Methionine regulates copper/hydrogen peroxide oxidation products of Aβ

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Abstract: Metal-catalysed oxidation (MCO) may play a causative role in the pathogenesis of Alzheimer’s disease (AD). Amyloid β peptide (Aβ), the major biomarker of AD, in the presence of copper ions reduces Cu^{2+} to Cu^{+} and catalyses the formation of H_{2}O_{2} that subsequently induces radicals through Fenton chemistry. Aβ is also subject to attack by free radicals, where the presence of Cu^{2+} in conjunction with H_{2}O_{2} catalyses oxygenation, primarily at the methionine sulfur atom. This work investigates MCO of Aβ, to gain further insight into the role of oxidative stress in AD. By combining a fluorescence assay with gel electrophoresis to monitor MCO reactions of Aβ (1–28) in the presence and absence of methionine it was determined that methionine can both protect some residues against MCO and promote the oxidation of Tyr(10) specifically. Electrospray ionization mass spectrometric analysis of methionine MCO products indicated the formation of methionine sulfoxide, methionine sulfone and related hydroxylated products. Similar products could be formed from the oxidation of Met(35) of Aβ and may relate to changes in properties of the peptide following MCO. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Alzheimer’s disease; amyloid β-peptide; Aβ; copper; metal catalysed oxidation; methionine oxidation; hydrogen peroxide; oxidative stress

INTRODUCTION

Metal-catalysed oxidation (MCO) gives rise to highly reactive intermediates such as hydroxyl radicals which lead to damage of bio-molecules and are implicated in aging and the pathogenesis of neurodegenerative diseases, including Alzheimer’s disease (AD) [1,2]. AD is characterized by the accumulation of senile plaques mainly composed of aggregated amyloid β-peptide (Aβ). Aβ is primarily a 40–42 amino acid peptide that is a proteolytic product derived from the β amyloid precursor protein (APP) (for review see [3–5]).

There are many indications that oxidative damage plays a causative role in AD [6], including: metal ions, especially iron, copper and zinc, being elevated in the brains of AD patients [7–10] and implicated in the catalytic activity that may produce free radicals; antioxidants and metal chelators have shown promise as potential treatments for AD [11–15]; and the toxicity of Aβ is eliminated by free radical scavengers in vitro [16,17].

Different studies have indicated that the formation of free radicals by Aβ occurs through interactions with Cu^{2+} where the peptide reduces Cu^{2+} to Cu^{+} [18–23]. This catalytic process leads to the formation of H_{2}O_{2} with the subsequent production of radicals such as the hydroxyl radical through Fenton chemistry [18,20,24]. Additionally, Aβ itself is subject to attack by free radicals, where the presence of Cu^{2+} can catalyse oxygenation, mainly at the methionine sulfur atom Met(35) [25,17], and dityrosine formation [26,23]. The most common source of reactive oxygen species (ROS) is H_{2}O_{2}, which in the presence of redox active metal ions Fe^{2+} or Cu^{+} generates the hydroxyl radical (Fenton reaction).

Met(35) has been reported to play an important role in the redox mediated toxicity of Aβ [1,2,17]. As reviewed by Varadarajan et al. [1], the accumulated data support a vital role for the methionine residue of Aβ peptide in oxidative stress and neurotoxicological properties of this peptide. Evidence includes: elevation of methionine oxidation in senile plaques which recently has been shown by Raman spectroscopy [28]; formation of ROS by the shorter fragments of β-amyloid peptide that contain the methionine residue, such as Aβ(25–35) and Aβ(32–35) [29]; substitution of the methionine by norleucine prevents toxicity [30]; and replacement of a single sulfur atom in the methionine residue of the peptide with a methylene (CH_{2}) has been reported to inhibit the toxicity of Aβ and the associated oxidative
stress to cultured hippocampal neurons [31]. Therefore, it has been hypothesized that the oxidative state of the Met(35) residue sulfur in Aβ has implications for initiation of free radicals and oxidative stress [32,2], and the neurotoxicity of Aβ in AD [32,31,33].

