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Kinetics of oxidation of cysteine and captopril via $Cs_3[Mo(CN)_8]$ and $Cs_3[W(CN)_8]$

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Abstract

The kinetics of the oxidation of cysteine and captopril via octacyanomolybdate(V) and octacyanotungstate(V) in a buffered acidic media (pH range 2.20–4.80) have been studied spectrophotometrically. The rate law for the oxidation is: Rate $= k$ [RSH] [Ox] $[H^+]^{-1}$, where RSH is cysteine or captopril and Ox is $Cs_3[Mo(CN)_8]$ or $Cs_3[W(CN)_8]$. The activation parameters (E_a, $\Delta H^{\#}$, $\Delta G^{\#}$, $\Delta S^{\#}$) for the oxidation of cysteine and captopril via $Cs_3[Mo(CN)_8]$ or $Cs_3[W(CN)_8]$ have been determined. The results indicate that $Cs_3[Mo(CN)_8]$ is more reactive than $Cs_3[W(CN)_8]$ as an oxidizing agent. Effects of pH, ionic strength, temperature, dielectric constant of the reaction medium and copper(II) ions on the oxidation rate have been studied. Mechanisms for the oxidation of cysteine to cystine and captopril to the corresponding disulfide have been proposed.

Keywords: Cysteine; Captopril; Octacyanomolybdate(V) ion; Octacyanotungstate(V) ion; Copper catalysis; Copper inhibition

1. Introduction

Thiol-containing compounds are effective antioxidants and act as scavengers for reactive oxygen species [1]. In biological systems, cysteine (an amino acid with a thiol group) plays an important role as an antioxidant and thus helps in the protection of cells from the destructive effect of free radicals [2]. Additional interest has been focused on cysteine in biological systems due to the effect of its oxidation on cell immune function [3]. Kinetic studies of the oxidation of cysteine *in vitro* are expected to shed light on its oxidation in biological systems. A large variety of oxidizing agents, such as cobalt(III) complexes [4–6], iron(III) complexes [6,7], vanadium(V) complexes [8], per-manganate ion [9], copper(II) complexes [10], sodium *N*-chlorotoluenesulphonamide [11], penta-cyanonitrosyl ferrate(III) ion [12], and manganese(III) ion [13], have been employed in the study of the kinetics of oxidation of cysteine. Oxidizing agents involving molybdenum have been used [14–16] in an attempt to mimic enzymatic oxidation of cysteine in biological systems. The catalytic role of trace amounts of copper(II) ions in the oxidation of cysteine has been reported by many researchers [17–19].

Herein, we report on the oxidation of cysteine and captopril [20] in acidic media (pH 2.20–4.80). Captopril is a thiol compound (1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline) widely used as antihypertensive drug [21–25].

2. Experimental

2.1. Materials

The cyano complexes, $K_4[Mo(CN)_8]\cdot 2H_2O$ [26], $K_4[W(CN)_8]\cdot 2H_2O$ [27], $Cs_3[Mo(CN)_8]\cdot 2H_2O$ and $Cs_3[W(CN)_8]\cdot 2H_2O$ [28,29], were prepared following the literature procedures. The above cesium salts were recrystallized from water and were used without further purification.

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Analytical grade reagents and distilled water were used. The aqueous solutions of cysteine, captopril and oxidizing agents were freshly prepared. The desired buffer solutions for varying the pH of the reaction medium were prepared by mixing suitable volumes of 0.10 M HCl and 0.10 M potassium hydrogen phthalate to give pH values in the range (2.20–3.80), while for the pH range (4.20–4.80) suitable volumes of 0.10 M NaOH and 0.10 M potassium hydrogen phthalate were mixed. The pH was measured with a WTM 3000 pH meter.

