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Kinetics of Os(VIII)-catalysed Oxidation of Some α-Amino Acids by Ferricyanide

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The rates of Os(VIII)-catalysed ferrocyanide oxidation of α-amino acids such as glycine, L-leucine, L-phenylalanine, DL-α-alanine and DL-valine have been measured spectrophotometrically. The reactions follow complex kinetics being first order with respect to both amino acid and the catalyst. The effect of alkali is slightly positive at high concentration (>0·2*M*). Although the rate is independent of ferricyanide ion concentration, it shows specific ferrocyanide ion catalysis. The energies of activation have been found within the range 12·9-14·3 kcal mole^{-1}. A suitable mechanism involving the formation of an intermediate amino acid-ferrocyanide complex is proposed.

OXIDATION of α-amino acids by a variety of oxidants has been studied in detail by several investigators[1-5]. Lambert and Jones[6] studied the uncatalysed oxidation of some amino acids by alkaline ferricyanide and reported that the reaction was extremely slow even at high ionic strengths (4·5*M* KNO_3). The strong catalytic effect of Os(VIII) on ferricyanide oxidations in alkaline media is well documented[7-9]. In this paper the kinetics of Os(VIII)-catalysed oxidation of glycine, L-leucine, L-phenylalanine, DL-α-alanine and DL-valine are reported and a mechanism of the reaction is suggested. It has been observed that the presence of a trace amount of Os(VIII) ($10^{-6}M$) is sufficient to catalyse the oxidation of α-amino acids by alkaline ferricyanide to a reasonable rate.

Materials and Methods

The reagents employed were glycine, DL-α-alanine, L-leucine, DL-valine, L-phenylalanine (all BDH samples) potassium ferricyanide (recrystallized) and sodium hydroxide (both BDH, AR samples) and osmium tetroxide (Johnson Matthey grade). All the solutions were prepared in doubly distilled water. Other reagents used were of AR grade.

The kinetics were followed by determining ferricyanide spectrophotometrically at various intervals of time on a Bausch and Lomb spectronic-20 spectrophotometer at 420 nm and keeping the ferricyanide ion concentration below $5{\cdot}0\times10^{-4}M$.

Stoichiometry — The reaction mixture containing a known excess of ferricyanide over amino acid was kept at 40° in the presence of $3{\cdot}9\times10^{-6}M$ Os(VIII) and 0·2*M* alkali for 12 hr. The amount of ferricyanide left revealed 1:2 stoichiometry between amino acid and ferricyanide. The reaction may be represented by the following equation:

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

where R represents H, CH_3, $(CH_3)_2CHCH_2$, $(CH_3)_2CH$ and $C_6H_5CH_2$ for glycine, α-alanine, leucine, valine and phenylalanine respectively.

Product analysis — The presence of keto acids in the reaction products was detected by various spot tests[10], and the results are in agreement with earlier work on the oxidation of amino acids by Os(VIII)[11,12].

Results

Effect of varying [ferricyanide] — There was a gradual increase in the rate of disappearance of ferricyanide at high [amino acid], and absorbance versus time plots represented curves with increasing slope value. However, at different concentrations of ferricyanide these curves were parallel to each other, and the half-life periods in ferricyanide were dependent on the initial [ferricyanide] (Table 1). The oxidation rate appears to be independent of [ferricyanide], the catalytic influence of ferrocyanide formed is also evident. It was verified by carrying out investigation in the presence of excess ferrocyanide where the zero order plots were reasonably linear curves (Fig. 1).