In general, MCO of methionine (methionyl) residues results in oxidation of the sulfur to methionine sulfoxide Met(O) [34,35]. This oxidation product is known to cause formation of higher oligomers of Aβ and to protect His of Aβ from oxidation [36,37]. MCO is suggested to result from direct oxidation [38], with a higher rate at pH 8.5 [39], and Cu²⁺ catalysed this oxidation only at low reactant concentration [39]. The rate of oxidation appears to be higher in proteins than for the free amino acid [40]. Oxidation of the sulfur increases the hydrophilicity of the protein and can induce conformational changes [41] in a variety of proteins including Aβ as shown by us and other groups [42,43,17]. Met(35) oxidation causes partial conversion of Aβ from β-sheet to random coil and decreases Aβ aggregation [42–44]. Additionally, Met(35) oxidized Aβ is both soluble and toxic to neuronal cells [17] and may prove to play an important role in AD. Methionine also has been reported to protect other residues from MCO [45]. Hence, investigation of MCO of methionine is important, as is the identification of stable and suitable MCO methionine peptides that may be used as a biomarker for MCO of proteins and peptides in biological systems.

The goal was to study changes to Aβ peptides under conditions of MCO, and to investigate the role that methionine plays in regulating MCO, to gain further insights into the role of oxidative stress in AD. To this end, techniques were developed to study MCO of amino acids and the study was extended to Aβ(1–28), a shorter version of the amyloid β-peptide. It was demonstrated that: (i) MCO of amino acids and peptides can be monitored using a fluorescence plate reader, with His and Tyr able to be selectively monitored depending upon the wavelength chosen; (ii) His, Phe and Tyr are most sensitive to MCO and also most readily monitored using this technique; and (iii) Met appears to protect His of Aβ(1–28) but promotes Tyr oxidation during MCO. This report shows that in the presence of exogenous methionine, MCO of Aβ(1–28) generated fluorescent products specific for tyrosine modification and caused formation of higher oligomers of Aβ(1–28), while in the absence of methionine, MCO of Aβ(1–28) generated fluorescent products mainly due to histidine and phenylalanine modifications, illustrating the role of methionine in regulating the oxidation products of Aβ.

EXPERIMENTAL PROCEDURES

Materials

All chemicals were purchased from BDH Chemicals (Poole, England), Sigma Chemical Co. (St Louis, USA) and Musashi (Melbourne, Australia) with the highest available quality and purity. All solvents were HPLC grade. Aβ peptides were synthesized using manual solid-phase (Boc) amino acid chemistry as described previously [46].

Electrophoretic molecular weight markers and reagents for enhanced chemiluminescence (ECL) were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Precast Tris-tricine gels (Novex) were obtained from Invitrogen Life Technologies (Carlsbad, CA, USA) and Trans-Blot nitrocellulose membrane were purchased from BioRad Laboratories (Hercules, CA, USA).

Instrumentation

High performance liquid chromatography (HPLC) was performed using a Waters system with dual 510 pumps and a model 996 photodiode array detector controlled using Millennium software (Milford, USA). Data were recorded on a Millennium 32 data collection package.

A fluorescence microplate reader (fMax) with incubator and SOFTmaxPRO software (Sunnyvale, USA) were used. Fluorescence plate reader measurements were carried out at 25°C using the following filters: λex 355 nm/λem 460 nm, λex 485 nm/λem 538 nm, λex 544 nm/λem 590 nm and λex 584 nm/λem 612 nm.

Electrospray ionization mass spectrometry (ESI-MS) was performed using a Micromass Quattro II triple quadrupole instrument (Manchester, UK) using positive ion mode. Samples were introduced to the source via a Hewlett Packard (HP1100) LC system (Palo Alto, USA) equipped with solvent degasser, binary pumps and auto-sampler. Different buffer solutions (either 0.1% formic acid or ammonium formate in MilliQ water: acetonitrile (50:50)) were fed at 40 μl/min to the MS probe. ESI-MS samples were dissolved in appropriate volumes of 1% formic acid. Chelex 100 was used for removal of Cu²⁺ to avoid suppression of the MS signal by the metal ion. Data were acquired in MCA mode from 90 to 1000 Da in 10 s scans, with an ion source at 80°C and sampling cone voltages of 25 or 30, 40 or 60 V. The voltages were selected and the high voltage value (60 V) used for fragmentation of tested samples and further identification.

Methods

Amino acid-/metal catalysed oxidation. 5 mg of each amino acid was dissolved in 10 ml of PBS (pH 7.4); 4 ml of each solution was mixed with 46 μl of 20 mM CuCl₂ by stirring at 37°C; the samples were sonicated for 2 min and kept in closed vials, bubbled with nitrogen gas for 3 min to ensure removal of oxygen then 40, 30, 20 and 10 μl of 3% H₂O₂ was added over 3 days. Excess amounts of Chelex 100 were added to the flasks at reaction end to ensure removal of any trace copper ions. The reaction was quenched by freezing under liquid nitrogen and freeze-dried. The dried samples and the controls were redissolved in 4 ml of MilliQ water and analysed using a fluorescence microplate reader and Fmoc amino acid analysis.

