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KINETICS OF OXIDATION OF AMINO ACIDS BY ALKALINE HEXACYANOFERRATE(III)

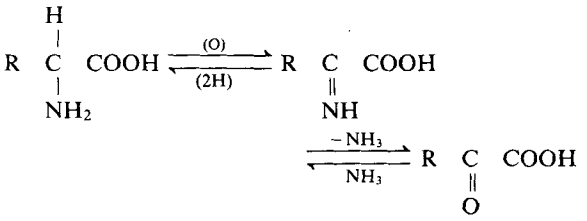
DIDCY LALOO AND MAHENDRA K. MAHANTI*

Department of Chemistry, North-Eastern Hill University, Shillong 793 003, India

The kinetics of the oxidation of α-amino acids (glutamic acid and aspartic acid) by alkaline hexacyanoferrate(III) were studied at constant ionic strength and over the temperature range 323–348 K. The rate was dependent on the first power of the concentrations of substrate and oxidant, but was independent of the concentration of alkali in the range studied. The value of k_H/k_D was in the range 8·1–8·3 for the slow step, indicating the loss of a hydrogen atom from the C—H bond, giving a radical species which was characterized by ESR spectroscopy. The reaction proceeds by way of the α-imino acid, formed in a fast step, which undergoes hydrolysis to give the corresponding α-keto acid.

INTRODUCTION

Amino acids are the building blocks in protein synthesis. The fact that the important naturally occurring amino acids have α-hydrogen atoms suggests that the biosynthesis and degradation of amino acids occur by way of the α-imino acids and α-keto acids, thus



Earlier work on the oxidation of amino acids by potassion hexacyanoferrate(III) had shown that the rate was dependent on the structure of the amino acid and the nature of the cation in the inert electrolyte.[1] Subsequently, the kinetics of the oxidation of amino acids by potassium hexacyanoferrate(III), catalysed by osmium(VIII), was reported.[2–4]

The oxidation of amino acids is of importance both from a chemical point of view and with regard to the mechanism of amino acid metabolism. This investigation forms part of a broad programme of studies on the mechanistic aspects of the oxidation of amino acids by potassium hexacyanoferrate(III) in alkaline media. A detailed investigation of the kinetics of the oxidation of glutamic acid and aspartic acid by alkaline hexacyanoferrate(III) in alkaline medium at constant ionic strength under a nitrogen atmosphere is reported in this paper.

EXPERIMENTAL

The substrates (BDH, Poole, UK) were found to be chromatographically pure, but were further assayed by the acetic acid–perchloric acid method,[5] and their aqueous solutions were used for kinetic studies. All other compounds employed were obtained from E. Merck (Darmstadt, FRG). The ionic strength of the system was kept constant at a high value (0·5 M) using concentrated sodium perchlorate solution. Triply distilled water was used throughout the kinetic runs. All reactions were performed under a nitrogen atmosphere.

Kinetic measurements. The rate studies were made under pseudo-first-order conditions by keeping an excess of amino acid over the oxidant. Aqueous solutions of the amino acids were prepared. Solutions of potassium hexacyanoferrate(III) were prepared in aqueous sodium hydroxide, and sodium perchlorate solution was added to adjust the ionic strength. The two solutions were separately thermostated at 338 K for 1 h under nitrogen and then mixed in equal volumes by syringing into the spectrophotometric cell. The kinetics were followed by monitoring the disappearance of $[Fe(CN)_6^{3-}]$ spectrophotometrically at 420 nm (Beckman UV 26 spectrophotometer). At this wavelength, the absorption due to hexacyanoferrate(II) was negligible.[6] The course of the reaction was followed for five half-lives. The rate constants were evaluated from

* Author for correspondence.

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the linear ($r = 0{\cdot}994$) plots of log[oxidant] vs time, and were reproducible to within ±3%.

