Identification of Peptide Oxidation by Tandem Mass Spectrometry

KEVIN L. SCHEY* AND ERIC L. FINLEY
Department of Cell and Molecular Pharmacology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425

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ABSTRACT
Oxidative reactions play important roles in a variety of biochemical events ranging from normal metabolism to aging and disease processes. Proteins represent major targets for modification in these reactions, and identification of sites and structures of modifications may lead to mechanistic understanding and approaches for prevention. In this Account, the utility of mass spectrometry and its advantages are described for the identification of oxidative modifications to protein targets. A variety of examples are provided to illustrate how modifications are accurately identified and quantitated using modern methods of ionization coupled with HPLC and tandem mass spectrometry.

Introduction

Oxidative processes play critically important roles in a variety of life events ranging from normal metabolic events to aging and to the onset of disease.[1,2] Indeed, an increasing number of physiological disorders and disease states are being attributed in some way to oxidative mechanisms, including ischemia, atherosclerosis, inflammation and rheumatoid arthritis, Alzheimer's disease and other mental disorders, etc.[3] A variety of reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygen, superoxide, and peroxide radicals, have been suggested to play a role in the etiology of some disorders, and these ROS can be generated by a variety of mechanisms.[4−6] Regardless of the source of reactive oxygen species, biopolymers represent major targets for modification by these highly reactive compounds, often resulting in decreased molecular function or molecular sequestration/degradation, perhaps leading to cellular dysfunction. Proteins, in particular, are susceptible to oxidative damage, and resulting products of protein and amino acid oxidative damage have been studied for most of this century.[7−9]

Traditional methods of protein analysis such as gel electrophoresis, isoelectric focusing, Edman sequence analysis, and amino acid analysis have been the primary tools employed to assess oxidative damage to proteins. Although much has been learned using these techniques, specific sites and extents of modifications in the protein structures are difficult to determine, and, as such, many studies have been limited to model studies on amino acids or small peptides. Examination of protein modification in general is challenging, given the limitations of these tools. For a successful outcome using amino acid analysis, a specific modified structure needs to be stable to hydrolysis conditions, and some products of tryptophan and histidine are likely to be unstable to such conditions.[10] Moreover, both Edman sequence analysis and amino acid analysis require some idea of the modified structure in order to prepare a synthetic standard for positive identification of an isolated product. We, and others, have used the relatively new tools of biological mass spectrometry to examine protein modification. State-of-the-art mass spectrometry now has better limits of detection than Edman sequencing approaches, and with modern MS techniques it is fairly straightforward to determine sites and structures of modifications on peptides, even when the peptides are N-terminally blocked to Edman sequencing. The focus of our work is to identify protein modifications using mass spectrometry with the goal of understanding oxidative mechanisms and the effect of oxidation on protein structure and function. Below we discuss the techniques involved and the application of mass spectrometry for identification of oxidative modifications to lens proteins.

Biological Mass Spectrometry

With the advent of ionization methods for biopolymers such as matrix-assisted laser desorption/ionization (MALDI)[11] and electrospray ionization,[12] the field of mass spectrometry has blossomed into a powerful and valuable tool for examining posttranslational modifications to proteins. Further development of ultralow, nanoliter-per-minute flow electrospray sources, termed nanospray sources, has pushed detection limits for peptide sequencing to attomole levels.[13] Tandem mass spectrometry (MS/MS) was originally developed for molecular structure analysis of mass-selected ions by employing collision-induced dissociation of selected ions followed by mass analysis of resulting fragments.[14] The combination of MALDI or electrospray sources with tandem mass spectrometers has allowed detailed molecular level structures to be elucidated for a wide variety of molecules, including modified peptides.[15] Specifically, tandem mass spectrometry can be employed to determine the site(s) and nature of modification(s), and, when coupled to high-perfor-

Kevin L. Schey was born September 11, 1962, in Abington, PA. He obtained a B.S. Degree in chemistry from Muhlenberg College in Allentown, PA, and a Ph.D. degree in analytical chemistry with R. Graham Cooks at Purdue University in West Lafayette, IN. He began his academic career at the Medical University of South Carolina in 1990 at the level of Assistant Professor in the Department of Cell and Molecular Pharmacology and Experimental Therapeutics and was promoted to Associate Professor in 1996. His research interests are in the development and application of mass spectrometry for biomolecular structure determination. Specific applications of interest include lens protein modification in cataractogenesis, integral membrane protein structure determination, and protein−protein interactions in signal transduction.

Eric L. Finley was born in 1970 in Charleston, SC. He received a B.S. degree in biology from the College of Charleston in 1992. He is currently a graduate student with Kevin L. Schey at the Medical University of South Carolina. He plans to do postdoctoral work in the laboratory of Ken Tomer at the National Institue of Environmental Health Sciences in Research Triangle, NC.

* Corresponding author. Phone: (843) 792-2471. Fax: (843) 792-2475. E-mail: scheykl@musc.edu.

