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Correct Identification of Oxidized Histidine Residues Using Electron Transfer Dissociation

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Abstract

Oxidative modification to the side chain of histidine can noticeably change the collision-induced dissociation (CID) pathways of peptides containing this oxidized residue. In cases where an oxidized peptide consists of two or more isomers differing only in the site of modification, oxidation to histidine usually causes the other oxidized sites to be mis-assigned in CID spectra. These spectral mis-assignments can sometimes be avoided by using multiple stages of MS/MS (MS^n) or via specially-optimized liquid chromatographic separation conditions. In this manuscript, we demonstrate that these mis-assignments can be more readily and easily avoided by using electron-transfer dissociation (ETD) to dissociate the oxidized peptides. Furthermore, we find that the relative insensitivity of ETD to side chain chemistry allows the extent of oxidative modification to be determined readily for peptide isomers having more than one site of oxidation. The current results along with previous studies of oxidized peptides suggest that ETD is probably a better technique than CID for obtaining correct sequence and modification information for oxidized peptides.

Keywords

electron transfer dissociation; oxidized peptides; MS/MS; peptide sequencing; protein oxidation

Introduction

Mass spectrometry (MS) is the technique of choice to determine amino acid modifications in peptides and proteins. Numerous studies have used MS to identify and locate protein post-translational modifications,[1-3] and increasingly MS is being used in combination with covalent labeling reactions to study protein structures. An emerging set of methods to study protein structures are those that rely on oxidative modifications as indicators of structure. These methods use radicals (e.g. $^{\bullet}OH$) to modify solvent-exposed[4-8] or metal-bound amino acids.[9-20] After oxidation, modifications to amino acid side chains are usually identified by digesting the protein of interest with a proteolytic enzyme and subjecting the modified peptides to tandem MS (MS/MS) analyses. The pattern of amino acid modifications is then used to obtain information about the protein structure or changes in its structure upon binding a ligand or another protein. During the MS/MS analyses, determination of the modified amino acids relies on finding product ions whose m/z ratios are shifted from expected values.

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Determining the individual amino acid modifications is usually straightforward because oxidative modifications often do not change peptide ion dissociation patterns, but there are several examples in which they do. Oxidative modifications to cysteine and methionine residues have very noticeable effects on peptide ion dissociation patterns. For instance, oxidation of cysteine to cysteic acid can lead to very selective peptide dissociation in some cases and to more efficient overall peptide dissociation in other cases.[21-24] The presence of cysteic acid enhances peptide bond dissociation at its C-terminal side and can allow mobilization of an additional proton that initiates cleavages more efficiently at other peptide bonds as well. Oxidation of cysteine to cysteine sulfinic acid also gives rise to selective dissociation on the C-terminal side of this residue when the number of arginine residues equals or exceeds the peptide charge state.[25, 26] The prevalence of selective dissociations can ultimately limit the structural information obtained from CID studies. Oxidation of methionine to methionine sulfoxide can also have a notable effect on peptide ion dissociation patterns.[27-32] When the number of protons on the peptide does not exceed the number of basic residues, product ion spectra of peptides containing methionine sulfoxide are dominated by a neutral loss of methane sulfenic acid (CH_3SOH).[31] Indeed, in many cases no other sequence information is present, highlighting the effect that this oxidative modification can have.

While cysteine and methionine residues are readily oxidized, other amino acids such as those with aromatic side chains are also susceptible to oxidation.[33] Histidine, in particular, can be easily oxidized, and our group recently reported that oxidation of histidine to 2-oxo-histidine can change peptide dissociation patterns very noticeably.[34] In some cases, we have found that histidine oxidation can result in tandem mass spectra with misleading information, causing oxidized amino acids to be incorrectly identified. Clearly, such misleading information is very problematic for methods that rely on oxidative modifications as indicators of protein structure. These incorrect assignments during CID analyses could have broader significance as well. Oxidized histidine residues are commonly found in proteins from cells that have undergone oxidative stress.[35-37] 2-oxo-histidine is even considered to be a marker of cellular oxidative stress.[38]

Given the spectral interpretation difficulties that oxidized histidines can cause when peptides containing this oxidized residue are subjected to CID, we set out to explore the merits of using electron transfer dissociation (ETD) as an alternative. The success of ETD, and the analogous technique electron-capture dissociation, in obtaining sequence information from post-translationally modified peptides is well established.[39-44] We have also previously shown that ETD provides more useful sequence information for peptides containing oxidized cysteine or methionine residues.[45] Thus, we predicted that for peptides with oxidized histidines ETD would be able to provide product ion spectra that are less prone to misinterpretation. In addition, for peptides in which more than one residue is modified, we were interested in investigating whether ETD could provide accurate, semi-quantitative data about the degree to which individual sites are modified. Such data are very difficult to obtain with CID but could be useful for improving the accuracy of protein structural analysis methods that rely on oxidative labeling.