An alternative method was used to suit the conditions required for ESI-MS analysis. The size of the samples was multiplied by 10; PBS was replaced by ammonium acetate buffer with the same pH and H₂O₂/amino acid 10:1 molar ratio was added immediately. In addition, the incubation time was reduced to 1 day. Excess amounts of Chelex 100
Aβ oxidation

**RESULTS**

**Metal Catalysed Oxidation of Amino Acids**

As a prelude to attempting to understand MCO modifications to Aβ, initially MCO mediated changes to the individual amino acids were investigated. The object of this study was to develop an assay for monitoring the changes in peptide residues due to Cu²⁺ and H₂O₂ oxidation. Fmoc amino acids analysis was used to monitor the impact of MCO on the different amino acids. Selected amino acids were assayed using a fluorescence plate reader after MCO. Two of the readily available plate filters were found to be specific for monitoring the monoclonal antibody WO2 species using the monoclonal antibody WO2 which recognizes residues 5–8 [51].

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**Table 1**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Solution added (molar ratio)</th>
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<tr>
<td></td>
<td>H₂O₂&lt;b&gt;³ (√)</td>
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<sup>a</sup> All the samples were made up to 1 ml by addition of 10 mM ammonium acetate buffer (pH 7.4) and the control samples included the same compositions as 1–8 but CH₃CN : H₂O replaced the peptide solution.

<sup>b</sup> 10 μl of 3.0 mM H₂O₂.

<sup>c</sup> 100 μl of 3.0 mM CuCl₂.

<sup>d</sup> 100 μl of 3.0 mM CuCl₂.

<sup>e</sup> 1 μl of 300 μm methionine.

<sup>f</sup> 10 μl of horseradish peroxidase solution (0.1 mg in 1 ml of ammonium acetate buffer).

<sup>g</sup> 100 μl of 30 μM Aβ<sub>1–28</sub> (0.1 mg dissolved in H₂O : CH₃CN, 1 : 1 v/v).

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**Fmoc-amino acid analysis.** Fmoc (fluorenylmethoxycarbonyl) amino acid analysis was carried out using a slight modification of Haynes’s method [48,49]. 100 μl of amino acid with MCO reaction mixture or the control sample [with the same concentration of the amino acid but without MCO components] in 1.5 ml Eppendorf tube was mixed with 5 μl of borate buffer (pH 8.5, 16 mM), followed by addition of 100 μl of Fmoc reagent solution (6 mg in 1.5 MilliQ water), agitated for 90 s and followed by the addition of 60 μl cleavage reagent (680 μl of 0.1 M EDTA and 20 μl NaOH +300 μl of hydroxylamine hydrochloride in 1 ml MilliQ water + 80 μl of 2-(methylthio)ethanol) prepared fresh daily. The reaction components were mixed and allowed to stand for 3.5 min. The reaction was stopped by addition of 140 μl of the quenching reagent (2 ml glacial acetic dissolved in 8 ml acetonitrile acid). 20 μl of the reaction mixture was injected into the HPLC system.

**Polyacrylamide gel electrophoresis (PAGE) and Western blotting.** MCO of Aβ<sub>1–28</sub> was monitored by SDS–PAGE. The dried samples were dissolved in sample buffer (8 mM urea, 8% sodium dodecyl sulfate (SDS), 30% glycerol, 100 mM tricine, 0.01% phenol red and 10% mercaptoethanol), and subjected to PAGE and calibrated Western blot according to previously described procedures [50]. Western blots were probed for Aβ species using the monoclonal antibody WO2 which recognizes residues 5–8 [51].

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were added to the flasks at reaction end to ensure removal of any trace copper ions. The reactions were decanted into clean tubes and frozen in liquid nitrogen and lyophilized. The dried samples were analysed using ESI-MS and fractions were collected from HPLC for Fmoc-amino acid analysis.