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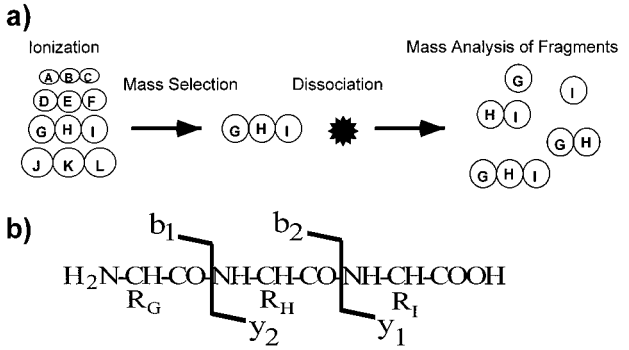

FIGURE 1. (a) Schematic diagram indicating the steps in a tandem mass spectrometry experiment for a sample consisting of four hypothetical tripeptides and (b) fragment ion nomenclature for peptide dissociation in a tandem mass spectrometer.[15]

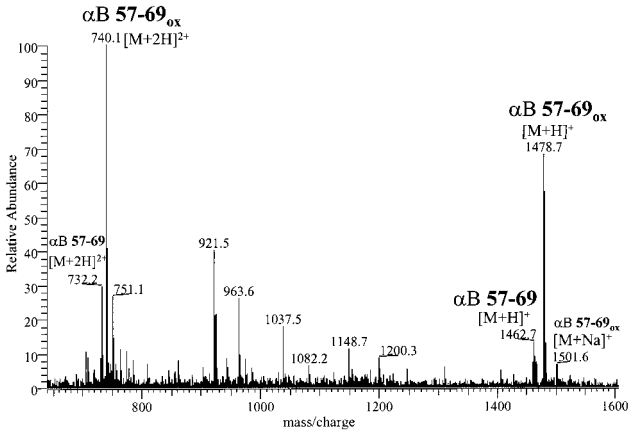

FIGURE 2. Electrospray mass spectrum of an HPLC fraction containing native and oxidized tryptic peptides of bovine α-crystallin.

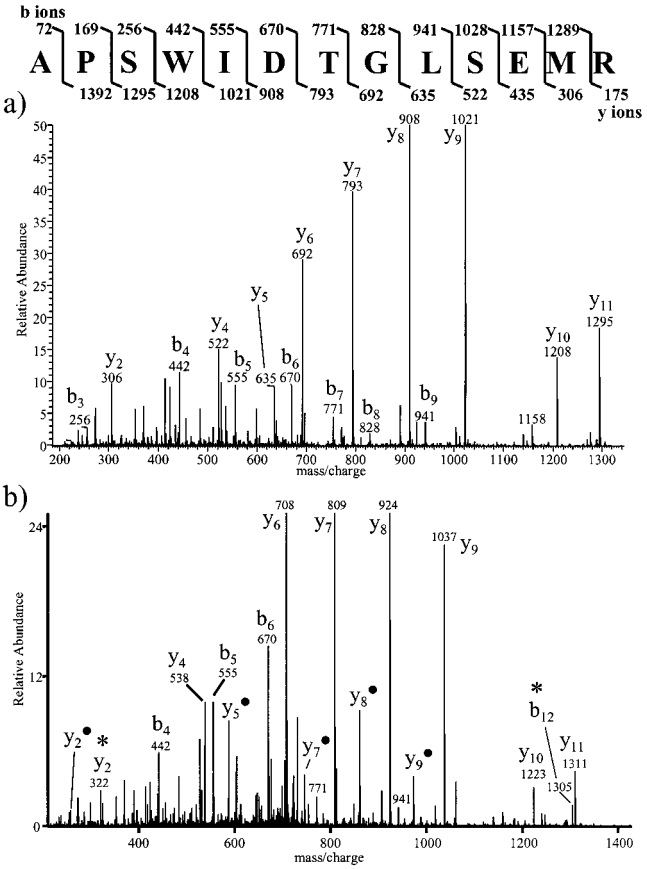

FIGURE 3. Tandem mass spectra of (a) native bovine αB-crystallin residues 57−69 and (b) oxidized bovine αB-crystallin residues 57−69, indicating the observed fragmentation pattern and the sequence ion assignments. The label ● indicates loss of CH_3SOH. Asterisks indicate ions which identify the site of oxidation. The predicted fragment ions are displayed at the top of the figure.

mance liquid chromatography (HPLC), quantitation can be accomplished as well. This experiment is now often performed in an automated HPLC−MS/MS fashion for separation of peptides; i.e., automated sequence analysis is carried out in real time for each eluting peptide, resulting in hundreds to thousands of determined peptide sequences in each LC/MS/MS experiment.[16] This approach is proving indispensable as the proteome is being deciphered.[17]