Experimental

Materials

Hydrogen peroxide (30%), formic acid, tris(hydroxymethyl)-aminomethane (Tris), and tris (hydroxymethyl)aminomethane hydrochloride (Tris-HCl) were obtained from EM Science (Gladstone, NJ). Ascorbic acid and copper(II) sulfate were purchased from the Sigma-Aldrich Corporation (St. Louis, MO). Acetic acid and HPLC-grade methanol were obtained from Fisher Scientific (Fair Lawn, NJ). All reagents were used as provided. Distilled, deionized water was generated with a Millipore (Burlington, MA) Simplicity 185 water purification system.

The peptides angiotensin I (DRVYIHPFHL), OVA peptide 323-339 (ISQAVHAAHAEINEAGR), β-Amyloid 10-20 (YEVHHQKLVFF), Myelin Proteolipid Protein 139-151 (HSLGKWLGHPDKF), and Brain Derived Acidic Fibroblast Growth Factor 102-111 (HAEKHWFVGL) were obtained from American peptide company (Sunnyvale, CA).

Peptide oxidation

All peptides were oxidized using metal-catalyzed oxidation (MCO) reactions as described previously.[12-17] Briefly, MCO reactions were performed at room temperature in aqueous solutions containing 250 μM peptide, 250 μM $CuSO_4$, 10 mM ascorbate, 2 mM H_2O_2, and 50 mM Tris-HCl/Tris, buffered to a pH of 7.4. In all cases the reactions were initiated by the addition of ascorbate, H_2O_2, or both. The reactions were stopped after 30 minutes by the addition of 1% (by volume) glacial acetic acid.

Instrumentation

Mass spectral analyses were performed on a Bruker (Billerica, MA) Esquire LC quadrupole ion trap mass spectrometer and a Bruker HCTultra PTM Discovery System quadrupole ion trap mass spectrometer. On both mass spectrometers the source conditions were chosen to maximize the ion signal of the desired ions. For direct injection experiments on the Esquire LC, the sample was delivered at 1 μL/min using a syringe pump. HPLC-MS analyses on this mass spectrometer were conducted using an HP1100 (Agilent, Wilmington DE) system with a Discovery C18 column (2.1 × 150 mm; Supelco). The LC effluent was split in a 1:4 ratio with the smaller outlet being fed into the electrospray ionization (ESI) source of the mass spectrometer. For separation of the oxidized peptide isomers, isocratic separations were performed. The two mobile phases that were used were (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. The mobile phase composition during the separation of the oxidized isomers consisted of between 30 and 40% of mobile phase B and 60 to 70% of mobile phase A, depending on the peptide to be separated.

Tandem mass spectrometry (MS/MS) experiments using electron transfer dissociation (ETD) were carried out on a Bruker HCTultra PTM Discovery quadrupole ion trap mass spectrometer. Samples were infused at a flow rate of 1 to 2 μL/min using a Cole Palmer syringe pump (Vernon Hills, IL). ETD was performed using total reaction times between 50 and 200 ms and low mass cutoffs ranging from m/z 30 to 200; these values were chosen to maximize dissociation efficiency. Accumulation times for the fluoranthene radical anion, which was used as the ETD reagent, were optimized for each experiment but were typically between 3 ms and 5 ms. In some cases, collisional activation was used to aid the ETD of doubly-charged ions. When this procedure was performed, the collision activation process used a low-amplitude resonance excitation voltage to resonantly excite the singly-charged radical cations that did not dissociate upon ETD of their doubly-charged precursors in a manner similar to previously described.[46]

Results and Discussion

Histidine oxidation: misassignments of oxidized residues using CID but correct assignments using ETD

Oxidation of histidine can lead to very noticeable changes in the dissociation patterns of peptide ions containing this residue as we demonstrated recently.[33] The preferential cleavage at the C-terminal side of histidine, which is often observed in product ion spectra of peptides with basic residues that equal or exceed the number of protons on the peptide,[47-52] is reduced or eliminated upon oxidation of histidine. Furthermore, tandem mass spectra of peptides with oxidized histidine residues can easily be misinterpreted, especially when the oxidized peptide is a mixture of more than one isomer differing in the site of oxidation.[34] For example, by comparing the percentages of oxidized forms for the product ions from CID (Figure 1a) of oxidized

