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SHORT COMMUNICATION

OXIDATION OF L-CYSTINE BY CHROMIUM(VI) - A KINETIC STUDY

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ABSTRACT. The kinetics of the title reaction was studied spectrophotometrically in $HClO_4$ medium at 380 nm. The data suggested that the order with respect to cystine is fractional, whereas chromium(VI) follows first order kinetics. The reaction was second order in $[H^+]$. Cysteic acid was found to be the main product of oxidation. A suitable mechanism was proposed leading to the rate law. The activation parameters were computed using linear least squares method.

KEY WORDS: Oxidation, Cystine, Chromium(VI)

INTRODUCTION

The literature survey reveals that kinetic studies on the oxidation of L-cystine are limited, using oxidants like iodine [1], alkaline permanganate [2], potassium ferrate [3], chlorite and chlorine dioxide [4] and hypochlorous acid [5]. L-Cystine is contained in many proteins and makes an important contribution to the determination of protein structure. It is first incorporated in the protein chain in the form of a monomer, L-cysteine [$SHCH_2CH(NH_2)COOH$] and when two such monomers come into close proximity in the folding of a polypeptide chain, the –SH groups of two cysteine molecules will be oxidized and converted into a disulfide bond, namely L-cystine [$-SCH_2CH(NH_2)CO_2H]_2$. Cystine is required for proper vitamin B_6 utilization and is also helpful in healing of burns and wounds, breaking down mucus deposits in illnesses such as bronchitis as well as cystic fibrosis. The reaction of chromium(VI) with L-cysteine in perchlorate media was reported by Mc Cann and Mc Auley [6]. They reported a 1:1 chromate ester formation between cysteine and chromium(VI). The reaction was also investigated by Srivatsava and co-workers [7]. They found the reaction to be first order each in oxidant, reducing substrate and $HClO_4$ concentrations. The present study is carried out to investigate the oxidation of L-cystine, the dimer of L-cysteine with chromic acid to get a further insight into the mechanism.

EXPERIMENTAL

A 0.3 M solution of L-cystine in 0.25 M $HClO_4$ was prepared from 99.9 % pure L-cystine (E.Merck). A 0.01 M solution of chromium(VI) was prepared from 99.9 % chromic acid (E. Merck) by dissolving it in water and its concentration was checked against standard iron(II) solution. A 8.0 M solution of $NaClO_4$ was prepared by neutralizing Na_2CO_3 (BDH, A.R.) with 70 % $HClO_4$ (Merck, ProAnalysis). All other chemicals used in this investigation were of A.R. grade.

The kinetic measurements were carried out at 40 ± 0.1 ^oC in 1.0 M $HClO_4$ medium under the conditions $[H^+]$ >> [cystine] >> [Cr(VI)]. The progress of the reaction is followed by measuring the absorbance of chromium(VI) at 380 nm using a Milton-Roy, Spectronic-1202 UV-Visible spectrophotometer with 1 cm path length silica cells. The temperature was maintained constant using a SEW Lab liquid circulatory bath S-36. The observed rate constants were found to be reproducible within ± 4 %.

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The product analysis was carried out chromatographically [8]. The chromatographic plate was spotted with the reaction product and it was saturated with vapours of phenol and then it was run in water-saturated phenol. After the development of the chromatogram, the plate was removed from the tank and dried. It was then sprayed with 1 % solution of ninhydrin in n-butanol and heated in an oven for five minutes at 100 ^{0}C. The r.f. value was found to be 0.17 which was in good agreement with the value of 0.1 reported by Dixit and Srivastava [8] which confirms the presence of cysteic acid. Further, the absence of sulfate as evidenced by the lack of a precipitate with barium chloride clearly indicated that the oxidation did not proceed to cleavage of the carbon-sulfur bond.

The test for free radicals was carried out taking cystine and $HClO_4$ in a Thumberg tube and acrylonitrile, chromium(VI) solution in a bent tube. After evacuating the system, the solutions were mixed by tilting the tube. The reaction mixture was kept aside and even after 24 h no precipitate was observed.

RESULTS AND DISCUSSION

Known amounts of cystine were reacted completely with a known excess of chromium(VI) at 40 ^{o}C in 1.0 M $HClO_4$ and after 12 h the residual [chromium(VI)] in each case was determined spectrophotometrically at 380 nm. The stoichiometry of the reaction was found to correspond to the equation

$$3 \text{ cystine} + 10\ Cr^{VI} \xrightarrow{H_2O} 6 \text{ cysteic acid} + 10\ Cr^{III}$$

Chromium(III), one of the products has negligible effect on the rate of the reaction.

