Tetrahedron 72 (2016) 7770–7789

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Neighboring amide participation in the Fenton oxidation of a sulfide to sulfoxide, vinyl sulfide and ketone relevant to oxidation of methionine thioether side chains in peptides

Olivier Mozziconacci [a], Ganga Viswanathan Bhagavathy [c], Takuhei Yamamoto [c], George S. Wilson [b], Richard S. Glass [c,*], Christian Schöneich [a,*]

[a] Department of Pharmaceutical Chemistry, 2095 Constant Avenue, University of Kansas, Lawrence, KS 66047, USA
[b] Department of Chemistry University of Kansas, Lawrence, KS 66047, USA
[c] Department of Chemistry and Biochemistry, The University of Arizona, Tucson, AZ 85721, USA

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 20 August 2016
Accepted 27 August 2016
Available online 3 September 2016

Keywords:
Two center–three electron (2c–3e) bond
Sulfide radical cation
Conformationally constrained sulfide
Fenton oxidation
Neighboring group effect
methionine

ABSTRACT

Oxidation of Met affects the stability of proteins, and was identified as a step in the beta amyloid-dependent pathogenesis of Alzheimer's disease. One-electron oxidation of Met is facilitated through stabilization of sulfide radical cations with electron-rich heteroatoms. The formation of such 2-center-3-electron bonds, formed between sulfide radical cations and amides, leads to pronounced product selectivity during biologically relevant oxidation conditions. Conformationally constrained methionine analogs embedded within a norbornane framework, i.e., 2,6-*endo*, *endo*- and 2,6-*exo*, *endo*-pyrrolidine amide thiomethyl bicyclo[2.2.1]heptanes were synthesized. Oxidation of both methionine analogs in the Fenton oxidation yielded some sulfoxide. In addition, the oxidation of the endo, endo-derivative generated a vinyl sulfide while the exo, endo-derivative was converted into a ketone, indicating a selective influence of a sulfur-oxygen 2-center-3-electron bond on product formation. Mechanistic details of product formation were investigated through the incorporation of stable isotopes.

1. Introduction

Redox processes of methionine (Met) have attracted considerable attention in the biomedical sciences, as protein Met oxidation can affect enzyme activity,[1,2] protein turnover,[3] and/or protein-protein interactions.[4,5] Methionine (Met) serves important functions in proteins including (i) the maintenance of conformational stability, e.g., through interaction of the sulfur with π-systems,[6] (ii) the binding of transition metal-containing ligands, and (iii) the control of redox processes.[7–10] Met residues in proteins have been referred to as endogenous antioxidants,[11] based on their facile oxidation to Met sulfoxide,[12] which can be reversed through the methionine sulfoxide reductase enzymes.

In particular, the metal-catalyzed one-electron oxidation reaction of Met appears to play a role in the neurotoxicity of β-amyloid peptide (βAP),[13,14] involved in the pathogenesis of Alzheimer's disease. However, the molecular features of β-amyloid peptide, which supports the one-electron oxidation of Met are poorly understood. This motivated our experimental work on metal-catalyzed oxidation of specifically designed organic Met analogs, described in the present paper. βAP contains a Met residue at position 35, Met[35], which appears to be involved in the generation of reactive oxygen species through the reduction of Cu^{2+}.[14–20] Thermodynamically, the one-electron oxidation of Met by βAP-bound Cu^{2+} is unfavorable.[11,21,22] However, the one-electron oxidation of Met by Cu^{2+} (Eq. 1) may be driven by exergonic follow-up reactions, such as the deprotonation of an intermediary sulfide radical cation,[23,24] representatively shown for the Met ε-CH_3 group (Eq. 2).

$$\mathrm{Met}(>\mathrm{S}) + \mathrm{Cu}^{2+} \rightarrow \mathrm{Met}(>\mathrm{S}^{\bullet+}) + \mathrm{Cu}^{+} \quad (1)$$

$$\mathrm{Met}(>\mathrm{S}^{\bullet+}) \rightarrow \mathrm{Met}(-\mathrm{S}-{}^{\bullet}\mathrm{CH}_2) + \mathrm{H}^{+} \quad (2)$$

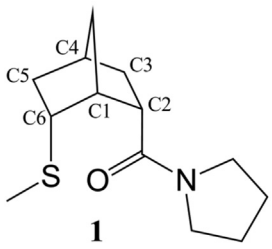
In addition, any reduction of the one-electron reduction potential of Met may promote the one-electron oxidation by Cu^{2+}. Theoretical calculations on βAP, where Met[35] is incorporated into a C-terminal α-helix (based on the NMR structure of βAP1-40 in a micelle-containing aqueous solution[25]), show that the one-

* Corresponding authors. Fax: +1 (520) 621 8407 (R.S.G.); tel: +1 7858644880 (C.S.); e-mail addresses: rglass@email.arizona.edu (R.S. Glass), schoneic@ku.edu (C. Schöneich).

