Mechanism of Oxidation of L-Cysteine by Tetraoxoiodate(VII) in Aqueous Acid Medium

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The kinetics and mechanism of the oxidation of L-cysteine by tetraoxoiodate(VII) ion in aqueous acid medium has been studied at $0.03 \leq [H^+] \leq 0.1$ mol dm^{-3} under pseudo-first order conditions of an excess of tetraoxoiodate(VII) concentration at $1 = 0.11$ mol dm^{-3} (NaClO$_4$). The reaction obeys the rate expression:

$$-d\,[IO_4^-]/dt = \{k_3K_1K_2[H^+] + k_5\}\,[RSH][IO_4^-]$$

Addition of AcO$^-$ and NO$_3^-$ had no effect on the reaction but the rate of reaction decreased with increase in ionic strength of the medium. Increase in dielectric constant decreased the rate of reaction. The rates are consistent with a mechanism which involves the formation of free radicals which subsequently dimerized into disulfides. The reaction has been rationalized on the basis of the inner-sphere electron transfer mechanism.

Keywords: L-Cysteine, Oxidation, Mechanism, Tetraoxoiodate(VII).

INTRODUCTION

L-Cysteine, is a naturally occurring sulfur-containing non-essential amino acid found in living cells[1] and an important structural and functional component of many proteins and enzymes. It is unique among the twenty amino acids required by human because it contains a sulfhydryl (-SH) group which is involved in the formation of disulfide bonds that are crucial in defining the structures of many proteins. The sulfhydryl group is a nucleophilic center and therefore can undergo addition and substitution reactions. It is an important precursor in the intra-cellular manufacture of the antioxidant, glutathione, which protects cells from toxins such as free radicals[2] through its ability to regenerate and prolong the activity of vitamin E[3]. In human biological system L-cysteine is often involved in electron transfer reactions, helps in enzyme-catalyzed reactions and is also oxidized by oxygen to give cysteine sulfonic acid[4]. L-Cysteine is also able to bind to heavy metal ions in biological systems thus acting as a scavenger in the removal of toxic metal ions from the human body[5]. L-cysteine and its derivatives form stable metal complexes[6-8] with 3*d* metal fragments and therefore can potentially be employed as new agents for metal chelation therapy[9,10].

The thiol group undergoes nucleophilic attack, electron transfer, hydride transfer, hydrogen radical transfers and oxygen atom transfer reactions[11,12]. Kinetic information has been reported on the oxidation of L-cysteine and closely related thiols by metal complexes[13-15]. These reports indicate that oxidation of the thiols to disulphide may involve binuclear complex formation prior to the electron transfer step. There were also cases where the involvement of binuclear complexes were not established in the redox process[16,17]. Iyun *et al.*[18] reported a 2:1 stoichiometry for the reaction of L-cysteine with oxobridged ruthenium ion (RuORu)$^{4+}$. Also the reaction showed inverse acid dependence and negative Bronsted-Debye salt effect. The reaction operated by an outer-sphere mechanism. However, oxidation of L-cysteine and DL-penicillamine by Cr(VI) in both high and low pH medium showed a sulphur-linked chromate ester as possible intermediate and followed innersphere-pathway[19,20].

In this report we have for the first time followed the course of the electron transfer reaction of L-cysteine, a biologically important thiol with an oxyanion. We are interested in its reaction with oxygen donors outside the body to ascertain whether the reaction will conform to the mechanism assumed when reacting with pure oxygen in living cells leading to formation of cystine sulphonic acid or otherwise. Also we hope this study will extend the frontier of knowledge in understanding the role assumed by thiols as electron transfer enzymes[21,22].

EXPERIMENTAL

L-Cysteine (Koch-light Lab. MP 220 °C) hereafter denoted as RSH was ascertained pure by melting point determination. KIO$_4$ (East Anglia Chemicals, 99.8 %) was standardized iodimetrically. All other reagents were AnalaR grade are used without further purification unless otherwise stated.

Kinetic studies were conducted *via* spectrophotometry by monitoring the increase in absorbance of the reaction mixture at 480 nm (λ_{max} of aqueous iodine)[23] as a function of time using Milton Roy Spectronic 2ID Spectrophotometer. All measurements were made under pseudo-first order conditions of excess periodate and at constant ionic strength of. 0.11 mol dm^{-3} (NaClO$_4$) unless otherwise stated. Under such conditions, kinetic curves were exponential and rate constants were obtained from logarithmic plots of absorbance differences against reaction time. Specific rates from replicate runs were in close agreement (std dev. = 0.01). Second order rate constants, k$_2$, were derived as ratios of k$_{obs}$/[IO$_4^-$] for each run.