When the kinetic runs were made with isolation of [chromium(VI)] by taking cystine in excess, plots of log(absorbance) versus time were found to be linear even beyond 85 % completion of the reaction, indicating that the reaction is first order in [chromium(VI)]. Furthermore, when [chromium(VI)] was varied from 2.0-15.0 x 10^{-4} M, the pseudo-first order rate constants, k^I were found to be invariable, indicating the order with respect to [chromium(VI)] to be one (Table 1).

The order with respect to cystine was determined by carrying out kinetic runs in 1.0 M $HClO_4$ in the presence of 4.0 x 10^{-4} M chromium(VI) and varying the cystine concentration over the 1.0-10.0 x 10^{-2} M range. The pseudo-first order rate constants were found to increase with increase in [cystine] (Table 1). When the logarithms of k^I values were plotted against the logarithms of the corresponding [cystine], a linear plot (Figure 1) with a slope of 0.60 was obtained indicating the order with respect to cystine to be fractional.

Also, the plot of $1/k^I$ versus 1/[cystine] (Figure 2) were found to be linear with positive intercept on the 1/k-axis, thus obeying Micahelis-Menten kinetics. But no spectrophotometric evidence for complexation was observed between cystine and chromium(VI). However, in the oxidation of L-cysteine, its monomer by chromic acid in $HClO_4$ medium, Mc Cann and Mc Auley [6] noticed the formation of a transient orange red 1:1 chromate ester, spectrophotometrically at 420 nm. Since the –SH groups of the two cysteine molecules are involved in the formation of disulfide bridge (-S-S-) in cystine, there may not be spectrophotometric evidence for complexation between cystine and chromium(VI). But the carboxylic and amino groups may also coordinate to chromium(VI), which supports the kinetic evidence for complexation between L-cystine and chromium(VI).

To study the effect of ionic strength on the rate of the reaction, kinetic runs were carried out keeping the concentrations of all other reactants constant and varying the ionic strength over the range 1.25-3.5 M using $NaClO_4$ solution (Table 1). The rate constants thus obtained show that the rate of reaction increases slightly with increase in ionic strength of the medium.

Table 1. Effect of [Cr(VI)], [cystine], [H^+], [$NaClO_4$] on the pseudo first order rate constant, k^I at 40 °C.

$[Cr^{VI}] \times 10^4$ (M)	[Cystine] x 10^2 (M)	[H^+] (M)	[$NaClO_4$] (M)	$k^I \times 10^4$ (s^{-1})
2.00	4.00	1.00	1.25	10.36
4.00	4.00	1.00	1.25	9.97
6.00	4.00	1.00	1.25	11.13
8.00	4.00	1.00	1.25	11.51
10.00	4.00	1.00	1.25	11.13
15.00	4.00	1.00	1.25	12.28
4.00	1.00	1.00	1.25	3.68
4.00	2.00	1.00	1.25	6.64
4.00	4.00	1.00	1.25	9.97
4.00	6.00	1.00	1.25	13.20
4.00	8.00	1.00	1.25	14.58
4.00	10.00	1.00	1.25	16.88
4.00	4.00	0.50	3.00	2.76
4.00	4.00	0.75	3.00	6.14
4.00	4.00	1.00	3.00	9.97
4.00	4.00	1.25	3.00	14.58
4.00	4.00	1.50	3.00	24.94
4.00	4.00	1.75	3.00	33.77
4.00	4.00	2.00	3.00	48.59
4.00	4.00	1.00	1.25	9.97
4.00	4.00	1.00	1.50	9.21
4.00	4.00	1.00	2.00	9.53
4.00	4.00	1.00	2.50	9.76
4.00	4.00	1.00	3.00	9.97
4.00	4.00	1.00	3.50	10.23

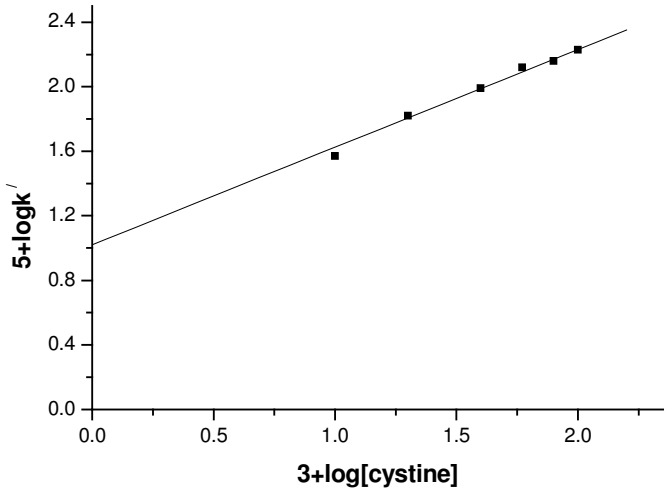


Figure 1. Plot of log $k^/$ versus log [cystine].