angiotensin I (DRVYIHPFHL), one concludes that His6, Pro7, Phe8, and His9 are all likely oxidation sites (Table 1). A series of MS^n experiments on the y_4+O, $(b_9+O)^{2+}$, and the b_8+O product ions determines that oxidation is limited to only His6 and His9 (Figures S1, S2, and S3 in Supplemental Information). Separation of the oxidized isomers by LC and subsequent MS/MS analysis also confirms the presence of only two isomers with oxidation at His6 and His9, respectively (Figure S4, Supplemental Information). It should be noted that isocratic separation conditions are necessary to separate these two isomers (Figure 1b). Such conditions are atypical for most LC-MS studies of peptides from, for example, protein digests. The misleading interpretation of the initial CID spectrum (i.e. data in Table 1) arises because oxidative modifications at His6 and His9 suppress the normally prominent dissociation pathways adjacent to these residues. Shut down of these preferential dissociation pathways occurs at different places in the two different oxidized isomers, which is what causes the spectral misinterpretation.[34]

Because the dissociation pathways in ETD are less sensitive to side-chain chemistry,[45] we predicted that ETD of oxidized angiotensin I might avoid misassignments of the oxidized residues. So, ETD was performed on the doubly protonated version of oxidized angiotensin I without prior LC separation of the oxidized isomers. This ETD spectrum shows unoxidized c_2-c_5 ions, unoxidized and oxidized forms of the c_7 and c_8 product ions, and only an oxidized form of the c_9 product ion in addition to only oxidized forms of the z_7-z_9 product ions (Figure 1c). By comparing the percentages of oxidized forms for each product ion, it is clear that the same misassignments that arise during interpretation of the CID spectrum (Figure 1a and Table 1) do not arise upon interpretation of the ETD spectrum (Table 2). The oxidation percentages indicate that His6 and His9 are oxidized. The ring structure of proline at residue 7 prevents the formation of a c_6 product ion, so the actual ETD data itself cannot alone confirm whether His6 or Pro7 is oxidized, but in this case we know that His6 is oxidized from the previous LC-CID-MS/MS data. Moreover, the percent oxidation of the c_7 and c_8 ions indicate that oxidation at His6 accounts for about 65% of the oxidized peptide, which is consistent with the LC data shown in Figure 1b. The integrated peak areas for the two oxidized isomers from the UV chromatogram indicate that His6 accounts for about 60% of the oxidation. As expected, oxidation of His6 and His9 does not significantly influence the extent to which the c_7-c_9 product ions are formed from the two different isomers.

Another example of the effect of oxidized histidine on peptide CID patterns involves the OVA peptide (ISQAVHAAHAEINEAGR). The CID spectrum of the $(M+O+2H)^{2+}$ species of the OVA peptide is difficult to interpret correctly (Figure 2a). Upon examining the percentages of oxidized forms for each product ion in the y- and b-series, there appears to be a mixture of perhaps four isomeric peptide ions differing by the site of oxidation. This conclusion arises from the series of y ions from y_9 to y_{12} and b ions from b_6 to b_9 that have increasing oxidation percentages (Table S1, Supplemental Information). Complete oxidation (100%) of the product ions from y_{12} to y_{14} and b_9 to b_{16}, along with increasing oxidation percentages in the b_6, b_7, and b_8 ions and the y_9, y_{10}, and y_{11} ions suggest that His6, Ala7, Ala8, and His9 are all oxidized. LC-MS data of the oxidized OVA peptide, again using atypical isocratic conditions, provides evidence for only two oxidized isomers (Figure 2b). MS/MS of the two individual oxidized isomers after LC separation clarifies that oxidation is limited to only His6 and His9 (Figures S5a and b, Supplemental Information). Presumably the difficulty in correctly interpreting the CID data arises from a similar effect observed with angiotensin I.

The ETD spectrum of the unseparated $(M+O+2H)^{2+}$ species of the OVA peptide shows unoxidized z_5-z_8 product ions, unoxidized and oxidized forms of z_9-z_{11} product ions, and oxidized z_{12}-z_{16} and $c_{9\text{-}16}$ product ions (Figure 2c). Comparing the percentages of the oxidized product ions, it becomes clear that only His6 and His9 are oxidized and Ala7 and Ala8 are not (Table 3). Moreover, the percent oxidation of the z_9, z_{10}, and z_{11} product ions indicate that

oxidation at His9 accounts for about 46% of the oxidized peptide, which is consistent with the LC data shown in Figure 2b, which indicates His9 accounts for about 47% of the oxidation. The ETD spectrum of the triply-charged version of the oxidized peptide also shows very similar oxidation percentages, indicating that the charge state has little effect on the information that is gathered (Figure S6, Supplemental Information). Just as in the case of angiotensin I, ETD appears to provide more accurate and straightforward information than CID for peptides containing oxidized histidine residues.