Stoichiometry and product analysis. Various ratios of hexacyanoferrate(III) to amino acid in the presence of 0·5 M sodium hydroxide (ionic strength adjusted by the addition of sodium perchlorate) were equilibrated at 338 K for 24 h under nitrogen. Estimation of residual oxidant showed that 1 mol of amino acid consumed 2 mol of hexacyanoferrate(III), corresponding to the stoichiometric equation

$$RCH(NH_2)COOH + 2Fe(CN)_6^{3-} + 2OH^- \rightarrow RCOCOOH + 2Fe(CN)_6^{4-} + NH_3 + H_2O$$

Using the same experimental conditions as for the kinetic determinations, solutions of substrate and oxidant in sodium hydroxide (ionic strength adjusted by the addition of sodium perchlorate) were mixed and kept at 338 K for 24 h under nitrogen. The evolution of ammonia was shown by partial distillation of the reaction mixture. The ammonia formed was absorbed in an excess of 0·5 M hydrochloric acid. The excess acid was then back-titrated against base in the presence of methyl red indicator.[7]

The reaction mixture was extracted with diethyl ether, washed with water, dried over anhydrous magnesium sulphate and then concentrated. The product obtained was the corresponding keto acid, detected by spot tests,[8] in agreement with earlier work.[9]

The product obtained was treated with an acidic solution of sodium hydrogen-sulphite and cooled in ice. A 25 ml volume of 0·05 M 2,4-dinitrophenylhydrazine solution was added and the mixture kept overnight at 0 °C. The solid compound formed was filtered, dried, recrystallized from a mixture of ethyl acetate and light petroleum and weighed as the 2,4-dinitrophenylhydrazone (DNP) derivative of the corresponding keto acid (Table 1). The yields were *ca* 75–80%.

ESR measurements. Using the appropriate reaction conditions, the radicals were generated by mixing the substrate and oxidant, by volume, in an ESR tube just outside the cavity of the spectrometer (Varian E4). The mixture was placed under high vacuum in order to expel dissolved oxygen, and the sample tube was placed in the cavity of the spectrometer. The conditions for obtaining the spectra at 77 K were as follows: scan range, 4000 G; field set, 3300 G; modulation amplitude, 6·3 G; microwave frequency, 9·45 GHz; time constant, 0·3 s; and scan time, 4 min.

Table 1. Characterization of the product

Amino acid	Keto acid (oxidation product)	2,4-DNP derivative: m.p. (°C)
Glutamic acid	Oxaloacetic acid	222
Aspartic acid	α-Ketoglutaric acid	218

RESULTS AND DISCUSSION

Kinetic results

The rate data are given in Table 2. Under the experimental conditions, the rate law could be expressed as

$$\text{Rate} = -d[Fe(CN)_6^{3-}]/dt = k[\text{amino acid anion}]\,[Fe(CN)_6^{3-}].$$

When [amino acid anion] ⩾ [oxidant], the second-order rate constant can be represented approximately by k_{obs}(first order)/[amino acid anion]$_0$. The presence of an intervening methine group in glutamic acid could lead to a diminution of the $+I$ effect, which may be responsible for the difference in the rates of oxidation of these two amino acids.

A linear correlation ($r = 0{\cdot}992$) between log k_{obs} and the reciprocal of temperature in the range 323–348 K was observed for the amino acids. The rate data and the activation parameters are given in Table 3. The near constancy of the free energy of activation points to a common mechanism for the oxidation of both the amino acids.

The addition of hexacyanoferrate(II) ions in the concentration range 1×10^{-4}–1×10^{-3} M did not have any effect on the rates of these oxidation reactions, indicating that the reaction between the substrate and

Table 2. Rate data for the oxidation of amino acids at 338 K ($\mu = 0{\cdot}5$ M)

[Substrate] (10^2M)	[$K_3Fe(CN)_6$] (10^3M)	[NaOH] (M)	$10^3 k_{obs}$(s^{-1}) Glutamic acid	$10^3 k_{obs}$(s^{-1}) Aspartic acid
5·0	1·0	1·0	2·1	4·7
10·0	1·0	1·0	4·5	9·3
25·0	1·0	1·0	10·5	24·0
50·0	1·0	1·0	21·0	47·0
100·0	1·0	1·0	43·0	95·0
25·0	0·75	1·0	10·5	23·5
25·0	0·50	1·0	10·5	25·0
25·0	0·25	1·0	10·0	24·0
25·0	0·10	1·0	10·5	24·0
25·0	0·05	1·0	10·0	24·5
25·0	1·0	0·75	10·5	24·0
25·0	1·0	0·50	10·0	25·0
25·0	1·0	0·25	10·5	24·0
25·0	1·0	0·10	10·2	23·5
25·0	1·0	0·05	10·0	25·0
25·0	1·0	0·01	10·5	24·0