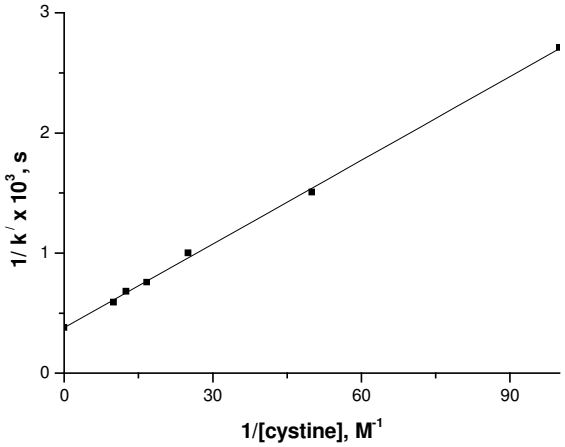


Figure 2. Plot of $1/k^{/}$ versus 1/[cystine].

In order to study the effect of $[H^+]$ on the pseudo-first order rate constant, k^{I}, kinetic runs were carried out at various $HClO_4$ concentrations over the 0.5-2.0 M range, keeping the ionic strength and other reactant concentrations constant. The pseudo-first order rate constants were found to increase with increase in $[H^+]$ (Table 1).

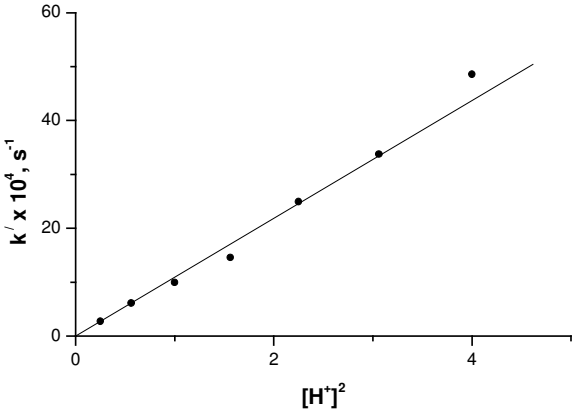


Figure 3. Plot of $k^{/}$ versus $[H^+]^2$.

Further, the plot of k^{I} versus $[H^+]^2$ (Figure 3) was found to be a straight line passing through origin indicating second order dependence on hydrogen ion concentration. Therefore, the rate law may be given as

$$\frac{-d[Cr^{VI}]}{dt} = k_{obs}[Cr^{VI}] = a\,[H^+]^2\,[\text{cystine}]^x\,[Cr^{VI}]$$

where $[Cr^{VI}]$ represents the total chromium(VI) concentration and [cystine] represents the total cystine concentration and 'x' is less than unity.

The effect of temperature on the rate of the reaction was studied by carrying out the reaction at four different temperatures, 35, 40, 45 and 50 ^{o}C, respectively. The pseudo-first order rate constants thus obtained were tabulated in Table 2.

Table 2. Effect of temperature on the pseudo-first order rate constant, k^{I}
([cystine] = 4.0 x 10^{-2} M; [Cr^{VI}] = 4.0 x 10^{-4} M; [$NaClO_4$] = 1.25 M; [H^+] = 1.0 M).

Temperature, K	308	313	318	323
$10^4 k^I (s^{-1})$	7.41	9.97	13.05	16.50

The energy of activation E_a and the entropy of activation $\Delta S^{\#}$ were calculated at 40 °C employing the linear least-squares method and were found to be 54.0±3.7 $kJmol^{-1}$ and -40.8±6.0 $JK^{-1}mol^{-1}$, respectively. The energy of activation indicates that the reaction is moderately slow. Similarly, negative value of entropy of activation suggests that the activated complex is rigid in nature. The corresponding Eyring plot was shown in Figure 4.

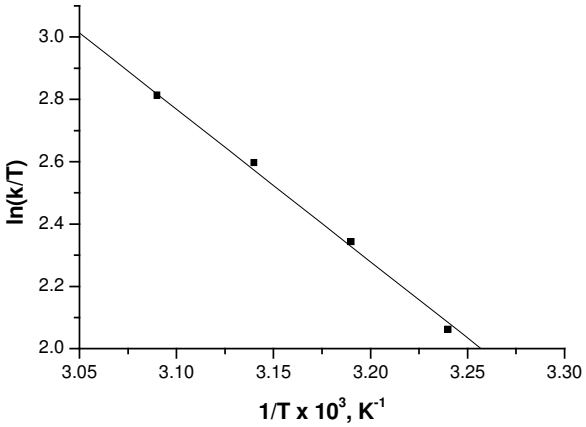


Figure 4. Plot of 1/T versus ln(k/T) (Eyring plot).