2.2. Kinetic measurements

Reactions were carried out in buffered acidic media using thermostatted bath (HAAK, MD-BRUE/PU) to regulate the temperature to ±0.2 °C. Kinetic measurements were carried out in aqueous solutions unless otherwise specified. The pH was measured with a WTM 3000 pH meter. The pH meter was calibrated using standard pH solutions. The measured pHs were corrected for 50% v/v aqueous/methanol using the relationship [30,31]: $PH_{corr.} = PH_{meas.} + 0.08$. The ionic strength was adjusted to a constant value of 0.0800 M or 0.100 M by the addition of $NaClO_4$. The progress of the reaction was followed by measuring the absorbance (A_t) of the unconsumed $[Mo(CN)_8]^{3-}$ or $[W(CN)_8]^{3-}$ at 390 and 356 nm, respectively. The absorption measurements were carried out with use of a thermostatted Jasco V530 UV/Vis spectrophotometer. Solutions of cysteine, captopril and the cyano complexes were protected from light. By maintaining stoichiometric amounts of (1:1) of the thiol (RSH = cysteine, captopril) and the oxidizing agent ($Cs_3[Mo(CN)_8]$, $Cs_3[W(CN)_8]$) and performing spectrophotometric measurements at the proper wavelength. The following stoichiometric equation was found to hold for the two oxidants:

$$2RSH + 2[M(CN)_8]^{3-} = RSSR + 2[M(CN)_8]^{4-} + 2H^+$$

where M = Mo or W; RSSR = cystine or the corresponding disulfide of captopril. The disulfide compounds, cystine (m.p. = >250 °C) and the corresponding disulfide of captopril (m.p. = 220–224 °C) precipitated from reacted solutions were identified by their melting points. Formation of $[Mo(CN)_8]^{4-}$ and $[W(CN)_8]^{4-}$ in the oxidation reactions was evidenced in the oxidation reaction by the gradual increase of the absorption bands at $\lambda = 410$ and 428 nm, respectively.

3. Results and discussion

3.1. Effect of thiol concentration

The oxidation of cysteine and captopril were investigated at a constant pH and ionic strength. The concentration of the oxidants, $[Mo(CN)_8]^{3-}$ or $[W(CN)_8]^{3-}$, was kept constant at 1.00×10^{-3} M, while the concentration of cysteine or captopril was varied to study the effect of thiol concentration on reaction rate. For each run a plot of ln A versus time gave a good straight line, and the corresponding observed first-order rate constant (k) was obtained. The results for the oxidation of cysteine via the two oxidants are shown in Table 1. The effect of cysteine concentration on the reaction rate indicates a first-order kinetics with respect to cysteine.

However, when the reaction was carried out at stoichiometric amounts of the reactants (thiol-to-$[M(CN)_8]^{3-}$ = 1:1, where thiol = cysteine or captopril; M = Mo, W), absorption measurements for about 70% completion indicated second-order kinetics as shown in Fig. 1.

3.2. Effect of H^+ concentration

With other variables constant, the rate constant for the oxidation of cysteine has been found to decrease with increasing acidity (Table 2).

Plotting the second-order rate constant (k') versus $1/[H^+]$ for the oxidation of cysteine by $[Mo(CN)_8]^{3-}$, in the pH range 2.20–2.80, gave a straight line indicating first-order kinetics with respect to $1/[H^+]$ (Fig. 2). Similar behavior was observed for the oxidation of captopril by both oxidants.

3.3. Effect of ionic strength

The variation of the rate constant with ionic strength was investigated in the presence of $NaClO_4$ over a range of ionic strength $I = 0.100$–0.250 M. The results are shown in Table 3. A Plot of log k versus $\sqrt{I}$ gave a straight line in both cases. From the slope of such plots, values of $Z_A Z_B$

Table 1
Observed first-order rate constants for the oxidation of cysteine via $[Mo(CN)_8]^{3-}$ and $[W(CN)_8]^{3-}$[a]

103 [cys]	$[Mo(CN)_8]^{3-}$ 10^3 k (s^{-1})	$[W(CN)_8]^{3-}$ 10^3 k (s^{-1})
4.00	16.9	4.42
5.00	21.4	5.50
6.00	25.6	6.63
7.00	30.0	7.70

[a] $[Mo(CN)_8]^{3-} = [W(CN)_8]^{3-} = 1.00 \times 10^{-3}$ M, $T = 15.0$ °C, $I = 0.0800$ M, pH 2.20.

