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Reactivity of Ferrate(VI) and Ferrate(V) with Amino Acids†

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The oxidation of the essential amino acids by ferrate(VI) (Fe(VI)) and ferrate(V) (Fe(V)) has been studied by stopped-flow and pulse radiolysis techniques at pH 12.4 and 23–24 °C. FeO_4^{3-} was formed in these studies by reduction of Fe(VI) with radiation-generated reducing radicals. Both Fe(VI) and Fe(V) react preferentially with amino acids in which the α-amino group is protonated ($RCH(NH_3^+)COO^-$). Rate constants (k_5) for reactions of Fe(VI) with $RCH(NH_3^+)COO^-$ range from 10 to 10^3 M^{-1} s^{-1}. The corresponding k_6 values for Fe(V) are orders of magnitude higher: $k_6 = (0.01\text{–}5.0) \times 10^7$ M^{-1} s^{-1}. Cysteine, a reducing species, reacts with Fe(VI) at a rate of $k = 760 \pm 49$ M^{-1} s^{-1} and with FeO_4^{3-} at a nearly diffusion-controlled rate of $k = (4.0 \pm 0.8) \times 10^9$ M^{-1} s^{-1}; both rates were computed on the basis of $[\text{cysteine}]_{tot}$. The ratio k_6/k_5 varies from 5×10^3 to 3×10^5; for cysteine this ratio is 5×10^6. The oxidation process of amino acids initiated by Fe(V) proceeds in presence of Fe(VI) by a chain reaction in which amino acid free radicals and Fe(V) are the chain carriers.

Introduction

Elucidation of the aqueous chemistry of the hypervalent oxidation states of iron is of interest because of their role in biological and industrial catalytic processes. While significant progress has been made in the elucidation of the properties of the ferryl ion ($Fe{=}O^{2+}$) as an active center in enzymes, oxygen carriers, porphyrins, and complexes soluble in nonaqueous solvents, research on ferrate(IV), ferrate(V) and ferrate(VI) and their protonated forms has been sporadic.[1-19] A reason for this may be that with the exception of the Fe(VI) ion, which is stable in alkaline solutions, all other hypervalent oxidation states are relatively short-lived and hence require for their study fast kinetic techniques, such as pulse radiolysis (pr), premix pulse radiolysis (ppr), or a combination of the stopped-flow technique with pulse radiolysis (sf-pr).

While the overall objective of this project is the study of the reaction mechanisms by which the various hypervalent iron species oxidize organic and inorganic compounds of biological and industrial interest, the more immediate goal is to obtain clues which would help solve a problem that has perplexed researchers in the field of superoxide chemistry for some time. The issue is whether the ferryl/perferryl ion, or some other strong oxidizing iron–peroxide complex, is formed in the superoxide-driven Fenton reaction near neutral pH:[20,21]

$$HO_2 + O_2^- + H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

$$Fe(III) + O_2^- \rightarrow Fe(II) + O_2 \quad (2)$$

$$F(II) + H_2O_2 \rightarrow F(III) + OH^\bullet + OH^- \quad (3)$$

$$Fe(II)/Fe(III) + H_2O_2/HO_2^-/OH \rightarrow \text{Fe(hypervalent)? or Fe(peroxy complex)?} \quad (4)$$

Earlier evidence for postulating the formation of a powerful oxidizing species of iron in reaction 4 was based mainly on observations from in vitro experiments in which biological damage, due to exposure to a flux of O_2^- radicals, was only partially supressed by OH radical scavengers, superoxide dismutases which very efficiently destroy O_2^-, catalase which destroys H_2O_2, and/or a combination of some or all of these protective systems.[22] Since O_2^-/HO_2 radicals are themselves not very reactive,[23-25] these deleterious effects have been ascribed to the catalytic effect of trace amounts of transition metals (Fe, Cu, Mn, etc.).[21,26]

Some more recent reports[27-29] have shown that reaction of hydrogen peroxide with ferrous polyamino carboxylate complexes at neutral pH yields an oxidizing species that does not behave like the typical OH radical; e.g., the unknown species does not react with some well-known OH radical scavengers such as *tert*-butyl alcohol, while when it does react, the rate constants are different from well-established values for the OH radical.[20,27-30] Hence, it was concluded that the unknown oxidizing species is either some iron–peroxide complex or a ferryl species.[21,22,26-29]

In the present investigation, Fe(V) and Fe(VI) serve as model compounds for ferryl and perferryl complexes. Amino acids were chosen as substrates because (a) as building blocks of proteins,

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† Abbreviations used: AA, amino acid; $RCH(NH_3^+)COO^-$, protonated amino acid; AA• amino acid radical; Ala, A, alanine; Arg, R, arginine; Asn, N, asparagine; Asp, D, aspartic acid; Cys, C, cysteine; $(Cys)_2$, cystine; Glu, E, glutamic acid; Gln, Q, glutamine; Gly, G, glycine; His, H, histidine; Ile, I, isoleucine; Leu, L, leucine; Lys, K, lysine; Met, M, methionine; Phe, F, phenylalanine; Pro, P, proline; Ser, S, serine; Thr, T, threonine; Trp, W, tryptophan; Tyr, Y, tyrosine; Val, V, valine.