Table 3. Effect of temperature and activation parameters[a]

Temperature (K) (±0·1 K)	$10^3 k_{obs}(s^{-1})$ Glutamic acid	Aspartic acid
323	3·9	6·7
328	5·7	9·5
333	7·2	16·8
338	10·5	24·0
343	14·2	31·0
348	21·3	48·0
E (kJ mol^{-1})	27 ± 2	22 ± 2
$\Delta H^{\neq}$ (kJ mol^{-1})	24 ± 2	19 ± 2
Log A	4·7	4·5
$\Delta S^{\neq}$ (J K^{-1} mol^{-1})	−155 ± 5	−165 ± 5
$\Delta G^{\neq}$ (kJ mol^{-1})	76 ± 2	74 ± 2

[a] [Substrate] = 0·25 M; [$K_3Fe(CN)_6$] = 1×10^{-3} M; [NaOH] = 1·0 M; μ = 0·5 M.

oxidant (the electron-abstraction step) was an irreversible step.

Variations in the ionic strength of the medium using sodium-perchlorate (μ = 0·01–0·5 M) did not have any effect on the rates of these reactions.

The addition of salts such as NaCl, $NaNO_3$, KNO_3, Na_2SO_4 and $MgSO_4$ (concentration range 1×10^{-4}–5×10^{-3} M) did not have any influence on the rates of these reactions. Striking specific cation effects had been observed in an earlier investigation of the oxidation of amino acids by hexacyanoferrate(III) in alkaline medium, where changing the cation from lithium to caesium resulted in a > 100-fold increase in the rate of the reaction.[1] In the present investigation, salt effects were not observed. It seems possible that any effects due to the addition of salts, in the concentration range studied, may be compensated for by the high ionic strength of the medium, thus vitiating any observed effect of the addition of salts.

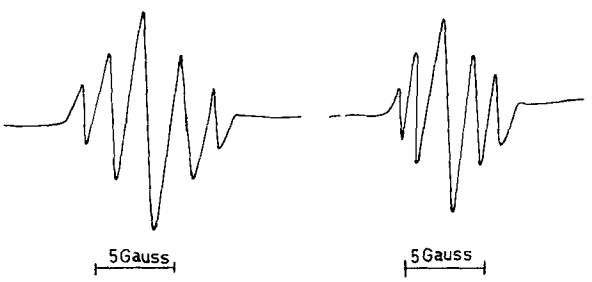

Figure 1. ESR spectra for the oxidation of (a) glutamic acid and (b) aspartic acid. Conditions: scan range, 4000 G; field set, 3300 G; modulation amplitude, 6·3 G; microwave frequency, 9·45 GHz; time constant, 0·3 s; scan time, 4 min; temperature 77 K

Table 4. Solvent isotope effect at 338 K[a]

Amino acid	$k_{H_2O}10^3 k_{obs}(s^{-1})$	$k_{D_2O}10^3 k_{obs}(s^{-1})$	k_{H_2O}/k_{D_2O}
Glutamic acid	10·5	11·0	0·95
Aspartic acid	24·0	25·0	0·96

[a] [Substrate] = 0·25 M; [$K_3Fe(CN)_6$] = 1×10^{-3} M; [NaOH] = 1·0 M; μ = 0·5 M.

ESR of the corresponding radicals generated from the oxidation of each of the substrates (Varian E-4), gave five-line spectra with intensity ratios of 1 : 2 : 3 : 2 : 1 (Figure 1).

Solvent isotope studies in D_2O medium gave k_{H_2O}/k_{D_2O} values close to unity (Table 4). Even though OD^- is a stronger base than OH^-, the k_H/k_D value indicated that OD^- did not influence the kinetics of the reaction. Since the rate of the reaction was independent of the concentration of the alkali in the range studied, the ratio k_{H_2O}/k_{D_2O} should be close to unity, as was observed.