Figure 1 illustrates the tandem mass spectrometry experiment, both instrumental sequence and data interpretation. Panel a indicates the sequence of events employed to generate sequence information by tandem mass spectrometry, including ionization of all components in a hypothetical mixture of four tripeptides, mass selection of the peptide to be sequenced, dissociation of the selected peptide via collision with an inert gas, and finally mass analysis of the peptide fragments. Peptides tend to fragment at amide bonds in low-energy collisions, resulting in a predictable fragmentation pattern leading to sequence information (panel b). A nomenclature has been developed to label fragment ions containing N-terminal (b ions) and C-terminal (y ions) amino acids.[15] Since all amino acids have unique masses, with the exceptions of leucine/isoleucine and lysine/glutamine pairs, primary sequence information can be obtained from mass differences of signals measured in the MS/MS data. Furthermore, with a few exceptions, a modified amino acid will have an unnatural molecular weight and be readily identifiable in the fragmentation pattern. One exception is oxidized methionine, which has a nominal mass equivalent to that of phenylalanine. Fortunately, in this case, facile loss of sulfinic acid from oxidized methionine in the MS/MS spectra distinguishes the residues.

The use of mass spectral data for the identification of peptide modifications is illustrated in Figures 2 and 3. Figure 2 shows an electrospray mass spectrum of an HPLC fraction from a tryptic digest of bovine α-crystallin. Note that both singly charged $[M + H]^+$ and multiply charged $[M + 2H]^{2+}$ ions are observed for both the native αB 57−69 peptide and the oxidized (+16 Da) peptide. Tandem mass spectrometry was carried out on both peptides in turn by selection of their corresponding $[M + 2H]^{2+}$ ions. After isolation and fragmentation of the unmodified ion at m/z 732, the tandem mass spectrum in Figure 3a was recorded. After isolation and fragmentation of the oxidized peptide at m/z 740, the tandem mass spectrum in Figure 3b was recorded. Details of the interpretation of these data are presented below; however, a brief examination of the fragmentation pattern provides a clear indication that these peptides are related (b ions are identical) and that they differ at their C-termini (y ions are shifted).

The predictability of peptide fragmentation in the tandem mass spectrometry experiment has led to a number of sequencing algorithms to aid in the interpretation of MS/MS spectra.[18−20] An advantage of the MS/MS approach to peptide sequencing is that most structures are stable to digestion, sample preparation, and analysis conditions. An additional advantage of mass spectrometric sequencing, as shown in Figure 3, is the ability to sequence modified peptides, including N-terminally blocked peptides, even when they are present in complex mixtures. The sensitivity for sequencing using tandem mass spectrometry is now unparalleled by traditional sequencing methods and is often reported in the femtomole to attomole range.[13] The major limitation in sequencing by mass spectrometry is the inability to determine atomic positions or exact molecular structures equivalent to those obtained by NMR.

Because of the aforementioned advantages, tandem mass spectrometry has been used to examine oxidative modifications to amino acids, peptides, and proteins.[6,21−26] Not only have important discoveries been made in regard to protein structure, but also novel oxidative products have been reported using the mass spectrometry approach. One cautionary note is that oxidation can occur during sample preparation or during HPLC or mass spectrometric analysis which can complicate interpretation.[27] In our laboratory, sample preparation is typically carried out under argon with degassed solvents and buffers. Control unmodified samples are then run through the HPLC−MS instrument to ensure that oxidation is sample related and not an instrumental artifact.

The focus of the work in this laboratory has been on oxidative damage to lens protein structures as it relates to lens aging and cataractogenesis. A large body of evidence suggests that oxidative stress in the lens plays an important role in lens aging and opacification.[28] We are applying mass spectrometric methods in an attempt to identify and quantitate molecular-level changes in the lens. Below we present a series of examples from our lens protein work to illustrate the power of tandem mass spectrometry for protein structure elucidation in general, and specifically for determination of products of protein oxidation. In model studies, bovine α-crystallin, consisting of two 20-kDa subunits (αA and αB), was exposed to either photooxidation (λ > 295 nm), singlet oxygen (from UV-irradiated uroporphyrin), Fenton reaction products (from $FeCl_2$, H_2O_2, EDTA), or hydroxyl radical (generated by radiolysis under N_2O). Examples of structural analysis of resulting oxidized peptides and determined structures are presented below. The data were acquired using a Finnigan LCQ ion trap mass spectrometer. Ions were created by electrospray ionization of peptides eluting from a standard reverse-phase HPLC separation.

Determination of Oxidized Peptide Structures

Oxidation of a peptide, for the purposes of this report, results from addition of oxygen atoms to susceptible amino acids in a peptide (e.g., methionine, cysteine, histidine, or tryptophan). This reaction can produce either a simple shift in the peptide mass in increments of 16 Da due to sequential additions of oxygen atoms or other mass shifts due to oxidation followed by subsequent reaction. Figure 4 shows proposed structures of oxidized methionine, tryptophan, and histidine residues observed in our model studies of bovine α-crystallin oxidation. Examples of structural characterization by tandem mass spectrometry of oxidized peptides are shown in Figure 3b for single oxidation (addition of one oxygen atom) of methionine 68 from αB-crystallin and in Figure 5 for single oxidation of histidine 97 from αA-crystallin. The sequence of the peptides and the predicted fragment ion masses are shown at the top of each tandem mass spectrum. The key fragment ions which define the sites of modification are indicated with asterisks.