**Aβ peptide metal catalysed oxidation.** Aβ<sub>1–28</sub> (0.01 mg/ml or 3 μM) in ammonium acetate buffer (0.01 M, pH 7.4) was incubated with H₂O₂ (1 μg/ml or 30 μM) in CuCl (3 μg/ml or 31 μM) or CuCl₂ (4 μg/ml or 31 μM), with and without methionine (0.46 μg/ml or 3.1 μM) (see Table 1). These solutions were vortexed for 10 s and incubated for 1 day at 37°C. Chelex 100 was added to remove Cu ions and the samples freeze-dried. The samples and the controls were redissolved in MilliQ water or MilliQ water: acetonitrile (1 : 1) and analysed by ESI-MS, UV/Vis, fluorescence microplate reader and gel electrophoresis-polyacrylamide gel electrophoresis utilizing the WO2 antibody as described below.

All MCO reactions were done in the absence of light to avoid photochemical reactions [47].

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to reduce polarity and prevent the amino acid from eluting with the solvent peak. Furthermore, the Fmoc group enhances the sensitivity of amino acids to UV-Vis and fluorescence detectors. This method was applied to quantify amino acid oxidation in the Cu²⁺/H₂O₂ system. Oxidation was quantified by calculating the difference in peak area for the starting Fmoc-amino acid before and after reaction (Figure 1). The results show that aliphatic amino acids have the greatest stability before and after reaction (Figure 1). The results show that aliphatic amino acids have the greatest stability against MCO, especially alanine, isoleucine, leucine and glycine. The remaining amino acids, except tryptophan, are modified under conditions of MCO.

Fluorescence microplate reader analysis of MCO of amino acids. A fluorescence microplate reader was used to determine any changes in fluorescence of amino acids following oxidation conditions as a possible assay for oxidation of Aβ residues. The four available filters with the microplate reader (λ_ex 355 nm/λ_em 460 nm, λ_ex 485 nm/λ_em 538 nm, λ_ex 544 nm/λ_em 590 nm and λ_ex 584 nm/λ_em 661 nm) were tested; but only the first two filters showed positive results (Figure 2).

Filter (λ_ex 355 nm/λ_em 460 nm) showed an increase in fluorescence from MCO of histidine, phenylalanine and tyrosine. Both histidine and phenylalanine MCO products produced the largest increase in fluorescence (see Figure 2(a)). Consequently, this filter can be used to monitor MCO of histidine and phenylalanine in peptides and proteins. The filter (λ_ex 485 nm/λ_em 538 nm) was the most sensitive to the fluorescent products of tyrosine oxidation (Figure 2(b)). Aromatic residues are known to fluoresce, so it is not surprising that His, Phe and Tyr showed the strongest fluorescence after MCO. It should be noted that the presence of Cu²⁺ quenched the fluorescence signal of some amino acids, especially tyrosine products (such as dityrosine) since Cu²⁺ was not removed from the crude solutions; this indicated that the MCO products of some amino acids formed strong ligands for Cu²⁺.

ESI-MS identification of MCO of methionine. ESI-MS analysis of the Cu²⁺-MCO reaction mixtures of methionine showed fragmented forms of the amino acids (data not shown) even at low cone voltage (20 V). Methionine showed a peak with loss of a CO₂Hg group at 45.1 mu. Other masses for fragmented methionine were detected and could be due to the catalytic activity of copper ions causing fragmentation of amino acids in the ESI [52–54]. Fmoc-amino acid analysis was used to isolate the modified forms of methionine by MCO. ESI-MS data indicated the formation of the usual oxidation products as found for H₂O₂ without Cu²⁺, i.e. formation of Met(O), but also showed formation of methionine sulfone (dioxide) Met(O)₂. In addition, the hydroxylated form also appeared in the MS, as indicated by the additional proton (i.e., O + 1 mu) and/or sodium mass (i.e., O + 23 mu).

Metal catalysed oxidation of Aβ(1–28). Aβ(1–28) was used as a model for Aβ(1–40) and Aβ(1–42) because it is more soluble, aggregates less, contains all the non-aliphatic amino acids (except Met (35)) and all the residues required for metal binding [55,22]. The addition of exogenous methionine has previously been shown to induce redox reactions [22] with Aβ(1–28) and Cu²⁺; and was, therefore, added (1 : 1 molar ratio) to some samples to test its role in MCO of the peptide. Also Cu⁺ and Cu²⁺ were used to identify the importance of the metal ion oxidation state to the conversion and formation of reactive oxygen species (ROS) in MCO and Fenton reaction (conversion of Cu⁺ to Cu²⁺).

