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THE OXIDATION OF AMINO ACIDS BY FERRATE(V). A PRE-MIX PULSE RADIOLYSIS STUDY

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The forms of ferrate(V) which are derived from the one-electron reduction of potassium ferrate (K_2FeO_4) by ethanol radicals react with representative amino acids (glycine, methionine, phenylalanine and serine) at rates that are greater than $10^5 M^{-1}s^{-1}$ near pH 10. The predominant interaction in the alkaline pH range is between the protonated ferrate(V) species, $HFeO_4^{2-}$, and the amino acid anion.

$$Fe(V) + \text{amino acid} \rightarrow Fe(III) + NH_3 + \alpha\text{-keto acid}$$

The rate-determining process is the two electron reduction of ferrate(V) to iron(III) with oxidation and subsequent deamination of the amino acid. The reaction appears to involve an entry of the amino acid into the inner coordination sphere of ferrate(V). In all cases, ferrate(V) exhibits preferred attack on the amino group in contrast to the OH radical which attacks the thioether site of methionine and the phenyl ring of phenylalanine.

KEY WORDS: ferrate(V), ferrate(VI), amino acids, glycine, methionine, phenylalanine, serine.

INTRODUCTION

Higher oxidation states of iron play a unique role in the function of biochemical oxidants and hydroxylating agents. The activity of iron in its +4 and +5 oxidation states is of course modulated to a considerable extent by the protein environment and porphyrinic ligand systems. In addition, the oxidizing equivalents are ultimately supplied by dioxygen or peroxides which interact in complex ways with the catalytic iron system.[1,2,3] Higher valence states of iron are also frequently implicated in oxidations which are catalyzed by simple iron(II/III) complexes. These may involve the hydroxyl radical (Fenton systems) or possibly iron(IV) and iron(V) intermediates.[3-5] We are currently engaged in attempting to elucidate some of the fundamental characteristics of iron(V) in its simplest state as an oxyanion in solution.

Ferrate(V) is generated by the pulse radiolytic reduction of ferrate(VI), K_2FeO_4, with reducing free radicals.[6] In the pH range 8-14, ferrate(V) decays by relatively slow second-order kinetics involving the interactions of the two forms $HFeO_4^{2-}$ and FeO_4^{3-} and its reactions with biological substrates may be studied.[7-9] Using rapid pre-mix pulse

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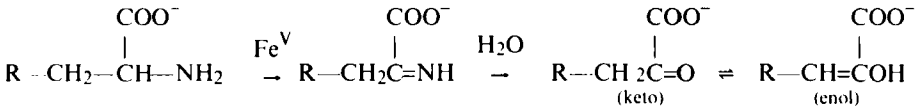
radiolysis, it is possible to measure the rates at which ferrate(V) reacts with substrates which are themselves quite reactive towards the ferrate(VI) precursor. Previously, studies of the reaction between Fe(V) and the amino acids were conducted in alkaline solutions where potassium ferrate and the amino acids react slowly.[8]

A study of the interaction of ferrate(V) with aspartic acid revealed that oxidation caused deamination of the amino acid via an imino acid intermediate ($RCH_2C(=NH)COOOH$).[9]



The hydrolysis of the initial imine oxidation product to oxaloacetic acid was found to be the rate-limiting step in establishing of the keto-enol equilibrium. The high absorptivity of the (enol) form could be detected spectrophotometrically even with the small amounts (≈ 10 μM) of ferrate(V) generated by pulse radiolysis. In the present study we examine the pH dependences of the reactions of ferrate(V) with glycine, methionine, phenylalanine and serine using rapid pre-mix pulse radiolysis. This permits us to make a more detailed analysis of the factors controlling ferrate(V) reactivity with amino acids.

