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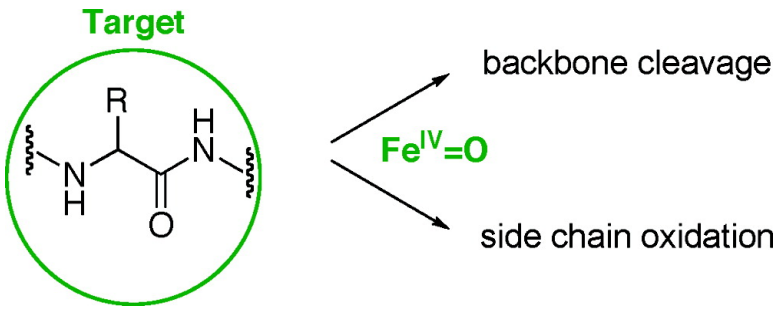
J. Am. Chem. Soc., **2007**, 129 (41), 12390-12391• DOI: 10.1021/ja075075i • Publication Date (Web): 26 September 2007



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Published on Web 09/26/2007

Targeting Peptides with an Iron-Based Oxidant: Cleavage of the Amino Acid Backbone and Oxidation of Side Chains

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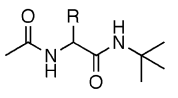
Proteins are an integral part of the chemistry of life. In an oxygen-rich environment, the amino acid residues of proteins are susceptible to attack by reactive oxygen species (ROS) such as singlet oxygen, superoxide, or hydroxy radical.[1,2] This damage can disable proteins, and peptide oxidation has been implicated in numerous disease states, as well as in the progression of aging. How ROS damage proteins is well understood, and the reaction products of amino acid degradation by ROS have been characterized.[3] In contrast, much less is understood about how metal-based oxidants react with amino acid residues.[4,5] This is unfortunate because metal-based oxidants have greater potential than ROS to modify amino acid residues of proteins with high selectively and specificity, considering they are typically less reactive, nondiffusible, and could be directed to sites on a protein. Recent achievements regarding the generation of high-valent iron complexes,[6,7] coupled with the high abundance of iron in biological systems, make iron-based oxidants attractive for targeting proteins of interest. Toward this end, herein we describe the oxidation of amino acids by an iron-based oxidant, which results in cleavage of the amino acid backbone and modification of side chains.

To study how iron-based oxidants might react with amino acid residues in a protein, a series of protected amino acid substrates (**1**−**7**, Figure 1) was constructed that were designed to model individual residues within a polypeptide chain. Starting from the parent amino acid, the N-terminus was acetylated. On the C-terminus, a *tert*-butyl amide was installed to block the weak C−H bonds that would be equivalent to the α-position of the next residue in a polypeptide chain.

Next, using substrates **1** and **4**, conditions of the oxidation reaction were optimized for iron-based catalyst and stoichiometric oxidant. Water was chosen as a solvent because it is most relevant to biological systems, and MeCN was incorporated to solublize the amino acid substrates. For these studies, a series of catalysts were picked that are known to generate iron-based oxidants, including $[Fe^{II}(N4Py)(MeCN)](ClO_4)_2$ (**8**) that can generate a reactive Fe^{IV}═O species which is stable in aqueous media[8] and is powerful enough to oxidize a C−H bond of cyclohexane[9,10] as well as complexes $Fe^{II}(TPA)(OTf)_2$ (**9**)[11] and $[Fe^{II}(BPMEN)](OTf)_2$ (**10**) (Figure S1).[12] For optimization, substrates **1** and **4** were screened against catalysts **8**−**10** and oxidants H_2O_2, CH_3CO_3H, PhIO, and $KHSO_5$ (Oxone). The combination of **8** and $KHSO_5$ was found to be most effective so these conditions were adopted for the following studies.

