Oxidation of Zinc Finger Cysteines to Thiolsulfinate

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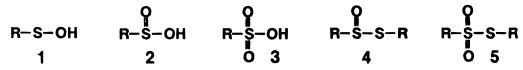
Transcription factors that contain zinc finger motifs constitute a major class of DNA-binding proteins.[1] In these motifs, Zn(II) binds to four conserved Cys and His residues in a tetrahedral coordination that stabilizes a unique peptide structure[2] consisting of a β-hairpin turn and an α-helix that is competent to bind to DNA.[3] While metal ion binding to these sequences in transcription factors and to individual zinc finger peptides has been studied,[4] redox reactions that may damage the transcription factor or DNA when transition metals other than Zn(II) are bound to these motifs have received little attention.[5,6] This reactivity may be important in metal toxicity and/or carcinogenicity,[7] since oxidation of zinc finger sequences in transcription factors reduces or eliminates their ability to bind to DNA, even in the presence of Zn(II).[8]

In a recent study of a peptide corresponding to the third zinc finger of the transcription factor Sp1 (Sp1-3), we found that metal ions bind tightly to the peptide and affect the rate of Cys oxidation by dioxygen, with Zn(II) suppressing and Ni(II) enhancing the loss of free thiols, relative to the metal-free peptide.[5] Cysteines that are oxidized by one electron to the disulfide can be reduced under physiological conditions to regenerate thiols capable of binding Zn(II). However, further oxidation to sulfenic acid **1**, sulfinic acid **2**, sulfonic acid **3,** or, in the case of two thiols, thiolsulfinate **4** or thiolsulfonate **5** is not reversible under



biological conditions, and transcription factors with zinc finger cysteines that are oxidized to these species are irretrievably incompetent to bind to DNA.

Oxidation of organic thiols to these species has been well studied,[9] but their biological occurrence is rare. Cysteines in proteins and peptides are typically oxidized only to intra- and intermolecular disulfides,[10] as has been reported for zinc finger sequences.[8a,11] The sulfenic acid of Cys has been chemically characterized in papain and glyceraldehyde-3-phosphate deyhdrogenase,[12] bovine serum albumin,[13] and NADH peroxidase and NADH oxidase from *Streptococcus faecalis*,[14] and it is proposed as an intermediate in peroxide oxidation of the OxyR transcription factor[15] and in substrate reduction by the alkyl hydroperoxide reductase system from *Salmonella typhimurium*.[16] Recently, higher oxidation states of S have been implicated in the insulin mimetic properties of peroxovanadate compounds, which irreversibly oxidize the essential Cys at a protein−tyrosine phosphatase active site.[17] The few known biological cases of thiolsulfinates,[18] however, are in natural products, such as β-lipoic acid,[19] the antibacterial species allicin from garlic,[20] and the antitumor antibiotic leinamycin.[21] We report the oxidation of the two cysteines in Sp1-3 to the thiolsulfinate, which, we believe, is the first demonstration of thiolsulfinate formation in a protein or peptide.

Table 1. Quantitative Reverse-Phase HPLC Analysis of Peptide Products[a] from 24 h Reactions of Sp1-3

	peptide percentage			
reaction[b]	Sp1-3	SpS-S	SpS-S═O	other[c]
Sp1-3, O_2		100		
Sp1-3, O_2, Zn(II)	95 ± 3	5 ± 3		
Sp1-3, O_2, Ni(II)		60 ± 7	10 ± 3	30 ± 4
Sp1-3, H_2O_2, Ni(II)		45 ± 3	30 ± 3	25 ± 6
Sp1-S, H_2O_2, Ni(II)		59 ± 3	28 ± 4	13 ± 7
Sp1-3, H_2O_2		80 ± 5	20 ± 5	
SpS-S, H_2O_2		80 ± 4	20 ± 4	
Sp1-3, H_2O_2, Zn(II)	56 ± 4	36 ± 2	8 ± 2	

[a] Sp1-3, SpS-S, and SpS-S═O are the reduced, disulfide, and thiolsulfinate forms of Sp1-3 peptide, respectively. The mobile phase contains 0.1% TFA and metal ions do not remain bound to the peptides during chromatographic separation. [b] Each reaction consisted of ∼60 μM Sp1-3 or SpS-S and, where indicated, 66 μM metal ion (introduced as a solution of the sulfate salt) and ∼200 μM H_2O_2. Dioxygen was gently bubbled through solutions where O_2 was the oxidant. [c] Sum of minor species detected by HPLC including primarily the species found at 31.2 and 34.1 min.

