Os(VIII) & Ferrocyanide Catalysed Oxidation of Alanine, Phenylalanine & Valine by Ferricyanide

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The kinetics of Os(VIII)-catalysed oxidation of alanine, phenylalanine and valine by ferrocyanide have been studied. As the rates of the reactions are strongly dependent on ferrocyanide formed, the studies have been made at large [ferrocyanide] to avoid autocatalysis. The rates are independent of [ferricyanide] while the order in [amino acid] and [Os(VIII)] is nearly unity in each case. It is observed that the Os(VIII)-catalysed oxidation of α-amino acids by ferricyanide shows a very complex kinetics. The main oxidizing species has, however, been found to be Os(VIII) and ferricyanide is merely used up in regenerating the Os(VIII) species from the Os(VI) formed. A suitable mechanism involving formation of a transient complex (α-amino acid-ferricyanide) has been proposed.

The oxidation of some α-amino acids has been studied by several investigators1-7. It has been observed that the oxidation of α-amino acids by alkaline ferricyanide, though extremely slow even at high ionic strength8, is catalysed by Os(VIII) to give the corresponding keto acids. The keto acids as the end products have been reported in literature9,10 for the oxidation of α-amino acids by alkaline Os(VIII).

An interesting feature of the reaction of α-amino acids by ferricyanide in the presence of Os(VIII) is the strong catalytic influence of ferrocyanide ion11. The results of kinetic investigation of Os(VIII) amino acid-ferricyanide system in the presence of excess of ferrocyanide are reported in this paper and a suitable mechanism is advanced.

Materials and Methods

Aqueous solutions of DL-α-alanine, L-phenylalanine and DL-valine were freshly prepared from BDH reagents. Stock solutions of potassium ferricyanide and sodium hydroxide were prepared from analar BDH reagents. Osmium tetroxide used was from Johnson Matthey. Potassium ferrocyanide solutions were prepared from a recrystallized reagent. Doubly distilled water was used throughout. All other reagents used were of AR grade.

Kinetic measurements — The rate constants were measured by determining the concentration of ferrocyanide spectrophotometrically at 420 nm using a Bausch & Lomb spectronic-20 spectrophotometer. The [ferrocyanide ion] was kept below 5.0×10^{-4} M.

Identification of complex — Measurements were made on a Beckman model DU spectrophotometer at 290 nm. At this wavelength, where ferrocyanide absorbed fairly strongly, it was observed that addition of the increasing amount of amino acid in the presence of alkali gradually decreased the absorbance due to ferrocyanide (up to about 30%) until the [amino acid]:[Fe(CN)_{4}^{2-}] ratio became nearly unity. This decrease in absorbance was, however, small when the [amino acid]:[Fe(CN)_{4}^{2-}] ratio was greater than unity. As the absorbance due to amino acid was negligible at this wavelength, the existence of a transient {amino acid-Fe(CN)_{4}^{2-}} complex is indicated.

Stoichiometry — A reaction mixture containing a known excess of ferricyanide over amino acid was kept at 40°C in the presence of 0.1 M NaOH, 5.0×10^{-3} M Fe(CN)_{4}^{2-} and 3.90×10^{-4} M Os(VIII) (in case of phenylalanine Os(VIII) conc. = 2.34×10^{-4} M) for 6 hr. The amount of ferricyanide left was consistent with the stoichiometric Eq. (1)

\[\text{RCHNH}_{2}\text{COOH} + 2\text{Fe(CN)}_{4}^{2-} + 2\text{OH}^- = \text{RCOOCOOH} + \text{NH}_3 + \text{H}_2\text{O} + 2\text{Fe(CN)}_{4}^{2-}\] ... (1)

where R represents CH_{3}O, (CH_{3})_{2}CH- and C_{6}H_{5}CH_{2}- for α-alanine, valine and phenylalanine, respectively.

The presence of keto acids was detected by spot tests12.

Results

The α-amino acid-ferricyanide reactions were investigated at several initial [reactants]. It was observed that concentration-time plots (Fig. 1) in ferricyanide were linear in excess of ferrocyanide suggesting that the rate is independent on [ferricyanide]. Pseudo-zero order rate constants (k_{0}) in ferricyanide were nearly constant at all [ferricyanide] and thus further established zero order dependence in ferricyanide (Table 1).

The results of the effect of a change in concentration of amino acids and Os(VIII) are summarized in Tables 1 and 2 respectively. The plots of k_{0} versus [amino acid] (Fig. 2) showed a deviation from linearity at higher [amino acid] while a plot of 1/k_{0} vs 1/[amino acid] was linear with an intercept. It, therefore, appears that the order in amino acid falls from unity at higher [amino acid]. The plots of k_{0} vs Os(VIII)
were linear, establishing first order dependence in Os[VIII].

As the ferrocyanide is a product of the reduction of ferricyanide, its accelerating influence is encountered in every kinetic run. At low initial [ferrocyanide] the half-life periods \((t_{1/2})\) of the reaction dropped sharply with increase in [ferrocyanide] and plots of \([Fe(CN)]^{2+}\) against \(1/t_{1/2}\) were linear with positive intercepts.