RESULTS AND DISCUSSION

The stoichiometry of the reaction was determined by spectrophotmeteric titration. The [H$^+$], [RSH] and ionic strength were kept constant at 0.1 mol dm^{-3}, 1.0×10^{-3} mol dm^{-3} and 0.11 mol dm^{-3} (NaClO$_4$) and 0.1 mol dm^{-3} respectively at 23.5 ± 0.5 °C. The concentration of periodate was varied from 1×10^{-4} to 1×10^{-2} mol dm^{-3}. Plot of absorbance at completion of reaction *versus* [RSH]/[IO$_4^-$] gave a sharp break at 6.80 ± 0.03. This result indicates a stoichiometry of one mole of IO$_4^-$ reacting with about seven moles of RSH as represented with eqn. 1.

$$2IO_4^- + 2H^+ + 14RSH \longrightarrow 7RSSR + 8H_2O + I_2 \quad (1)$$
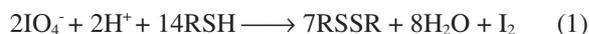
One of the products of the reaction, RSSR, a disulphide, has no effect on the rate of reaction in excess IO$_4^-$. However, when RSH is used in excess the rate of reaction is retarded. This could be attributed to product accumulation. Production of disulphide, RSSR, as a by-product of oxidation of RSH has been documented by other workers[17,18,23]. In this reaction evidence for disulphide being the organic product of reaction and not sulphoxide was confirmed by the method reported by McAuley *et al.*[24,25]. Evidence for iodine being of one of the products of the reaction was indicated by the sudden change of the reaction mixture from colourless to yellowish brown. The UV-visible spectra of this solution showed wavelength maxima of 480 nm which is very similar to the absorption maxima of aqueous iodine[26,27]. Test with starch solution was positive.

Under pseudo-first order conditions with [IO$_4^-$] at least 20-fold excess and isolation of L-cysteine at [H$^+$] = 0.1 mol dm^{-3}, plots of log (A$_\infty$–A$_t$) were linear to about 85 % extent of reaction indicating unit order with respect to RSH. Also, varying of [IO$_4^-$] from 8.0-10. 0 × 10^{-3} mol dm^{-3} at constant [RSH] at [H$^+$] = 0.1 mol dm^{-3}, the results (Table-1) indicate increase of pseudo-first order rate constants, k$_{obs}$, with [IO$_4^-$].

A plot of log k$_{obs}$ *versus* log [IO$_4^-$] (Fig. 1), was linear (R^2 = 0.997) with a slope of 0.997 indicating first order with respect [IO$_4^-$]. Also, a plot of k$_{obs}$ *vs*. [IO$_4^-$] was linear passing through the origin thereby confirming first order. This gives the rate law at 0.1 mol dm^{-3} [H$^+$] to be.

TABLE-1
SECOND ORDER RATE CONSTANTS FOR THE OXIDATION OF L-CYSTEINE BY PERIODATE ION IO$_4^-$ IN AQUEOUS ACID MEDIUM AT 23.5 ± 0.5 °C AT (RSH) = 0.001 mol dm^{-3}

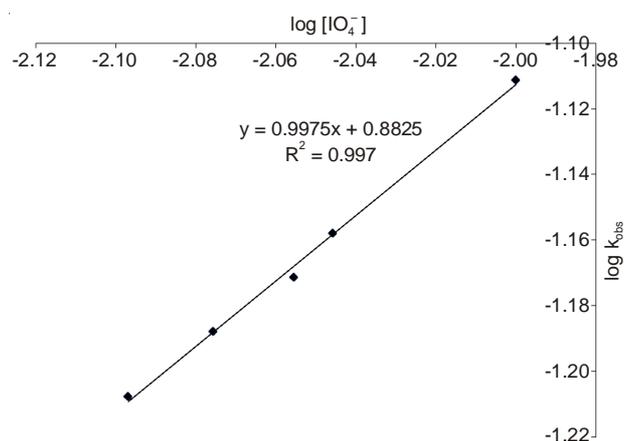| 10^3 [IO$_4^-$] (mol dm^{-3})	[H$^+$] (mol dm^{-3})	I (NaClO$_4$) (mol dm^{-3})	10^3 k$_{obs}$ (s^{-1})	k$_2$ (dm^3 mol^{-1} s^{-1})
8.0	0.10	0.11	6.20	7.75
8.4	0.10	0.11	6.49	7.73
8.8	0.10	0.11	6.74	7.66
9.0	0.10	0.11	6.95	7.72
10.0	0.10	0.11	7.74	7.74

