Fe–3S]^{3+}$, $[3Fe–2S]^{5+}$, $[3Fe–1S]^{7+}$, and $[3Fe]^{7+}$. Additional peaks in this mass spectrum arise from sodium attachment and from oxygen adducts. From these data, the [3Fe–4S] cluster appears much more susceptible to degradation during electrospray ionization in the positive mode. The decrease in the stability of the [3Fe–4S] cluster may result from the lability of the inorganic sulfide in this cluster compared to the [4Fe–4S] form. As can be seen in Fig. 4, the [4Fe–4S] cluster contains all trivalent sulfur, whereas the [3Fe–4S] cluster contains mostly divalent sulfur. Other iron–sulfur proteins with divalent sulfur exhibit similar losses, for example, the [2Fe–2S] cluster of iron hydrogenase γ subunit [15]. Fig. 5 also shows the 5− charge state for [3Fe–4S] ferredoxin that was electrosprayed as a negative ion and shows a large peak corresponding to the intact [3Fe–4S] cluster with almost no degradation products, in strong contrast to the 5+ charge state of the same protein. Although the positive mode spectrum demonstrates the formation of many product ions, the negative mode spectrum contains only one small peak corresponding to the loss of one sulfur atom from the cluster. It has been suggested that during negative mode ESI the protein collapses into a highly compact conformation that shelters the cluster from degradation; however, this is not supported by the increased number of lower charge states that are obtained for ubiquitin in prior research [27] or for ferredoxins in negative ion mode as compared to spectra obtained during positive ion mode. Although the mass spectrum obtained in positive ion mode provides information about degradation products and possible intermediates in the formation of iron–sulfur clusters, these data suggest that the protein degrades during ionization rather than degrading by air oxidation while in solution.

Proteins that contain mixed metal clusters provide challenging targets for analysis. The ESI-FTICR mass spectrum of a mixed metal center containing a [3FeNi–4S] cluster from Thermococcus litoralis is shown in Fig. 6. This cluster is ligated to the protein through four cysteine sulfur–metal bonds. The appearance of the distribution of charge states for the positive and negative mode spectra is similar to that observed for other metalloproteins. The negative

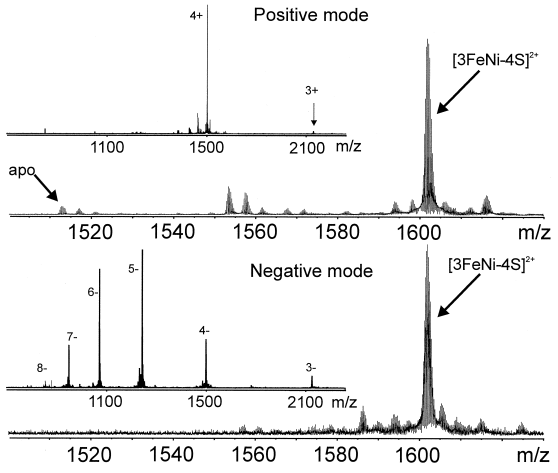

Fig. 6. Expansion of the peaks corresponding to the 4+ and 4− charge states in the ESI-FTICR mass spectra of ferredoxin from P. furiosus with a nickel-substituted [3FeNi–4S] cluster. The insets show the entire mass range. Adducts of sodium and oxygen are observed at lower abundance.