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Table I. Reactivity of $RCH(NH_3^+)COO^-$ with Fe(VI) and Fe(V) Determined under Anaerobic Conditions in 0.1 M Phosphate (pH 12.4, 23–24 °C)[a]

amino acid	pK_a	$\log (K/K_o) = \sigma$	$10^{-1}k_5$, M^{-1} s^{-1}	$10^{-6}k_6$, M^{-1} s^{-1}	$10^{-4}k_6/k_5$
Ala (A)	9.87	−0.090	3.1 ● 0.4	3.1 ± 0.20	9.1
Arg (R)	9.04	0.740	25.2 ± 2.3	19.8 ± 0.30	7.9
Asn (N)	8.72	1.060	43.1 ± 10.0	36.4 ± 0.30	8.4
Asp (D)	9.82	−0.041	3.8 ± 0.8	2.6 ± 0.10	6.8
Glu (E)	9.47	0.310	17.0 ± 0.8	4.8 ± 0.30	2.8
Gln (Q)	9.13	0.650	67.0 ± 7.4	15.2 ± 0.30	2.3
Gly (G)	9.78	0.000	9.7 ± 0.3	8.4 ± 0.60	8.7
His (H)	9.18	0.600	15.0 ± 1.7	22.2 ± 0.10	1.5
Ile (I)	9.76	0.020	4.8 ± 0.9	2.8 ± 0.10	5.8
Leu (L)	9.74	0.040	3.2 ± 0.4	3.0 ± 0.20	9.4
Lys (K)	8.95	0.825	110.0 ± 20.0	27.5 ± 0.90	2.5
Met (M)	9.28	0.500	38.1 ± 2.6	14.4 ± 0.80	3.8
Phe (F)	9.31	0.470	9.6 ± 0.3	9.5 ± 0.40	9.9
Pro (P)	10.60	−0.820	1.1 ± 0.1	0.1 ± 0.01	0.9
Ser (S)	9.21	0.570	14.0 ± 4.8	37.2 ± 2.80	26.6
Thr (T)	9.10	0.679	14.0 ± 2.0	46.8 ± 3.20	33.4
Trp (W)	9.39	0.389	25.5 ± 2.0	9.3 ± 0.40	3.6
Tyr (Y)	9.11	0.670	150.4 ± 21.7	8.1 ± 0.20	0.5
Val (V)	9.74	0.040	4.6 ± 0.5	2.9 ± 0.20	6.3

[a] Concentration ranges of amino acids used in studies with Fe(VI): $[Trp]_0$, $[Tyr]_0$ = 0.05–0.2 M; $[Ala]_0$, $[(Cys)_2]_0$, $[Glu]_0$, $[Gly]_0$, $[Lys]_0$, $[Met]_0$, $[Phe]_0$, $[Ser]_0$, $[Thr]_0$, $[Val]_0$ = 0.1–0.4 M; $[Arg]_0$, $[Asp]_0$, $[Asn]_0$, $[Gln]_0$, $[His]_0$, $[Ile]_0$, $[Leu]_0$, $[Pro]_0$ = 0.2–0.4 M. Concentration ranges of amino acids used in studies with Fe(V): $[Ala]_0$ = 2–10 mM; $[Arg]_0$, $[(Cys)_2]_0$, $[Gln]_0$, $[His]_0$, $[Ile]_0$, $[Leu]_0$, $[Lys]_0$, $[Met]_0$, $[Thr]_0$ = 5–20 mM; $[Asn]_0$ = 3–15 mM; $[Asp]_0$, $[Glu]_0$, $[Pro]_0$, $[Val]_0$ = 2.5–15 mM; $[Gly]_0$ = 2.5–17.75 mM; $[Phe]_0$ = 10.9–54.5 mM; $[Ser]_0$ = 5–50 mM; $[Trp]_0$ = 6.25–33.12 mM; $[Tyr]_0$ = 2.5–18.85 mM.

they constitute a very large fraction of the total mass in living cells, (b) they are known to complex transition metals which could become site-specific targets for the Fenton reaction, (c) they do not react with either superoxide radicals[24] or hydrogen peroxide, and (d) their chemistry with the OH radical is relatively well established.[31] It has been shown, that while both ferrate(V) and ferrate(VI) oxidize amino acids, Fe(V) is the more powerful oxidant, and its reactivity exceeds that of Fe(VI) by many orders of magnitude. Although, to the best of our knowledge, formation of the perferryl species in biological systems is yet to be discovered or proven unequivocally (nitridoiron(V) porphyrin transients have been reported recently),[32] studies of the reactivity of isolated hypervalent iron species may illuminate the role of iron in catalytic processes.