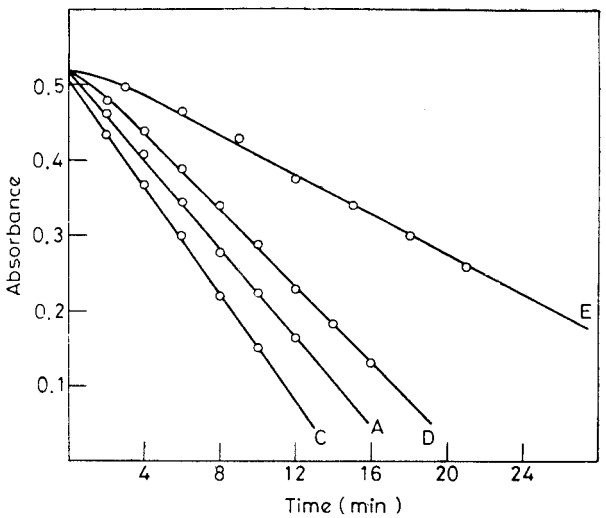

Fig. 1 — Zero order rate plots in ferricyanide at 35° and in the presence of $5\times10^{-3}M$ $Fe(CN)_6^{4-}$ for (A) leucine, (C) phenylalanine, (D) alanine and (E) valine {$[Fe(CN)_6^{3-}] = 5{\cdot}0\times10^{-3}M$; [amino acid] = $4{\cdot}0\times10^{-3}M$; $[OH^-] = 0{\cdot}2M$; $[OsO_4] = 3{\cdot}9\times10^{-6}M$}

TABLE 1 — EFFECT OF VARYING [REACTANTS] ON RATE AT 35° IN THE PRESENCE OF $3.9 \times 10^{-6}M$ OsO_4

$10^4 \times [Fe(CN)_6^{3-}]$ M	$10^3 \times$ [amino acid] M	$10 \times [OH^-]$ M	$t_{1/2}$ (min)				
			Leucine	Glycine	Phenylalanine	Alanine	Valine
3·0	4·0	2·0	27	28	37	40	67
3·5	4·0	2·0	31	32	42	50	74
4·0	4·0	2·0	35	36	51	65	84
4·5	4·0	2·0	39	41	56	69	101
5·0	4·0	2·0	43	46	62	74	120
5·0	2·4	2·0	64	75	92	103	195
5·0	3·2	2·0	53	59	68	88	153
5·0	5·6	2·0	33	35	45	60	94
5·0	8·0	2·0	26	28	33	45	70
5·0	4·0	0·4*	49	53	65	82	140
5·0	4·0	0·8	40	40	64	76	—
5·0	4·0	1·2	35	31	62	69	120
5·0	4·0	1·6	28	26	60	66	105
5·0	4·0	2·0	26	23	56	62	100
5·0	4·0	2·8	21	17	48	54	80

*Effect of alkali on the rate of oxidation of glycine and leucine was studied at $\mu = 1{\cdot}2M$ ($NaClO_4$) while on the rest of amino acids it was studied at $\mu = 0{\cdot}4M$ ($NaClO_4$).

Effect of varying [amino acid] — Studies carried out at various initial concentrations of the amino acids revealed that an increase in [amino acid] resulted in a decrease in half-life period ($t_{1/2}$) in ferricyanide (Table 1). The plots of log ($1/t_{1/2}$) (proportional to pseudo zero order rate constant in ferricyanide) versus log [amino acid] were linear with slope values of ~1 thereby establishing first order dependence with respect to amino acid.

Effect of varying $[OH^-]$ — The effect of increasing [alkali] was less pronounced in oxidation of phenylalanine, α-alanine and valine. However, in the case of glycine and leucine $t_{1/2}$ decreased significantly with increasing [alkali] (Table 1).

Effect of varying [Os(VIII)] — There was a marked catalytic influence of osmium tetroxide on the reaction rate. The results in Table 2 show a near proportional decrease in $t_{1/2}$ in ferricyanide with increasing in [Os(VIII)] indicating a direct dependence on the [catalyst].

Effect of ferrocyanide ions — The oxidation was found to be highly susceptible to ferrocyanide ions which strongly accelerated the rate (Table 3). As ferrocyanide is also a product of the reaction its accelerating influence is encountered in every kinetic run. The plot of $[Fe(CN)_6^{4-}]$ against $1/t_{1/2}$ was almost linear with a positive intercept on Y-axis.

Effect of varying ionic strength — The effect of varying ionic strength was studied by carrying out the investigations in the presence of different amounts of $NaClO_4$. The results (Table 4) show a slight positive salt effect.