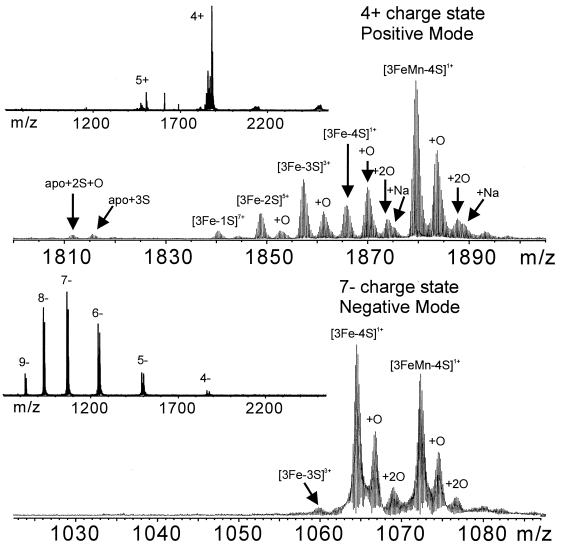

Fig. 7. Expansion of the most abundant peaks in the ESI-FTICR mass spectra of ferredoxin from P. furiosus with a manganese-substituted [3FeMn–4S] cluster in positive mode (4+ charge state, top) and negative mode (7− charge state, bottom). The insets show the entire mass range. Adducts of sodium and oxygen are observed at lower abundance.

mode mass spectrum shows a wider distribution of charge states than for the protein electrosprayed in the positive mode. The metalloprotein is less stable in the positive ion mode, evidenced by the appearance of apoprotein, however, the extent of cluster degradation is not significant. The expansion of the 4+ and 4− charge states shows the intact [3FeNi–4S] cluster. The apparent mass for the protein is 6400.40 Da and the calculated mass is 6404.36 Da, which results in a derived oxidation state for the cluster as 2+ and one disulfide bond known to exist between C20 and C43 [28,29]. The observed oxidation state of 2+ corresponds to that measured previously for this metalloprotein under oxidizing conditions in its biological environment. These data for this mixed metal cluster are consistent with our hypothesis that metal–sulfur clusters that contain only trivalent sulfur are stable toward degradation in both the positive and negative modes of ionization.

The mass spectrum of [3FeMn–4S] is shown in Fig. 7. This mixed metal cluster has been studied by other spectroscopic methods. In prior studies, the presence of this mixed metal center was inferred from the absence of a signal in its magnetic circular dichroism (MCD) spectrum, as this cluster is MCD silent, whereas the [3Fe–4S] center from which it is derived produces a clear signal [30]. Fig. 7 shows the positive and negative mode mass spectra for [3FeMn–4S] ferredoxin. Both positive and negative ion mass spectra exhibit a peak corresponding to the [3FeMn–4S]-containing protein. This is the first direct evidence of the existence of this mixed metal cluster in this protein. As is the case for the other iron–sulfur proteins, the negative ions show a wider distribution of charge states, and exhibit higher average charge than the positive ions. Also, the positive ions show greater complexity in their mass spectrum, due to the products [3Fe–nS] (n = 1–4). Interestingly, the negative ion mass spectrum also exhibits an intense peak for [3Fe–4S] in addition to the expected [3FeMn–4S] peak. We interpret these results as evidence that some of the protein had degraded to the [3Fe–4S] compound during sample handling. Based on our experience with iron–sulfur protein mass spectra, the data appear to result from a mixture of [3FeMn–4S] and [3Fe–4S]. The sum of the intensities of the [3Fe–nS] (n = 1–4) peaks in the positive mode is roughly

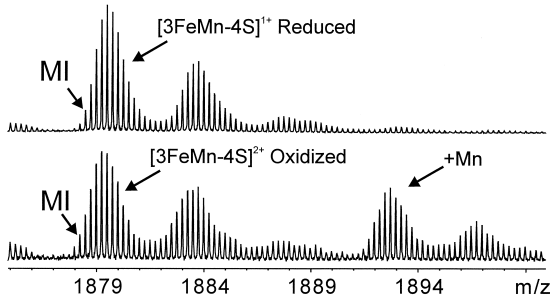

Fig. 8. Positive ion ESI-FTICR mass spectra of the reduced (top) and oxidized (bottom) [3FeMn–4S] containing P. furiosus ferredoxin. The oxidized form of the protein contains adducts of oxygen and manganese, presumably from other protein molecules that have lost the metal from the cluster during oxidation. The assigned oxidation states are derived from the mass measurement.

