Free Radical Biology and Medicine 70 (2014) 278–287

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original Contribution

Cross-linking methionine and amine residues with reactive halogen species

Graziella E. Ronsein [a,*], Christine C. Winterbourn [b], Paolo Di Mascio [a,*], Anthony J. Kettle [b]

[a] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, 05508-900 São Paulo, SP, Brazil
[b] The Centre for Free Radical Research, Department of Pathology, University of Otago at Christchurch, Christchurch, New Zealand

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form
3 January 2014
Accepted 20 January 2014
Available online 28 January 2014

Keywords:
Hypochlorous acid
Hypobromous acid
Sulfilimine cross-links
Methionine
Amine
Free radicals

ABSTRACT

Irreversible cross-links are increasingly being recognized as important posttranslational oxidative protein modifications that contribute to tissue injury during oxidative stress and inflammation. They also have a structural function in extracellular matrix proteins such as collagen IV. Likely contenders for forming such cross-links are the reactive halogen species that are generated by neutrophils and eosinophils, including hypochlorous acid, hypobromous acid, and their related haloamines. Methionine residues are kinetically preferred targets for these oxidants and oxidation can potentially result in sulfilimine (> S = N–) bonds with amines. Therefore, we investigated whether oxidation of methionine in the model peptide formyl-Met-Leu-Phe-Lys (fMLFK) produces cross-links with lysine residues, using mass spectrometry to characterize the products. As expected, the sulfoxide was the major product with each reactive halogen species. However, intra- and intermolecular cross-linked products were also formed. Isomers of an intramolecular sulfilimine were readily produced by hypobromous acid and bromamines, with hypochlorous acid forming lesser amounts. The predominant cross-link with chloramines was an intermolecular bond between the sulfur of fMLFK and the amine derived from the chloramine. Reactive halogen species also formed these sulfilimine cross-links in other peptides that contain methionine. We propose that protein cross-links involving methionine and amine residues will form via this mechanism when granulocytes are activated at sites of inflammation. Our results also support the proposal that reactive halogen species generated by the peroxidase peroxidasin could be responsible for the sulfilimine bonds that are integral to the structure of collagen IV.

Phagocytic cells contain heme peroxidases that catalyze the formation of hypohalous acids from hydrogen peroxide and a halide. These highly reactive oxidants play a central role in host defense but also contribute to inflammatory tissue damage [1,2]. Myeloperoxidase is an abundant protein in neutrophils and is also present in monocytes. It catalyzes the oxidation of chloride (Cl^-), bromide (Br^-), and the pseudo-halide thiocyanate (SCN^-) to hypochlorous acid (HOCl), hypobromous acid (HOBr), or hypothiocyanite (^-OSCN), respectively [3–6]. Eosinophil peroxidase generates ^-OSCN and HOBr but not HOCl [7–9]. It is becoming apparent that another mammalian heme peroxidase, peroxidasin 1 (also known as vascular peroxidase 1), is capable of oxidizing chloride and bromide [10,11].

HOCl and HOBr react rapidly with cysteine and methionine residues on proteins to give predominantly disulfides and methionine sulfoxide, respectively [12–16]. Chloramines and bromamines, formed by the reaction of these oxidants with amine residues, are less reactive but show a similar preference [12,17,18]. There is increasing evidence that HOCl and HOBr can promote cross-linking of biological molecules via non-disulfide bonds. For example, numerous investigators have reported that reactive halogen species promote the nonreducible cross-linking of proteins including fibronectin [19], lysozyme [20], calprotectin [21,22], and those in erythrocyte membranes [23]. Such cross-links also occur between DNA and protein [24]. Oxidation of methionine residues by HOCl was implicated in the facile oligomerization of myoglobin [25]. Although it is generally believed that oxidation of methionine by hypohalous acids yields methionine sulfoxide [26,27], it was recently demonstrated that dehydromethionine and azasulfonium salts from *N*-terminal methionine residues are major products in these reactions [28,29]. These species contain a five-membered ring joined by an $>N–S^+<$ bond. A related

Abbreviations:: GYGGFM, Gly-Tyr-Gly-Gly-Phe-Met; GYGGFM(S = O), Gly-Tyr-Gly-Gly-Phe-Met sulfoxide; fMLFK, formyl-Met-Leu-Phe-Lys; fM(= O)LFK, formyl-Met-Leu-Phe-Lys sulfoxide; f**M**LF**K**, intramolecular adduct of formyl-Met-Leu-Phe-Lys; *NAc*-K-fMLFK, intermolecular adduct between N_α-acetyl-lysine and formyl-Met-Leu-Phe-Lys; LC–MS, liquid chromatography–mass spectrometry; EMS, enhanced mass spectrum; EPI, enhanced product ion; SRM, selected reaction monitoring

* Corresponding authors. Fax: +55 11 3815 5579.
E-mail addresses: ronsein@iq.usp.br (G.E. Ronsein), pdmascio@iq.usp.br (P. Di Mascio).

0891-5849/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2014.01.023

sulfilimine cross-link (–N=S<) between hydroxylysine-211 and methionine-93 of adjoining protomers in collagen IV has also been identified [30]. The collagen cross-links have been shown to be catalyzed by peroxidasin 1 and a reaction involving hypohalous acids has been proposed. However, the species generated *in vivo* has yet to be clearly defined [10]. These novel bonds are vital for correct folding of collagen IV. They also mask the collagen IV autoantigen in human Goodpasture disease, and failure to form them contributes to the pathogenesis of this autoimmune disease [31]. Recent data indicate that this sulfilimine cross-link arose 500 Mya and is evolutionarily conserved throughout Metazoa [32]. In view of the importance of the Met-Lys in extracellular matrix formation and autoimmunity, it is important to determine whether it can be formed by hypohalous acids and to understand the mechanism of the process.