Fig. 1. A plot of log k$_{obs}$ *versus* log [IO$_4^-$] for the oxidation of RSH by IO$_4^-$

$$-d[IO_4^-]/dt = k_2[IO_4^-][RSH] \quad (2)$$

Pseudo-first order rate constants have a linear dependence on [IO$_4^-$] at constant [H$^+$] (Table-1). It is from these results that second order rate constants, k$_2$, were evaluated as the ratio k$_{obs}$/[IO$_4^-$]. The k$_2$ values are fairly constant (7.72 ± 0.035 dm^3 mol^{-1} s^{-1}). Within the range 0.03 ≤ [H$^+$] ≤ 0.1 mol dm^{-3} and other parameters kept constant, the rate of reaction increased with [H$^+$] as shown in Table-2.
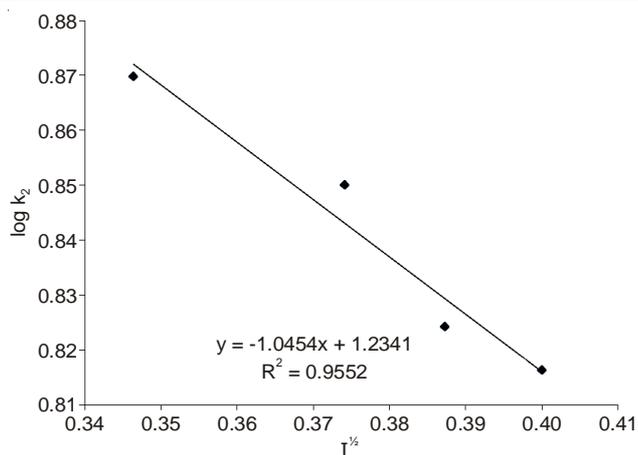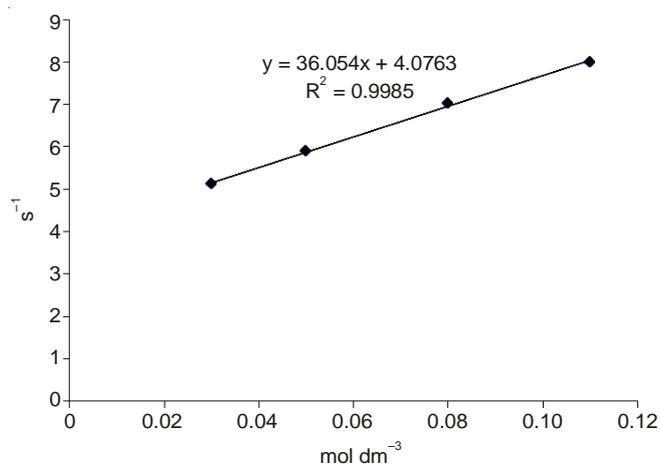
TABLE-2
EFFECT OF ACID CONCENTRATION ON RATE OF OXIDATION OF L-CYSTEINE BY IO$_4^-$ IN AQUEOUS ACID MEDIUM AT 23.5 ± 0.5 °C

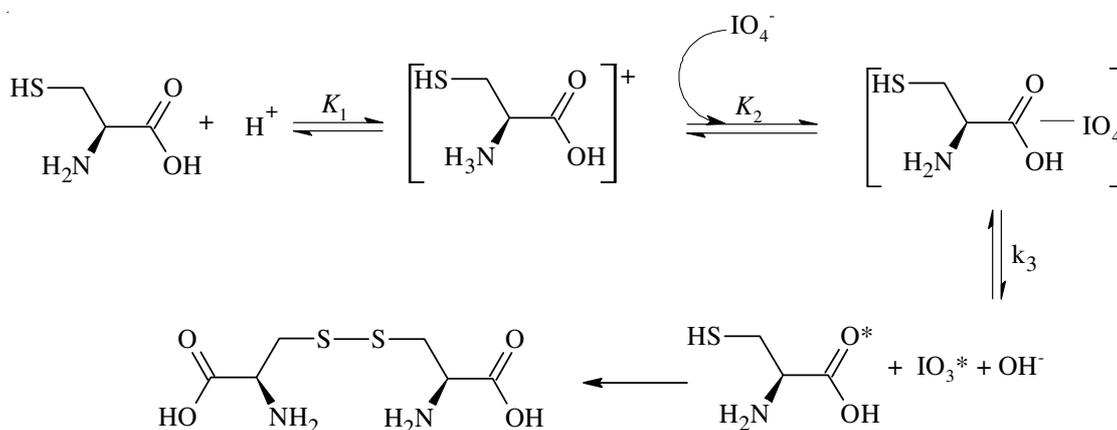| 10^3 (IO$_4^-$) (mol dm^{-3})	[H$^+$] (mol dm^{-3})	I (NaClO$_4$) (mol dm^{-3})	k$_2$ (dm^3 mol^{-1} s^{-1})
8.0	0.03	0.11	5.12
8.0	0.05	0.11	5.90
8.0	0.08	0.11	7.02
8.0	0.11	0.11	8.00
8.0	0.10	0.11	7.75

