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Kinetics of Os(VIII)-catalysed Alkaline Hexacyanoferrate(III) Oxidation of Some α-Amino Acids in Presence of Excess of Ferrocyanide

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The Os(VIII)-catalysed oxidation of some α-amino acids such as L-leucine, L-glutamic acid, L-glutamine and L-asparatic acid by alkaline hexacyanoferrate(III) in the presence of excess ferrocyanide has been studied spectrophotometrically. The oxidation follows complex kinetics being first order in amino acid and zero order in hexacyanoferrate(III) at lower concentrations of amino acid. At extremely large concentration of amino acid ([AA] : $[Fe(CN)_6^{3-}]$ = 80) the reaction becomes nearly independent of [amino acid] and almost second order in $[Fe(CN)_6^{3-}]$. The order in Os(VIII) has been found to be unity in every case. A small variation in ferrocyanide ion concentration is best expressed as rate $\propto$ {constant + $[Fe(CN)_6^{4-}]$}. A suitable mechanism consistent with the kinetic data has been proposed.

EARLIER, while studying the kinetics of Os(VIII)-catalysed oxidation of α-amino acids by ferricyanide, it was observed that concentration versus time plots were not linear due to strong catalytic influence of ferrocyanide. As the ferrocyanide is a product of reduction of ferricyanide, its accelerating influence was encountered in every kinetic run which showed curves with increasing slopes. Therefore, in the present study the kinetics of Os(VIII)-catalysed oxidation of some α-amino acids such as L-leucine, L-glutamic acid, L-glutamine and L-asparatic acid by ferricyanide were investigated spectrophotometrically in the presence of excess of ferrocyanide to avoid auto-catalysis.

Materials and Methods

Solutions of L-leucine, L-glutamic acid, L-glutamine L-asparatic acid and potassium ferrocyanide (all from BDH) were freshly prepared in doubly distilled water. Preparation of all other solutions and the experimental procedure adopted were identical to those reported earlier[1].

Stoichiometry — The reaction mixture containing a known excess of ferricyanide over amino acids was kept at 40° in the presence of 0·1*M* alkali and $3{\cdot}90\times10^{-6}M$ Os(VIII) (in case of leucine, [Os(VIII)] $= 1{\cdot}95\times10^{-6}M$) for 6 hr. The amount of ferricyanide left revealed 1:2 stoichiometry between amino acid and ferricyanide as shown in Eq. (1),

$$RCHNH_2COOH + 2\,Fe(CN)_6^{3-} + 2OH^- = RCOCOOH + NH_3 + H_2O + 2Fe(CN)_6^{4-} \quad ...(1)$$

where R represents $(CH_3)_2CHCH_2$, $-CH_2COOH$, $-CH_2CH_2COOH$ and $-CH_2CH_2CONH_2$ groups for leucine, asparatic acid, glutamic acid and glutamine respectively.

Product analysis — The presence of corresponding keto acids as the reaction products was detected by spot tests[2], in agreement with the earlier work[3,4].

Results

Effects of varying [ferricyanide] and [amion acid] — The kinetics of oxidation of α-amino acids (AA) were investigated at several initial concentrations of the reactants in excess ferrocyanide. The concentration versus time plots in ferricyanide were linear (Fig. 1) suggesting an independent nature of the rate in ferricyanide. The pesudo-zero order rate constants (k_0) in ferricyanide were nearly constant (Table 1) at all [ferricyanide], which further established zero order dependence in ferri-cyanide.

The results of varying [amino acid] are given in Table 1. The plots of k_0 versus [amino acid] showed a deviation from linearity (Fig. 1) at higher [amino acid], while the plots of $1/k_0$ versus 1/[amino acid] were linear (Fig. 2). It, therefore, appears that the order in amino acid falls from unity at higher [amino acid].