equal to the intensity of the [3Fe–4S] peak in the negative mode mass spectrum. Prior condensed phase studies of the [3FeMn–4S] protein have shown that the cluster readily loses manganese to yield [3Fe–4S] under oxidizing conditions [30]. The [3Fe–4S] cluster is produced upon oxidation of the [3FeMn–4S] center, as manganese is lost from the cluster [30]. The apparent mass for the protein with the manganese cluster is 7509.89 Da and the calculated mass for the protein is 7512.88 Da. The difference of 3 Da between apparent and calculated masses is consistent with one disulfide bond in the protein (C21 and C48) and a 1+ charge on the metal cluster, which is the known oxidation state for the metal cluster in its reduced form [30]. As in the case of the [3Fe–4S] spectra shown in Fig. 5, the cluster remains intact as a negative ion, but becomes unstable and degrades during positive mode ionization. Upon loss of manganese from the [3FeMn–4S] cluster as it is oxidized, the [3Fe–4S] cluster is formed and degrades further by losing sulfur. The [3Fe–4S] cluster appears in its oxidized form (1+), but the [3FeMn–4S] cluster is found to be in its reduced form (1+). Prior research has shown that the oxidized form of a metal containing species appears in its highest oxidation state when ionized by ESI [31]. After exposure to air, the oxidized [3FeMn–4S] cluster (oxidation state of 2+) is detected, Fig. 8. Both the reduced and the oxidized forms of the manganese-containing cluster are shown in Fig. 8. The apparent mass for the protein with the oxidized cluster intact is 7508.86 Da and the calculated mass is 7512.88 Da. The difference is accounted for by having one disulfide bond and an oxidation state of 2+ for the cluster [30]. As the cluster becomes fully oxidized, a manganese adduct peak appears in the mass spectrum, however, the same peak does not appear in the reduced sample. The source of the extra manganese is presumably from protein that has lost this metal during degradation of the mixed metal cluster.

4. Conclusion

The data presented here for iron–sulfur proteins illustrate the possibilities for analysis using both positive ions and negative ions from electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Introducing these metalloproteins to the mass spectrometer leads to considerable degradation of the open cuboidal [3Fe–4S] cluster in positive ion mode, however, these proteins appear more stable when detected as negative ions. Among the metalloproteins studied in this research, generally those metals or metal clusters that are ligated to the protein through four cysteine residues appear the most stable during ESI. These include iron and zinc rubredoxin from Clostridium pasteurianum and the [3FeNi–4S] cluster for Thermococcus litoralis ferredoxin. The most stable form of wild-type ferredoxin is the [4Fe–4S] cluster, which is as stable as the rubredoxins, even though it is ligated to the protein through only three cysteine ligands and an aspartic acid residue. The [3Fe–4S] version of the same protein is much less stable than its [4Fe–4S] counterpart in positive mode, however, in negative mode, the intact cluster is detected, illustrating an increased stability as a negative ion. The [3FeNi–4S] cluster, as previously discussed, has similar stability in both positive and negative modes of ionization, which may be due to the increase in stability provided by its fourth cysteine ligand. Manganese substitution into the [3Fe–4S] cluster forming a [3FeMn–4S] cluster does increase cluster stability. Degradation of the manganese cluster

to the [3Fe–4S] cluster occurs through oxidation of the protein [30] and probably exists in solution prior to ionization in either mode. The intact manganese substituted cluster is detected in both positive and negative mode ionization while the [3Fe–4S] is stable only in the negative ion mode.

Acknowledgements

The authors gratefully acknowledge funding provided by the National Science Foundation, CHE 9974579 (I.J.A.) and MCB 9809060 (M.W.A.). They thank Professor John R. Eyler at the University of Florida for providing the heated metal capillary interface used in these experiments. They also thank Michael Johnson of the University of Georgia for discussions and insights regarding iron–sulfur proteins, as well as for providing several of the samples used for these studies.