Materials and Methods

Materials. All chemicals purchased (Sigma, Aldrich) were of reagent grade or better and were used without further purification. Nitrogen and nitrous oxide used for purging/saturation of solutions were of UHP grade (MG Gases Ltd.). Potassium ferrate (K_2FeO_4) of high purity (98% plus) was prepared by the method of Thompson et al.[33] A molar absorption coefficient ϵ_{510nm} = 1150 M^{-1} cm^{-1} was used for the calculation of $[FeO_4^{2-}]$ in the pH range between 10.0 and 12.4.[16]

Solutions were prepared with water that had been distilled and then passed through a Millipore ultrapurification system. All solutions contained phosphate, which served not only as a buffer but also as a complexing agent for Fe(III), which otherwise precipitates rapidly as a hydroxide that not only interferes with the optical monitoring of the reaction but also accelerates the spontaneous decomposition of Fe(VI). Anaerobic solutions of amino acids (see footnote *a* of Table I for concentration ranges of amino acids) in 0.2 M Na_3PO_4, adjusted to pH 12.4 with NaOH, were prepared in a glass apparatus in which they were purged of oxygen with either N_2 or N_2O and transferred to airtight stopped-flow syringes. The Fe(VI) solutions (80–150 μM) were prepared by addition of solid samples of K_2FeO_4 to deoxygenated 0.005 M Na_2HPO_4/0.001M borate, pH 9.0, where they are most stable.

Methods. Stopped-flow experiments were carried out with a Durrum Model D-110 stopped-flow apparatus interfaced to a data acquisition and analysis system designed by On-Line Instruments System (Olis), Inc. A series of experiments was routinely initiated with the measurement of the spontaneous decay of Fe(VI) under the given conditions, the rate of which was used as a correction factor for runs in which Fe(VI) was mixed with a given amino acid. The reaction was monitored at 510 nm, the absorption maximum of Fe(VI). All experiments were carried out under pseudo-first-order conditions at 23–24 °C. Routine spectral measurements were taken on a Cary Model 210 instrument. All rate constants (k_5), corrected where required for the spontaneous decay of Fe(VI) under similar conditions, represent averages of nine experimental runs per $[RCH(NH_3^+)COO^-]$ concentration; most amino acids were studied over an approximately 4-fold concentration range (see footnote *a* of Table I).

Premix Pulse Radiolysis. Although the amino acids react with Fe(VI) at very moderate rates (with the exception of cysteine), the rates are too fast for routine pulse radiolysis studies, in which solutions are stored for a longer time period in a reservoir from which the pulsing cell is automatically filled. Hence, a gravity premixing apparatus was used in which two solutions are mixed in a jet-mixer before flowing into the pulsing cell. The dead time of this setup is approximately 5–7 s, a time sufficiently short if the experiments are carried out in the range between pH 10.0 and 12.4. In order to be able to compare the chemistry of Fe(V) with that of Fe(VI), most of the studies were carried out at pH 12.4, where the rate of reaction of Fe(VI) with the amino acids is the lowest. A new apparatus under construction will allow us to carry out similar experiments down to pH 6. The pulse radiolysis experiments were carried out using the BNL 2-MeV Van de Graaff accelerator. Pulse lengths of 100–850 ns with doses ranging from 0.2 to 3.0 krad were used. Dosimetry was carried out using KSCN assuming $G((SCN)_2^-)$ = 6.13 and ϵ_{472nm} = 7950 M^{-1} cm^{-1}. The optical path length was either 2 or 6.1 cm.

Steady-State Studies. The chain oxidation of amino acids by Fe(V) in the presence of excess Fe(VI) was studied under steady-state conditions using ^{60}Co γ rays. Two ^{60}Co sources were used with a γ-ray flux equivalent to 0.74 and 0.108 krad/min, respectively. The irradiations and optical measurements of Fe(VI) disappearance were carried out at 23–24 °C.

Results

Reactivity of Fe(VI) with Amino Acids. The rates of reaction of Fe(VI) with the essential amino acids studied at pH 12.4 by the stopped-flow technique are listed in Table I. Studies of the pH effect on the oxidation rate of several amino acids showed a marked increase in reactivity with increasing acidity (slope ≈ −1) between pH 12.4 and 10.0. A typical example, shown in Figure 1 for glycine, illustrates the change in the observed first-order rate constant with pH, for which the slope is −1.12. Similar results were obtained for alanine (slope ≈ −0.91), lysine (slope ≈ −0.94), phenylalanine (slope ≈ −0.96), and serine (slope ≈ −0.88). Taking into consideration that all amino acids, except proline, have as a common denominator a free carboxyl group ($pK_a \approx 2$) and a free unsubstituted amino group on the α-carbon atom ($pK_a \approx$