Misassignments of other oxidized residues can be corrected using ETD

Even when residues other than histidine are oxidized, CID data is often ambiguous with regards to the sites of oxidation. An example is the CID data of singly-oxidized β-amyloid peptide (YEVHHQKLVFF) (Figure 3a and Table S2, Supplemental Information). The oxidation percentages for the y_2, b_9, b_9^{2+}, and b_{10}^{2+} product ions indicate that Phe10 and Phe11 are oxidized and even suggest that oxidation at these two residues accounts for about 10-15% of the peptide's oxidation. The failure to observe oxidized versions of the y_3, y_5, and y_6 ions, however, bring this conclusion into question. Similarly confusing are the changes in the oxidation percentages of the b-series of ions from the b_9 ion to the b_5 ion. The oxidation percentage drops by about 30% from the b_9 to b_8 but the percentage is successively higher (73%, 78%, and 83%) for the b_7, b_6, and b_5 ions, respectively; note that the oxidation percentage for the b_9 and b_5 ions are essentially the same. These reproducibly inconsistent oxidation percentages, along with the unoxidized y_3, y_5, and y_6, make it impossible to confidently conclude which residues are oxidized in the β-amyloid peptide. LC-MS data of the oxidized peptide, however, provide evidence for five different oxidized isomers (Figure 3b). Just like in the examples above, atypical LC conditions were necessary to fully separate these isomers. MS/MS of each individual oxidized isomer after LC separation indicates that oxidation occurs at Tyr1, His4, His5, Phe10 and Phe11 (Figure S7, Supplemental Information).

The ETD spectrum of oxidized β-amyloid shows unoxidized and oxidized forms of z_5-z_{10} ions and c_5-c_{10} product ions (Figure 3c). The presence of unoxidized and oxidized z_5-z_{10} and c_5-c_{10} product ions and increasing percentages of oxidized forms indicate that Tyr1, His4, His5, Phe10 and Phe11 are oxidized (Table 4). For example, 84% of the z_{10} product ions are oxidized, indicating that the extent of oxidation at Tyr1 is 16%. Similarly, 91% of the c_{10} product ions are oxidized, indicating that the extent of oxidation at Phe11 is 9%. If a similar analysis is done for the other residues, the oxidation percentages for Phe10 (9%), His4 (30%), and His5 (36%) can be determined. The oxidation percentages obtained from the ETD data are consistent with the UV absorbances from LC (Figure 3b). The ETD spectrum of the triply-charged version of oxidized β-amyloid (Figure S8, Supplemental Information) also gives very similar extents of oxidation for each of the oxidized residues.

Another example in which CID experiments are unable to provide confident assignment of the oxidation sites involves the Myelin Proteolipid Protein (MPP) 139-151 (HSLGKWLGHPDKF). The CID spectrum of the triply-charged oxidized peptide (Figure 4a and Table S3), without prior LC separation, suggests a number of oxidation sites, with definitive assignments difficult to make. LC-MS data of the oxidized peptide indicate that three oxidized isomers exist (Figure 4b), and MS/MS of each isomer points to His1, Trp6, and His9 as the oxidized residues. Unlike the CID spectrum, the ETD spectrum of triply-charged oxidized peptide from an unseparated sample correctly identifies the three oxidation sites (Figure 4c) and provides the correct oxidation percentages for each residue. The presence of unoxidized and oxidized z_5-z_{12} and c_2-c_8 product ions and increasing percentages of these oxidized forms provide clear evidence that His1, Trp6, and His9 are modified (Table 5). Moreover, the degree of oxidation at each residue (i.e. His1 = 20%; Trp6 = 30%; His9 = 50%) is consistent with the LC data for this peptide (Figure 4b). Yet again, ETD more accurately

identifies oxidation sites than CID. We have obtained similar results for other peptides that contain both oxidized histidine residues and other oxidized residues. These peptides include HAEKHWFVGL and WGQGGGTHNQ.

