Chapter-4
Kineitecs and Mechanism of deamination and decarboxylation of 2-aminopentanedioic acid by quinoliniumdichromatre (QDC) in aqueous perchloric acid

INTRODUCTION

Oxidation of amino acids is of great importance both from chemical view point and from its bearing on the mechanism of amino acid metabolism. Amino acids not only act as building blocks in protein synthesis but also play a significant role in the metabolism. They are subjected to many reactions and can supply precursors for many endogenous substances like hemoglobin in blood. They can undergo many kinds of reactions, depending on whether a particular amino acid contains a polar or non-polar substituent. 2-Aminopentanedioic acid / L-Glutamic acid (glu-e) is one of the most abundant amino acids, especially high in cereal proteins and can be oxidized by different oxidants [1-4]. A salt of glu-e is used in the production of monosodium glutamate and nutritional supplements. L-glutamate itself can be used as medicine and promotes oxidation process. It combines with ammonia as a drug-free glutamine. It is mainly used for the treatment of hepatic coma and severe liver dysfunction, but the response is not satisfactory. Racemic
glutamate is used for the production of drugs. Physiologically, it plays a role in the metabolism of amino groups and is the precursor of neurotransmitter, gamma-aminobutyric acid, L-glutamic acid. Being acidic in nature it is important in determining 3-D conformation of proteins.

It is well established that the reduction of chromium(VI) to chromium(III) with a variety of organic and inorganic reductants can occur by a multiplicity of mechanisms which depend on the nature of the reducing agent [5]. The existence of different species of chromium(VI) in acid solutions, unstable oxidation states [chromium(IV) and chromium(V)] and the tendency of chromium(III) to form a variety of complexes, all combine to give systems of considerable complexity [6]. Attempts have been made to confirm the intermediacy of chromium(IV) and chromium(V) by use of competitive experiments [7–8]. In acid solution, the reported reduction potential of the Cr(VI)/Cr(III) couple is 1.33V [9]. The reagent, quinolinium dichromate (QDC) is a versatile oxidant that deserves further investigation and some kinetic studies of the oxidation of inorganic substrates by QDC are available [10]. A number of reports on the mechanism of oxidation of several substrates by quinolinium dichromate are available which is shown to oxidize primary and secondary alcohols to the corresponding aldehydes [11-12], cyclic alcohols to cyclic ketones [13], bicyclic alcohols [14] and benzyl alcohol [15]. The α-hydroxyacids [16] and α-ketoacids [17] are oxidized with QDC and their
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reactions are studied kinetically. QDC oxidizes cinnamic and crotonic acids smoothly in N,N-dimethylformamide in the presence of an acid to give aldehydes [18-27].

Since there are no reports on the kinetics of oxidation of 2-aminopentanedioic acid by QDC we are reporting the kinetics of its oxidation by QDC in perchloric acid, in order to identify the chromium(VI) intermediate and to propose a suitable mechanism. The activation parameters and thermodynamic quantities have been determined and discussed.

EXPERIMENTAL

Stock solutions of L-glutamic acid (Merck, Mumbai, India). quinolinium dichromate (QDC) (Sigma-Aldrich) were prepared in double distilled water and standardized iodometrically [12]. HClO$_4$ and NaClO$_4$ were employed to maintain the required acidity and ionic strength, respectively.

Kinetic Studies

The reaction was initiated by mixing the previously thermostated solutions of Glu-e and QDC, which also contained the required amount of perchloric acid, sodium perchlorate and double distilled water. The reaction was followed spectrophotometrically at 360 nm. The spectral changes during
the reaction under standard conditions at room temperature are given in Fig.1. Application of Beer's law under the reaction conditions was verified between $1.0 \times 10^{-4}$ and $1.0 \times 10^{-3}$ mol dm$^{-3}$ of QDC and the extinction coefficient was found to be $\varepsilon = 1,246 \pm 12$ dm$^3$mol$^{-1}$cm$^{-1}$. The pH of the reaction mixture in the beginning and in the end remained constant. The kinetic runs were followed for more than 80% completion of the reaction and first order kinetics was observed. The pseudo-first order rate constants, $k_{obs}$ obtained from the slope of the plots of log(absorbance) versus time were linear (Fig.2). The $k_{obs}$ were reproducible within ±5% and are the average of at least three independent kinetic runs (Table 1).