Fluorescence microplate reader analysis of MCO of Aβ(1–28). The fluorescence microplate results
enhanced by the addition of free methionine. The peptide does not contain a Met residue, yet MCO of Tyr(10) is not caused an increase in fluorescence as oxidized Met surprisingly, this modification was inhibited by the addition of methionine. However, the presence of methionine appeared to promote the modification of tyrosine (Tyr(10)) as indicated by the increase in the fluorescence intensity using the λex 485 nm/λem 538 nm filter (see Figure 2(b) and Figure 3(b)). The methionine itself does not cause an increase in fluorescence as oxidized Met products do not fluoresce (Figure 2) but the addition of methionine results in an increase in oxidized tyrosine products. These results illustrate the importance of methionine in MCO, in facilitating the modification of Tyr(10) in Aβ(1–28). Unlike full-length Aβ, Aβ(1–28) does not contain a Met residue, yet MCO of Tyr(10) is enhanced by the addition of free methionine.

**Gel electrophoresis-polyacrylamide gel electrophoresis.** Gel electrophoresis was used to determine the aggregated state of Aβ(1–28) after MCO modification.

**Figure 3** Fluorescence intensity of Aβ(1–28) and H2O2 after incubation with Cu2+ or Cu2+ and methionine. The samples are: 1 (H2O2 + Aβ(1–28)), 2 (H2O2 + Aβ(1–28) + Cu2+), 3 (H2O2 + Aβ(1–28) + Cu2+ + methionine), 4 (H2O2 + Aβ(1–28) + Cu2+), and 5 (H2O2 + Aβ(1–28) + Cu2+ + methionine). (a) Plate reader filter (λex 355 nm/λem 460 nm); (b) Plate reader filter (λex 485 nm, λem 538 nm).

The results are summarized in Figure 4 and show that Aβ(1–28) in the presence of H2O2 and Cu ions aggregated more readily to form higher oligomers. The peptide with H2O2 and Cu2+ showed some aggregation but with H2O2 and Cu2+ showed little intensity on the gel, suggesting that modifications to the epitope of the WO2 antibody had occurred. The published epitope for this antibody is residues 5–8 (RHDS) [51]. In the presence of methionine, H2O2 and Cu2+ a similar loss of intensity was observed suggesting modifications to the antibody epitope, i.e. MCO of histidine. The addition of methionine to the H2O2 and Cu2+ reaction with Aβ(1–28) changed the profile of the reaction such that massive aggregation was observed and there was no evidence of any interference with the antibody epitope as immunoreactivity to the WO2 antibodies was still observed, i.e. MCO of tyrosine, which would not affect the RHDS residues. These differences in the effects of Cu2+ and Cu2+ reflect the formation of different oxidation products of Aβ(1–28) with modification of histidines and formation of dityrosines [22], respectively.

**DISCUSSION**

Monitoring the modification of Aβ by MCO is difficult partly because a variety of residues can undergo oxidation and each can form multiple oxidation products (see Figure 1). In addition, binding of metal ions such as Cu2+ to a specific region may cause changes in the conformation of the peptide that promote aggregation [56] making analysis more difficult. Nevertheless, specific changes were observed, allowing conclusions to be made about the role of specific residues such as methionine and tyrosine in MCO of Aβ.

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Under oxidative conditions the Cu$^{2+}$ bound to the histidine residues of Aβ [22] induces modifications to a variety of different residues. NMR results showed that the imidazole ring is modified in the case of histidine, and the results of amino acid analysis and HPLC showed that phenylalanine formed Tyr, o-Tyr and m-Try following MCO (data not shown). However, the presence of methionine alters the profile of products from the MCO, possibly by reducing Cu$^{2+}$ to Cu$^{+}$ as shown by Curtail et al. [22], such that modification of tyrosine residues is promoted (see Figures 2–4). It was recently shown that when Cu$^{2+}$ bound to Aβ(1–42) is reduced to Cu$^{+}$ by a reductant this process is facilitated via a proton-coupled electron transfer (PCET) involving Tyr(10). As the electron passes from the reductant to the copper there is a concerted transfer of a proton to Tyr(10), resulting in the phenol oxygen being protonated and Tyr(10) being susceptible to oxidative modification [57,23]. Reactions involving PCET have become increasingly implicated in a range of biological systems including charge transport in DNA and enzymatic oxygen production [58].