Conclusions

Histidine oxidation influences peptide dissociation patterns in such a way that CID data from these peptides often leads to incorrect assignments of oxidized residues. ETD, however, overcomes this problem because dissociation patterns from this technique are relatively insensitive to side chain chemistry. This relative insensitivity to side-chain chemistry also allows the amount of oxidation occurring at given residues to be accurately determined from ETD spectra of peptides that have more than one oxidation site. In contrast, CID spectra of such peptides rarely reflect the accurate oxidation extent. LC-MS can help overcome this problem, but atypical LC conditions are needed to fully separate the oxidized isomers. ETD provides a way to get the correct information by dissociating the oxidized isomers simultaneously. With this dissociation technique, typical LC conditions can be used without having to be concerned about the inability to separate oxidized isomers. Finally, with an increasing number of researchers using oxidative surface mapping to study protein structure, ETD would appear to be the dissociation technique of choice to avoid misidentification and mis-quantitation of oxidation sites that are quite common with CID data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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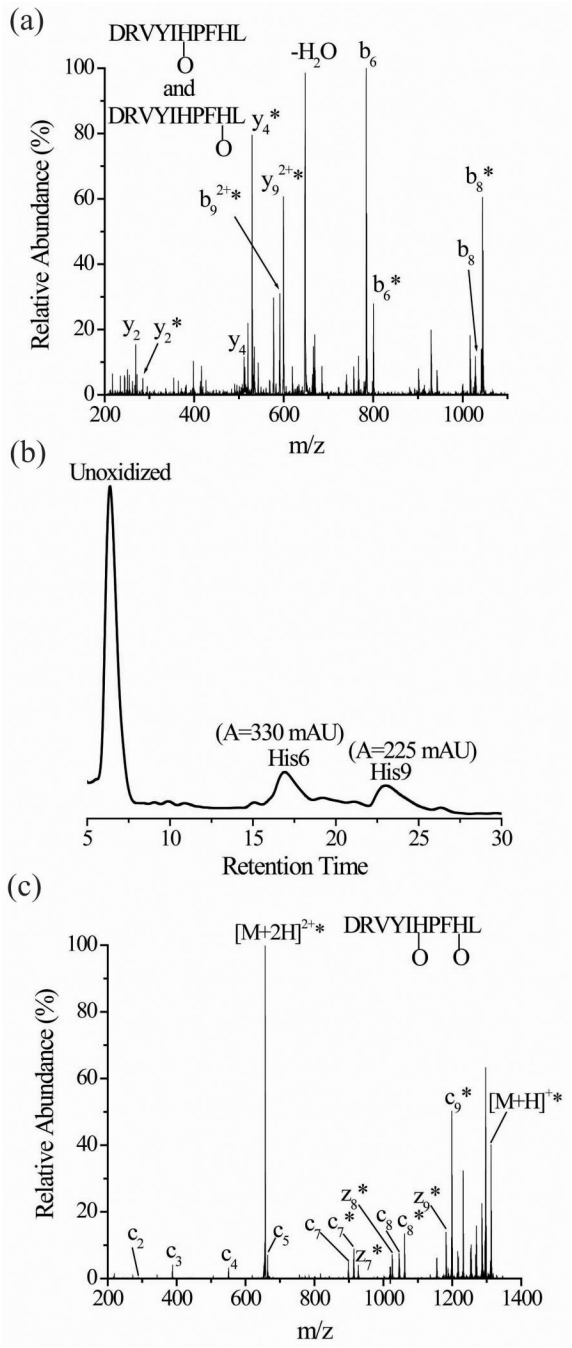

Figure 1.

(a) CID of singly oxidized angiotensin I $(M+O+2H)^{2+}$ with prior LC separation. (b) UV trace of the two oxidized isomers after LC separation. The His6 and His9 labels in the inset correspond to peptides that are oxidized at these residues. (c) ETD of singly oxidized angiotensin I $(M+O+2H)^{2+}$ after simultaneously subjecting the isomeric His6- and His9-oxidized forms to ETD without prior separation. The asterisks indicate the product ions that are oxidized.