References

[1] T.D. Veenstra, Biophys. Chem. 79 (1999) 63.
[2] J.A. Loo, Mass Spectrom. Rev. 16 (1997) 1.
[3] S.A. Lorenz, E.P. Maziarz, T.D. Wood, Appl. Spectrosc. 53 (1999) 18A.
[4] M.A. Kelly, M.M. Vestling, C.C. Fenselau, P.B. Smith, Org. Mass Spectrom. 27 (1992) 1143.
[5] B.A. Mansoori, D.A. Volmer, R.K. Boyd, Rapid Commun. Mass Spectrom. 11 (1997) 1120.
[6] L. Konermann, D.J. Douglas, J. Am. Soc. Mass Spectrom. 9 (1998) 1248.
[7] Q.P. Lei, X.Y. Cui, D.M. Kurtz, I.J. Amster, I.V. Chernushevich, K.G. Standing, Anal. Chem. 70 (1998) 1838.
[8] H. Troxler, T. Kuster, J.A. Rhyner, P. Gehrig, C.W. Heizmann, Anal. Biochem. 268 (1999) 64.
[9] L. Konermann, D.J. Douglas, Biochemistry 36 (1997) 12296.
[10] P.F. Hu, J.A. Loo, J. Mass Spectrom. 30 (1995) 1076.
[11] O. Henin, B. Barbier, F. Boillot, A. Brack, Chem.-Eur. J. 5 (1999) 218.
[12] O. Nemirovskiy, R. Ramanathan, M.L. Gross, J. Am. Soc. Mass Spectrom. 8 (1997) 809.
[13] Y. Petillot, E. Forest, I. Mathieu, J. Meyer, J.M. Moulis, Biochem. J. 296 (1993) 657.
[14] H. Remigy, M. Jaquinod, Y. Petillot, J. Gagnon, H. Cheng, B. Xia, J.L. Markley, J.K. Hurley, G. Tollin, E. Forest, J. Protein Chem. 16 (1997) 527.
[15] K.A. Johnson, M. Verhagen, M.W.W. Adams, I.J. Amster, Anal. Chem. 72 (2000) 1410.
[16] J.L. Breton, J.L.C. Duff, J.N. Butt, F.A. Armstrong, S.J. George, Y. Petillot, E. Forest, G. Schafer, A.J. Thomson, Eur. J. Biochem. 233 (1995) 937.
[17] J. Armengaud, J. Gaillard, E. Forest, Y. Jouanneau, Eur. J. Biochem. 231 (1995) 396.
[18] K. Natarajan, J.A. Cowan, J. Am. Chem. Soc. 119 (1997) 4082.
[19] Y.T. Li, Y.L. Hsieh, J.D. Henion, B. Ganem, J. Am. Soc. Mass Spectrom. 4 (1993) 631.
[20] D. Fabris, J. Zaia, Y. Hathout, C. Fenselau, J. Am. Chem. Soc. 118 (1996) 12242.
[21] F. He, C.L. Hendrickson, A.G. Marshall, J. Am. Soc. Mass Spectrom. 11 (2000) 120.
[22] P.S. Brereton, M.F.J.M. Verhagen, Z.H. Zhou, M.W.W. Adams, Biochemistry 37 (1998) 7351.
[23] R.C. Conover, A.T. Kowal, W. Fu, J.B. Park, S. Aono, M.W.W. Adams, M.K. Johnson, J. Biol. Chem. 265 (1990) 8533.
[24] S. Koenig, K. Haegele, H. Fales, Anal. Chem. 70 (1998) 4453.
[25] M.W. Senko, S.C. Beu, F.W. McLafferty, J. Am. Soc. Mass Spectrom. 6 (1995) 229.
[26] V. Katta, B.T. Chait, J. Am. Chem. Soc. 113 (1991) 8534.
[27] D. Douglas, L. Konerman, J. Am. Soc. Mass Spectrom. 9 (1998) 1248.
[28] P.L. Wang, A. Donaire, Z.H. Zhou, M.W.W. Adams, G.N. LaMar, Biochemistry 35 (1996) 11319.
[29] R.C. Conover, J.B. Park, M.W.W. Adams, M.K. Johnson, J. Am. Chem. Soc. 112 (1990) 4562.
[30] M.G. Finnegan, R.C. Conover, J.B. Park, Z.H. Zhou, M.W.W. Adams, M.K. Johnson, Inorg. Chem. 34 (1995) 5358.
[31] G.J. Van Berkel, G.E. Giles, J.S. Bullock, L.J. Gray, Anal. Chem. 71 (1999) 5288.