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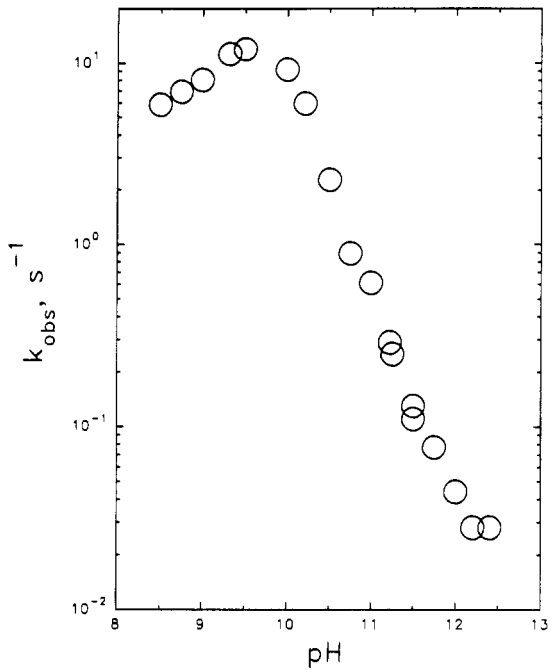

Figure 1. k_{obs} as a function of pH for the reaction of 0.1 M glycine with 37.5–60 μM Fe(VI) in N_2-saturated 0.1 M phosphate/0.001 M borate at 24 °C.

8.7–10.5), it is assumed, in view of the 10-fold increase in rate per pH unit, that it is the protonated α-amino acid group ($RCH(NH_3^+)COO^- \rightleftharpoons RCH(NH_2)COO^- + H^+$) that is responsible for the observed pH effect in the range where the slope is −1.0. Denoting the radical derived from $RCH(NH_3^+)COO^-$ as AA•, we can illustrate the Fe(VI) reaction as follows:

$$Fe(VI) + RCH(NH_3^+)COO^- \rightarrow Fe(V) + AA^\bullet \quad (5)$$

Reaction 5 is defined by $-d[Fe(VI)]/dt = k_5[Fe(VI)][RCH(NH_3^+)COO^-]$, where k_5 is obtained by dividing the measured first-order rate constant by $[RCH(NH_3^+)COO^-]$, which is evaluated from $[AA]_{tot}$ in a given experiment and the pK_a values listed in Table I.

Reactivity of Fe(V) with Amino Acids. Studies of k_{obs} as a function of pH (10.0–12.4) for glycine, yielded a slope of −1.0, which suggests that, similar to Fe(VI), Fe(V) reacts faster with increasing acidity because the net charge on the substrate molecule is either neutral or negative in this pH range:

$$Fe(V) + RCH(NH_3^+)COO^- \rightarrow Fe(IV) + AA^\bullet \quad (6)$$

As Fe(V) reacts with $RCH(NH_3^+)COO^-$ approximately 10^3–10^5 times faster than Fe(VI) (see Table I, column 6), the kinetic measurements had to be carried out by the premix–pulse radiolysis technique. Fe(VI) solutions (0.005 M phosphate/0.001 M borate buffer, pH 9.0) were mixed in a jet-mixer with amino acid solutions of variable concentrations (see footnote *a* of Table I) containing 0.2 M phosphate buffer, pH 12.4, and 2 M ethanol. Both solutions were saturated with nitrous oxide; $[N_2O]$ = 0.026 M. The 1:1 mixture of the two solutions was pulse-irradiated within 5–7 s. The sequence of reactions, following the electron pulse (eq I), that lead to Fe(V) formation are shown in eqs 7–11. The numbers

$$H_2O \rightsquigarrow H(0.55), e_{aq}(2.65), OH(2.75), H_3O^+(2.65), H_2O_2(0.72), H_2(0.45)^{34} \quad (I)$$

