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Cu(II) catalysed oxidation of o-mercapto-benzoic acid by methylene blue in acidic medium: A kinetic study

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Kinetics of oxidation of *o*-mercaptobenzoic acid by methylene blue catalyzed by Cu(II) in presence of sulphuric acid and in aqueous ethanol medium (4% *v/v*) have been studied. Two moles of the substrate are oxidized by one mole of methylene blue forming the disulphide and the leuco dye as the end products. The order in methylene blue is 3/2 which increases to two at lower concentrations of Cu(II). The order in *o*-mercaptobenzoic acid is nearly zero. The rate is independent of variation in $[H^+]$ but at higher concentrations, the rate decreases to give a limiting value. The rate is not influenced on varying [Cu(II)] at higher levels but at lower concentrations (*ca.* $< 6.0 \times 10^{-6}$ *M*), there is a linear relationship between the rate and [Cu(II)]. The addition of reaction products does not influence the rate. Activation parameters have been evaluated and a plausible reaction scheme, presuming the reaction between metal – TSA complex and MB dimer as the rate determining step, has been proposed.

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In recent years, sulphur chemistry and metal ion catalysis have attracted considerable attention mainly due to the multifacet involvement of these systems in metabolic pathways[1-7]. The substrate chosen presently is *o*-mercaptobenzoic acid, which has been widely used as a model for papain whereas methylene blue has been employed as a model electron receptor for mitochondrial electron transfer reactions [8,9]. It may be mentioned that in an earlier communication[10], we have reported the kinetics of the uncatalyzed oxidation of TSA by MB but in recent past[11-20], considerable attention has been given to kinetic studies on metal – thiolate ligation. Thus, attempt was made to probe metal ion catalysis in these systems especially those involving metal ion acting as micronutrients. The substrates chosen are preferably bioactive molecules or those, which are involved in sulphur metabolism. A comparison of uncatalyzed and Cu (II)-catalyzed oxidation of TSA by MB reveals that besides the difference in order of reaction in the principal reactants, the uncatalyzed oxidation involves an ionic mechanism whereas radical mechanism is followed by the catalyzed reaction.

Further, activation parameters obtained for the two reactions suggest that the catalyzed reaction is largely kinetically controlled because the entropy of activation is found to be comparatively more negative in this case. These diversities in kinetic features make these reaction systems highly specific and fascinating. In continuance of our studies on sulphur analogues[21-25], the kinetics of Cu(II)-catalyzed interaction of TSA with MB have been studied in acidic medium.

Experimental

Solution of *o*-mercaptobenzoic acid (TSA, RSH; Evans Chemetics Inc., USA) was prepared in methanol (Qualigens GR). Solution of the oxidant methylene blue (MB; E. Merck, Germany) was prepared in doubly distilled water. The solutions were prepared afresh for each run. The oxidation product diphenyl disulphide 2,2'-dicarboxylic acid (the disulphide) was prepared by oxidizing TSA with hydrogen peroxide and extracting the product with methanol. Dihydromethylene blue (leuco base) was prepared by reducing MB solution with tin-hydrochloric acid couple[21]. Excess of hydrogen gas was boiled off and the solution was stored under a nitrogen atmosphere. The reaction was carried out in presence of ethanol (4% *v/v*) and sulphuric acid (*ca.* 4.0×10^{-3} *M*) in vessels (Pyrex, England) coated black from outside.

The progress of the reaction was followed spectrophotometrically by measuring the decrease in concentration of MB employing an ATI-Unicam UV Vis-100 spectrophotometer set at wavelength 664 nm since MB shows maximum absorbance at 664 nm ($\varepsilon_{max} = 6.76 \times 10^4\ M^{-1}\ cm^{-1}$).

Results and discussion

The stoichiometric investigations revealed that two moles of the substrate were oxidized by one mole of MB to form corresponding disulphide.

$$2\ RSH + MB \longrightarrow RSSR + H_2MB$$

The formation of the corresponding disulphide was confirmed spectrophotometrically since the peak obtained at 220 nm is in good agreement with the value of λ_{max} obtained for known sample of the disulphide.

The order in methylene blue is 3/2, which was confirmed by van't Hoff differential method. The average value of rate constant (0.36 $M^{-1/2}$ s^{-1}) remains practically unaffected on varying [TSA] indicating zero order kinetics in the substrate.

The rate constant increases on decreasing the initial concentration of MB. The rate constant remains unaffected on varying the ionic strength of the system as well as dielectric constant of the medium. However, reaction shows a transition in order in MB on adding KCl, NaCl and K_2SO_4 from 3/2 to 2 which may be attributed to environmental effects on the chromophoric nature of MB. The average value of second order rate constant (77.5 $M^{-1}s^{-1}$) remains practically unaffected on varying $[H^+]$ up to 1.0×10^{-2} M. Beyond this concentration, the value of rate constant decreases abruptly to 14.3 $M^{-1}s^{-1}$ although the magnitude of second order rate constant remains practically unchanged subsequently. It may be emphasized that in these reaction systems, the relationship between the rate and $[H^+]$ is normally found to be complex.