Effect of varying temperature — The oxidation of amino acids was studied at 30°, 35°, 40°, 45° and 50° and $1/t_{1/2}$ values were obtained. The activation energies ($\Delta E^{\ddagger}$) calculated from the Arrhenius plots (log $1/t$ versus $1/T$) were $12{\cdot}9 \pm 0{\cdot}1$, $13{\cdot}8 \pm 0{\cdot}1$, $13{\cdot}5 \pm 0{\cdot}2$, $14{\cdot}6 \pm 0{\cdot}2$ and $14{\cdot}3 \pm 0{\cdot}2$ kcal mole^{-1} for the oxidation of leucine, glycine, phenylalanine, alanine and valine, respectively.

Discussion

Mechanism — The results show that the oxidation of glycine, alanine, valine, leucine and phenyl-

TABLE 2 — EFFECT OF VARYING $[OsO_4]$ ON THE REACTION RATE AT 35°

$\{[Fe(CN)_6^{3-}] = 5{\cdot}0 \times 10^{-4}M$; $[OH^-] = 0{\cdot}2M$; [amino acid] = $4{\cdot}0 \times 10^{-3}M\}$

$10^6 \times [OsO_4]$ M	$t_{1/2}$ (min)				
	Leucine	Glycine	Phenyl-alanine	Alanine	Valine
2·34	70	—	105	125	208
3·12	53	63	81	93	150
3·90	43	46	62	74	120
5·46	31	32	44	43	84
7·80	22	23	28	32	57

TABLE 3 — EFFECT OF VARYING $[Fe(CN)_6^{4-}]$ ON THE REACTION RATE AT 35°

$\{[Fe(CN)_6^{3-}] = 5{\cdot}0 \times 10^{-4}M$; $[OH^-] = 0{\cdot}2M$; $[OsO_4] = 3{\cdot}9 \times 10^{-4}M$; [amino acid] = $4{\cdot}0 \times 10^{-6}M\}$

$10^4 \times [Fe(CN)_6^{4-}]$ M	$t_{1/2}$ (min)				
	Leucine	Glycine	Phenyl-alanine	Alanine	Valine
None	43	46	62	74	120
3·0	23	—	30	33	60
4·0	21	—	28	28	54
5·0	20	14	24	26	41
7·0	16	—	20	23	39
10·0	14	—	18	20	32

TABLE 4 — EFFECT OF ADDED SODIUM PERCHLORATE AT 35°

$\{[Fe(CN)_6^{3-}] = 5{\cdot}0 \times 10^{-4}M$; $[OH^-] = 0{\cdot}2M$; $[OsO_4] = 3{\cdot}9 \times 10^{-6}M$; [amino acid] = $4{\cdot}0 \times 10^{-3}M\}$

$[NaClO_4]$ M	$t_{1/2}$ (min)				
	Leucine	Glycine	Phenyl-alanine	Alanine	Valine
—	43	46	62	75	120
0·2	37	34	51	65	100
0·4	33	32	50	52	94
0·6	27	27	48	46	81
0·8	26	25	44	45	68

alanine proceeds by a common mechanism. It is well known that amino acids exist as dipolar ions in aqueous solution. These dipolar ions dissociate to amino acid anions and the pK values for $RCHNH_3^+COO^- \rightleftharpoons RCHNH_2COO^- + H^+$ system are reported[13] to be ~9·60 for all amino acids studied here except phenylalanine for which the value is 9·13 at 25°. Since studies were carried out in 0·2M NaOH, it may be assumed that the amino acids were completely dissociated into their anions. At the same time Os(VIII) in such media is reported[14] to exist as $HOsO_5^-$. Now, as experimental results show first order dependence in catalyst and amino acid, their interaction appears to constitute a primary rate determining reaction. It is mentioned in literature[12,15] that Os(VIII) forms complexes with amino acid in alkaline solution which are subsequently degraded to keto acids and ammonia. It is also mentioned that liberation of ammonia is a function of the rate of oxidation and, therefore, the reaction may be represented in general by Eqs. (1) and (2) (Scheme 1).