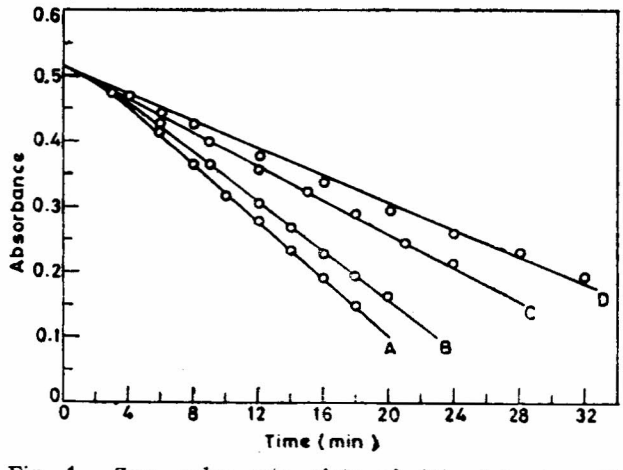

Fig. 1 — Zero order rate plots of (A) glutamic acid, (B) asparatic acid, (C) leucine and (D) glutamine in [ferricyanide] at 35° {Initial $[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-4}M$; $[Fe(CN)_6^{4-}] = 5{\cdot}0\times10^{-3}M$; $[OH^-] = 0{\cdot}1M$; $[AA] = 4{\cdot}0\times10^{-3}M$; and $[OsO_4] = 3{\cdot}90\times10^{-6}M$ for A, B and D and $1{\cdot}95\times10^{-6}M$ for C}

TABLE 1 — EFFECT OF VARYING $[Fe(CN)_6^{3-}]$ AND [AMINO ACID] AT 35°

{$[OH^-] = 0{\cdot}1M$; $[Fe(CN)_6^{4-}] = 5{\cdot}0\times10^{-3}M$; and $[Os(VIII)] = 3{\cdot}90\times10^{-6}M$ for glutamic acid, glutamine and asparatic acid and $1{\cdot}95\times10^{-6}M$ for leucine}

$10^4[Fe(CN)_6^{3-}]$ M	$10^2[AA]$ M	10^5k_0 (mole litre^{-1} min^{-1})			
		Leucine	Glutamic acid	Asparatic acid	Glutamine
3·0	4·0	1·18	1·70	1·60	1·19
3·5	4·0	1·30	1·77	1·67	1·20
4·0	4·0	1·28	1·73	1·66	1·16
4·5	4·0	1·32	1·85	1·64	1·24
5·0	4·0	1·23	1·84	1·67	1·15
5·0	2·4	0·90	1·13	1·10	0·64
5·0	3·2	1·15	1·40	1·36	0·80
5·0	5·6	1·58	2·48	2·04	1·33
5·0	8·0	1·86	2·95	2·34	1·55

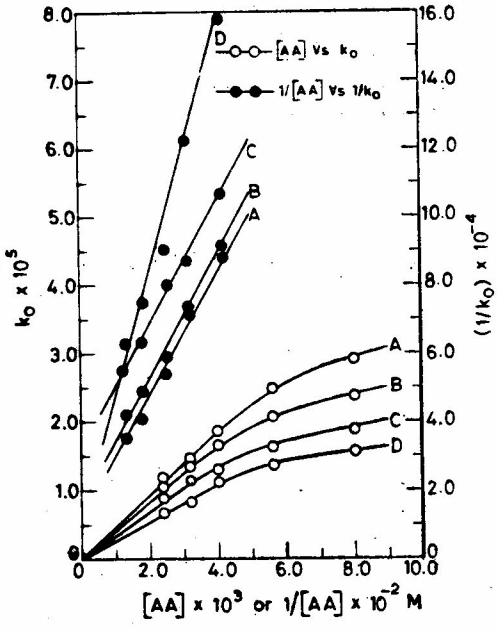

Fig. 2 — Plots of k_0 or $1/k_0$ versus [AA] or 1/[AA] at 35° {Initial $[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-4}M$; $[Fe(CN)_6^{4-}] = 5{\cdot}0\times10^{-3}M$; $[OH^-] = 0{\cdot}1M$; [AA] = $4{\cdot}0\times10^{-2}M$; and $[OsO_4] = 3{\cdot}90\times10^{-6}M$ for glutamic acid (A), asparatic acid (B) and glutamine (D) and $1{\cdot}95\times10^{-6}$ M for leucine (C)]