A plot of log k$_2$ against I$^{1/2}$ (Fig. 2) was linear (R^2 = 0.9552) and gave a slope of -1.05 suggesting that the product of the charges at the rate determining step is negative. Plot of rate constant against [H$^+$] in this acid range was linear and fitted eqn. 3:

$$k_2 = a + b [H^+] \quad (3)$$

The values of a and b as determined from Fig. 3 are 4.08 dm^3 mol^{-1} s^{-1} and 36.05 dm^6 mol^{-2} s^{-1} respectively. Enhancement of rate of reaction on increasing [H$^+$] may be resulting from the various equilibria established by IO$_4^-$ in aqueous solution[28] as shown below.

Fig. 2. log k_2 *versus* $I^{1/2}$

Fig. 3. k_2 *versus* $[H^+]$

$$IO_4^- + 2H_2O \longrightarrow H_4IO_6^- \qquad (4)$$

$$H_4IO_6^- + H^+ \longrightarrow H_5IO_6 \qquad (5)$$

These protonated species contributed meaningfully to the overall reaction rate since they are likely to be more reactive than IO_4^-. Also under acidic conditions (pH < 7) amino acids are known to possess charged groups resulting from the protonation of the amino group[29] to $-NH_3^+$. **Scheme-I** shows the proposed sequence of protonation of L-cysteine, reaction with IO_4^- and formation of L-cystine.

Within the acid range of this reaction (pH < 1.0), L-cysteine (RSH) instead of being in the Zwitterionic form will exist as the cationic form $HRSH^+$. This proposition was also made in the reaction between RSH and trisoxalatocobaltate(III) ion[23]. It was suggested as the reason for the dependence of the reaction on $[H^+]$. Electrostatically, $HRSH^+$ is favoured over RSH as the reactive intermediate since the oxidant is negatively charged. It is our opinion that the reactive species in the title reaction is $HRSH^+$.

Variation of ionic strength of the medium from 0.11-0.18 mol dm^{-3} (NaClO$_4$) (Table-3) resulted in negative Brønsted-Debye primary salt effect. The rate of reaction decreased as ionic strength increased. Least mean square plot of log k_2 against $I^{1/2}$ gave a slope of -1.05 suggesting that the product of charges at the rate determining step is negative. This indicates that oppositely charged species with unit charges respectively are interacting[30]. Also, the rate of reaction decreased as the dielectric constant (acetone-H$_2$O mixture) of the medium increased. Change in dielectric constant from 69.74 to 77.71 caused a reduction in the second order rate constant from 5.25-1.05 dm^3 mol^{-1} s^{-1}. At dielectric constant of 78.94 there was no noticeable reaction for up to 2 h. This corroborates operation of anion-cation interaction at the rate determining step[31]. Addition of varying concentrations (0.01-0.5 mol dm^{-3}) of acetate and nitrate ions in the reacting system did not affect the rate of the reaction. Second order rate constant k_2 values were within the range of 7.69 to 7.75 dm^3 mol^{-1} s^{-1}. This is suggestive of an inner-sphere mechanism[32]. However, no detectable intermediates were observed. This seems to be in agreement with the observation of Abdel-Khalek[33] in other reactions of IO_4^- where inner-sphere pathway was reported although with no detectable intermediates. Based on the acid

TABLE-3
EFFECT OF IONIC STRENGTH ON THE RATE OF
OXIDANTION OF L-CYSTEINE BY IO_4^- IN AQUEOUS
ACID MEDIUM AT 23.5 ± 0.5 °C AT (RSH) = 0.001 mol dm^{-3}