We propose that cross-links in and between proteins, formed via the covalent attachment of methionine and amine residues, are important products of the reactions of hypohalous acids generated by mammalian peroxidases. The aim of this study was to demonstrate that reactive halogen species promote either intramolecular cross-links in peptides containing both methionine and lysine residues or intermolecular cross-links between peptides when one has a methionine residue and the other a free amine group.

Materials and methods

Materials

L-Methionine, N_α-acetyllysine, glycine, and taurine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gly-Tyr-Gly-Gly-Phe-Met (Gly-Met-enkephalin, GYGGFM)[1] and formyl-Met-Leu-Phe-Lys (fMLFK) were from Bachem (Bubendorf, Switzerland). All the reagents for buffers were of analytical grade. Hypochlorous acid solution was prepared daily by diluting the concentrated stock solution and calculating its concentration using an ε_{292} of 350 $M^{-1}\,cm^{-1}$ at pH 12 [33]. HOBr solution was generated by mixing Br^- (10 mM) with OCl^- (2 mM) at pH 9 [19,33]. Hypobromous acid formation was followed spectrophotometrically

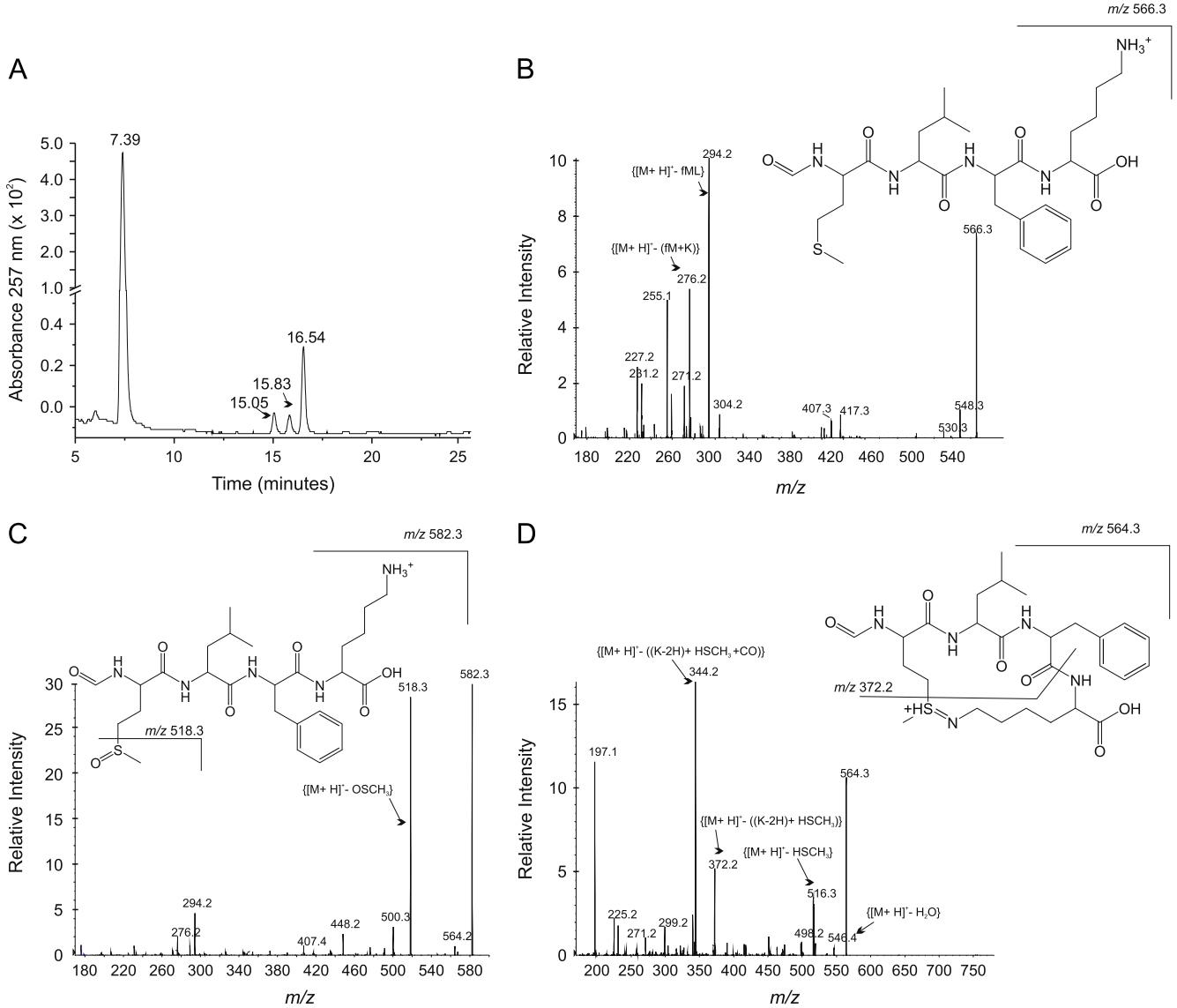

Fig. 1. Analysis of products formed in the reaction between HOBr and fMLFK. HOBr (200 μM) was added with vigorous vortexing to fMLFK (200 μM) in 20 mM phosphate buffer, pH 7.4, containing 100 μM DTPA. (A) UV chromatogram at 257 nm of the products and reactants. (B) MS/MS fragmentation pattern for the parent fMLFK (retention time of 16.54 min in (A)). (C) Proposed structure and MS/MS fragmentation pattern for the sulfoxide (fM(=O)LFK, retention time of 7.39 min in (A)). (D) Proposed structure and MS/MS fragmentation pattern for the intramolecular sulfilimines (f**M**LF**K**, retention times of 15.05 and 15.83 min in (A)).