**RESULTS AND DISCUSSION**

**Reaction Orders**

The order of reaction was determined from a plot of log $k_{obs}$ versus log(concentration) of QDC ($2.0 \times 10^{-4}$ to $2.0 \times 10^{-3}$ mol dm$^{-3}$) at constant concentration of Glu-e ($2.0 \times 10^{-2}$ mol dm$^{-3}$, HClO$_4$ = 3.0 mol dm$^{-3}$) and ionic strength, $I = 2.80$ mol dm$^{-3}$, and was found to be unity. Negligible variation in $k_{obs}$ occurs with varying concentration of QDC (Table 1). The plot of log $[a/(a-x)]$ versus time for different concentrations of QDC is linear and uniform (Fig.2). The Glu-e concentration was varied in the range of ($5 \times 10^{-3}$ to $5 \times 10^{-1}$).
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2 mol dm\(^{-3}\) at constant concentration of QDC, HClO\(_4\) and ionic strength was found to be uniform (Table 1).

**Product Analysis**

Different sets of reaction mixtures containing glu-e, an excess of QDC with constant ionic strength and acidity were left to stand for 12 h in an inert atmosphere at 25°C. The succinic semialdehyde produced was also identified from FT-IR spectrum. The \(v(C=O)\) appears at 1696 cm\(^{-1}\) (Fig.3) which is the characteristic band of aldehyde. It does not undergo further oxidation. Ammonia was identified by Nessler's reagent [29]. The CO\(_2\) liberated was qualitatively detected [30] by passing the gas into lime water. The results indicated that two moles of QHCrO\(_4\) (in term of Cr (VI)) consumed four mole of glu-e (Scheme 1.)

\[
4 \text{COOHCH}_2\text{CH}_2\text{CHNH}_2\text{COOH} + 2 \text{QHCrO}_4 + 2 \text{H}^+ \\
\downarrow \\
4 \text{COOHCH}_2\text{CH}_2\text{CHO} + 2\text{CrO}_2^- + 4\text{CO}_2 + 4\text{NH}_3 + 4 \text{H}^+ + 2\text{Q}
\]

Scheme 1
**Kinetic Study of 2-aminopentanedioic acid by quinolinium dichromate**

**Effect of Temperature**

The rate of reaction was measured at different temperature with varying concentration of perchloric acid and keeping other conditions constant. The rate constant, $k$ obtained from the intercept of the plots of $1/k_{obs}$ versus $1/\text{glu-e}$ was found to increase with temperature. The values of rate constant of glu-e at 293 K, 298 K, 303 K, and 308 K, was found to be 6.50 s$^{-1}$, 7.36 s$^{-1}$, 11.32 and 13.28 s$^{-1}$, respectively. The energy of activation corresponding to these constants was evaluated from a plot of log $k$ versus $1/T$ (Table 2).

**Effect of perchloric acid Concentration**

The effect of acidity on the rate of reaction was studied by varying the concentration of the perchloric acid (2.5-4.5 mol dm$^{-3}$). The $k_{obs}$ was found to be directly proportional to the concentration of the acid (Table 1).

**Effect of Ionic Strength and Dielectric Constant**

When the ionic strength was varied the rate constant was found to be proportional to the ionic concentration. The plot of log $k_{obs}$ versus $I$ was linear with positive slope (Fig.5) ($r > 0.9985$, $\sigma < 0.0836$). The effect of relative permittivity ($D$) on the rate constant was studied by varying the acetic acid content (v/v). Attempt to measure the relative permittivity was unsuccessful. However, they were computed from the values of pure liquids [31]. The rate
constant was found to increase with increase in the dielectric constant of the medium. A plot of $\log k_{\text{obs}}$ versus $1/D$ was linear with negative slope (Fig.6) ($r > 0.9614, \sigma < 0.0791$).