The rate of reaction increases on increasing [Cu(II)] and the rate constant ($k_{3/2}$) increases from 0.36 to 1.07 $M^{-1}s^{-1}$ on increasing [Cu(II)] from 6.0×10^{-6} M to 10.0×10^{-6} M (Table 1).

The rate of reaction remains unaffected on external addition of the reaction products, viz., the corresponding disulphide and the leucobase. Incidentally, these findings are in contrast with those obtained for the uncatalysed oxidation of TSA by MB. In that case, the rate was retarded on adding the disulphide and the leucobase and in presence of leucobase, order in MB changes from unity to zero at higher [H_2MB].

Table 1 — Effect of variation of [Cu(II)] on rate {[TSA] = 5.0×10^{-4} M; [MB] = 2.0×10^{-5} M; [H_2SO_4] = 4.0×10^{-3} M; Ethanol = 4% (v/v), Temp. = 35°C}

$[Cu(II)]\times10^6$, M	k_2, $M^{-1}s^{-1}$	$k_{3/2}$, $M^{-1/2}s^{-1}$
2.0	15.4	-
4.0	33.0	-
6.0	-	0.36
7.0	-	0.47
8.0	-	0.81
9.0	-	0.91
10.0	-	1.07

Further, the rate is not found to be influenced by varying the time of equilibration of the reaction system from 5-20 min as was noticed earlier in the oxidation of cysteine and 2-mercaptoethylamine hydrochloride by MB catalyzed by Cu(II). The enthalpy of activation was determined using the Arrhenius plots and was found to be 23.5 kJ mol^{-1}. The entropy (ΔS^*) and free energy of activation (ΔG^*) were evaluated in the usual manner and were found to be -77.9 $JK^{-1}mol^{-1}$and 47.1 $kJmol^{-1}$ respectively. Activation parameters for second order reaction were calculated by using van't Hoff isochore equation. ΔH^* was found to be 31.5 $kJmol^{-1}$, whereas ΔS^* and ΔG^* were found to be -66.5 $JK^{-1}mol^{-1}$ and 51.7 kJ mol^{-1} respectively.

Methylene blue exists as protonated species in acidic medium[21] while sulphydryl compounds are known to form complexes with metal ions[26]. Further, Cu(II) metal ion has been used as catalyst in the oxidation of a variety of sulfhydryl substrates[27] and here too, a complex represented as C is presumed to be formed between Cu(II) and *o*-mercaptobenzoic acid. Thus,

$$MB + H^+ \overset{k_1}{\rightleftharpoons} MBH^+ \qquad \ldots(1)$$

$$Cu + RSH \overset{k_2}{\rightleftharpoons} C \qquad \ldots(2)$$

The kinetic results may also be interpreted by postulating the participation of Cu(I) produced *in situ*, due to reduction of Cu(II) by TSA but such a possibility is ruled out in the light of the observations of Ehrenberg and coworkers[27] on the oxidation of cysteine by molecular oxygen catalyzed by Cu(II) in alkaline medium. These authors have explained the reaction mechanism by postulating the participation of Cu(II)-cysteine-O_2 complex and from EPR spectroscopy, have shown that 100 ± (2) % of copper is present as Cu(II). This obviously rules out the possibility of Cu(I) acting as a catalyst in the reaction system.

It may be mentioned here that the reaction was not catalyzed by adding Cu-TSA complex prepared externally to the reaction system which suggests the participation of some time - dependent species during the course of reaction. Incidentally, the existence of

reactive species eluding an easy stereochemical interpretation has been reported in literature and such species have been termed as reactive intermediates.

The protonated methylene blue (MBH^+) is shown to react with another methylene blue molecule forming a dimer D, as has been reported earlier[21]. Hence,

$$MBH^+ + MB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D + H^+ \qquad \ldots(3)$$

The complex C may, in turn, react with the dimer forming a transient species C* which may subsequently dissociate to give $RS^{\cdot}$ and half reduced methylene blue radical ($HM^{\cdot}$). It may be mentioned here that the participation of $RS^{\cdot}$ radical has been widely reported in the oxidation of thiols while the formation of half reduced methylene blue ($HM^{\cdot}$) radical (analogous to semiquinone radical) by the action of reducing substrates such as ascorbic acid on MB, has been mentioned in the literature. The participation of free radicals in the reaction system has been qualitatively confirmed by the positive polymerization test with acrylonitrile. This will lead to the following sequence of steps ultimately giving the end products.