$$N_2O + e_{aq}^- + H_2O \rightarrow OH + OH^- + N_2 \quad (7)$$

$$H + OH^- \rightleftharpoons e_{aq}^- + H_2O \qquad pK_a = 9.6^{34} \quad (8)$$

$$OH + CH_3CH_2OH \rightarrow CH_3\dot{C}HOH + H_2O \quad (9)$$

$$CH_3\dot{C}HOH + Fe(VI) \rightarrow CH_3CHO + Fe(V) + H^+ \quad (10)$$

$$CH_3CH_2OH + Fe(V) \rightarrow Fe(IV) + \text{radical} + H^+ \quad (11)$$

in parentheses in eq I are *G* values, that is the number of radicals formed per 100 eV of energy dissipated in the aqueous solution. The formation ($k_{10} = 8 \times 10^9$ M^{-1} s^{-1}) and decay ($k_{11} = 9.8 \pm 0.7$ M^{-1} s^{-1}) of Fe(V) were monitored in presence of 1 M EtOH at its absorption maximum, $\epsilon_{380nm} = 1475$ M^{-1} cm^{-1}.[16] The k_6 values for the reaction of Fe(V) with the various amino acids listed in Table I were measured under first-order conditions by varying $[Fe(V)]_0$ from 2 to 10 μM at a given $[RCH(NH_3^+)COO^-]_0$. A direct proportionality of the first-order disappearance of Fe(V) (corrected for its decay in the presence of 1 M EtOH) to the $[RCH(NH_3^+)COO^-]_0$ values was observed in all cases. A comparison of the total amount of Fe(VI) consumed per reducing radical generated in the pulse suggested that a chain reaction may be operational in these systems. As the chain propagation step involves most likely the reduction of Fe(VI) by a substrate radical, e.g. Gly• + Fe(VI), appropriate experiments were carried out to verify such a step. Using the ppr method, the reaction was monitored by observing the disappearance of Fe(VI) at 510 and 600 nm and formation of Fe(V) at 380 nm (N_2O-saturated 0.1 M glycine solutions adjusted to pH 12.4 with NaOH, 60.0–155.0 μM Fe(VI), conditions under which the Gly• radical was generated by the OH radical). The rates of disappearance of Fe(VI) and appearance of Fe(V) were similar within experimental error; $k(Fe(VI) + Gly^\bullet)_{obs} = (1.42 \pm 0.22) \times 10^9$ M^{-1} s^{-1}. In the absence of Fe(VI), the observed rates of formation and decay, as well as the spectral properties of the glycyl radical, are within experimental error consistent with earlier published results at pH 13.2.[35] Also, since in most of the experiments, Fe(V) was generated by reduction of Fe(VI) with the $CH_3\dot{C}HOH$ radical, it became important to establish if the latter also reacts with the amino acids. Pulse experiments using N_2O-saturated 1.5 M ethanol solutions, pH 12.4, containing 0.1–0.5 M glycine gave negative results.

Chain Oxidation of Amino Acids by Fe(V). Chain reactions are studied more conveniently under conditions where the free radicals ($CH_3\dot{C}HOH$ or AA•) and Fe(V) are at very low steady-state concentrations. Two ^{60}Co γ-ray sources were used in the irradiation of 1–5 mM glycine solutions (0.1 M phosphate, N_2O saturated, pH 12.4, 24 °C) containing 188–330 μM Fe(VI). One source, with a reducing radical flux of 7.5×10^{-2} μM/s (assuming $G(Gly^\bullet) = 6.1$), gave chain lengths for the disappearance of Fe(VI) from 2.7 to 6.0; a weaker ^{60}Co source, with a flux of 1.09×10^{-2} μM/s of Gly•, yielded chain lengths from 13.5 to 17.0.

Oxidation of Cysteine and Cystine by Fe(VI) and Fe(V). An exception to the overall behavior of the amino acids studied is that of cysteine and cystine. Cysteine is a reducing agent ($E° = 0.080$ V)[36] and undergoes oxidation at the –SH group ($pK_a = 8.37$; $pK_a(\alpha\text{-}NH_3^+) = 10.70$).[37] The rate constant for Fe(VI), $k = 760 \pm 49$ M^{-1} s^{-1}, was determined under first-order conditions and computed on the basis of the total concentration of cysteine present at pH 12.4. The corresponding value for the Fe(V) reaction, determined by the ppr method, is nearly diffusion controlled; $k = (4.0 \pm 0.8) \times 10^9$ M^{-1} s^{-1}. Cystine reacts with Fe(VI) at pH 12.4 at a rate of $k = 118 \pm 7$ M^{-1} s^{-1} (based on the p*K* of 10.25 for $\alpha\text{-}NH_3^+$), while the rate for Fe(V) is $k = (1.95 \pm 0.02) \times 10^6$ M^{-1} s^{-1}.

Carr et al.[37] found in an earlier study that the rate of oxidation of glycine by Fe(VI) decreases from 60.6 M^{-1} s^{-1} at pH 8.5 to 46.3 M^{-1} s^{-1} at pH 9.4. As their experimental conditions differ from ours, we can compare the results only at pH 8.5, where the pH effect on the acid–base equilibrium of glycine is relatively negligible. By dividing our first-order k_{obs} (s^{-1}) by the fraction of glycine present in the protonated form, we find that our second-order value $k = 61.8$ M^{-1} s^{-1} compares very favorably with Carr's value of 60.6 M^{-1} s^{-1}.