The QDC reacts with two moles of HClO$_4$ to give H$_2$CrO$_4$ [32-33]. It H$_2$CrO$_4$ reacts with glu-e forming a complex, which is reduced to an intermediate chromium(IV) and a product of glu-e. Subsequently, another molecule of acid chromate reacts with one molecule of glu-e producing another chromium(IV) intermediate, and a product of glu-e. In a further fast step, one molecule of chromium(IV) reacts with one more molecule of glu-e giving chromium(III) and final product of succinic semialdehyde (Scheme 2).

The results indicate the formation of a complex between glu-e and chromium(VI) in presence of perchloric acid. The formation of this complex was proved kinetically by Michaelis–Menten plot, i.e., a non-zero intercept of the plot of $1/k_{\text{obs}}$ versus $1/[\text{glu-e}]$ (Fig.4). The colour indicates the formation of Cr(VI)-species and appearance of Cr(III)-species with the isosbestic point at $\lambda=530$ nm.
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$$Q^+ DC + 2H^+ \overset{K_1}{\rightleftharpoons} H_2CrO_4 + Q \text{(Quinoline)}$$

$$H_2CrO_4 + COOHCH_2CH_2CHNH_2COOH \overset{K_2}{\rightleftharpoons} \text{Complex}$$

$$\text{Complex} \overset{k}{\xrightarrow{\text{Slow}}} COOHCH_2CH_2CHO + H_2CrO_3 + CO_2 + NH_3 \quad \text{Cr(IV)}$$

$$H_2CrO_4 + COOHCH_2CH_2CHNH_2COOH \overset{\text{Fast}}{\rightarrow} COOHCH_2CH_2CHO + H_2CrO_3 \quad \text{Cr(IV)}$$

$$\quad + CO_2 + NH_3$$

$$H_2CrO_3 + COOHCH_2CH_2CHNH_2COOH \overset{\text{Fast}}{\rightarrow} COOHCH_2CH_2CHO + CrO_2 \quad \text{Cr(III)}$$

$$\quad + CO_2 + NH_3 + 2H^+$$

Scheme 2
Appearance of isosbestic point indicates very low concentration of the probable intermediates like Cr(V) Cr(IV) [34] and their gradual decay to [Cr(III)]. The characteristic of electronic spectrum of Cr(III)-species lies in the range 320–600 nm. The original absorption maxima, (560 and 430 nm) was replaced by a single peak at 360 nm due to the formation of chromium(VI). Similar results have also been obtained by the oxidation of 2-propanol from chromium(VI) in aqueous acetic acid and, oxidation of thallium(I) by QDC in aqueous acetic acid-chloride media [35-36].

\[
\text{Rate} = k \text{ Complex C}
\]

\[
\text{Rate} = -\frac{d[QDC]}{dt} = kK_1K_2[\text{Glu-e}]_f [QDC]_f [H^+]_f^2 \quad (1)
\]

The total concentration of QDC is given by,

\[
[QDC]_t = [QDC]_f + [H_2CrO_4] + [\text{Complex C}]
\]

\[
[QDC]_t = [QDC]_f + K_1[QDC]_f [H^+]_f^2 + K_1K_2[\text{Glu-e}]_f [QDC]_f [H^+]_f^2
\]

\[
= [QDC]_f + \{ 1 + K_1 [H^+]_f^2 + K_1K_2[\text{Glu-e}]_f [H^+]_f^2 \} \]
Therefore

\[
[QDC]_f = \frac{[QDC]_t}{1 + K_1 [H^+]_f^2 + K_1 K_2 [\text{Glu-e}]_f [H^+]_f^2}
\]

(2)

where ‘t’ and ‘f’ stand for total and free concentration of QDC.

\[
[\text{Glu-e}]_t = [\text{Glu-e}]_f + [\text{Complex C}]
\]

\[
= [\text{Glu-e}]_f + K_1 K_2 [\text{Glu-e}]_f [QDC]_f [H^+]_f^2
\]

\[
= [\text{Glu-e}]_f \{ 1 + K_1 K_2 [QDC]_f [H^+]_f^2 \}
\]

Therefore,

\[
[\text{Glu-e}]_t = \frac{[\text{Glu-e}]_f}{1 + K_1 K_2 [QDC]_f [H^+]_f^2}
\]

(3)
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In view of the low concentration of QDC used in the experiment (Eq. 3) the term $K_1K_2 [QDC]_f [H^+]_f^2$ can be neglected in comparison to unity.