$$C + D \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C^* + Cu(II) + MB \qquad \ldots(4)$$

$$C^* \overset{k_3}{\rightarrow} RS^{\cdot} + HM^{\cdot} \qquad \ldots(5)$$

$$HM^{\cdot} + RSH \overset{fast}{\rightarrow} H_2M + RS^{\cdot} \qquad \ldots(6)$$

$$RS^{\cdot} + RS^{\cdot} \rightarrow RSSR \qquad \ldots(7)$$

On presuming step (4) as the rate determining step, the rate of reaction may be given by the expression,

$$\frac{-d[MB]}{dt} = k_2[C][D] - k_{-2}[C^*][Cu^{II}][MB^{\cdot}] \qquad \ldots(8)$$

On presuming steady state for the species C* and D and substituting $[C] = K_2[Cu^{II}][RSH]$, the rate of reaction is given by Eq. 9.

$$\frac{-d[MB]}{dt} = \frac{k_1k_2k_3\,K_1K_2[Cu^{II}][RSH][MB][H^+]}{k_{-1}k_{-2}[Cu^{II}][MB][H^+] + k_{-1}k_3[H^+] + k_2k_3K_2[Cu^{II}][RSH]} \qquad \ldots(9)$$

The rate expression (Eq. 9) explains the near zero dependence of rate on $[H^+]$ and [TSA], while it predicts a transition in order in MB from one to two. Under the given conditions, the reaction may exhibit an intermediate order of 3/2 in MB as has been observed. Further, at higher $[H^+]$, step (3) will be predominantly reversible giving a larger value of k_{-1}. This may lead to retardation in rate under such conditions as has been actually observed. The rate expression also explains a near zero order dependence of rate on [Cu(II)] at higher concentrations while there should be a linear dependence of rate on [Cu(II)] at lower concentrations of the catalyst. Moreover, at lower concentrations of Cu(II), the order in MB should be two which has also been verified experimentally (Table 1).

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References

1 Murahashi S I, Komiyo N, Terai H & Nakae T, *J Am Chem Soc*, 125 (2003) 15312.

2 Oritz de Montellano P R, *Cytochrome P-450 Structure, Mechanism and Biochemistry*, 2nd Edn., (Plenum Press, New York) 1995.

3 Singh A K, Rahmani S, Singh B, Singh R K & Singh M, *J Phy Org Chem*, 17 (2004) 249.

4 Singh A K, Singh V, Rahmani S, Singh A K & Singh B, *J Mol Catal*, 197 (2003) 91.

5 Robertson A & Shinkai S, *Coord Chem Rev*, 205 (2000) 157.

6 Qui L, Xie An-Jian & Shen Yu-Hua, *J Mol Catal A: Chem*, 244 (2006) 58.

7 Yatsimirsky A K, *Coord Chem Rev*, 249 (2005) 1997.

8 Muthakia G K & Jonnalgadda S B, *J Phys Chem*, 93 (1989) 4751.

9 Lehninger A L, *Biochemistry*, (Kalyani Publishers, India) 1978, p. 487.

10 Bhargava R & Mishra K K, *Phosph Sulf Silicon*, 54 (1990) 39.

11 Xie Z, *Coord Chem Rev*, 250 (2006) 259.

12 Arbona R M, Thorden J B, Holden H M & Raushel F, *Bioorg Chem* 33 (2005) 448.

13 Jain S L & Jain B, *J Mol Catal A: Chem*, 212, (1) (2004) 91.

14 Agboola B, Ozoemena K I & Nyokong T, *J Mol Catal A: Chem*, 227 (2005) 15.

15 Longhinothi E, Domingos J B, Szpoganicz B, Neves A & Nome F, *Inorg Chim Acta*, (2005) 358.

16 Wilker J J & Lippard S J, *Inorg Chem*, 38 (1999) 3569.

17 Bierbach U, Hambley T W & Farrel N, *Inorg Chem*, 37 (1998) 708.

18 Vargek M, Zhao X, Lai Z, Mclendon G L & Spiro T G, *Inorg Chem*, 38 (1999) 1372.

19 Reichel F, Ashton P R & Boons G J, *Chem Commun*, 21 (1997) 2087.

20 Muller B, Schneider A, Tesmer M & Vahrenkamp H, *Inorg Chem*, 38 (1999) 1900.

21 Mishra K K, Bhargava R & Chansoria K, *Oxid Commun*, 22 (1999) 87.

22 Mishra K K & Agrawal R, *Z Phys Chem*, 211(1999) 91.

23 Mishra K K, Agrawal R & Sylvester J, *Oxid Commun*, 25 (2002) 251.

24 Mishra K K, *J Indian Chem Soc*, 80 (2003) 297.

25 Mishra K K & Sylvester J, *Oxid Commun*, 29, (2006) 78.

26 Patai S, *The Chemistry of Thiol Group*, Vol II, (Wiley Interscience, New York) 1974, p. 817.

27 Ehrenberg L, Harms-Ringdahl M, Fedresak I & Granath F, *Acta Chem Scand*, 43 (1989) 177.