EXPERIMENTAL

Kinetic Studies

Pulse radiolysis experiments were conducted on a 2 MeV van de Graaff accelerator which is computer interfaced with a pre-mixing apparatus consisting of three Hamilton Precision Liquid Dispenser(PDL II) units. The apparatus has a dead time of 100-200 ms and is operated by remote control. Solutions of ferrate(VI) are premixed with amino acids (AA) and pulsed before substantial reaction between them can occur. The solutions were argon saturated and contained ≈ 0.1 M ethanol. Under these conditions, the primary oxidizing radicals are converted into reducing species within a fraction of a microsecond. In Eq.I, the numbers in parentheses represent G-values, that is, the number of molecules/radicals formed per 100 eV of energy dissipated in water.[10]

$$H_2O \rightsquigarrow OH(2.65),\ e_{aq}^-(2.75),\ H(0.65),\ H_2O_2(0.72),\ H_2(0.45) \qquad \text{I}$$

$$OH + CH_3CH_2OH \rightarrow H_2O + CH_3\dot{C}HOH \qquad 1$$

$$H + CH_3CH_2OH \rightarrow H_2O + CH_3\dot{C}HOH \qquad 2$$

Both e^-_{aq} and $CH_3\dot{C}HOH$ react at near diffusion controlled rates ($k_3 = 2.0 \times 10^{10}\ M^{-1}s^{-1}$; $k_4 = 2\times 10^9 M^{-1}s^{-1}$)[6,7] with $FeO_4^{2-}/HFeO_4^-$ yielding ferrate(V) which absorbs in the visible range and in the UV.

$$e^-_{aq} + FeO_4^{2-} \rightarrow FeO_4^{3-} \qquad 3$$

$$CH_3\dot{C}HOH + FeO_4^{2-} \rightarrow CH_3CHO + FeO_4^{3-} + H^+; \quad 4$$

Equilibria (5) and (6) account for the speciation of ferrate(V) in the near neutral and alkaline pH range.[7]

$$H_2HeO_4^- \rightleftharpoons HFeO_4^{2-} + H^+ \quad pK_5 \approx 7.2; 7.5^7 \quad 5$$

$$HFeO_4^{2-} \rightleftharpoons FeO_4^{3-} + H^+ \quad pK_6 \approx 10.1^7 \quad 6$$

In the pH range 8-13, ferrate(V) decay is predominantly second-order via reactions (7) and (8) ($k_7 = 1.5 \times 10^7\ M^{-1}s^{-1}$; $k_8 = 1.0 \times 10^7 M^{-1}$)[7]:

$$HFeO_4^{2-} + HFeO_4^{2-} \rightarrow 2\ Fe(III) + O_2/H_2O_2 \quad 7$$

$$HFeO_4^{2-} + FeO_4^{3-} \rightarrow 2\ Fe(III) + O_2/H_2O_2 \quad 8$$

In the presence of oxidizable substrates such as amino acids, ferrate(V) decay becomes first-order and dependent on the amino acid concentration. Typically, amino acid concentrations were varied in the range of 2-10 mM which provided adequate scavenging of the ferrate(V) without complications from too rapid reaction between ferrate(VI) and the amino acid. The kinetic analysis was performed by an on-line non-linear least squares fitting routine.

Potassium ferrate of high purity was prepared by methods previously described.[11] Molar absorption of 1150 $M^{-1}cm^{-1}$ at 510 nm was used to determine its concentration. The amino acids were purchased from Sigma Chemical Co. and used without further purification. Other chemicals were of reagent grade. The water was doubly-distilled and Milli-Q filtered. The argon blanket gas was of 99.999% purity (Liquid Carbonic Specialty Gas Corp., Chicago).

Product Analysis

Ammonia analysis was carried out with solutions containing 0.02M HCOONa, 0.1M NaOH, $[\text{Amino acid}]_0 = 400\ \mu M$ (glycine, lysine, methionine, phenylalanine, serine), $[K_2FeO_4]_0 \approx 100\ \mu M$, argon saturated. The solutions were irradiated for 2 minutes in a 6.3 Gy/min. ^{60}Co gamma-ray source and compared directly with unirradiated samples. Organic products were determined in solutions similar to those in which the reactivity of Fe(V) with amino acids was studied by pre-mix pulse radiolysis. Products were studied only of those amino acids which undergo keto-enolization. The enol forms absorb strongly near 260 nm and are formed at pH ≥ 12. The rate of enolization of oxaloacetate was monitored for 20 seconds after the oxidation of aspartate by Fe(V).[9] Similarly pyruvate and phenylpyruvate were determined to be products of alanine and phenylalanine oxidation respectively. These latter products were also confirmed by HPLC. Overall, total yields of α-keto acids and ammonia were consistent with their being exclusive products.