As [Os(VIII)] present in the reaction mixture is 10^{-3} times that of amino acid, reactions (1) and (2) represent nearly 0·1% oxidation of amino acids only. However, in the presence of ferricyanide a fast interaction between Os(VI) species and $Fe(CN)_6^{3-}$ takes place to regenerate Os(VIII) as shown in step (3) in Scheme 1.

$$AA + Os\ (VIII) \underset{k_1}{\overset{k_{-1}\ (slow)}{\rightleftharpoons}} Complex\ (X) \qquad ...(1)$$

$$(X) \xrightarrow{k_2\ (fast)} Oxidation\ products + Os(VI) \qquad ...(2)$$

$$Os(VI) + 2Fe(CN)_6^{3-} \xrightarrow{k_3\ (fast)} 2\ Fe(CN)_6^{4-} + Os(VIII) \qquad ...(3)$$

$$Amino\ acid + Fe(CN)_6^{4-} \underset{k_4}{\overset{k_{-4}\ (fast)}{\rightleftharpoons}} Complex\ (Y) \qquad ...(4)$$

$$(Y) + Os(VIII) \xrightarrow{k_5\ (slow)} Oxidation\ products + Os(VI) + Fe(CN)_6^{4-} \qquad ...(5)$$

Scheme 1

Formation of $Fe(CN)_6^{4-}$ in step (3) poses problems in the reaction as it is found to catalyse the rate of oxidation. It appears that $Fe(CN)_6^{4-}$ takes part in the reaction by forming complexes with amino acids which are again prone to oxidation by oxidizing agents. Iron(II) complexes of amino acids are reported in literature[16] whereas iron(III) does not apparently form complexes with amino acids above pH 7. In some cases complexes of $Fe(CN)_6^{4-}$ with amino acids are also reported[17].

It is more probable for the Os(VIII)-catalysed reaction to proceed with the formation of mixed ligand ferrocyanide-amino acid complex (step-4) which may get oxidized in a rate determining step by Os(VIII) (step-5). Os(VI) formed is then reoxidized to Os(VIII) by ferricyanide in a fast step (step-3).

Applying the steady state condition with respect to (X), (Y) and Os(VI), the rate law equation for the disappearance of $Fe(CN)_6^{3-}$ may be obtained as:

$$d/dt[Fe(CN)_6^{3-}] = [\text{Amino acid}][Os(VIII)]\{[k_1 + k_4k_5/k_{-4} \times [Fe(CN)_6^{4-}]\} \qquad ...(6)$$

where $k_{-1} \ll k_2$ and $k_{-4} \gg k_5$ [Os(VIII)] have been taken as suitable approximations.

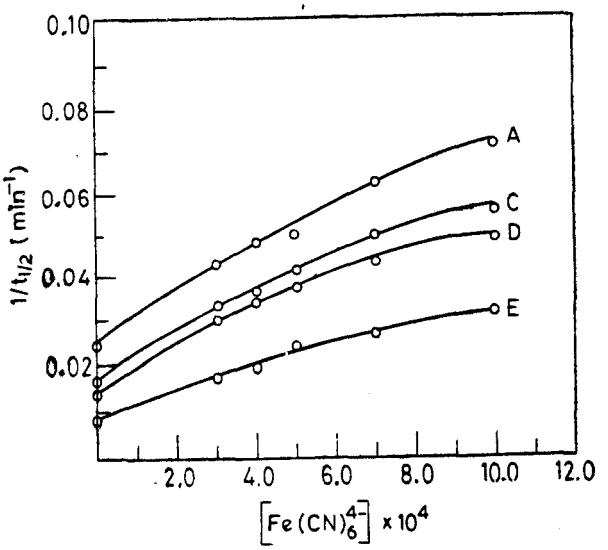

Fig. 2 — Plot of $1/t_{\frac{1}{2}}$ versus $[Fe(CN)_6^{4-}]$ for (A) leucine, (C) phenylalanine, (D) alanine and (E) valine {$[Fe(CN)_6^{3-}] = 5·0 \times 10^{-4}M$; [amino acid] = $4·0 \times 10^{-3}M$; $[OH^-] = 0·2M$; $[OsO_4] = 3·9 \times 10^{-6}M$}

The rate law (6) predicts that Os(VIII)-catalysed oxidation of amino acids by alkaline ferricyanide will follow a first order dependence with respect to amino acid and Os(VIII). The effect of ferrocyanide on reaction rate would be proportional to {constant + $[Fe(CN)_6^{4-}]$}. As has been mentioned in the earlier part of discussion, the rate determining reaction would involve interaction between negatively charged amino acid anion and $HOsO_5^-$ (step-1). For this step a positive salt effect is expected. Our experimental results are in complete agreement with the above facts.