An aqueous solution of chromic acid contains the following ions such as CrO_4^{2-}, $HCrO_4^-$ and $Cr_2O_7^{2-}$. In addition, it may possibly contain other species such as $HCr_2O_7^-$, $H_2Cr_2O_7$ or H_2CrO_4 [9]. Under the present experimental conditions (1.0 M [H^+]), the protonated chromium(VI) (H_2CrO_4) is presumed to be the reactive species.

L-Cystine, [$-SCH_2CH(NH_2)(COOH)]_2$ is a sulfur containing amino acid and it possess four pK_a values. Two corresponding to the carboxylic group $(COOH)_1$ = 1.51; $(COOH)_2$ = 2.79 and the other two for amino group $(NH_3^+)_1$ = 8.25; $(NH_3^+)_2$ = 8.97. Under the present experimental conditions ([H^+] = 1.0 M), cystine exists in the form of $^-OOC(NH_3^+)CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH(NH_3^+)COOH$ (cystine$^+$) to the extent of 3 % and as $HOOC(NH_3^+)CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH(NH_3^+)COOH$ (cystine^{2+}) to the extent of 97 %. Basing on these observations, cystine^{2+} is presumed to be the reactive species and the following mechanism has been proposed:

$$H_2CrO_4 + \text{cystine}^{2+} \underset{}{\overset{K}{\rightleftharpoons}} \text{Complex (C)} \quad (1)$$

$$C \xrightarrow[\text{slow}]{k} Cr^{IV} + \text{Products} \quad (2)$$

$$Cr^{IV} + \text{cystine}^{2+} \xrightarrow[\text{fast}]{} \text{products} \quad (3)$$

$$2Cr^{IV} \xrightarrow[\text{fast}]{} Cr^{V} + Cr^{III} \quad (4)$$

$$Cr^{V} + \text{cystine}^{2+} \xrightarrow[\text{fast}]{} \text{products} \quad (5)$$

where cystine^{2+} = $HOOC(NH_3^+)CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH(NH_3^+)COOH$

$$\text{Rate} = -d\frac{[Cr^{VI}]}{dt} = K[C] = KK[H_2CrO_4]_e[\text{cystine}^{2+}]_e \quad (6)$$

Since $[Cr^{VI}]_t = [H_2CrO_4]_e + [C]$;

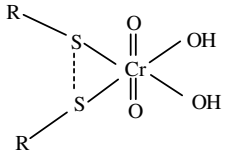
$$\text{Rate} = -d\frac{\left[Cr^{VI}\right]}{dt} = KK\frac{\left[Cr^{VI}\right]\left[\text{cystine}\right]}{1+K\left[\text{cystine}\right]} \quad (7)$$

The above rate equation explains the observed first order in [chromium(VI)] and fractional order in [cystine]. Equation (7) may therefore be transformed into;

$$\frac{1}{K_{obsd}} = \frac{1}{KK[\text{cystine}]} + \frac{1}{K} \quad (8)$$

From equation (8), a plot of $1/k^l$ versus 1/[cystine] should be a straight line with a positive intercept on y-axis. Exactly, similar plot was obtained experimentally thus supporting the proposed mechanism. Further, the values of k and K were determined and found to be 2.6 x 10^{-3} ± 0.1 s^{-1} and 16.2 ± 0.8 M^{-1}, respectively.

In most of chromium(VI) oxidations $HCrO_4^-$ is the reactive species. But in the present investigation H_2CrO_4 is active. The following transition state is proposed.



Further, the oxidation of L-cystine by chromium(VI) is slow compared to the oxidation of L-cysteine which was studied earlier by Mc Cann and Mc Auley [6] and also by Srivatsava *et al.* [7]. These authors reported the values of E_a as 29.28±12.55 $kJmol^{-1}$ and 41.84 $kJmol^{-1}$. However, in the present investigation the E_a value was found to be 54±4 $kJmol^{-1}$ since the breakage of S-S bond and further oxidation requires greater activation energy while the 'SH' group of L-cysteine can easily be oxidized with lower activation barrier.

From this observation it is concluded that sulfur atom is the primary centre of attack in the oxidation of cystine or cysteine. Further, the oxidation of cystine was found to proceed slowly when compared to the oxidation of cysteine. This may be due to the disulfide (S-S) bond present in cystine molecule, whereas the thiol group is free in cysteine. Hence, the oxidation of cysteine is much faster compared to that of cystine.

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