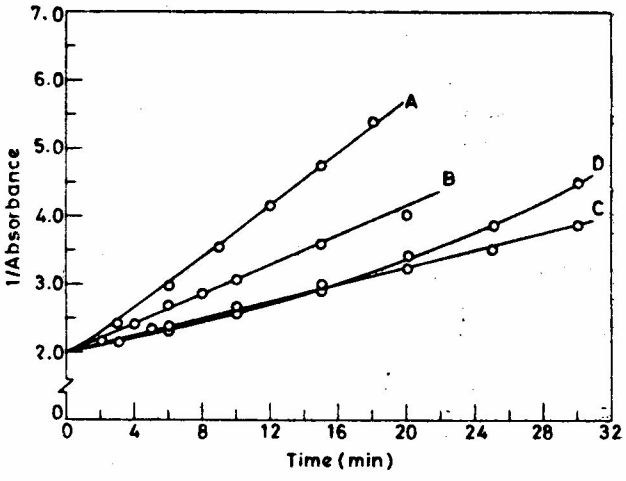

Fig. 3 — Plots of 1/absorbance versus time at 35° for (A) glutamic acid, (B) asparatic acid, (C) leucine and (D) glutamine at initial $[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-4}M$, $[Fe(CN)_6^{4-}] = 5{\cdot}0\times10^{-3}M$, $[OH^-] = 0{\cdot}1M$; and $[OsO_4] = 3{\cdot}90\times10^{-6}M$ for A, B and D and $1{\cdot}95\times10^{-6}M$ for C {[Glutamic acid] = $3{\cdot}0\times10^{-2}M$; [asparatic acid] = $2{\cdot}0\times10^{-2}M$; [leucine] = $4{\cdot}0\times10^{-2}M$; and [glutamine] = $4{\cdot}0\times10^{-2}M$}

It was observed that at very high [amino acid], the concentration versus time plots were not linear, while 1/absorbance versus time plots were almost linear (Fig. 3) suggesting a second order dependence in ferricyanide. Thus, it seems that the order in amino acid falls from unity to zero while that in ferricyanide increases from zero to two with large increase in amino acid concentrations.

Effect of varying [Os(VIII)] — Studies were carried out at various concentrations of Os[(VIII)]. The plots of k_0 versus [Os(VIII)] were linear emerging from the origin and established first order dependence in Os(VIII).

Effect of varying [ferrocyanide] — The oxidation of α-amino acids was studied over a wide range of [ferrocyanide]. At low initial [ferrocyanide] where the concentration versus time plots were non-linear with increasing slope value, the half-life periods

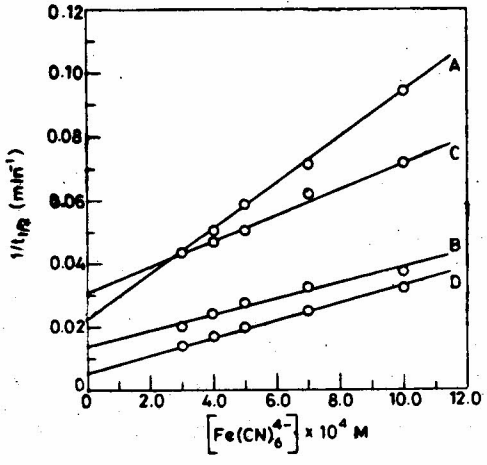

Fig. 4 — Plots of $1/t_{1/2}$ of (A) glutamic acid, (B) asparatic acid, (C) leucine and (D) glutamine versus $[Fe(CN)_6^{4-}]$ at 35° {$[OH^-] = 0{\cdot}2M$; $[Os(VIII)] = 3{\cdot}90\times10^{-6}M$; [AA] = $4{\cdot}0\times10^{-3}M$ and $[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-4}M$}

TABLE 2 — EFFECT OF VARYING $[Fe(CN)_6^{4-}]$ ON THE RATE CONSTANT AT 35°

{$[OH^-] = 0{\cdot}1M$; $[AA] = 8{\cdot}0\times10^{-3}M$; $[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-4}M$; and $[Os(VIII)] = 3{\cdot}90\times10^{-6}M$ for glutamic acid, glutamine and asparatic acid and $[Os(VIII)] = 2{\cdot}80\times10^{-6}M$ for leucine}