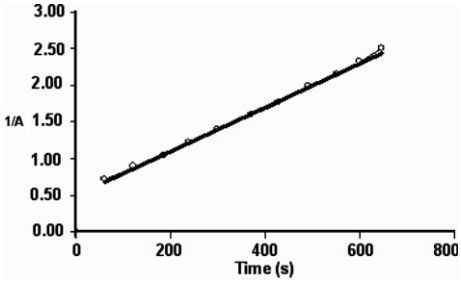

Fig. 1. A plot of $1/A$ vs. t for the oxidation of cysteine: $[Mo(CN)_8]^{3-} = [Mo(CN)_8]^{3-} = [cys] = 1.00 \times 10^{-3}$ M, pH 2.20, $I = 0.100$ M, $T = 15.0$ °C.

Table 2
Acid dependence of the kinetics of oxidation of cysteine using $[Mo(CN)_8]^{3-}$ and $W(CN)_8]^{3-}$[a]

pH	$[Mo(CN)_8]^{3-}$ k' (M^{-1} s^{-1})[b]	$[W(CN)_8]^{3-}$ k' (M^{-1} s^{-1})[b]
2.20	1.70	0.604
2.40	3.39	1.00
2.60	6.40	1.70
2.80	10.3	3.09

[a] $[Mo(CN)_8]^{3-} = [W(CN)_8]^{3-} = [cys] = 1.00 \times 10^{-3}$ M, $T = 15.0$ °C, $I = 0.0800$ M, 50% aqueous methanol.
[b] k' = second-order rate constant from plot of $1/A$ vs. time at different pH values.

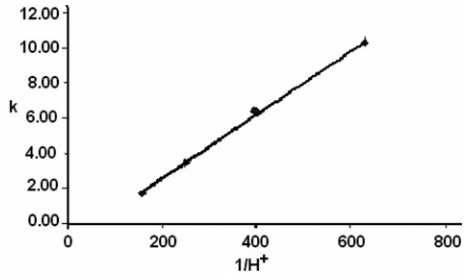

Fig. 2. A plot of k vs. $1/[H^+]$ for the oxidation of cysteine by $[Mo(CN)_8]^{3-}$ in 50% aqueous methanol. $[Mo(CN)_8]^{3-} = [cys] = 1.00 \times 10^{-3}$ M, $I = 0.0800$ M, $T = 15.0$ °C.

Table 3
Ionic strength dependence of the kinetics of oxidation of cysteine via $[Mo(CN)_8]^{3-}$ and $[W(CN)_8]^{3-}$

I (M)	$[Mo(CN)_8]^{3-}$[a] 10^3 k (s^{-1})	$[W(CN)_8]^{3-}$[b] 10^3 k (s^{-1})
0.100	22.3	5.50
0.150	25.0	6.54
0.200	27.5	7.44
0.250	30.5	8.54

[a] $[Mo(CN)_8]^{3-} = 1.00 \times 10^{-3}$ M, $[cys] = 5.00 \times 10^{-3}$ M, $T = 15.0$ °C, pH 2.20.
[b] $[W(CN)_8]^{3-} = 1.00 \times 10^{-3}$ M, $[cys] = 5.00 \times 10^{-3}$ M, $T = 15.0$ °C, pH 2.20.

for the reacting species at the rate determining step were obtained. These values are: +1.61 (cystein/$[Mo(CN)_8]^{3-}$), +1.00 (cystein/$[W(CN)_8]^{3-}$) and +0.570 (captopril/$[W(CN)_8]^{3-}$). This is an indication of a positive salt effect which implies that the reacting species at the rate determining step are RS- and the ion-pairing of the form $Na_n[M(CN)_8]^{n-3}$, where M = Mo or W. Values of n in the range 1.4–2.4 were experimentally obtained. Attempts to study the effect of ionic strength on the oxidation of captopril by $[Mo(CN)_8]^{3-}$ were unsuccessful due to precipitation problems.