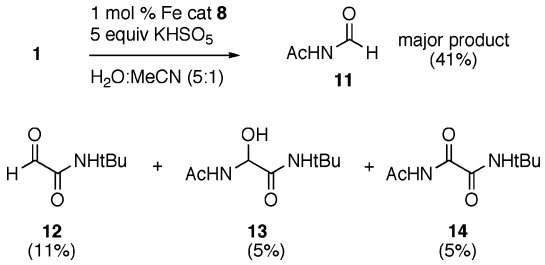
Results with the glycine substrate **1** prove that an iron-based oxidant can attack the amino acid backbone and lead to its scission. Treatment of **1** with 1 mol % of the Fe^{II} catalyst **8** and 5 equiv of $KHSO_5$ in a mixture of H_2O/MeCN (5:1) resulted in formation of aldehyde **11** as the major product (41%), along with recovered **1** (17%), aldehyde **12** (11%), the α-hydroxyglycine derivative **13** (5%), and glyoxamide **14** (5%) as lesser components (Scheme 1). Importantly, no reaction occurred between **1** and $KHSO_5$ in the absence of iron catalyst **8**, nor when using $Fe(ClO_4)_2$ or Fe^{II}(EDTA) as the catalyst, which indicates that the ligand N4Py, which produces a well-characterized Fe^{IV}═O species, is required. Also important was that similar product mixtures were formed in the reactions of **1** under aerobic and anaerobic conditions, which excludes autoxidation by atmospheric O_2 acting as the dominant reaction pathway.



1) Ac-Gly-NHtBu
2) Ac-Ala-NHtBu
3) Ac-Val-NHtBu
4) Ac-Phe-NHtBu
5) Ac-Tyr-NHtBu
6) Ac-Trp-NHtBu
7) Ac-Met-NHtBu

Figure 1. Model amino acid substrates for iron-catalyzed oxidation reactions.

Scheme 1. Oxidation of the Amino Acid Backbone in **1** by $KHSO_5$ and 1 mol % of **8**.



Because products **11**−**14** are characteristic of a peptide cleavage pathway that involves alkoxyradical intermediates,[2,13] more support was sought for the hypothesis that an Fe^{IV}═O species attacks the backbone. Therefore, a green solution of the iron-based oxidant $[Fe^{IV}(O)(N4Py)]^{2+}$ was generated,[8,9] then treated with 10 equiv of substrate **1** ([Fe] = 1 mM, 1:1 H_2O/MeCN) and the reaction was monitored by UV−vis spectroscopy. Decay of absorbance at 680 nm fit well to a single-exponential equation, furnishing a pseudo-first-order rate constant of $5.8(2) \times 10^{-5}$ s^{-1} (Supporting Information). Decay of $[Fe^{IV}(O)(N4Py)]^{2+}$ under the same conditions was slower with the 2,2-d_2 isotopomer of **1** (96% D), giving a kinetic isotope effect of 4.8, which is consistent with the Fe^{IV}═O species cleaving an α-CH bond of **1**. An inverse deuterium kinetic isotope effect of 0.95 was observed for the reaction of **1** in D_2O/CD_3CN solvent, which rules out the possibility that oxidation of **1** is initiated by NH abstraction. Taken together, these observations are most consistent with an Fe^{IV}═O attacking the glycine backbone and initiating a radical chain process carried by $KHSO_5$ that leads to formation of **11**−**14** in the catalytic reaction of Scheme 1.

The same backbone attack that occurred with **1** was not observed with alanine and valine substrates **2** and **3**. These aliphatic amino acids do not react under the same catalytic conditions used with **1**, nor do they promote decomposition of the Fe^{IV}═O species faster than the control experiment without substrate added. On the basis of first principles, **2** and **3** would be expected to react faster because the α-position is a tertiary carbon. However, these results are consistent with the observations of others,[14] that H-atom abstraction

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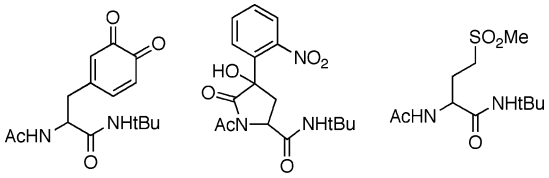

15 16 17

Figure 2. Major oxidation products from the reactions of substrates **4**, **6**, and **7** with Fe catalyst **8** and $KHSO_5$.

is slower with substituted amino acids because their carbon-centered radicals, which should enjoy greater stablization, are actually destabilized relative to the glycine radical because of nonbonding interactions that disrupt captodative stablization. It is worth noting that the reactivity of the Fe^{IV}═O species is also quite different from organic oxidants like dioxiranes that are known to hydroxylate the β-position of valine residues.[15]