Hence,

$$[\text{Glu-e}]_f = [\text{Glu-e}]_f$$ (4)

and

$$[H^+]_f^2 = [H^+]_f^2 + [H_2CrO_4] + [\text{Complex C}]$$

$$= [H^+]_f^2 + K_1[QDC]_f[H^+]_f^2 + K_1K_2[\text{Glu-e}]_f [QDC]_f [H^+]_f^2$$

$$= [H^+]_f^2 \left\{ 1 + K_1 [QDC]_f + K_1K_2 [\text{Glu-e}]_f [QDC]_f \right\}$$

$$[H^+]_f^2 = \frac{[H^+]_f^2}{1 + K_1 [QDC]_f + K_1K_2 [\text{Glu-e}]_f [QDC]_f}$$ (5)

Similarly,

$$[H^+]_f^2 = [H^+]_f^2$$ (6)

Substituting Eqs. 2, 4, and 6 in Eq. 1, we get Eq. 7, which explains all the experimentally observed orders with respect to different species of reaction.
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\[
\text{Rate} = - \frac{d[\text{QDC}]}{dt} = \frac{kK_1K_2[\text{Glu-e}][\text{QDC}][H^+]^2}{1 + K_1[H^+]^2 + K_1K_2[\text{Glu-e}][H^+]^2}
\]

Or

\[
\frac{\text{Rate}}{[\text{QDC}]} = K_{\text{obs}} = \frac{kK_1K_2[\text{Glu-e}][H^+]^2}{1 + K_1[H^+]^2 + K_1K_2[\text{Glu-e}][H^+]^2}
\]

The rate law (8) can be rearranged to Eq. 9, which is suitable for verification.

\[
\frac{1}{K_{\text{obs}}} = \frac{1}{kK_1K_2[\text{Glu-e}][H^+]^2} + \frac{1}{K_1K_2[\text{Glu-e}]} + \frac{1}{k}
\]

According to Eq. 8, plots of 1/kobs versus 1/[glu-e] and 1/kobs versus 1/[H^+]^2 should be linear (Fig. 4). The slopes and intercepts of such plots determine the values of \( K_1, K_2, k \) and the thermodynamic quantities for the first and second equilibrium steps of Scheme 2 (Table 2).

For example at 35 °C:

(i) From the plot of 1/kobs versus 1/[glu-e]

(Intercept) \(_1 = 1 / k\)
Therefore, \( k = \frac{1}{(\text{Intercept})_1} = 1.328 \times 10^{-2} \text{sec}^{-1} \)

(Slope) \( _1 = \frac{1}{kK_1K_2[H^+]^2} + \frac{1}{k} \)

(ii) From the plot of \( 1/k_{obs} \) versus \( 1/[H^+]^2 \)

(Intercept) \( _2 = 91.37 = \frac{1}{kK_1K_2[\text{Glu-e}]} + \frac{1}{k} \)

From (Intercept) \( _1 \), the value of \( k = 1.328 \times 10^{-2} \text{sec}^{-1} \)

Hence,

\[ 91.37 = \frac{1}{1.328 \times 10^{-2} \times K_2 \times 2.0 \times 10^{-2}} + \frac{1}{1.328 \times 10^{-2}} \]

\[ 1 + K_2(2.0 \times 10^{-2}) = 91.37 \times 1.328 \times 10^{-2} K_2 \times 2.0 \times 10^{-2} \]

\[ 1 + 0.02 K_2 = 0.0242678 K_2 \]

\[ K_2 = 234.31 \text{dm}^3 \text{mol}^{-1} \]

(Slope) \( _2 = 812.2 = \frac{1}{k K_1 K_2 [\text{Glu-e}]} \)

\[ 812.2 = \frac{1}{1.328 \times 10^{-2} \times 234.31 \text{dm}^3 \text{mol}^{-1} \times 2.0 \times 10^{-2} K_1} \]

\[ K_1 = 1.978 \times 10^{-2} \text{dm}^6 \text{mol}^{-2} \]