3.4. Effect of dielectric constant

The influence of the solvent dielectric constant has been studied by varying the dielectric constant using aqueous methanol solutions with different compositions. The results show no significant effect on the reaction rate with the dielectric constant of the reaction medium.

3.5. Evaluation of E_a, $\Delta H^{\#}$, $\Delta G^{\#}$ and $\Delta S^{\#}$

Reactions were investigated at 10.0, 15.0, 20.0 and 25.0 °C for the oxidation of captopril by $[Mo(CN)_8]^{3-}$ at stoichiometric amounts of reactants, constant pH (4.80) and constant ionic strength (0.100 M). Values of $\Delta H^{\#}$ and E_a (Table 4) were evaluated from plots of $\ln k$ versus $1/T$, where k is the second-order rate constant (Fig. 3). Applying Eyring equation at $T = 25.0$ ° C values of $\Delta G^{\#}$ and $\Delta S^{\#}$ were calculated.

The higher reactivity of $[Mo(CN)_8]^{3-}$ as an oxidizing agent in comparison with $[W(CN)_8]^{3-}$ is shown clearly from the values of the rate constants for the oxidation of cysteine via both oxidizing agents. The rate constant for the former is about four times greater than that for the latter under the same conditions (Table 1). This is consistent with the higher reduction potential [32] of $[Mo(CN)_8]^{3-}$ and lower activation energy for the oxidation process (Table 4).

3.6. Effect of trace metal ions

Trace elements, that are usually present in the reagents or buffers used in the kinetic studies, seem to play a catalytic role in the oxidation process of cysteine and related thiols [17–19]. Reports about the catalytic role of copper ions in the oxidation of cysteine and the low values of activation energies for the oxidation of cysteine determined in this study, encouraged us to study the catalytic

Table 4
Activation parameters for the kinetics of oxidation of cysteine and captopril via $[Mo(CN)_8]^{3-}$ and $[W(CN)_8]^{3-}$

Activation parameters	Cysteine[a]		Captopril[c]	
	$[Mo(CN)_8]^{3-}$[b]	$[W(CN)_8]^{3-}$	$[Mo(CN)_8]^{3-}$	$[W(CN)_8]^{3-}$
E_a (kJ mol^{-1})	30.1	38.8	6.43	9.59
$\Delta S^{\#}$ (J mol^{-1} K^{-1})	−144	−162	−212	−204
$\Delta G^{\#}$ (kJ mol^{-1})	43.0	84.8	67.2	67.9
$\Delta H^{\#}$ (kJ mol^{-1})	27.6	36.4	3.95	7.11

[a] $[Mo(CN)_8]^{3-} = [W(CN)_8]^{3-} = 1.00 \times 10^{-3}$ M, $[cys] = 5.00 \times 10^{-3}$ M, pH 2.20, $I = 0.0800$ M.
[b] 50% aqueous methanol.
[c] $[Mo(CN)_8]^{3-} = [W(CN)_8]^{3-} = [captopril] = 1.00 \times 10^{-3}$M, pH 4.80, $I = 0.100$ M.

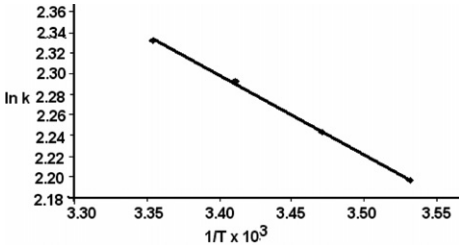

Fig. 3. A plot of $\ln k$ vs. $1/T$ for the oxidation of captopril by $[Mo(CN)_8]^{3-}$. $[Mo(CN)_8]^{3-} = [\text{captopril}] = 1.00 \times 10^{-3}$ M, pH 4.80, $I = 0.100$ M.