RESULTS

In the presence of glycine, ferrate(V) decays rapidly by a process which is first order

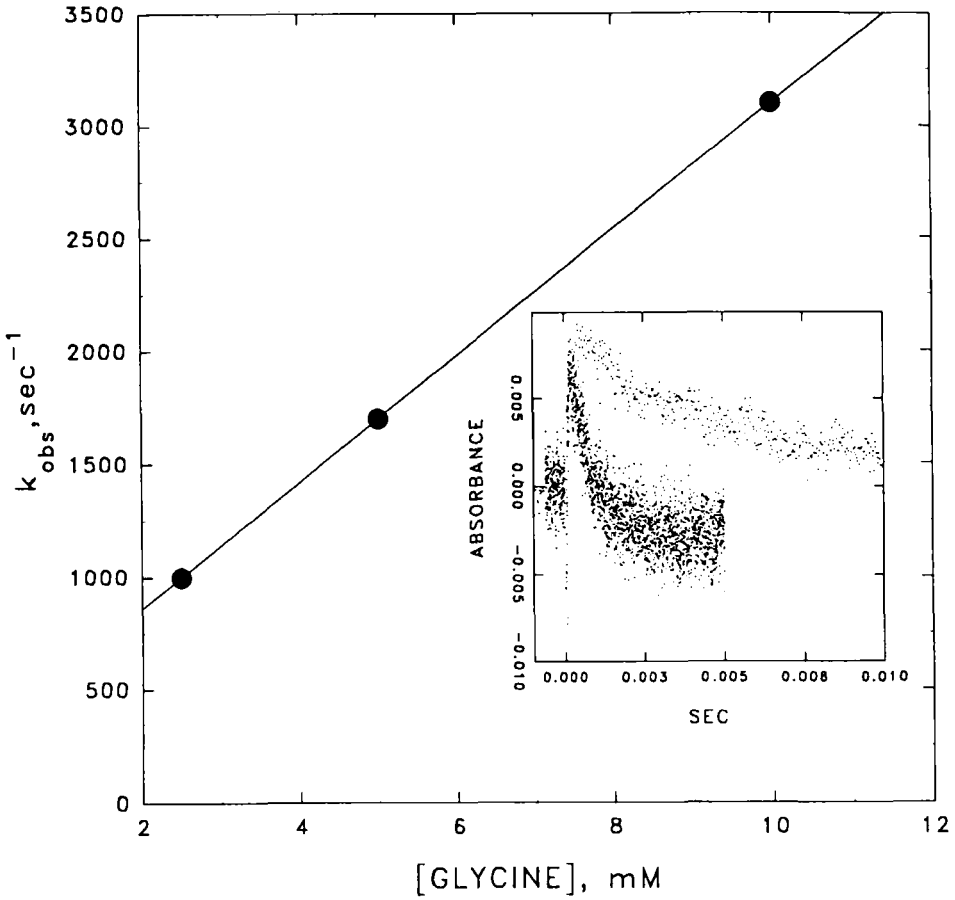


FIGURE 1 Observed rate constants for the disappearance of ferrate(V) at pH 8.8 in 0.025 M phosphate and ≈ 23°C as a function of $[Glycine]_0$. Inset: The two traces recorded at 380 nm are examples of the slow second-order spontaneous disappearance of $HFeO_4^-$ and its fast first-order reaction with 2.5 mM glycine.