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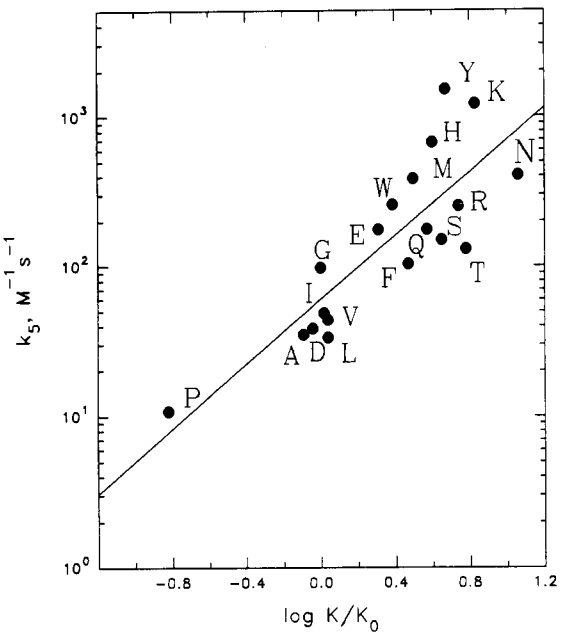

Figure 2. Hammett plot of log k_5 as a function of log $(K/K_0) = \sigma$. The values for k_5 and pK_a and the symbols for the various amino acids are listed in Table I. K_0 is the dissociation constant for glycine (NH_3^+-CH_2COO^-), while K is the corresponding dissociation constant of other amino acids.

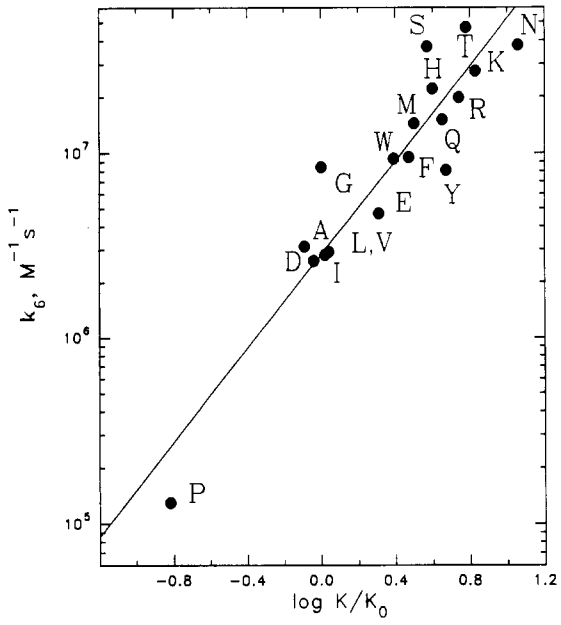

Figure 3. Hammett plot of log k_6 vs log $(K/K_0) = \sigma$. For explanation see caption to Figure 2 and text.

Discussion

While Fe(VI) is a very strong oxidizing agent in acidic solutions ($FeO_4^{2-} + 8H^+ + 3e^- \rightarrow Fe^{3+} + 4H_2O$; $E^\circ = 1.9$ V),[38] in alkaline solutions its reaction with organic compounds is rather sluggish, unless a given compound is a reducing agent.[11,19] Its stability in aqueous solutions, $pK_1 = 3.5$ and $pK_2 = 7.8$, decay kinetics as a function of pH, and reactivity with a number of organic compounds were reported earlier.[11] Overall, $RCH(NH_3^+)COO^-$ species react with Fe(VI) at pH 12.4 at rates that vary from 10 to 1.5×10^3 M^{-1} s^{-1} (Table I). On the basis of Hammett relation[39] log K = log K_0 + $\rho\sigma$, which is an empirical correlation that describes trends in rates and equilibrium constants for a set of similar reactions, the change in k_5 as a function of σ is shown in Figure 2. The plot was obtained by setting ρ arbitrarily equal to unity; thus σ = log (K_i/K_{i0}), where K_{i0} is the ionization constant for the unsubstituted amino acid (glycine, $CH_2(NH_3^+)COO^-$) and K_i the corresponding dissociation constant of amino acids in which the α-H atom is replaced by different R groups.

The effect of the R groups on the reactivity with Fe(VI) is shown by the following trend: aromatic (Phe, Trp, Tyr; $k_5 \approx (0.01\text{–}1.5) \times 10^3$ M^{-1} s^{-1}) ≥ diamino dicarboxylic (Lys, Arg, His; $k_5 \approx (0.15\text{–}1.2) \times 10^3$ M^{-1} s^{-1}) ≥ carboxamide (Asn, Gln; $k_5 \approx (4.3\text{–}6.7) \times 10^2$ M^{-1} s^{-1}) > thioether (Met; $k_5 = 3.8 \times 10^2$ M^{-1} s^{-1}) > hydroxy (Ser, Thr; $k_5 \approx 1.5 \times 10^2$ M^{-1} s^{-1}) ≥ monoamino dicarboxylic (Asp, Glu; $k_5 \approx (0.4\text{–}1.7) \times 10^2$ M^{-1} s^{-1}) ≥ aliphatic (Ala, Gly, Ile, Leu, Val; $k_5 \approx (0.3\text{–}1.2) \times 10^2$ M^{-1} s^{-1}) > cyclic (Pro; $k_5 \approx 10$ M^{-1} s^{-1}). The observed overlap between groups and the often wide spread within a group suggest that many factors (steric, inductive, electrostatic, etc.) contribute to the observed trend.