Table 5
Effect of $[Cu^{2+}]$ on the oxidation rate of captopril by $[Mo(CN)_8]^{3-}$

$[Cu^{2+}]$ (ppm)	k' ($M^{-1}\,s^{-1}$)
0.00	1.34
10.0	2.51
20.0	3.09
40.0	3.7

$[Mo(CN)_8]^{3-} = [\text{captopril}] = 1.00 \times 10^{-3}$ M, $T = 15.0$°C, pH 2.80, $I = 0.100$ M.

effect of $[Cu^{2+}]$. Table 5 shows how the rate constant for the oxidation of captopril by $[Mo(CN)_8]^{3-}$ increases with increasing Cu^{2+} ions concentration. Same effects have been observed for the oxidation of captopril by $[W(CN)_8]^{3-}$ and for the oxidation of cysteine by the two oxidants.

3.7. Effect of dipicolinic acid on the oxidation rate

Dipicolinic acid (a dibasic acid with $pK_{a_1} = 2.07$ and $pK_{a_2} = 4.66$) is known for its strong binding with Cu^{2+} and thus acts as an inhibitor for the Cu^{2+} catalytic role [17,18,33,34]. In this study it is observed that $t_{1/2}$ is 310 s for the oxidation of cysteine (1.00×10^3 M) via $[Mo(CN)_8]^{3-}$ (1.00×10^3 M) at pH 2.20 and 15.0 °C. Under these conditions, $t_{1/2}$ is increased up to 405 s upon using a 1.00×10^3 M concentration of dipicolinic acid.

Increasing the concentration of dipicolinic acid to 2.00×10^3 M indicates no further inhibition to the oxidation rate. It seems that a concentration of 1.00×10^3 M of dipicolinic acid is sufficient to mask the catalytic effect due to the impurity levels of Cu^{2+} ions.

In view of the above results the following mechanism is proposed:

$$RSH \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} RS^- + H^+$$

$$Cu^{2+} + RS^- \overset{k_2}{\rightarrow} Cu^+ + RS^{\cdot}$$

$$Cu^+ + [M(CN)_8]^{3-} \underset{k_{-3}}{\overset{k_3}{\leftrightarrows}} Cu^{2+} + [M(CN)_8]^{4-}$$

$$RS^- + [M(CN)_8]^{3-} \underset{\text{(slow)}}{\overset{k_4}{\leftrightarrows}} RS^{\cdot} + [M(CN)_8]^{4-}$$

$$2RS^{\cdot} \overset{k_5}{\rightarrow} RSSR$$

Assuming that the fourth step is the rate determining step and applying the steady state assumption for the thiolate anion (RS^-), the rate law can be derived as follows:

$$-\frac{d[M(CN)_8]^{3-}}{dt} = k_4[RS^-][M(CN)_8]^{3-}$$

$$k_1[RSH] = k_{-1}[RS^-][H^+] + k_2[Cu^{2+}][RS^-] + k_4[M(CN)_8]^{3-}[RS^-]$$

$$[RS^-] = \frac{k_1[RSH]}{k_2[Cu^{2+}] + k_{-1}[H^+] + k_4[M(CN)_8]^{3-}}$$

$k_{-1}[H^+] \gg k_2[Cu^{2+}] + k_4[M(CN)_8]^{3-}$, the $[H^+]$ is sufficiently high.

$$-\frac{d[M(CN)_8]^{3-}}{dt} = k_4k_1/k_{-1}[RSH][M(CN)_8]^{3-}[H^+]^{-1},$$

$$-\frac{d[M(CN)_8]^{3-}}{dt} = k_{obs}[RSH][M(CN)_8]^{3-}[H^+]^{-1}$$

where $k_{obs} = k_4k_1/k_{-1}$.

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