Spectrophotometric evidence for the formation of ferrocyanide-amino acid complex — At 290 nm where ferrocyanide absorbs fairly strongly, the addition of amino acids in the presence of alkali caused about 30% reduction in the absorbance due to ferrocyanide. This fall in absorbance is, however, not so marked when [amino acid]: $[Fe(CN)_6^{4-}]$ ratio is greater than unity. As amino acids have negligible absorbance at this wavelength, the formation of a transient [amino acid-$Fe(CN)_6^{4-}$] complex which is weakly absorbing in comparison to $Fe(CN)_6^{4-}$ is visualized.

It is reported in literature that Os(VIII)-catalysed oxidations by ferricyanide either show a retarding influence on the addition of ferrocyanide[7,8] or show a decrease in rate at the end of every kinetic run[18]. The slight deviation from linearity of the plots shown in Fig. 2 could therefore be attributed to the fall in [Os(VIII)] caused by the reverse of step (3) at high [ferrocyanide].

A slight increase in the rate of oxidation of glycine and leucine with an increase in [alkali] can be explained on the basis of the fact that at high [alkali] the uncatalysed oxidation of glycine and leucine may become prominent resulting in an increase in the reaction rate. In the case of alanine, valine and phenylalanine the uncatalysed oxidation even at high concentrations of alkali is negligible and therefore, the rates are almost independent of [alkali].

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References

1. BACON, R. G. R., HANNA, W. J. W. & STEWART, D., *J. chem. Soc.* (*C*), (1966), 1388.
2. CHANDRA, G. & SRIVASTAVA, S. N., *J. inorg. nucl. Chem.*, **34** (1972), 197.
3. SHEIKH, R. A. & WATERS, W. A., *J. chem. Soc.* (*B*), (1970), 988.
4. BOSE, A. K., MEHROTRA, R. M. & MUSHRAN, S. P., *Indian J. Chem.*, **11** (1973), 896.
5. CLARKE, T. G., HAMPSON, M. A., LEE, J. B., MORLEY, J. R. & SCANLON, B., *J. chem. Soc.* (*C*), (1970), 815.
6. LAMBERT, D. G. & JONES, M. M., *J. Am. chem. Soc.*, **88** (1966), 4615.
7. JINDAL, V. K., AGRAWAL, M. C. & MUSHRAN, S. P., *J. inorg. nucl. Chem.*, **33** (1971), 2469.
8. JINDAL, V. K., AGRAWAL, M. C. & MUSHRAN, S. P., *J. chem. Soc.* (*A*), (1971), 622.
9. SOLYMOSI, F., *Naturwiss*, **44** (1957), 374; *Chemist Analyst*, **51** (1962), 71.
10. FEIGL, F., *Spot tests in organic analysis* (Elsevier, London), 1960, 236: 382.
11. HAKE, T., *Lab. Invest.*, **14** Pt 2 (1965), 1208.
12. NYILASI, J. & ORSOS, P., *Acta chim. Acad. Sci., Hung.*, **75** (1973), 405.
13. GREENSTEIN, J. P. & WINITZ, M., *Chemistry of amino acids*, Vol. 1 (John Wiley, New York), 1961, 486.
14. GRIFFITH, W. P., *Q. Rev.*, **19** (1965), 254.
15. NYILASI, J. & SOMOGYI, P., *Ann. Univ. Sci., Budapest Rolando Nominatae*, **6** (1964), 139; *Chem. Abstr.*, **62** (1965), 16363g.
16. ALBERT, A., *Biochem. J.*, **47** (1950), 531.
17. MALIK, W. U. & ASLAM, M., *Indian J. Chem.*, **8** (1970), 736.
18. SINGH, N. P., SINGH, V. N., SINGH, H. S. & SINGH, M. P., *Aust. J. Chem.*, **23** (1970), 921.