10^3 $[IO_4^-]$ (mol dm^{-3})	$[H^+]$ (mol dm^{-3})	I (NaClO$_4$) (mol dm^{-3})	k_2 (dm^3 mol^{-1} s^{-1})
8.0	0.10	0.12	7.41
8.0	0.10	0.14	7.08
8.0	0.10	0.15	6.67
8.0	0.10	0.16	6.55

Scheme-I: Sequence of protonation of L-cysteine, reaction with IO_4^- and formation of L-cystine

dependence path of the reaction and substituting eqn. 3 into eqn. 2 we have the rate law to be

$$-d[IO_4^-]/dt = \{a + b\,[H^+]\}[IO_4^-][RSH] \qquad (6)$$

Eqn. 6 results from the following steps:

$$7RSH + 7H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} 7HRSH^+ \qquad (7)$$

$$HRSH^+ + IO_4^- \underset{}{\overset{K_2}{\rightleftharpoons}} [HRSH\text{-}IO_4] \qquad (8)$$

$$[HRSH\text{-}IO_4] \overset{k_3}{\longrightarrow} RS^* + IO_3^* + H_2O \qquad (9)$$

$$6HRSH^+ + IO_3^* \overset{k_4}{\longrightarrow} 6RS^* + 3H_2O^* + I^* + 6H^+ \qquad (10)$$

$$IO_4^- + RSH \overset{k_5}{\longrightarrow} RS^* + IO_3^* + OH^- \qquad (11)$$

$$6RSH + IO_3^* \overset{k_6}{\longrightarrow} 6RS^* + I^* + 3H_2O \qquad (12)$$

$$I^* + I^* \longrightarrow I_2 \qquad (13)$$

$$OH^- + H^+ \longrightarrow H_2O \qquad (14)$$

$$I_2 + I^- \longrightarrow I_3^- \qquad (15)$$

$$14RS^* \longrightarrow 7RSSR \qquad (16)$$

$$Rate = k_3[RSH_2\text{–}IO_4] + k_5[RSH]\,[IO_4^-] \qquad (17)$$

From eqn. 7:

$$[RSH_2^+] = K_1[RSH][H^+] \qquad (18)$$

From eqn. 8:

$$[RSH_2^-\,IO_4] = K_2[HRSH^+][IO_4^-] \qquad (19)$$

Substituting eqn. 18 into eqn. 19 gives

$$[RSH_2 - IO_4] = K_1K_2[RSH][H^+][IO_4^-] \qquad (20)$$

Therefore

$$Rate = k_3 K_1K_2[RSH][H^+] + k_5[RSH][IO_4^-] \qquad (21)$$

$$= k_3K_1K_2[H^+] + k_5[RSH][IO_4^-] \qquad (22)$$

Eqn. 21 is similar to eqn. 3, where $a = k_5$ and $b = k_3\,K_1K_2$

The presence of free radicals was indicated by the positive polymerization test on addition of acrylamide to partially oxidized mixture of the reactants in excess methanol. The formation of the intermediate as suggested in eqn. 8 is thought to be fast whereas its decomposition is the rate determining step. The reaction between IO_4^- and VO_2^+ showed similar characteristics[34]. Michaelis-Menten plot of $1/k_{obs}$ *versus* $1/(IO_4^-)$ was linear with positive intercept on the $1/k_{obs}$ axis (Fig. 4).
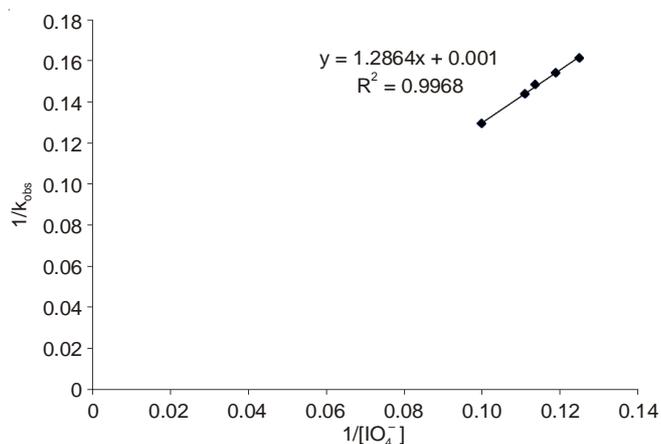

Fig. 4. Michaelis-Menten-type plot of $1/k_{obs}$ *versus* $1/[IO_4^-]$ for the title reaction

This suggests the possibility of intermediates or precursor complexes taking part in the reaction[18]. Above data strongly suggest that this reaction very likely follows the inner-sphere mechanism according to the proposed reaction scheme.

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