$10^3[Fe(CN)_6^{4-}]$ M	$10^5 k_0$ (mole litre^{-1} min^{-1})			
	Leucine	Glutamic acid	Asparatic acid	Glutamine
1·0	1·89	2·00	1·38	0·96
3·0	2·40	2·80	2·00	1·15
5·0	2·75	3·00	2·30	1·53
7·0	2·94	3·23	2·40	1·70
8·0	2·50	3·10	2·39	1·61
10·0	2·40	2·97	2·13	1·55

($t_{1/2}$) of the reactions dropped sharply and the plots of $[Fe(CN)_6^{4-}]$ versus $1/t_{1/2}$ were linear with positive intercepts (Fig. 4). At high [ferrocyanide] where $[Fe(CN)_6^{3-}]$ versus time plots were linear, k_0 values first levelled off and then showed a slight decreasing trend (Table 2).

Effect of varying ionic strength and $[OH^-]$ — Addition of $NaClO_4$ showed a negligible effect on the reaction rates up to $0{\cdot}4M$ $NaClO_4$, while a tenfold increase in [alkali] showed about 60% increase in k_0 values of leucine, glutamic acid and glutamine and about 4% increase in the case of asparatic acid at 35°.

Effect of varying temperature — The kinetics were measured at 35°, 40°, 45° and 50°. The energies of activation calculated from the Arrhenius plots were 9·21, 8·06, 8·29 and 13·15 kcal mole^{-1} for leucine, glutamic acid, asparatic acid and glutamine respectively.

Discussion

The Os(VIII)-catalysed oxidation of α-amino acids by ferricyanide in the presence of ferrocyanide follows complex kinetics. The zero order dependence in ferricyanide clearly suggests its involvement in the fast step. The strong catalytic influence of ferrocyanide at its low concentration also suggests the possible interaction between amino acids and ferrocyanide. Under the experimental condition the initial rates of oxidation are very small in comparison to those in the presence of ferrocyanide.

Fe(II) complexes of amino acids are reported in the literature[5] and in some cases[6] $Fe(CN)_6^{4-}$ complexes of amino acids are also mentioned. In the present case the more favourable path for the oxidation of amino acids seems to involve a primary reaction between the amino acids and $Fe(CN)_6^{4-}$ to give a complex. As the oxidation does not proceed in the absence of Os(VIII) even in the presence of ferrocyanide, the amino acid-ferrocyanide complex formed is oxidized by Os(VIII) in a slow step. Hence, a mechanism as shown in Scheme 1 may be proposed.

The proposed AA-$Fe(CN)_6^{4-}$ complex has already been characterized spectrophotometrically in our earlier studies[1]. Similar results were obtained for the amino acids studied presently.

$$AA + Fe(CN)_6^{4-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{complex (X)} \quad \text{..... fast} \qquad ...(2)$$

$$(X) + Os(VIII) \xrightarrow{k_2} Os(VI) + Fe(CN)_6^{4-} + \text{oxidation products} \quad \text{..... slow} \qquad ...(3)$$

$$Os(VI) + 2Fe(CN)_6^{4-} \xrightarrow{k_3} Os(VIII) + 2Fe(CN)_6^{4-} \quad \text{... fast} \qquad ...(4)$$

Scheme 1

$$AA + Os(VIII) \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{complex (Y)} \quad \text{..... slow} \qquad ...(5)$$

$$(Y) \xrightarrow{k_5} \text{keto acid} + NH_3 + Os(VI) \quad \text{..... fast} \qquad ...(6)$$

$$AA + Os(VI) \xrightarrow{k_6} \text{keto acid} + NH_3 + Os(IV) \quad \text{... slow} \qquad ...(7)$$

Scheme 2

It is mentioned in the literature[3,4] that amino acids form complexes with Os(VIII) which are subsequently degraded to keto acids, ammonia and Os(VI). The liberation of ammonia is a function of the rate of oxidation. It is also reported that at high [amino acid] Os(VI) is further reduced to Os(IV) by a fresh molecule of amino acid. The reactions may be represented as shown in Scheme 2.