The results of quantitative HPLC analysis of the peptide products from 24 h reactions of Sp1-3 [22] with O_2 or H_2O_2 in the absence or presence of Zn(II) or Ni(II) are presented in Table 1. The peptide was completely oxidized to the disulfide upon 24 h exposure to O_2 in the absence of metal ions, and, as reported earlier,[5] Zn(II) coordination to the peptide dramatically suppresses this oxidation. However, species other than the disulfide are detected when Ni(II) is bound to the peptide. Figure 1 shows

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(22) The Sp1-3 peptide, K-F-A-C-P-E-C-P-K-R-F-M-R-S-D-H-L-S-K-H-I-K-T-H-Q-N-K, was prepared by solid-phase peptide synthesis and purified by reverse phase HPLC as described in ref 5.

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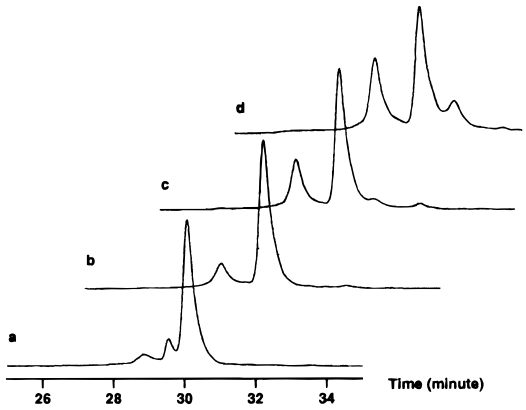


Figure 1. HPLC analysis of the reaction of 60 μM Sp1-3, 66 μM Ni(II), and 200 μM H_2O_2 in unbuffered Nanopure water (initial pH 7.5) after (a) 30 min, (b) 2 h, (c) 6 h, and (d) 24 h.

HPLC chromatograms of this reaction when H_2O_2 was used as the oxidant. One of these new species (28.9 min peak) appears within 30 min, before all of the reduced peptide (29.6 min peak) has been oxidized to the disulfide (30.1 min peak). By 24 h, this 28.9 min species has become a major reaction product and other new species (31.2 and 34.1 min peaks) have begun to appear. This reaction was also monitored by absorption spectroscopy, which showed gradual disappearance of the 305, 380, and ~400 (sh) nm thiolate-to-Ni(II) charge-transfer bands.[5] This loss of Ni(II)−thiolate bonding parallels loss of reduced peptide in the HPLC chromatograms, which is complete within 2 h.

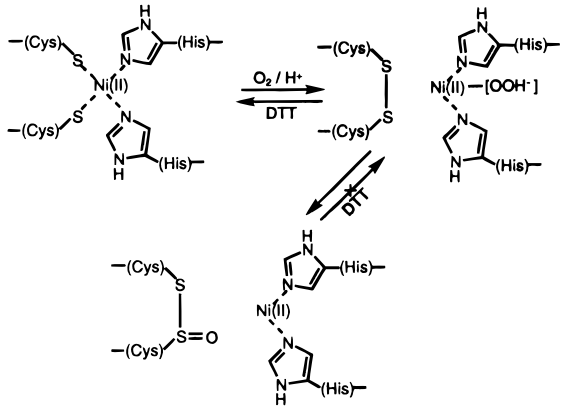
Reaction products from the 24 h reaction of Sp1-3 with H_2O_2 in the presence of Ni(II) were isolated and analyzed. MALDI mass spectrometry confirmed the identity of the 30.1 min species as the disulfide (*m*/*z* 3263; expected *m*/*z* 3266),[23] and the 28.9 min species was found to have a *m*/*z* 3280 parent peak, consistent with the thiolsulfinate (expected *m*/*z* 3282) of Sp1-3. This was confirmed by its IR spectrum, which has a 1077 cm^{-1} peak that is characteristic of the S═O stretching vibration of thiolsulfinates.[18] The identity, properties, and formation of the minor 31.2 and 34.1 min peptide species that begin to appear only after several hours of oxidation continue to be under investigation. Finally, when the mixture of reaction products is treated with excess dithiothreitol, only the disulfide is reduced to the dithiol.

