Cooperativity effect of mercaptosuccinic acid on the oxidation of thiourea by methylene blue in acidic medium

K.K. Mishra* and Jane Sylvester

Department of Postgraduate Studies and Research in Chemistry, Rani Durgavati University, Jabalpur, 482001, Madhya Pradesh, India

The principal substrate, thiourea (TU) interacts with methylene blue (MB) in a molar ratio of 2: 1 and the rate of oxidation is considerably enhanced in presence of mercaptosuccinic acid (TMA). The order of reaction is zero in MB and unity with respect to TU. The rate initially increases on increasing [TMA] but it is not affected at higher concentrations of TMA (*ca* $\geq 2.0 \times 10^{-4}$ M). The order in MB changes from zero to –½ at lower concentrations of MB (*ca* $\leq 1.0 \text{x} 10^{-5}$ M). The reaction is characterised by a high negative entropy of activation. The kinetic features have been explained in terms of a competitive reaction between the half-reduced MB radical ($HM^{\cdot}$) and the TU molecule. This postulate has been verified by making the runs in presence of EDTA. The possibility of the participation of metal ion(s) as an impurity was ruled out on the grounds that the reactions of TU and TMA with MB individually in acidic medium do not show a negative order.

Keywords: thiourea, oxidation, methylene blue, cooperativity effect

Electron transfer reactions are recognised as vital components of the complex biochemical matrix which sustains life. Photochemical processes involving electron transfer and excitation energy transfer are expected to occur very rapidly in organised molecular systems in which donor and acceptors are located within close proximity and optical orientation.[1] It has been pointed out that these processes are appreciably influenced by the steric organisation and participation of nuclear vibrations in the donor-electron receptor matrix[2] and such an interaction is facilitated by neighbouring groups in reacting molecules almost insensitive to the electron transfer reaction being studied. Incidentally, the chemistry of this proximity or cooperativity effect has not been understood properly and thus, attempt has been made to investigate the interaction of model biological electron receptor, methylene blue with thiourea mediated by biologically active substrates such as thiolacids.

Experimental

Thiourea (TU) and mercaptosuccinic acid (thiomalic acid, TMA) were obtained from Reanal, Budapest (Hungary) and Evans Chemetics Inc, USA respectively. The two solutions were prepared in doubly-distilled water. The thiourea solution was equilibrated with hydrochloric acid (0.01M) for about three hours so that TU attains its maximum reactivity as already noticed earlier.[3] The equilibrated TU has been used as the principal reactant in these investigations. The solution of methylene blue (E.Merck, Germany) was also prepared in double distilled water and kept in the dark because light and temperature affect its stability.[4] The runs were made in presence of HCl (*ca* 0.25M) and at a fixed ionic strength (*ca* 0.252M). Equilibrated TU solution and other ingredients of the reaction mixture including TMA were taken in one conical flask and the MB solution was taken in the other. The flasks (Pyrex, England), coated black on the outside to avoid the influence of light on the rate of reaction, were thermostatted for 30 minutes at the desired temperature in a water bath (Julabo, Germany, Model F-34; accuracy ± 0.02°C). The reaction was started by adding the requisite aliquot of MB to the reaction system.

The runs were also made under anaerobic conditions. For this, the reaction mixture was purged with purified nitrogen gas during the course of reaction. Since identical results were obtained under aerobic and anaerobic conditions; the kinetic studies were made under aerobic conditions. The disulfides of TU and TMA were prepared by oxidising them with equivalent amounts of hydrogen peroxide.[5] Methylene blue was reduced by the Sn–HCl couple and the leuco-base was stored in a nitrogen atmosphere.[6] All other reagents were either BDH AnalaR or E.Merck's GR grade samples.

The reaction was studied on an ATI-UNICAM UV2-100 spectrophotometer set at 664 nm by measuring the depletion in concentration of methylene blue with time. The interference due to leuco-base and the disulfides is ruled out because these species absorb strongly in the UV region of the spectrum. Beer's law was obeyed in the concentration range employed and the spectrometer was thus calibrated. The FTIR spectra were recorded on a Shimadzu FTIR spectrometer 8400 S using KBr pellets.

EPR, fast rate measurements and interface studies

The EPR studies were made on a Bruker Spectrometer, EMX 6/1, resonant frequency 9.81 GHz, while the interaction of TU and TMA was investigated by a stopped flow spectrometer (Applied Photophysics SX-18 MV). An attempt was made to probe the possibility of the formation of an interface between TU and TMA under the prevailing conditions by using a Langmuir-Blodgelt Interface System (Niha Technology, model 601S).

Stoichiometry and product analysis

The stoichiometry of the reaction was determined analytically as well as spectrophotometrically. These studies indicate that two moles of TU are oxidised by one mole of MB forming the disulfide and the leuco-base.