In Figure 3b, the tandem mass spectrum of a tryptic peptide of molecular weight 1479, ($[M + 2H]^{2+}$ ion at m/z 740 selected) is shown. Molecular weight comparison with predicted tryptic peptides of α-crystallin resulted in a tentative assignment of this peak as the doubly charged molecular ion of αB-crystallin 57−69 ($[M + H]^+$, m/z 1463) plus addition of one oxygen atom (predicted $[M + 2H]^{2+}$, m/z 740). Electrospray ionization of tryptic peptides typically results in doubly charged molecular ions, as shown in Figure 2, which fragment in the mass spectrometer to yield singly charged fragment ions. Since the starting protein sequence is known in this case, one simply needs to compare the observed fragmentation pattern with the predicted fragmentation patterns of tryptic peptides which match the measured molecular weight. In this case, the C-terminus-containing y_2−y_{11} fragment ion series (m/z 322, 538, 708, 809, 924, 1037, 1223, 1311) matched exclusively to the sequence αB 57−69 but were shifted by 16 Da above the predicted masses, indicating that the oxygen atom is contained in the C-terminus of the peptide. Since the masses of the b ions at m/z 442 (b_4), 555 (b_5), 670 (b_6), 771 (b_7), and 941 (b_9) were identical to the predicted fragment ion masses for the unoxidized αB 57−69 peptide, both the assignment of this peptide and the location of the oxidized residue to the C-terminus were confirmed. The b_{12} (m/z 1305) and y_2 (m/z 322) ions are produced by fragmentation on the C- and N-terminal sides, respectively, of the methionine 68 residue, and the masses of these ions are each shifted 16 Da higher. These two ions define the site of one oxygen atom addition as methionine 68 (present as methionine sulfoxide), and this assignment is further confirmed by the fragment ion peaks at m/z 258, 745, 860, 973, 1247 (labeled ●). The masses of these peaks are 64 Da lower than those of the y_2, y_7, y_8, y_9, and y_{11} ions, respectively, and are due to loss of sulfinic acid (CH_3SOH) from the oxidized methionine 68 residue. The loss of CH_3SOH occurs during collision-induced dissociation in the mass spectrometer and is diagnostic of oxidized methionine.[29] The tandem mass spectrum for a second putative oxidized tryptic peptide (m/z 659) of α-crystallin is shown in Figure 5. This peptide was tentatively assigned as the doubly charge molecular ion ($[M + 2H]^{2+}$) of αA-crystallin 89−99 ($[M + H]^+$, m/z 1301)