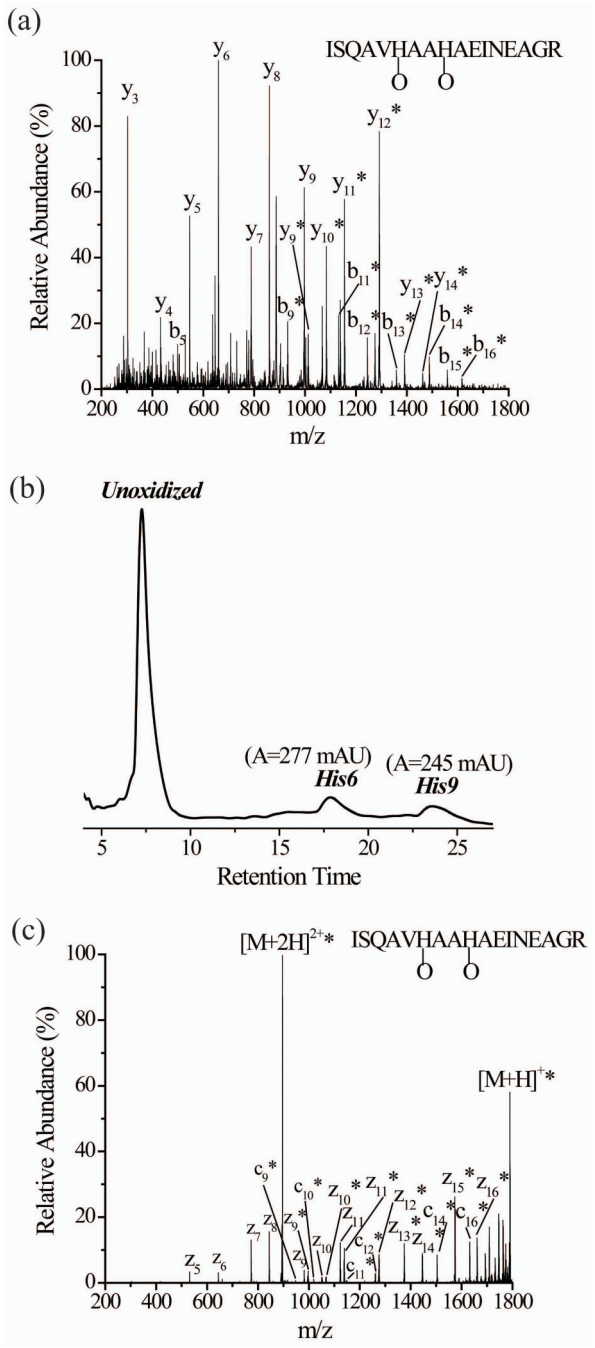

Figure 2.

(a) CID of singly oxidized OVA peptide $(M+O+2H)^{2+}$ after simultaneously subjecting the isomeric His6- and His9- oxidized forms to CID without prior separation. (b) The UV trace of the two oxidized isomers of singly oxidized OVA peptide after LC separation. The His6 and His9 labels correspond to peptides that are oxidized at these residues. (c) ETD of singly oxidized OVA peptide $(M+O+2H)^{2+}$ after simultaneously subjecting the isomeric His6- and His9-oxidized forms to ETD without prior separation. The asterisks indicate the product ions that are oxidized.

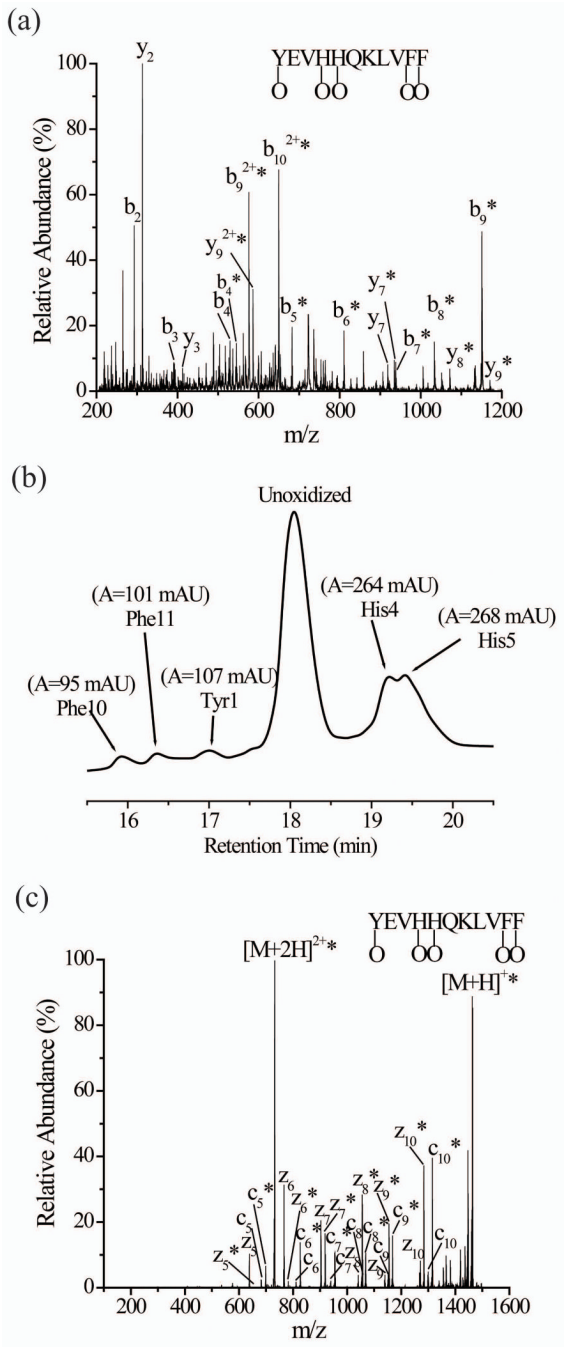

Figure 3.