Mechanism

The dissociation of amino acids depends on the pH of the medium. It is known that amino acids exist as zwitterions in aqueous solution. In strong acidic or alkaline media, the following equilibria exist:

$$\underset{(SH^+)\text{ cation}}{RCH(\overset{+}{N}H_3)COOH} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{(S^\circ)\text{ zwitterion}}{R.CH(\overset{+}{N}H_3)COO^-} \underset{H^+}{\overset{OH^-}{\rightleftharpoons}} \underset{(S^-)\text{ anion}}{RCH(NH_2)COO^-}$$

In alkaline solution, the zwitterion is converted to the anion, $RCH(NH_2)COO^-$, which is the reactive species under the present experimental conditions. The pK_a values for the system

$$RCH(\overset{+}{N}H_3)COO^- \rightleftharpoons RCH(NH_2)COO^- + H^+$$

have been reported.[10] Since all the kinetic studies were performed at high concentrations of sodium hydroxide, it may be assumed that the amino acids would be completely dissociated into their anions, $RCH(NH_2)COO^-$.

The radicals generated from the oxidation of each of the substrates gave five-line ESR spectra with intensity ratios of 1 : 2 : 3 : 2 : 1. The radical intermediate was formed by the loss of a hydrogen atom from the carbon atom of the methine group. The number of lines was accounted for by the radical species $R\dot{C}(NH_2)COO^-$,

Table 5. Kinetic isotope effect at the α-carbon atom[a]

Amino acid	$10^3 k_{obs}(s^{-1})$		
	$RCH(NH_2)COO^-$	$RCD(NH_2)COO^-$	k_H/k_D
Glutamic acid	10·5	1·3	8·1
Aspartic acid	24·0	2·9	8·3

[a] [Substrate] = 25×10^{-2} M; $[K_3Fe(CN)_6] = 1 \times 10^{-3}$ M; [NaOH] = 1·0 M; μ = 0·5 M.

which corresponds to a radical with two equivalent protons and a nitrogen atom, all having nearly equal coupling constants. The *g*-values were 2·002.

Since potassium hexacyanoferrate(III) is a one-electron oxidant, it would be justified to postulate that the reaction between the substrate and oxidant would give rise to a radical intermediate, analogous to enzymatic oxidation reactions which are also known to proceed via radical intermediates.[11] This would suggest that hexacyanoferrate(III), as chemical oxidant, is capable of simulating enzymatic behaviour.

The kinetic isotope effect caused by deuterating the α-carbon atom was studied. The k_H/k_D values were observed to be between 8·1 and 8·3 (Table 5), indicating that, in the rate-determining step, the C—H bond underwent fission to give a radical species, $R\dot{C}(NH_2)COO^-$, which has been characterized by ESR spectroscopy.

CONCLUSION

The rate of the reaction was dependent on the first power of the concentrations of substrate and oxidant, and was independent of the concentration of alkali in the range studied. The addition of hexacyanoferrate(II) ion had no effect on the rate of the reaction. The presence of radical intermediates was characterized by ESR spectroscopy. The kinetic isotope effect at the α-carbon atom indicates the fission of a C—H bond in the slow step of the reaction. Subsequent steps involved the rapid reaction of the radical intermediate with the oxidant, yielding the imino compound, which underwent hydrolysis to give the corresponding keto acid and ammonia.

The reaction sequence for the oxidation of these amino acids (glutamic acid and aspartic acid) by potassium hexacyanoferrate(III) in alkaline medium is as follows:

$$RCH(NH_3^+)COO^- + OH^- \rightleftharpoons RCH(NH_2)COO^- \xrightarrow[\text{slow}]{Fe(CN)_6^{3-}} R\dot{C}(NH_2)COO^- \xrightarrow[\text{fast}]{Fe(CN)_6^{3-}} RC(=NH)COOH \xrightarrow{H_2O} RC(=O)COOH + NH_3$$

The products obtained in each case (the corresponding α-keto acid) were isolated and characterized.

This mechanistic pathway for the oxidation of amino acids to the keto acids, via the intermediate formation of the imino acid, has been well established in the synthesis of α-keto acid esters.[12]

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