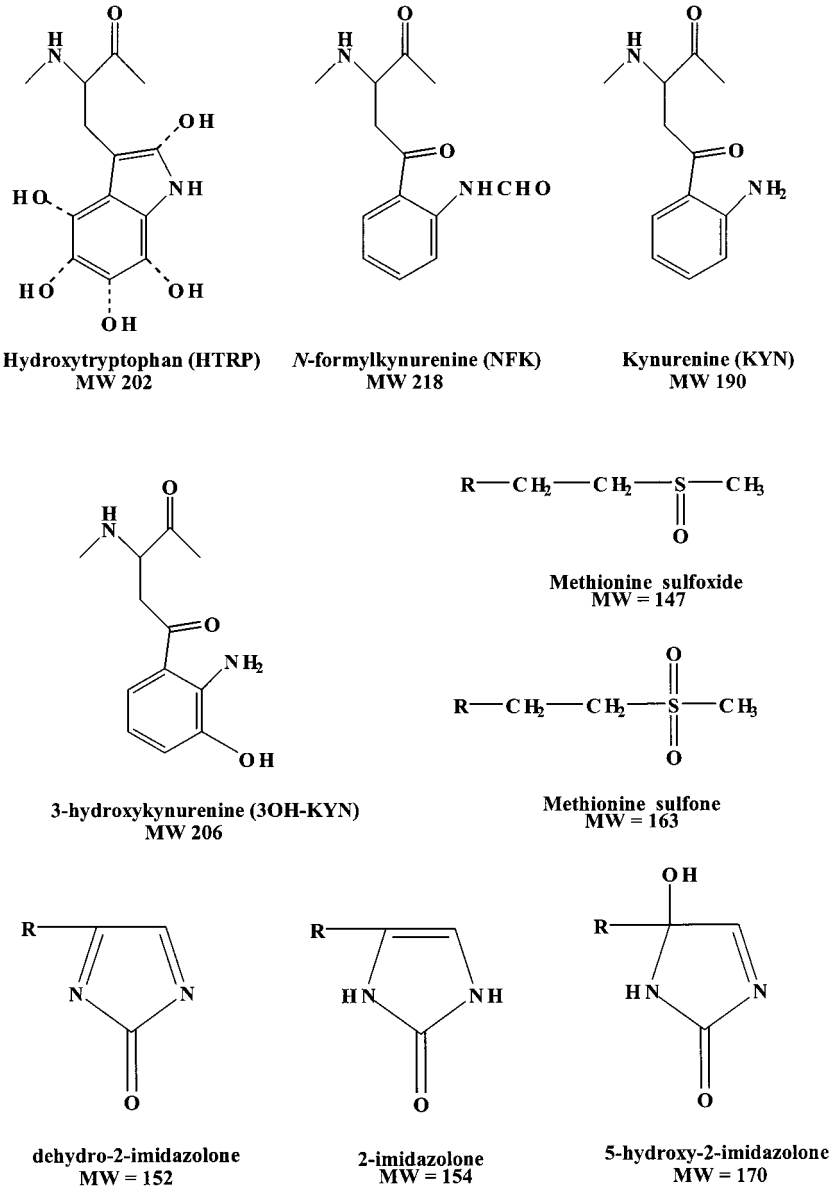

FIGURE 4. Proposed structures of oxidative products of methionine, tryptophan, and histidine observed in our model studies of oxidative modifications to bovine α-crystallin.

plus addition of one oxygen atom (predicted $[M + 2H]^{2+}$, m/z 659). The identity of this peptide was confirmed in the tandem mass spectrum by the masses of the b_2 (m/z 228), b_4 (m/z 472), b_6 (m/z 718), and b_7 (m/z 847) fragment ions, which were found to be identical to the predicted masses for the ions from αA 89−99. Since the masses of these fragment ions were identical to the predicted masses, the oxidation site is not contained in the N-terminus of the peptide. However, the masses of the b_9 (m/z 1113), b_{10} (m/z 1170), and y_2, y_4−y_7, y_9 (m/z 204, 470, 599, 698, 845, 1089) ions were shifted 16 Da above the predicted masses and provide evidence that the oxidized residue is in the C-terminus. In the spectrum, two measured masses correspond to the predicted masses for b and/or oxidized y fragment ions. The peak at m/z 357 could be either a b_3 or an oxidized y_3 ion, while the peak at m/z 960 has the same mass as a b_8 or an oxidized y_8 ion. Disregarding the information from these ions due to their ambiguity, b_9 (fragmentation on the C-terminal side of histidine 97), y_2 (fragmentation on the N-terminal side of histidine 97), and y_4 (fragmentation on the C-terminal side of isoleucine 96) ions limit the oxidation site to isoleucine 96 or histidine 97. On the basis of the oxidative susceptibility of these amino acids, the most probable site of oxidation is histidine 97, most likely present as 2-imidazolone. If the ions at m/z 357 and 960 are included in the analysis, the assignment of histidine 97 as the oxidation site is further strengthened. One advantage of the LCQ ion trap instrument is that ambiguity in fragment ion assignment can be resolved by mass selection of the fragment ion followed by fragmentation, otherwise known as an MS/MS/MS or an MS^3 experiment. These data reveal one of the weaknesses of tandem mass spectrometry in that the exact location of the oxygen atom on the histidine residue cannot be determined, and therefore specific structures must be assumed on the basis of previous

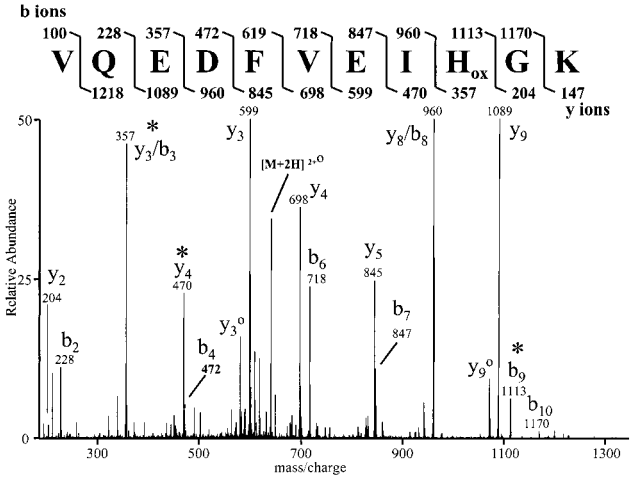

FIGURE 5. Tandem mass spectrum of oxidized bovine αA-crystallin residues 89−99, indicating the observed fragmentation pattern and the sequence ion assignments. The label ○ indicates loss of water, and asterisks indicate ions which identify the site of oxidation. The predicted fragment ions are displayed at the top of the figure.

chemical information typically obtained by NMR spectroscopy at the amino acid level.

Tandem mass spectra of multiply oxidized peptides can be interpreted in a manner similar to that described above to determine the presence of oxygen of atoms on one or more susceptible amino acids in the peptide. However, the potential exists for more complex chemistry to occur than that described above for single oxygen additions. A simple 32-Da mass shift can result from double oxidation of methionine to methionine sulfone, double oxidation of histidine to 5-hydroxy-2-imidazolone, double oxidation of tryptophan to form *N*-formyl kynurenine (NFK), or addition of one oxygen atom to two different amino acids (e.g., one on methionine and one on tryptophan). Depending on the type of oxidative exposure and subsequent reactions, addition of two oxygen atoms to histidine followed by hydrolysis can produce a shift in mass of 14 Da, and addition of two oxygen atoms to tryptophan followed by loss of carbon monoxide can result in kynurenine (a 4-Da shift). Obviously, the more highly oxidized the peptide becomes, the greater the chance for complex product formation resulting in complex tandem mass spectra.