The HPLC data in Figure 1 suggest that the thiolsulfinate results primarily from oxidation of the disulfide, and this was confirmed by treating the disulfide form of Sp1-3 with H_2O_2 in the presence of Ni(II). HPLC analysis of this 24 h reaction mixture showed a similar amount of thiolsulfinate as formed in the reaction of the reduced Sp1-3 peptide, although the amounts of remaining disulfide and other oxidation products were higher and lower, respectively, at this point in the reaction (Table 1).

We also observed that Ni(II) is not required for the formation of thiolsulfinate, which is found upon H_2O_2 oxidation of Sp1-3 in the absence of metal ions[24] and in the presence of Zn(II) (Table 1). In the former case, the distribution of thiolsulfinate and disulfide products at 24 h is identical whether starting with the reduced peptide or the disulfide form. Zinc protects the peptide thiols from oxidation by H_2O_2 and also reduces the amount of thiolsulfinate formed. Thus, as was reported earlier for metal ion effects on the loss of free thiols,[5] Ni(II) enhances and Zn(II) suppresses the formation of thiolsulfinate, relative to the metal-free peptide.

Oxidation of the Sp1-3 cysteines to thiolsulfinate appears to be a two-step reaction with initial two-electron oxidation to the disulfide, followed by a slower two-electron oxidation to the thiolsulfinate. The zinc finger peptide contributes to the formation of thiolsulfinate in this reaction, since analogous experiments with glutathione (γ-Glu-Cys-Gly) fail to yield detectable levels of oxidation products other than the disulfide under any of the reaction conditions in Table 1. Of particular interest is thiolsulfinate formation by O_2 oxidation, which is observed with Ni(II) but not Zn(II). We propose that Ni(II) remains coordinated to the two His residues in the disulfide form and binds the peroxide generated in the first step; the Ni(II)-bound peroxide is then responsible for disulfide oxidation to the thiolsulfinate, as shown in Scheme 1.

Scheme 1



Additional evidence supports this mechanism: (1) Sp1-3 does not react with dioxygen to generate the thiolsulfinate in the absence of Ni(II); (2) the thiolsulfinate is not formed when dioxygen and Ni(II) react with the disulfide form of Sp1-3; (3) even in the presence of catalase, which rapidly disproportionates free hydrogen peroxide, Sp1-3 still reacts with dioxygen in the presence of Ni(II) to generate the thiolsulfinate. Similar Ni(II)-bound oxidant species have been proposed for DNA oxidation[25] and protein cross-linking[26] and cleavage[27] by Ni(II)−peptide complexes.

In summary, we report the first example of cysteine oxidation to a thiolsulfinate in a peptide. This oxidation product is found in the reaction of Sp1-3 with H_2O_2 in the absence and the presence of Ni(II) or Zn(II), but only Ni(II) facilitates thiolsulfinate formation with dioxygen. This thiolsulfinate is not reduced by excess thiol reducing agents, suggesting that its formation in the zinc finger of a transcription factor would permanently eliminate its ability to bind zinc and interact with DNA. In contrast to this result with a peptide, oxidation reactions of Ni(II) *cis*-dithiolate complexes with square-planar N_2S_2 Ni(II) coordination give mono- and disulfenates and -sulfinates, or mixed sulfenate/sulfinate species, but not a stable thiolsulfinate;[28] steric constraints of the ligand and/or different Ni(II) structural and electronic properties may dictate this difference in reactivity between the peptide and these model complexes. Since Cys-X-Y-Cys sequences are found in numerous proteins,[29] in addition to those that contain zinc fingers, thiolsulfinate formation may be more prevalent than suspected. Current studies are examining this possibility and characterizing this oxidation reaction in more detail.

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(23) This difference is within the 0.1−0.2% error range typically encountered with MALDI mass spectrometry.

(24) Experiments run in the presence of the chelator diethylenetriaminepentaacetic acid (DETAPAC) gave similar results, suggesting that trace levels of Fe(II) are not responsible for thiolsulfinate formation.

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