The spectral and kinetic properties of Fe(V) that are pertinent to this report were published earlier:[17] $pK_2 = 7.5$, $pK_3 = 10.10$; $\epsilon_{380nm}^{max} = 1475$ M^{-1} cm^{-1} at pH 12.4; $FeO_4^{3-}/HFeO_4^{2-}$ disappears spontaneously by second-order kinetics in a pH-dependent fashion with rates that vary from $k_{obs} = 9 \times 10^7$ M^{-1} s^{-1} at pH 6 to 1×10^7 M^{-1} s^{-1} at pH 12. In strongly alkaline solutions Fe(V) disappears by a unimolecular process, $k_{obs} = 8 \pm 1$ s^{-1}.[17] Some earlier results[16] indicated that Fe(V) is 3–4 orders of magnitude more reactive toward hydrogen peroxide and formate than Fe(VI). A similar behavior has been observed in the present study where the ratio k_6/k_5 varies from 5×10^3 to 3×10^5 (see column 6, Table I). A Hammett plot of k_6 for Fe(V) as a function of σ is shown in Figure 3.

The effect of the R groups on the reactivity $RCH(NH_3^+)COO^-$ with Fe(V) decreases in the following order: hydroxy (Ser, Thr; $k_6 \approx 4 \times 10^7$ M^{-1} s^{-1}) ≈ carboxamide (Asn, Gln; $k_6 \approx 3 \times 10^7$ M^{-1} s^{-1}) ≈ diamino dicarboxylic (Lys, Arg, His; $k_6 \approx 2 \times 10^7$ M^{-1} s^{-1}) ≥ aromatic (Phe, Trp, Tyr; $k_6 \approx 10^7$ M^{-1} s^{-1}) ≈ thioether (Met; $k_6 = 10^7$ M^{-1} s^{-1}) ≥ aliphatic (Ala, Gly, Ile, Leu, Val; $k_6 \approx 5 \times 10^6$ M^{-1} s^{-1}) = monoamino dicarboxylic (Asp, Glu; $k_6 \approx 4 \times 10^6$ M^{-1} s^{-1}) > cyclic (Pro; $k_6 = 10^5$ M^{-1} s^{-1}). Similar to the case of the Fe(VI) reactions, the differences in k_6 are most likely due to inductive and/or steric effects of the various substituted groups on the α-carbon atom.

Although the OH radical induced decarboxylation of amino acids has been studied for some time, the mechanistic details, which are still under debate, lean currently in favor of an attack at the amino nitrogen[37] (previously, oxidation was thought to occur via H atom abstraction at the α-C).[31,40–44] Attack at the amino group is apparently favored in strongly alkaline media, where the amino group is deprotonated. The proposed amino acid decarboxylation mechanism,[31] induced by the OH radical, suggests the formation of a transient radical cation (reaction 12) which subsequently decarboxylates (reaction 13).

$$NH_2CH_2CO_2^- + OH \rightarrow {}^{+\bullet}NH_2CH_2CO_2^- + OH^- \quad (12)$$

$$^{+\bullet}NH_2CH_2CO_2^- \rightarrow NH_2CH_2^\bullet + CO_2 \quad (13)$$

As the pH dependence of the reactions of Fe(VI) and Fe(V) with amino acids shows a trend opposite to that of the OH radical, this could be due to an electrostatic attraction between the net doubly/triply negatively charged iron species and the positively charged α-NH_3^+ group or the easier approach to a net neutrally

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charged $RCH(NH_3^+)COO^-$ substrate molecule. Such course of action could be possible if one assumes that, in addition to several resonance structures, the Fe(V) species also has discrete electronic configurations like $Fe^{V}{=}O \leftrightarrow Fe^{IV}{-}O^{\bullet-}$, which would give it a partial radical character capable of H atom abstraction from the α-C atom or attack on the α-N nitrogen. That the ferryl species is capable of aliphatic H atom abstraction is well documented.[5-9] Formation of carbonyl products from amino acids exposed to the Fenton system reported recently[45] further supports such a reaction pathway. Hence, in summary, the following reactions of amino acids with Fe(VI)/Fe(V) must be taken into consideration: (a) oxidations similar to those of primary and secondary amines by $KMnO_4$, which lead to formation of nitroxides[46] ($RCH_2NH_2 \rightarrow RCH_2NO_2$), (b) oxidations similar to those observed for the OH radical, which yield carbonyl products, and (c) the possibility of an oxidative attack on some R groups. Since the kinetic parameters in this study were obtained from rates of disappearance of Fe(VI)/Fe(V), which do not give any specific clues, conclusions as to the site of attack and specific reaction steps in the overall mechanism would be highly speculative and hence are not discussed at this time.