After mass spectrometric analysis, two tryptic peptides of α-crystallin, αA 1−11, were found to be shifted in mass to 32 Da higher than predicted. Figure 6 shows examples of double oxidation involving two residues: methionine 1 and tryptophan 9 in panel a and methionine 1 alone in panel b. The sequence of the oxidized peptides and the corresponding fragment ion masses are shown at the top of each spectrum.

The tandem mass spectrum of a tryptic peptide (*m*/*z* 730.5) of α-crystallin is shown in panel a of Figure 6. On the basis of molecular weight, this peptide was initially assigned as the doubly charged ($[M + 2H]^{2+}$) molecular ion of N-terminally blocked αA-crystallin 1−11 with two additional oxygen atoms (predicted $[M + 2H]^{2+}$, *m*/*z* 730.5). In this peptide, one oxygen atom can be added to

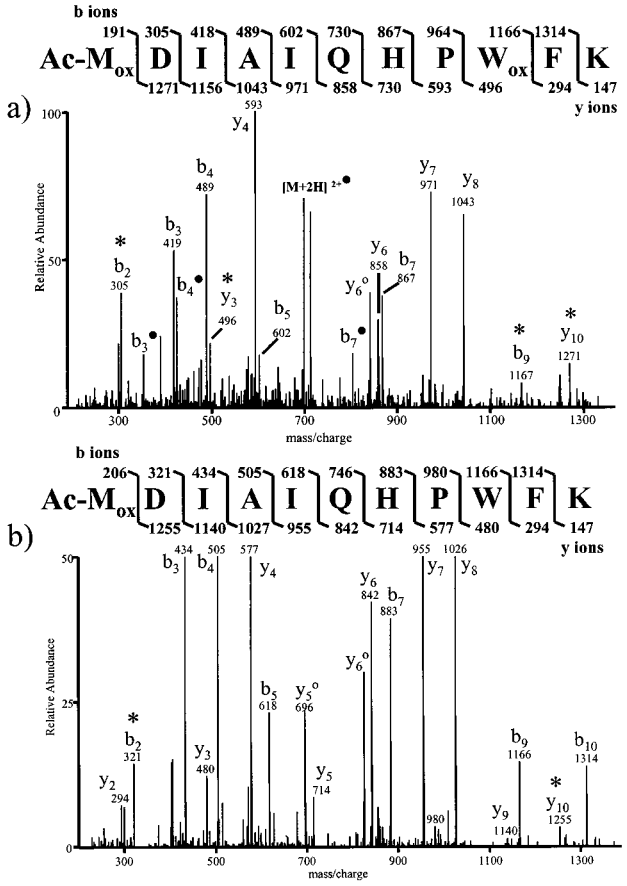

FIGURE 6. Tandem mass spectra of doubly oxidized bovine αA-crystallin residues 1−11, indicating the observed fragmentation pattern and the sequence ion assignments for (a) singly oxidized methionine 1 and singly oxidized tryptophan 9 and (b) doubly oxidized methionine 1. The label ● indicates loss of CH_3SOH, and the label ○ indicates loss of water. Asterisks indicate ions which identify the site of oxidation. The predicted fragment ions are displayed at the top of the figure.

two of three potential oxidation sites (methionine 1, histidine 7, and tryptophan 9), and each of these residues can be doubly oxidized. The masses of the b_2 (*m*/*z* 305), b_3 (*m*/*z* 419), b_4 (*m*/*z* 489), b_5 (*m*/*z* 602), and b_7 (*m*/*z* 867) fragment ions correspond to the masses of the b fragment ions of αA 1−11 plus 16 Da. This confirms the assignment of this peptide as αA-crystallin 1−11 and indicates that one oxygen atom has been added to a residue in the N-terminus-containing methionine 1. The occurrence of peaks with masses due to loss of sulfinic acid (labeled ●) from methionine sulfoxide contained in the b_3, b_4, and b_7 fragment ions verifies that one site of oxidation is methionine 1. This accounts for 16 Da of the 32-Da mass shift. The y_3, y_4, y_6−y_8, and y_{10} ions (*m*/*z* 496, 593, 858, 971, 1043, 1271) were shifted 16 Da higher than the predicted y fragment ion masses, indicating that another oxidized residue was in the tryptophan containing a C-terminus. The mass of the y_3 ion (*m*/*z* 496), due to fragmentation on the C-terminal side of tryptophan 9, was shifted by 16 Da and suggested the three C-terminal residues, Trp-Phe-Lys, as the second site of oxidation. The b_9 ion (*m*/*z* 1167), due to fragmentation after tryptophan 9, was shifted 32 Da higher than predicted (16 Da due to

methionine sulfoxide), which confirmed that tryptophan 9 is converted into hydroxytryptophan. Again, the exact position of oxygen atom attachment cannot be determined using this approach.