and much faster than the spontaneous decay rate. A representative plot of values of k_{obs} *vs* $[glycine]_0$ is shown in Figure1. The inset shows the kinetic traces of ferrate(V) decay in the presence and absence of an amino acid. It is noted that the OD_∞ of the ferrate(V)/amino acid reaction is the same as that of its spontaneous decay. This shows that reaction between ferrate(V) and amino acids is stoichiometric i.e. there is no chain decomposition of ferrate(VI) arising from the intermediate production of free radicals. Since the glycine radical reduces ferrate(VI) to ferrate(V) at a rapid rate ($k = 1.4 \times 10^9 M^{-1}s^{-1}$)[9], the latter would be detected spectrophotometrically in the catalytic degradation of Fe(VI) *via* the hypothetical reactions (9) and (10):

$$Fe^{V} + AA \rightarrow Fe^{IV} + AA^{\cdot} \qquad \text{(hypothetical)} \qquad 9$$

$$Fe^{VI} + AA^{\cdot} \rightarrow Fe^{V} + AA(\text{oxidized}) \qquad \text{(hypothetical)} \qquad 10$$

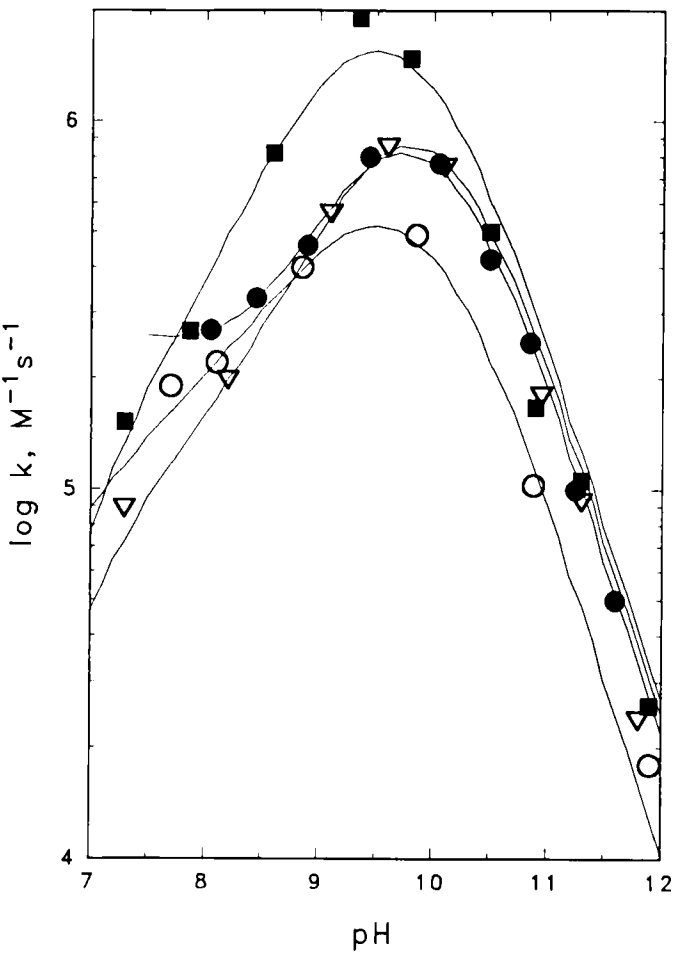


FIGURE 2 The pH dependences of the second order rate constants for reactions of ferrate(V) with glycine (▽), methionine (●), phenylalanine (o) and serine (■). The fitted curves are obtained using the rate constants given in Table 1.

The absence of such a chain suggests that reaction (11) proceeds *via* a concerted two-electron oxidation which converts Fe^{V} to Fe^{III} in a single step without intermediate production of free radicals or iron(IV):

$$Fe(V) + \text{amino acid} \rightarrow Fe(III) + NH_3 + \alpha\text{–keto acid} \qquad 11$$