With amino acid substrate **4**, side-chain oxidation was observed (Figure 2). Treatment of **4** with 10 mol % of **8** and 3 equiv of $KHSO_5$ produced orthoquinone **15** as the major oxidation product (17%). Formation of **15** from **4** involves three consecutive oxidations, and a logical progression would involve formation of a tyrosine derivative **5**, then a catechol DOPA derivative, then oxidation to form **15**. However, treatment of **5** under the same conditions as with **4** does not lead to formation of **15**, but instead leads to decomposition. Therefore, formation of **15** from **4** may involve a metastable iron-bound phenolate as an intermediate.[16] Analogous to our observations in the oxidation of **1**, control experiments showed that oxidation of neither **4** nor **5** occurred with $KHSO_5$ in the absence of catalyst **8**, nor when using $Fe(ClO_4)_2$ or Fe^{II}(EDTA) as the catalyst.

Oxidation of the amino acid side chains was also observed with substrates **6** and **7**. However, control experiments concluded that these substrates react with $KHSO_5$ in the absence of catalyst **8**. With tryptophan substrate **6**, oxidation with 1 mol % of **8** and 3 equiv of $KHSO_5$ led to a complex mixture of products. The major product formed, which was also unique to the iron-catalyzed reaction, was determined to the acyl lactam derivative **16** (18%) where oxidation of the aniline to a nitrobenzene had occurred. Treating the methionine substrate **7** with 1 mol % of catalyst **8** and 2 equiv of $KHSO_5$ led to formation of the sulfone **17** (97%), which is the same product obtained without **8**. Seeing that **6** and **7** react with $KHSO_5$ alone, evidence was gathered later on that these substrates will react with the iron-based oxidant $[Fe^{IV}(O)(N4Py)]^{2+}$ as well.

After identifying the major products from the oxidation reactions, the relative rates of reaction for substrates **1**−**7** were established. Reactions were performed under pseudo-first-order conditions in a 1:1 mixture of H_2O/MeCN to obtain full solubility of all substrates. These experiments established that the tyrosine substrate **5** causes decomposition of the Fe^{IV}═O species most rapidly out of all of the substrates, followed by **6** and then **7**. The glycine derivative **1** was of intermediate reactivity. Phenylalanine substrate **4** was significantly less reactive than **1**.[17] From reactions with **6** and **7**, the decrease of absorbance at 680 nm was fit satisfactorily to furnish pseudo-first-order rate constants (Table 1). The rate

Table 1. Pseudo-First-Order and Relative Rate Constants for the Decomposition of $[Fe^{IV}(O)(N4Py)]^{2+}$ ([Fe] = 1 mM in 1:1 H_2O/MeCN) by Amino Acid Substrates **1** and **4**−**7** (10 equiv) at 25 °C

substrate	k_{obs} (s^{-1})	k_{rel}
5	n.d.[a]	>293
6	$17.0(5) \times 10^{-3}$	293
7	$3.15(8) \times 10^{-3}$	54
1	$5.8(2) \times 10^{-5}$	1
4	n.d.[a]	<1

[a] n.d. = not determined.

constant measured for **7** agrees well with the previous observations in reactions of the $[Fe^{IV}(O)(N4Py)]^{2+}$ with aromatic sulfides.[8]

In conclusion, we have established that an iron-based oxidant can facilitate oxidative cleavage of the amino acid backbone and modification of side chains. Studies are now underway in our laboratory to characterize the chemoselectivty and order of reactivity of iron-based oxidants with all twenty natural amino acids, in addition to studies that will define the mechanism of these reactions. Furthermore, we anticipate that iron-based oxidants, like that derived from **8**, can be applied to the site-specific modification and/or cleavage of peptides and proteins, akin to extensive investigations regarding the cleavage of nucleic acids by bleomycin and its model complexes.

Acknowledgment. We thank Wayne State University for their generous financial support of this research and Melody Kelley for assistance with preparing substrates for these studies.

Supporting Information Available: Experimental procedures for preparation of **1**−**7**, **11**−**17**, including characterization data, UV−vis spectra and kinetic fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA075075I