Panel b of Figure 6 shows the tandem mass spectrum of a second peptide of the same mass (*m*/*z* 730.5) as the peptide described in panel a, but having a different HPLC retention time. This signal was also tentatively identified as the doubly charged molecular ion ($[M + 2H]^{2+}$) of doubly oxidized αA-crystallin 1−11. The masses of the y fragment ions (*m*/*z* 294, 480, 577, 714, 842, 955, 1026, 1140, 1255) were identical to the predicted masses for the y ions of αA 1−11. This confirmed the identity of the peptide, but, because the C-terminus was not oxidized, the peptide did not have the same structure as the oxidized αA 1−11 peptide described in panel a. The b_2−b_{10} fragment ion series (*m*/*z* 321, 434, 505, 618, 883, 980, 1166, 1314) was shifted by 32 Da above the predicted masses. The b_2 ion (*m*/*z* 321), which was shifted by 32 Da, limited the site of double oxidation to the first two N-terminal residues, Met 1-Asp 2. The mass of the y_{10} fragment ion (*m*/*z* 1255), produced by fragmentation on the C-terminal side of methionine 1, matches the predicted mass for this ion. This eliminates aspartic acid 2 and identifies methionine 1 as the site of double oxygen addition. Methionine sulfone is produced from methionine by the addition of two oxygen atoms. Interestingly, there is no corresponding diagnostic neutral loss in the fragmentation pattern for the sulfone as was described for the sulfoxide.

More extensive oxidation of a peptide can be characterized as described above with the obvious increase in complexity of interpretation. The same approach can be used for spectral interpretation, and MS^3 experiments can be used to resolve ambiguities in sequence assignment. A brief mention should be made of sequencing complete unknown oxidized peptides by this approach. Sequencing complete unknown peptides is accomplished by determining mass differences in the MS/MS data. Complementary agreement is sought for assigned b and y ions in the spectrum. Oxidation sites are identified by mass differences described above for susceptible amino acids and by diagnostic neutral losses such as sulfinic acid indicative of methionine sulfoxide.

Quantitative Analysis of Oxidized Peptides

Quantitative analysis by gas chromatography or liquid chromatography coupled to mass spectrometry has long been routinely employed for the analysis of small-molecular-weight compounds and is a necessity in any industrial analytical laboratory. The evolution of mass spectrometry into the analysis of larger biomolecules has led to the investigation of MALDI and ESI mass spectrometry as quantitative tools, although MALDI is not an ideal quantitative tool due to its small linear dynamic range and differences among peptides in matrix co-crystallization and ionizability. However, ESI, particularly when coupled to HPLC, provides an excellent tool for obtaining quantitative information on proteins and peptide samples.[30]

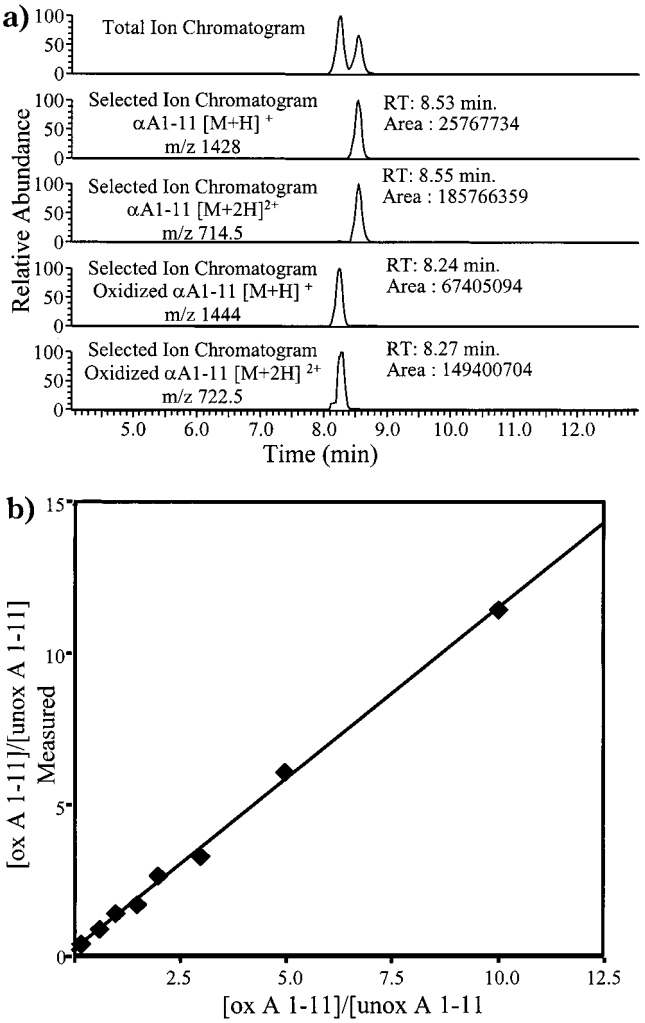

FIGURE 7. (a) Total ion and selected ion chromatograms (SIC) for molecular ions ($[M + H]^+$ and $[M + 2H]^{2+}$) of native and oxidized αA-crystallin residues 1−11 and (b) standard curve generated by determining the ratios of molecular ion signals (summed areas under the SIC) for synthetic native and oxidized αA crystallin residues 1−11 measured by HPLC electrospray mass spectrometry for mixtures of measured molar ratios.