As the concentration of Os(VIII) present in the reaction mixture is $\sim10^{-3}$ times to that of amino acid, the reaction in Scheme 2 represents nearly 0·1% oxidation of amino acids. However, in the presence of ferricyanide a fast interaction between Os(VI) species and $Fe(CN)_6^{3-}$ takes place to regenerate Os(VIII)[7].

Applying the steady state conditions with respect to (X), (Y) and Os(VI) in steps 2-7, the rate law equation for the disappearance of $Fe(CN)_6^{3-}$ is obtained as:

$$-\frac{d[Fe(CN)_6^{3-}]}{dt} = \frac{k_3[AA][Os(VIII)][Fe(CN)_6^{3-}]^2}{k_6[AA]+k_3[Fe(CN)_6^{3-}]^2}\left\{\frac{k_4k_5}{k_4+k_5} + \frac{k_1k_2[Fe(CN)_6^{4-}]}{k_{-1}}\right\} \qquad ...(8)$$

where $k_{-1} \gg k_2[Os(VIII)]$ has been taken as a suitable approximation.

Further step (4) is very fast in comparison to step (7), and therefore taking $k_3[Fe(CN)_6^{3-}]^2 \gg k_6[AA]$ the rate Eq. (8) reduces to Eq. (9).

$$-\frac{d[Fe(CN)_6^{3-}]}{dt} = [AA][Os(VIII)]\left\{\frac{k_1k_5}{k_4+k_5} + \frac{k_1k_2}{k_{-1}}[Fe(CN)_6^{4-}]\right\} \qquad ...(9)$$

The above rate law (Eq. 9) predicts that the rate of oxidation would be first order each in [AA] and [Os(VIII)], and zero order in [ferricyanide]. The effect of ferrocyanide on the rate would be proportional to {constant $+[Fe(CN)_6^{4-}]$} as was observed experimentally (Fig. 4, where $1/t_{\frac{1}{2}} \propto k_0$).

The fall in order of the reaction in amino acids may be explained as follows: since step (4) is very fast Os(VI) formed during the oxidation of amino acid is immediately converted into Os(VIII) by ferricyanide. It is only at very high [amino acid]

that some of Os(VI) is reduced to Os(IV) via the slow step (7). Evidently this will result in a decrease in [Os(VIII)] which in turn will decrease the rate of oxidation. However, in such a case the plots of $1/k_0$ versus 1/[AA] should be linear with intercept according to rate law (8) (Fig. 2).

Thus the fall in k_0 value at high [amino acid] has been interpreted in terms of the fact that the term k_6[AA] is not negligible in comparison to $k_3[Fe(CN)_6^{3-}]^2$ in Eq. (8). Consequently, at larger concentrations of amino acid a reaction independent of ferricyanide is not warranted. In fact, at such high amino acid concentrations where the rate of disappearance of ferricyanide would become independent of [amino acid], a second order dependence in ferricyanide should be obtained. Therefore, for moderately large concentrations of the amino acids the order in ferricyanide would be between 0 and 2. Inverse $[Fe(CN)_6^{3-}]$ versus time plots (Fig. 3) at high amino acid concentrations are almost linear suggesting a second order dependence in ferricyanide. It is further observed that in the case of oxidation of glutamine which shows minimum variation from the first order behaviour (Fig. 2), the order in ferricyanide at higher [glutamine] is minimum ($\sim$1). It, therefore, appears that the order in amino acid decreases from 1 to 0 while that for ferricyanide increases from 0 to 2 with a large increase in [amino acid]. This is in good agreement with the rate law (8).

The dependence on large ferrocyanide concentration seems to be more complicated than shown by Eq. (8). The fall in k_0 values (Table 2) at high ferrocyanide concentrations may be possibly due to the back conversion of Os(VIII) to Os(VI) through the reverse of step (4). As k_0 is proportional to [Os(VIII)], a substantial increase in [ferrocyanide] when accompanied by a decrease in [Os(VIII)], may result in a decrease in k_0 value.

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