That the oxidations of $RCH(NH_3^+)COO^-$ by Fe(VI) and Fe(V) proceed under anerobic conditions by a chain reaction has been demonstrated in experiments carried out under steady-state conditions. The increase in chain length with decreasing steady-state concentrations of transients suggests that the propagation step or steps are of second order. Whether the radical AA$^\bullet$ formed in reaction 6 is the same as the one generated by the OH radical in reaction 12 is yet unknown. Also, as the radical derived from a given amino acid most likely has a pK_a value below that of its parent compound,[47] the symbol AA$^\bullet$ is being used until the nature of the species is established. Hence, the initiation and propagation steps of the overall chain reaction can be described by the following reactions:

$$Fe(V) + RCH(NH_3^+)COO^- \rightarrow Fe(IV) + AA^\bullet \quad (6)$$

$$AA^\bullet + Fe(VI) \rightarrow Fe(V) + \text{product(s)} \quad (14)$$

$$Fe(IV) + RCH(NH_3^+)COO^- \rightarrow Fe(III) + AA^\bullet \quad (15)$$

$$Fe(V) + AA^\bullet \rightarrow Fe(IV) + \text{product(s)} \quad (16)$$

$$Fe(IV) + AA^\bullet \rightarrow Fe(III) + \text{product(s)} \quad (17)$$

$$Fe(III) + AA^\bullet \rightarrow Fe(II) + \text{product(s)} \quad (18)$$

That Fe(IV) is formed in reaction 6 can be deduced from the observation of a chain reaction which is propagated in reaction 14 by the free radical AA$^\bullet$. The latter can be formed only in a single-electron oxidation of $RCH(NH_3^+)COO^-$ with the simultaneous formation of Fe(IV) which, although not yet proven, can most likely also propagate the chain by reaction 15. A two-electron oxidation step ($Fe(V) + 2e^- \rightarrow Fe(III)$) would most likely not yield a free radical. Whether Fe(VI) and Fe(V) yield different amino acid radicals "AA$^\bullet$" will be determined from spectral studies with an improved premixing apparatus. The termination of the chain could involve reactions between various iron species and AA$^\bullet$ radicals (reactions 17 and 18) or disproportionation of AA$^\bullet$ which is of the order of 10^8 M^{-1} s^{-1}.[35] Because Fe(III) most likely accumulates as the reaction progresses, its reduction to Fe(II) may have an indirect effect on the propagation of the chain, since Fe(II) reduces Fe(VI) at a rate of $k \approx 10^5$ M^{-1} s^{-1} under these experimental conditions and its reaction with Fe(V) is most likely 1–2 orders of magnitude higher. As the overall chain mechanism is complex, its further study will be confined for the time being to glycine only.

From the linear relationship of the Hammett plot, one can infer that both hypervalent iron species react with these amino acids at the same sites (α-N and/or α-C-H) regardless of the nature of the R group. In contrast, the OH radical, which is known to react in general quite indiscriminately, not only reacts with glycine at the α-N and/or α-C-H but in reaction with tryptophan adds on almost exclusively to the indole moiety.[48-50] Trends such as those shown in the Hammett plots as well as the differences in reactivity with specific sites will be further explored.

Thus, if Fenton reactions conducted in vivo systems indeed generate hypervalent iron species as well as OH radicals, one approach to differentiate between these entities would be to combine product analysis with competition studies. Prerequisites to such a strategy are studies of the reactivity of hypervalent iron species with isolated amino acids, as well as establishment of experimental evidence for the site(s) of attack on the amino acids by the hypervalent iron species; relevant information regarding the OH radical is available in the literature.[31,51] Future studies will be extended to include Fe(IV), as only a comparison of the reactivity of all of the hypervalent iron species under similar conditions can serve to clarify the reactive iron species generated in Fenton chemistry at neutral pH.

Conclusion

The results presented in this paper and in a previous report[16] demonstrate that the oxidation of organic and inorganic compounds by Fe(VI) can be in principle significantly accelerated by addition of an effective reducing agent which converts ferrate(VI) to ferrate(V), as Fe(V) is shown to react 3–5 orders of magnitude faster than Fe(VI). Both hypervalent iron species (Fe(VI)/Fe(V)) react preferentially with the protonated forms of amino acids, and in the absence of dioxygen, the oxidation of organic compounds like amino acids proceeds by chain reactions. Finally, evidence exists to suggest that these hypervalent iron species react with amino acids at either the α-C or the α-N, whereas OH reacts indiscriminately with either atom or the R groups.

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