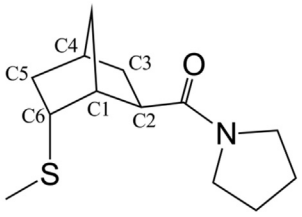
http://dx.doi.org/10.1016/j.tet.2016.08.075
0040-4020/

electron oxidation of Met[35] can result in bond formation between the Met sulfur and the oxygen of the peptide bond C-terminal of Ile [31].[26] Such sulfur-oxygen bond formation may result in the stabilization of a sulfide radical cation[27] and, consequently, reduce the oxidation potential of Met[35]. Time-resolved pulse radiolysis experiments have, indeed, provided evidence for the association of a sulfide radical cation with the peptide bond in model compounds such as *N*-acetyl-Met amide,[28] but also in a heptapeptide, N-Ac-$Gly_3MetGly_3$.[29] Importantly, when the C-terminal α-helix of βAP1-40 was disrupted through substitution of Ile[31] by Pro[31], the mutant βAP1-40(Ile[31]Pro) had a significantly lower efficiency at reducing Cu^{2+} as compared to wild-type βAP(1-40).[30] The mutant also showed a ca. 10-fold reduced efficiency to form a sulfur oxygen bond between Met[35] and Pro[31] as compared to Met[35] and Ile [31] in wild-type βAP(1-40),[26] consistent with a contribution of sulfur-oxygen bond formation to the oxidation of βAP(1-40) by Cu^{2+}.

The sulfur-oxygen bond formed between a sulfide radical cation and a peptide bond oxygen is best characterized as a 2-center-3-electron (2c–3e) bond, indicated through the symbol S∴O.[31] For a better characterization of such sulfur-oxygen three-electron bonds we applied pulse radiolysis to synthetic substituted norbornane derivatives,[32] including compounds **1** and **2**,[33] where the

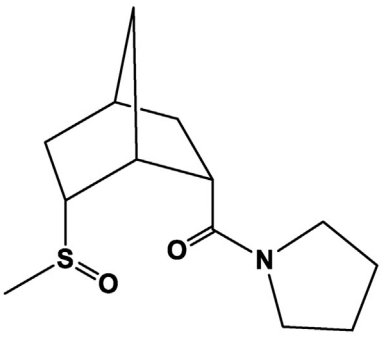


1



2

pyrrolidine amide substituent was either in the endo (**1**) or exo (**2**) position. These compounds were subjected to oxidation by hydroxyl radicals (HO•), and time-resolved UV-spectroscopy used to monitor the formation of three-electron sulfur-oxygen bonded species. Clear spectroscopic evidence for a sulfur-oxygen three-electron bond was obtained for **1** while such species could not be detected for species **2**. These results confirmed earlier pulse radiolysis experiments with simple amides.[32] Importantly, certain S-substituted endo norbornanes showed significantly lower oxidation potentials as compared to exo-substituted norbornanes, confirming a role of sulfur-oxygen bond formation in the lowering of these potentials.[32,33]



3

It can be expected that the formation of three-electron bonded sulfur-oxygen species is of relevance to product formation upon one-electron oxidation of Met derivatives, or, more generally, Met residues in proteins. Such formation of a sulfur-oxygen bond would rationalize the high propensity for β-amyloid peptide Met oxidation, associated with the formation of reactive oxygen species.[13,15–18] Therefore, the objective of this paper was a detailed product analysis for the oxidation of the synthetic substituted norbornane derivatives **1** and **2**, for which time-resolved kinetic experiments had previously revealed different transients induced by one-electron oxidation.[33] For product analysis, we subjected the model compounds **1** and **2** to oxidation by well-defined Fenton chemistry, and, for comparison, to electrochemical oxidation, and analyzed the products by HPLC-MS/MS using high-resolution mass spectrometry. Notably, specifically compound **1** serves as a model for proline-assisted Met oxidation in electron transfer processes such as proposed by Giese et al.[34,35]

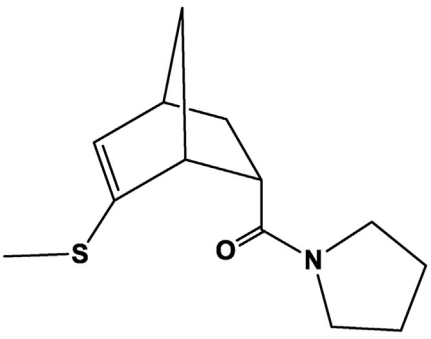
We shall demonstrate that the Fenton oxidation of 2,6-*endo*,*endo*- and 2,6-*exo*, *endo*-pyrrolidine amide thiomethyl derivatives of norbornane lead to the formation of sulfoxide, vinyl sulfide and/or ketone derivatives where the formation of the vinyl sulfide (and the ketone) are dependent on the intermediary formation of an $(>S\therefore O<)^+$ bond.

2. Results

The products formed on Fenton oxidation of compounds **1** and **2** were separated by LC. The structures of the products formed were determined by a combination of methods: MS, MS/MS analysis of gas-phase fragmentation pathways, chemical derivatization and comparison with chemically synthesized compounds. MS fragmentation pathways were based on literature precedent and theoretical calculations. A signature pathway has been identified as the retro-Diels–Alder reaction (RDA) if a double bond is present in the norbornyl ring or generated in a fragmentation pathway. This latter process is well illustrated in MS/MS analysis of compounds **1** and **2**. Their MS/MS spectra, analysis and proposed gas-phase fragmentation are presented in the Supplementary data (Fig. S1–S4).

2.1. Fenton oxidation of 1

2.1.1. LC-MS analysis. Shown in Fig. 1 is the LC-MS analysis of the products obtained by Fenton oxidation of **1**. In addition to unreacted **1** two products were found, referred to products **3** and **4** in Fig. 1. The structures of products **3** and **4** are shown below, and were determined by a combination of methods as presented in the following.



4

*2.1.2. CID of **3**.* The CID of **3** with assignments of the proposed structures of the major fragments is shown in Fig. 2. Since the parent ion of **3** has *m*/*z* 256.2 which is 16 mass units greater than **1**