(a) CID of singly oxidized β-amyloid peptide $(M+O+2H)^{2+}$ after simultaneously subjecting the isomeric oxidized forms to CID without prior separation. (b) The UV trace of the five oxidized isomers of singly oxidized β-amyloid peptide after LC separation. The Phe10, Phe11, Tyr1, His4, and His5 labels correspond to peptides that are oxidized at these residues. (c) ETD of singly oxidized β-amyloid peptide $(M+O+2H)^{2+}$ after simultaneously subjecting the isomeric oxidized forms to ETD without prior separation. The asterisks indicate the product ions that are oxidized.

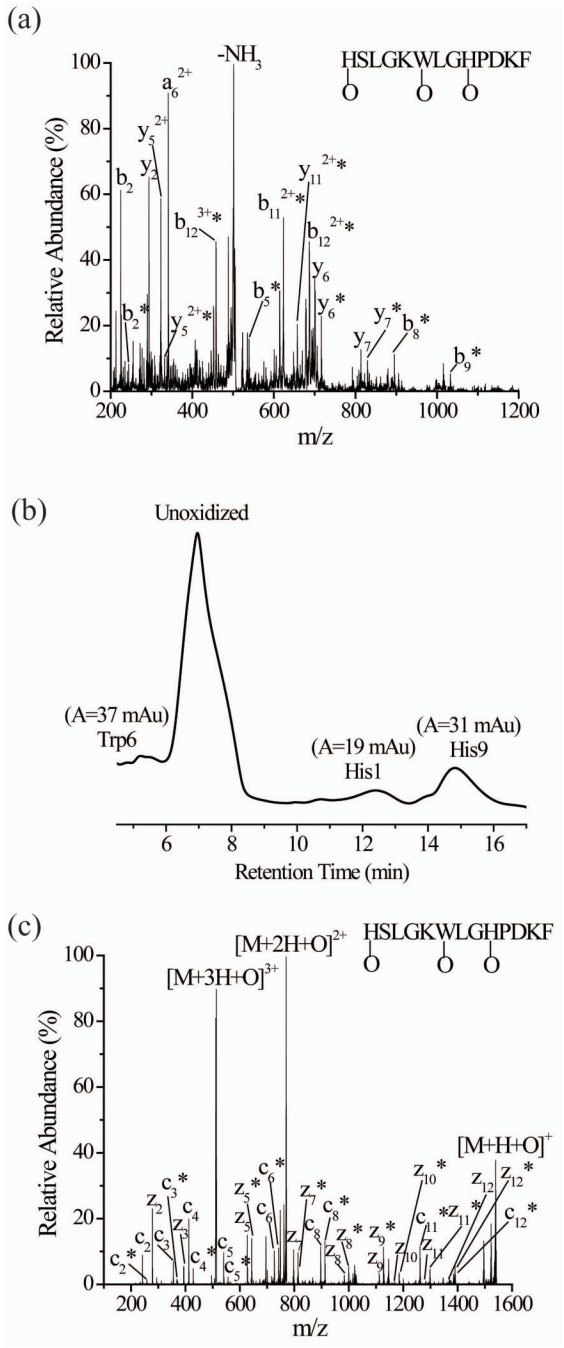

Figure 4.

(a) CID of singly oxidized MPP peptide $(M+O+3H)^{3+}$ after simultaneously subjecting the isomeric oxidized forms to CID without prior separation. (b) The UV trace of the three oxidized isomers of the singly oxidized MPP peptide after LC separation. The Trp6, His1, and His9 labels correspond to peptides that are oxidized at these residues. (c) ETD of singly oxidized MPP peptide $(M+O+3H)^{3+}$ after simultaneously subjecting the isomeric oxidized forms to ETD without prior separation. The asterisks indicate the product ions that are oxidized.

Table 1

Percentage of oxidized product ions observed in the CID spectrum of the doubly charged ion of oxidized angiotensin peptide $(M+O+2H)^{2+}$

Product Ion	$m/z_{unoxidized}$ [a]	$m/z_{oxidized}$ [a]	Percentage oxidized [b,c]
y_2	269	285	25 ± 2
y_3	416	432	15 ± 1
y_4	513	529	90 ± 5
y_5	649	665	100
y_6	763	779	100
y_7	926	942	100
y_8	1025	1041	100
y_9^{2+}	591	599	100
b_2	272	288	0
b_3	371	387	0
b_4	534	550	0
b_5	647	663	0
b_6	784	800	20 ± 6
b_7	881	897	45 ± 3
b_8	1028	1044	80 ± 5
b_9^{2+}	583	591	100

[a] These m/z ratios correspond to the nominal m/z ratio of the observed product ions.

[b] The percentage oxidized is obtained by dividing the ion abundance of the oxidized product ion by the sum of the oxidized and unoxidized product ions.