We have measured the relative response of synthetic native and oxidized αA-crystallin 1−11 to determine the utility of HPLC−ESI-MS in examining the extent of lens protein oxidation. A standard curve was generated by injecting different ratios of native and oxidized αA-crystallin 1−11 into the HPLC−ESI-MS instrument. Selected ion chromatograms (the intensity of a particular ion signal plotted as a function of time) for the molecular ions of native and oxidized peptides were generated and the areas under the curves determined. Because of the multiple charge states generated by electrospray ionization, each ion intensity ($[M + H]^+$ and $[M + nH]^{n+}$) must be selected, plotted, and measured.

An example of this approach is presented in Figure 7 for synthetic native and oxidized αA-crystallin 1−11. The calculated areas under the selected ion chromatograms in Figure 7a are used to derive a standard curve such as that shown in Figure 7b. The standard curve for native and oxidized αA-crystallin 1−11 indicates a linear re-

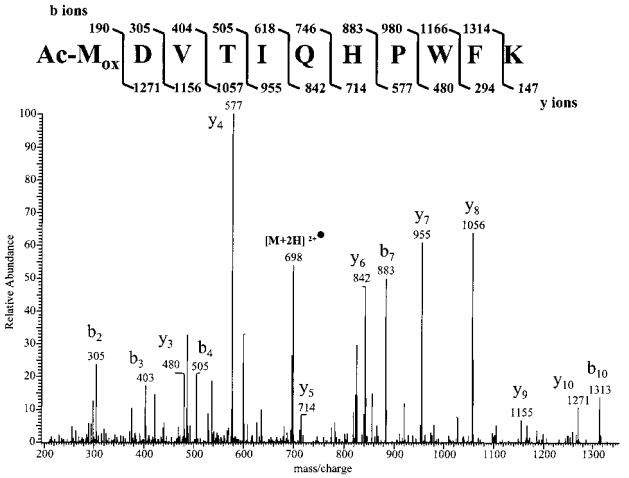

FIGURE 8. Tandem mass spectrum of oxidized human αA-crystallin residues 1−11 isolated from a 15-year-old lens, indicating the observed fragmentation pattern and the sequence ion assignments. The label ● indicates loss of CH_3SOH. The predicted fragment ions are displayed at the top of the figure.

sponse over 80−100-fold differences in relative concentrations of native and oxidized peptide. The data suggest that oxidized residues present in a mixture at 1% relative abundance can be quantitated by this approach. These results were used to semiquantitatively determine the extent of oxidation to α-crystallin in both in vitro reactions and protein isolated from human lenses. A more rigorous quantitative approach would be to use a stable isotope-labeled internal standard which would have the same sequence as the peptide to be quantitated in order to eliminate differences in peptide solubility or ionizability. However, when quantitating multiple modified peptides in a single protein, the production of an internal standard for each modification is prohibitive.

Identification of Oxidized Peptides from Human Lens Tissue

The methods described above are now being applied to examine the extent of oxidation to α-crystallin in human lenses as a function of age and cataract. As was found in the model studies on bovine α-crystallin, methionine 1 in αA- and αB-crystallin is extremely susceptible to oxidation. The tandem mass spectrometry approach has been used to identify methionine sulfoxide in human αA- and αB-crystallins.[21,31] Figure 8 shows a tandem mass spectrum of oxidized human αA-crystallin 1−11 isolated from a 15-year-old lens. The same interpretation described above applies to these data to determine the site of modification. The measured molecular weight shift of 16 Da from the predicted molecular weight ($[M + H]^+$, measured m/z 1460, predicted m/z 1445) suggested the sequence to be oxidized αA-crystallin 1−11. The b ions are shifted from the predicted (shown at top) while the y ions are not shifted, suggesting N-terminal oxidation. The loss of CH_3SOH is clearly present, providing conclusive evidence for methionine 1 sulfoxide as the structure present in the protein.

Conclusions

Protein oxidation has been shown to decrease enzymatic activity, accumulate with age, and possibly be involved in the etiology of numerous diseases.[1,2] The amino acids most susceptible to oxidation include methionine, cysteine, histidine, and tryptophan, and the products formed from oxidation of these residues have predictable shifts in molecular weights compared to the molecular weights of unoxidized structures. Tandem mass spectrometry provides a fast, sensitive, and accurate method for identifying the sites and extent of oxidation in proteins. Its advantages over conventional time- or sample-consuming methods make it a powerful tool in the examination of protein modification in general. Future use of tandem mass spectrometry for quantitative and qualitative analysis of modified proteins will be extensive because of the ability to obtain structural details on modified peptides present in complex mixtures at attomole levels as well as because of the potential for quantitative analysis. Thus, it is clear that tandem mass spectrometry will be a vital tool as we enter the proteome era.

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