Modifications to tyrosine residues include the formation of 3,4-dihydroxyphenylalanine (DOPA), dopamine, dopamine quinone, dityrosine (DT), isoDT and dihydroxyindol [57]. DT (Cortho − Cortho linkage) and isoDT (Cortho − O linkage) are the most stable structures of these products [59,60]. Recent results from Atwood et al. [26] have shown that Aβ(1–42) in the presence of Cu/H$_2$O$_2$ forms dityrosine. These different tyrosine linkages lead to the formation of higher oligomers and facilitate peptide aggregation as shown in Figure 4. In addition, Schoeneich and Williams [61] studying the reaction of Aβ 1–16 with Cu/H$_2$O$_2$ by HPLC-MS/MS detected 2-oxo-histidine, which is generated from the oxidation of histidine residues of Aβ 1–16. Our current study sits between these two reports and is able to explain the apparent disparity in the results since Aβ(1–28) does not contain Met(35). Without methionine, histidines are modified and when methionine is added to Aβ(1–28), modified forms of tyrosine are produced, including dityrosine linkages. Our results support recent work which showed a modification in both histidine and tyrosine residue contents of Aβ peptides by MCO of Cu$^{2+}$ + H$_2$O$_2$ [62]. In addition, the data support the suggestion that histidine and tyrosine residues are most vulnerable to metal mediated oxidative attack [62].

Oxidation of the sulfur atom of the methionine residue during MCO of Aβ peptides plays an important role in peptide modification as recently reported [25,2], where Met(35) is proposed to be the main source of ROS from Aβ [2]. It was previously shown [17] that oxidation of Met(35) leads to changes in Aβ structure and lipid interactions but that the peptide is still neurotoxic. Additionally Dong et al. have shown evidence that extensive side chain oxidation occurs in Met(35) of Aβ in the senile plaque of diseased brain [28].

The Western blot results revealed that the aggregation of Aβ(1–28) in the presence of copper ions induces modifications which prevent the antibody binding these forms (Figure 4) as seen for Aβ(1–28) + H$_2$O$_2$ + Cu$^{2+}$, which suggests that in this case residues 5–8 (RHDS) are modified. The presence of methionine inhibited these modifications, and instead promoted modification of Tyr10 (as shown by fluorescence plate reader results). The results support the hypothesis of methionine acting as a scavenger, which prevents the modification of amino acids such as histidine in Aβ but allows modification to tyrosine which is not part of the epitope of the WO2 antibody.

Methionine adducts modified by MCO were identified by ESI-MS. The modified forms of methionine were isolated as Fmoc amino acid derivatives (Figure 1(b)) and were not detected by direct RP-HPLC analysis as they are most likely to be of higher polarity than methionine. These products are summarized in Figure 5. Higher oxidized compounds of methionine may have an additional oxygen at the sulfur atom or to one of the carbon atoms, or addition of a perhydroxyl group (OOH) at the sulfur atom [63,64]. Sulfides oxidized to sulfoxides and then sulfones are known reactions for sulfur atoms in different compounds [65], but it has been shown by MS that copper ions catalysed the formation of these products in addition to the other active oxidation products. Some of these products have been identified recently in other oxidation reactions, e.g. oxohydroxysulfur produced from the photochemical reaction of H$_2$O$_2$ with DMSO [66]. Furthermore, the hydroxyl radical formed from a Fenton-like reaction [67] is known to react with sulfoxide compounds to

![Figure 5](https://example.com/figure5.png)

**Figure 5** Different forms of sulfur in methionine residue as a result of metal catalysed oxidation (MCO). These include formation of: (a) known sulfoxides or oxo-sulfur Me(O), (b) sulfones or dioxsulfur (methionine sulfone) Met(O$_2$), and (c) other hydroxyl sulfur compounds, such as (i) hydroxyl sulfur, (ii) oxohydroxysulfur, (iii) dihydroxyl and (d) their sodium salts. Low yields of higher oxidized forms could induce (e) methylene sulfoxonium ion, and (f) sulfonic acid derivative with additional oxygen atoms Met(O$_3$).
form oxohydroxysulfur derivatives which decay with a 100 ns half-life to form sullone derivatives [67], but our MS suggests that the oxohydroxysulfur derivative of methionine is more stable. Similar products may be formed from the oxidation of Met(35) of Aβ and have implications to conformational changes in the peptide [68,69]. Further, oxidized products of MCO of Aβ may be detected by fluorescence assays and used to study structure-function relationships.

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