The pH dependence of the reaction rates exhibits in all cases a bell shape with a maximum between pH 9 and 10 which coincides with the protonation equilibria of both ferrate(V) and of the amino acids. The data in Figure2 show the observed rate constants k_{11} as a function of pH for the reaction of ferrate(V) with glycine, methionine, phenylalanine and serine. Reaction 11 must be resolved into potential contributions from reactions (12)-(16):

$$FeO_4^{3-} + RCH(NH_2)CO_2^- \rightarrow Fe(III) + \text{products} \quad 12$$

$$HFeO_4^{2-} + RCH(NH_2^+)CO_2^- \rightarrow \text{"} \quad \text{"} \quad 13$$

$$HFeO_4^{2-} + RCH(NH_2)CO_2^- \rightarrow \text{"} \quad \text{"} \quad 14$$

$$FeO_4^{3-} + RCH(NH_3^+)CO_2^- \rightarrow \text{"} \quad \text{"} \quad 15$$

$$H_2FeO_4^- + RCH(NH_3^+)CO_2^- \rightarrow \text{"} \quad \text{"} \quad 16$$

It should be noted that reactions (14) and (15) create a proton ambiguity in the overall rate expression that arises from reactions (12)-(16) and the relevant proton equilibria. However Reaction (15) is expected to contribute negligibly (see Discussion). The rate constants (or their limiting values) for the above reactions are listed in Table 1. These rate parameters are derived from their respective fits to the pH dependences as shown in Figure2 and the rate law in expressions (II) and (III). The species H_2FeO_4 plays a relatively minor role under our conditions and contributes to the reaction only in the case of methionine. The following rate expression was used for ferrate(V) reduction, where K_a is the acid dissociation constant for the respective amino acid, $D = (1 + K_5/[H^+] + K_5K_6/[H^+]^2)$ and k_{15} is assumed to be negligible in determining the other rate constants:

$$d[Fe^V]/dt = k_{11}[Fe^V][AA] \quad II$$

$$k_{11} = \{1/D(1 + K_a/[H^+])\}\{k_{12}K_5K_6K_a/[H^+]^3 + k_{13}K_5/[H^+] +$$

$$+ (k_{14}K_5K_a + k_{15}K_5K_6)/[H^+]^2 + k_{16}\} \quad III$$

REACTIONS OF AMINO ACID DERIVATIVES.

The values of k_{13} and k_{14} listed in Table 1 show that the zwitterion reacts with $HFeO_4^{2-}$ an order of magnitude slower than the anion form of the amino acid. It has also been shown that deamination results from the interaction of aspartic acid and ferrate(V).[9] Therefore oxidation of the amino acid is presumably occurring by attack of ferrate(V) on nitrogen or the α-carbon atom. To distinguish some possibilities the effects of methyl substitution at either position using (A) N-methylglycine, (B) N-dimethylglycine, (C)

TABLE 1
Rate Constants for the Reaction of Ferrate(V) Species with Amino Acids at = 23°C in 0.025 M Phosphate.

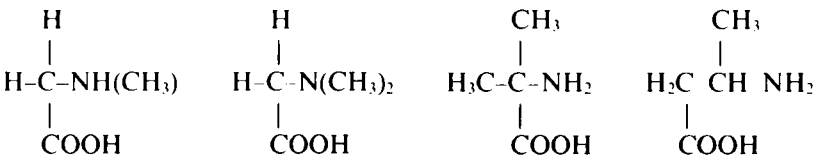
	SERINE	GLYCINE	PHENYLALANINE	METHIONINE
pK_a[15]	9.06	9.57	9.15	9.05
k_{12}*	$<10^3$	$<10^3$	$<10^3$	$<10^3$
k_{13}*	3×10^5	1.6×10^5	2.1×10^5	2×10^5
k_{14}*	8×10^6	4.5×10^6	2.6×10^6	2.8×10^6
k_{15}*	(estimated to be unimportant to the overall reaction)			
k_{16}*	$\leq3\times10^4$	$\leq3\times10^4$	$\leq3\times10^4$	$\approx3\times10^5$

* ($M^{-1}s^{-1}$)

TABLE 2
Observed Rate Constants for the Reactions of Methyl-Substituted Amino Acids (10 mM) with Ferrate(V) at pH 8.8 and ≈ 23°C in 0.025 M Phosphate.