[c] The error associated with oxidized percentage value corresponds to the standard deviation from at least three separate measurements

Table 2

Percentage of oxidized product ions observed in the ETD spectrum of the doubly charged ion of the oxidized angiotensin I peptide $(M+O+2H)^{2+}$

Product Ion	$m/z_{unoxidized}$ [a]	$m/z_{oxidized}$ [a]	Percentage oxidized [b,c]
c_2	289	305	0
c_3	388	404	0
c_4	551	567	0
c_5	664	680	0
c_7	898	914	66 ± 2
c_8	1045	1061	64 ± 1
c_9	1182	1198	100
z_7	910	926	100
z_8	1009	1025	100
z_9	1165	1181	100

[a] These m/z ratios correspond to the nominal m/z ratio of the observed product ions.

[b] The percentage oxidized is obtained by dividing the ion abundance of the oxidized product ion by the sum of the oxidized and unoxidized product ions.

[c] The error associated with oxidized percentage value corresponds to the standard deviation from at least three separate measurements

Table 3

Percentage of oxidized product ions observed in the ETD spectrum of the doubly charged ion of the oxidized Ova peptide $(M+O+2H)^{2+}$

Product Ion	$m/z_{unoxidized}$ [a]	$m/z_{oxidized}$ [a]	Percentage oxidized [b,c]
z_5	530	546	0
z_6	643	659	0
z_7	772	788	0
z_8	843	859	0
z_9	980	996	46.1 ± 0.3
z_{10}	1051	1067	45 ± 3
z_{11}	1122	1138	46 ± 1
z_{12}	1259	1275	100
z_{13}	1358	1374	100
z_{14}	1429	1445	100
z_{15}	1557	1573	100
z_{16}	1644	1660	100
c_9	932	948	100
c_{10}	1003	1019	100
c_{11}	1132	1148	100
c_{12}	1245	1261	100
c_{13}	1359	1375	100
c_{14}	1488	1504	100
c_{15}	1559	1575	100
c_{16}	1616	1632	100

[a] These m/z ratios correspond to the nominal m/z ratio of the observed product ions.

[b] The percentage oxidized is obtained by dividing the ion abundance of oxidized and unoxidized product ions.

[c] The error associated with oxidized percentage value corresponds to the standard deviation from at least three separate measurements.

Table 4

Percentage of oxidized product ions observed in the ETD spectrum of the doubly charged ion of the oxidized β-amyloid peptide $(M+O+2H)^{2+}$

Product Ion	$m/z_{unoxidized}$ [a]	$m/z_{oxidized}$ [a]	Percentage oxidized [b,c]
z_5	637	653	18 ± 1
z_6	765	781	19 ± 2
z_7	902	918	48 ± 2
z_8	1039	1055	84 ± 2
z_9	1138	1154	83 ± 4
z_{10}	1267	1283	84 ± 4
c_5	683	699	82 ± 3
c_6	811	827	81 ± 2
c_7	939	955	83 ± 1
c_8	1052	1068	82 ± 1
c_9	1151	1167	81.0 ± 0.4
c_{10}	1298	1314	91 ± 1

[a] These m/z ratios correspond to the nominal m/z ratio of the observed product ions.

[b] The percentage oxidized is obtained by dividing the ion abundance of the oxidized product ion by the sum of the oxidized and unoxidized product ions.

[c] The error associated with oxidized percentage value corresponds to the standard deviation from at least three separate measurements

Table 5

Percentage of oxidized product ions observed in the ETD spectrum of the triply charged ion of the oxidized MPP peptide $(M+O+3H)^{3+}$

Product Ion	$m/z_{unoxidized}$ [a]	$m/z_{oxidized}$ [a]	Percentage oxidized [b,c]
z_2	278	294	0
z_3	393	409	0
z_5	627	643	48.3 ± 0.3
z_7	797	813	47 ± 1
z_8	983	999	79 ± 2
z_9	1111	1127	78 ± 1
z_{10}	1168	1184	79 ± 2
z_{11}	1281	1297	80 ± 6
z_{12}	1368	1384	79 ± 2
c_2	242	258	21 ± 1
c_3	355	371	19 ± 2
c_4	412	428	20 ± 1
c_5	540	556	21 ± 1
c_6	726	742	51.8 ± 0.4
c_7	839	855	52 ± 5
c_8	896	912	53 ± 1
c_{10}	1130	1146	100
c_{11}	1245	1261	100
c_{12}	1373	1289	100

[a] These m/z ratios correspond to the nominal m/z ratio of the observed product ions.

[b] The percentage oxidized is obtained by dividing the ion abundance of the oxidized product ion by the sum of the oxidized and unoxidized product ions.

[c] The error associated with oxidized percentage value corresponds to the standard deviation from at least three separate measurements