At 35°C:

\[ K_1 = 1.978 \times 10^{-2} \text{dm}^6 \text{mol}^{-2} \]

\[ K_2 = 234.31 \text{dm}^3 \text{mol}^{-1} \]

\[ k = 1.328 \times 10^{-2} \text{sec}^{-1} \]

Similarly, \( k, K_1 \) and \( K_2 \) were calculated at different temperatures (Table 2). Van’t Hoff plots were made (log \( K_1 \) versus \( 1/T \) and log \( K_2 \) versus \( 1/T \)). The \( \Delta H, \Delta S \) and \( \Delta G \) were calculated for the first and second equilibrium steps (Scheme 2). A comparison of these values (Table 2) with those obtained for the
slow step refer to the rate limiting step, supporting the fact that the reaction before the rate determining step is fairly fast and involves a high activation energy [37-39]. An increase in the volume of acetic acid leads to an increase in the reaction rate. A plot of $\log k_{obs}$ versus $1/D$ was linear with positive slope which is contrary to the convention. Perhaps the effect is countered substantially by the formation of active species to a greater extent in a low dielectric constant medium leading to the net increase in the rate [40]. The mechanism is also supported by moderate values of $\Delta H$ and $\Delta S$. The negative $\Delta S$ value indicates that the complex (C) is more ordered than the reactants [41]. The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation, presumably occurs via an inner-sphere mechanism. This conclusion is supported by observations [42-44] made earlier.

CONCLUSION

The reaction between Glu-e and QDC is very slow in low perchloric acid concentration at room temperature. The oxidant, chromium(VI) exists in acid medium as $H_2CrO_4$, which takes part in the chemical reaction. The rate constant of slow step and other equilibrium constants involved in the mechanism are evaluated and activation parameters with respect to slow step of reaction were computed. The overall mechanistic sequence described here, is consistent with the products formed.
Fig. 1 Spectral changes during the oxidation of Glu-e by QDC in aqueous perchloric acid at 25°C; QDC = 8.0 × 10^{-2}/mol dm^{-3}, [Glu-e] = 2.0 × 10^{-2}/mol dm^{-3}, [HClO4] = 3.0, and I = 2.80/mol dm^{-3} (scanning time interval 1.0 min)
Fig. 2 Plot of log a/(a-x) versus time in aqueous perchloric acid at 25 °C,

Glu-e = 2.0 \times 10^{-2}, [HClO_4] = 3.0, I = 2.80 mol dm^{-3} and QDC = (A) 2.0
(B) 6.0, (C) 8.0, and (D) 10.0 mol dm^{-3}
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Fig. 3 FT-IR spectrum of the product of oxidation of Glu-e by QDC

Fig. 4 Verification of rate law (7) in the form of Eq. 9 (Table 1)
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Fig. 5 Effect of ionic strength on the oxidation of Glu-e by QDC in aqueous perchloric acid medium at 25°C

Fig. 6 Effect of dielectric constant on the oxidation of Glu-e by QDC in aqueous perchloric acid medium at 25°C
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Table 1. Effect of variation of QDC, Glu-e, and perchloric acid on the oxidation of glu-e by QDC in perchloric acid medium at \( I = 2.80 \text{ mol dm}^{-3} \) and at 25°C (Scheme 2)

<table>
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<tr>
<th>[QDC] ( \times 10^4 ) (mol dm(^{-3}))</th>
<th>[Glu-e] ( \times 10^2 ) (mol dm(^{-3}))</th>
<th>[HClO(_4)] (mol dm(^{-3}))</th>
<th>( k_{\text{obs}} \times 10^{-3} ) (s(^{-1}))</th>
<th>( k_{\text{cat}} \times 10^{-3} ) (s(^{-1}))</th>
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_Table 2. Activation parameters and thermodynamic quantities of the oxidation of glu-e by QDC in aqueous perchloric acid medium._

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<td>13.28</td>
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(a) Effect of temperature with respect to slow step of the Scheme 1

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<td>ΔH^± (kJ mol^-1)</td>
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<td>ΔG^± (kJ mol^-1)</td>
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(b) Activation parameters

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(d) Thermodynamic Parameters using K_1 values using K_2 values

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<td>ΔG^± (kJ mol)</td>
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Kineic Study of 2-aminopentanedioic acid by quinoliniumdichromatre


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