Amino Acid	(k_{obs})/[Amino Acid]
GLYCINE	$(3.0\pm1.0)\times10^{5}M^{-1}s^{-1}$
N-Methylglycine	$(1.2\pm0.3)\times10^{5}M^{-1}s^{-1}$
N-Dimethylglycine	$<5\times10^{3}M^{-1}s^{-1}$ (estimated upper limit)
α aminoisobutyric	$(1.2\pm0.3)\times10^{5}M^{-1}s^{-1}$
β-aminoisobutyric	$(7.0\pm0.2)\times10^{4}M^{-1}s^{-1}$

alpha-aminoisobutyric acid, and (D) beta-aminoisobutyric acid were measured. Their structures are given below and the corresponding observed second-order rate constants for reaction with ferrate(V) (measured at pH 8.8) are listed in Table 2.



(A) (B) (C) (D)

For (B) no reaction could be measured while for (A), (C), and (D), the rates are only slightly less than glycine at this pH.

CONCLUSIONS

The protonated form of ferrate(V), $HFeO_4^{2-}$, is much more reactive than FeO_4^{3-} toward amino acids. $HFeO_4^{2-}$ is about an order of magnitude more reactive toward the anion forms than the zwitterions which accounts for the maximum in the pH dependences. We assumed in evaluating expression (III) that the term involving k_{15} is negligible in comparison to k_{14} since the least reactive forms, FeO_4^{3-} and the zwitterion, are involved. The reaction is virtually suppressed when both amine hydrogens are substituted in N-dimethyl glycine from which we can conclude that the strong inhibition is due to the difficulty of oxidizing the (C–N) bond when a methyl group must be eliminated. We expect that the substitution of alpha-hydrogens in (C) is not significantly inhibiting because the system can decarboxylate if it is not possible to eliminate a proton from this site.

It should be noted that inhibition due to protonation of the amine ($k_{13}<k_{14}$) is greater than the slight decrease upon substitution of a single amine hydrogen by a methyl group. This suggests that the attack on nitrogen by ferrate(V) is inhibited more by the change in the nucleophilicity of the amine function caused by protonation than steric hindrance due to a single methyl substitution at this site.

An interesting aspect of ferrate(V) chemistry is that the $HFeO_4^{2-}$ ion reacts rapidly with itself (reaction (7)) or with FeO_4^{3-} (reaction (8)) ion. However FeO_4^{3-} does not interact with another FeO_4^{3-}.[7] We have previously considered two explanations for this: 1) that the oxygen atoms of $HFeO_4^{2-}$ have strong free-radical character or 2) that

relative to FeO_4^{3-}, $HFeO_4^{2-}$ is substitutionally labile and can expand its coordination sphere. This could be induced by a nucleophilic attack on ferrate(V) by an oxide ligand of another ferrate(V) in the second-order decay process or by an amine as in the present case. If an inner-sphere ferrate(V)-AA complex is involved, it is possible to account simply for the two-electron oxidation. The rate constants for reactions between the amino acid anion and $HFeO_4^{2-}$ ($>10^6 M^{-1}s^{-1}$) approach that observed for the second-order decay rates of this species (reactions (7), (8)).

Alternatively, the $Fe^V=O$ system might abstract hydrogen from nitrogen as does the hydroxyl radical.[12] The lower activity of the zwitterion vs anion forms of the aliphatic amino acids towards ferrate(V) is paralleled in their reactions with the hydroxyl radical. However, it is clear from the pH dependences of Fig.2 that methionine is not attacked at the sulfur site but rather, like the other amino acids, at nitrogen. The product analysis, which shows that NH_3 is one of the first oxidation products, corroborates the kinetic observations. This is in contradistinction to the OH radical which attacks the thioether site of methionine[13] at a near diffusion-controlled rate.[14] The same effect of amine protonation is seen in the case of phenylalanine. The electrophilic hydroxyl radical attacks the phenyl ring in preference to the amino acid side-chain.[13] It is likely therefore that ferrate(V) reacts by the substitutionally controlled process discussed above or it is, at least, much more selective in its reactions than the hydroxyl radical. We are currently trying to establish whether these simple high valent iron systems can mimic the important hydroxylating functions of biological iron enzymes in which the oxygen (or oxide) ligands of the iron are highly activated.

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