**Discussion**

The zero order dependence in [ferricyanide] clearly suggests its involvement in fast steps. The strong catalytic influence of low [ferrocyanide] also suggests a possible interaction between amino acids and ferrocyanide. Under the experimental conditions the initial rates of oxidation are very small in comparison to that in the presence of ferrocyanide ion.

Iron(II) complexes of amino acids are reported in the literature and in some cases Fe(CN)⁺ complexes with the amino acids are also mentioned. In the present case the more favourable path of...
The disappearance of Fe(CN)₆⁻ is given by Eq. (5): 

\[ \frac{d}{dt} \text{[Fe(CN)₆]} = \frac{k₄ \text{[amino acid]} \cdot \text{[Os(VIII)]} \cdot \text{[Fe(CN)₆]}^4}{k₅ \text{[amino acid]} + k₆ \text{[Fe(CN)₆]}^5} \times \left\{ \frac{k₇ k₈ + k₉ \text{[Fe(CN)₆]}^7}{k₄ + k₅} \right]\] ... (5)

where \( k₄ \gg k₅ \text{[Os(VIII)]} \) has been taken as a suitable approximation.

Further step (iii) (Scheme 2) is very fast in comparison to step (vi) and, therefore, taking \( k₅ \times \text{[Fe(CN)₆]}^3 \geq k₆ \text{[amino acid]} \) the rate law (5) reduces to rate law (6):

\[ \frac{d}{dt} \text{[Fe(CN)₆]} = \frac{\text{[amino acid]} \cdot \text{[Os(VIII)]}}{k₄ + k₅} \times \left\{ \frac{k₆ k₇ + k₈ \text{[Fe(CN)₆]}^7}{k₄ + k₅} \right]\] ... (6)

The above rate law predicts that the rate of oxidation would be of first order in [amino acid] and [Os(VIII)] and of zero order in the [ferricyanide]. The effect of ferricyanide on the rate constants would be proportional to \{constant + [Fe(CN)₆]²\} as was observed experimentally.

The decrease in order in amino acids may be explained as follows. Since step (iii) (Scheme 2) is fast, Os(VI) formed during oxidation of amino acids is immediately converted to Os(VIII) by ferricyanide. It is only at high [amino acid] that some of Os(VI) is reduced to Os(IV) state via a slow step (vi) (Scheme 2). Evidently this will result in a decrease in [Os(VIII)] which will subsequently decrease the rate of oxidation. However, in such a case the plot of \( 1/k₉ \) versus \( 1/[\text{amino acid}] \) should be linear with an intercept according to rate law equation (5). This expectation is supported by the plots in Fig. 2. The values of the intercept agree with the relative rates of oxidation of \( \alpha \)-alanine, phenylalanine and valine by Os(VI) reported in the literature. The oxidation of valine being slowest by Os(VI) shows a negligible intercept.

Thus the fall of \( k₉ \) at high [amino acid] has been interpreted in terms of the fact that the term \( k₉ \text{[amino acid]} \) is not negligible in comparison to \( k₈ \text{[Fe(CN)₆]}^3 \) in the rate law (5). Consequently, at high [amino acid] the independence of rate on ferricyanide is not warranted. In fact, at high [amino acid] where the rate of disappearance of ferricyanide would become independent of [amino acid] a second order dependence in [ferricyanide] should be obtained. For moderately high [amino acid] the order in ferricyanide would therefore be between 0 to 2. Experimental studies at higher [amino acid] were made and results show that absorbance-time plots are no more linear. It is further observed that in the case of oxidation of \( \alpha \)-alanine which shows maximum variation from first order behaviour (Fig. 2) the order in ferricyanide obtained at higher [\( \alpha \)-alanine] is maximum (~2), while the same obtained for valine is minimum (~1). It therefore appears that the order in [amino acid] decreases from 1 to 0 while that for ferricyanide increases from 0 to 2 at high [amino acid].
This is in good agreement with the rate law equation (5).

According to above mechanism an increase in ionic strength will result in an increase in the rates of slow processes (ii) and (vi) (Scheme 2), both being between similarly charged ions [Os(VIII) exists as $\text{HOsO}_2$; Os(VI) as $\text{HOSO}_4^-$ and amino acid as its anion in alkaline media]. But increase in rate of step (ii) increases the rate of disappearance of ferricyanide (step iii) while increase in the rate of step (vi) results in loss of catalyst and thus a decrease in the rate of amino acid oxidation. There will, therefore, be a negligible salt effect. This is in agreement with our observations recorded earlier. However, in the absence of the initial excess of ferrocyanide, formation of Os(VI) would be too little via step (ii) and thus step (vi) would be of no consequence. Here a first order dependence in amino acid and a positive salt effect is expected. This has been found to be true in our earlier investigations.41

The dependence on ferrocyanide seems to be more complicated than the one presented in rate law (5). The levelling off of the $k_0$ values (Table 3) at high [ferrocyanide] may be possibly due to the back conversion of Os(VIII) by Os(VI) by way of reverse step (iii) at high [ferrocyanide]. As the rate constant is proportional to [Os(VIII)] and (const + [Ferro.]), a large increase in [ferrocyanide] when accompanied by a decrease in [Os(VIII)] would not result in any increase in the rate constants.

References