The characterisation of the reaction products was made spectrophotometrically by recording the absorption spectrum of the reaction mixture in methanol. The spectrum of TMA gives significant peaks at 238 and 373 nm, but on equilibration with TU, only one peak at 242 nm is obtained. The kinetic findings recorded subsequently unequivocally establish that TU is the principal reactant and it reacts with MB in acidic medium in a mole ratio of 2: 1 forming the corresponding disulfide.[3]

Results and discussion

It was observed that at appreciably low concentration (*ca* 4.0×10^{-3}M), protonated TU reacts with MB in acidic medium in presence of mercaptosuccinic acid (TMA) and the extent of reaction is greatly enhanced under these conditions (Table 1). An attempt was made to explore the possibility of the influence of time of equilibration of protonated TU (RSH_2^+) with TMA on the rate, as was noticed for TU with hydrogen ions. The results show that there is no such influence on the rate of reaction (Table 2). The kinetics of the reaction were thus followed with zero minute equilibration of RSH_2^+ with TMA.

Influence of principal reactants on the rate

The reaction follows a zero order kinetics with respect to MB and the zero order rate constant varies between 3.1×10^{-9} and 11.0×10^{-9}M s^{-1} on varying [TU] between 4.0×10^{-3} to 10.0×10^{-3} M.

Table 1 Rate data exhibiting co-operativity effect of TMA

Substrate	Time/min	Extent of reaction/%
TU	100	33
TMA	100	4
TU + TMA	100	85

Solution condition
[TU] = 4.0×10^{-3}M; [TMA] = 4.0×10^{-4}M; [MB] = 2.0×10^{-5}M; [HCl] = 0.25M; ionic strength = 0.252M; temperature = 35°C.

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* Correspondent. E-mail: kkmishra_chem@indiatimes.com

Table 2 Rate data for different time of equilibration of protonated TU with TMA

Time of equilibration/min	$k_0 \times 10^9$/M s^{-1}
0	3.1
15	2.7
30	3.1
60	3.1
90	3.3

Solution condition
[TU] = 4.0 × 10^{-3}M; [TMA] = 4.0 × 10^{-4}M; [MB] = 2.0 × 10^{-5}M; [HCl] = 0.25 M; ionic strength = 0.252M; temperature = 35°C.

The rate initially increases on increasing [TMA](*ca* ≤ 2.0 × 10^{-4} M) but above this concentration, the rate constant attains a limiting value (Table 3). The zero-order rate constant shows a fractional dependence on [TMA] (0.3) as revealed by plots of log k_o and log [TMA]. An attempt was made to study the mode of interaction of TMA with TU by using a stopped flow spectrophotometer but the interaction was found to be too rapid. The addition of succinic acid in place of mercaptosuccinic acid to the reaction system did not exhibit the cooperativity effect under these conditions which indicates that the –SH group is involved in the interaction. No evidence was found for a stable interface between TU and TMA. The rate constant changes proportionately as a function of [TU] when different amounts of TU and TMA are added to the system.

The rate increases on increasing the initial concentration of MB and the order in MB changes from zero to –½ at lower [MB] (*ca* ≤ 1.0 × 10^{-5}M) (Table 4). The coefficeint of variance (c.v.) has been given in the parentheses against each value of the rate constant to highlight the transition in order in MB on varying its concentration.

The rate shows a linear dependence on [H$^+$], as indicated by the linear plot of log k_0 against log [H$^+$] with a slope of unity. In these variations, the ionic strength of the reaction system was maintained constant (*ca* 0.50M) by adding the requisite amount of potassium chloride to the reaction mixture.

The reaction shows an incoherent behaviour when the runs are made in presence of electrolytes such as KCl, NaCl, KNO_3, $Mg(NO_3)_2$ and $MgCl_2$.These observations may be attributed to the environmental effects as reported in case of the MB molecule.[7]

This is also corroborated by studying the effect of variation of dielectric constant of the medium on the rate. The rate slightly accelerates on adding the disulfide but it is not affected on adding the leuco-base. The activation parameters were evaluated by using the Arrhenius plot and Δ*H**, Δ*S** and Δ*G** are found to be 34.5 kJ mol^{-1}, -299.5 J deg^{-1}mol^{-1} and 126.8 kJ mol^{-1} respectively.

Mechanism

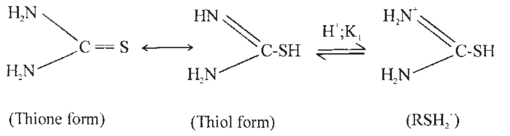
The kinetic findings significantly indicate a transition in order from zero to –½ with respect to methylene blue at lower concentrations. Incidentally, such a behaviour has been noticed by Laidler and Hoare[8] in the urease catalysed hydrolysis of urea where at low concentrations, the order in urea is + 1 which gradually becomes zero and finally, at high concentrations of urea the order in the substrate becomes –1. It has already been pointed out by Mishra and Agrawal that the order in MB changes from zero to minus half (–½) in the reaction of thiosulfate ion (TS) with methylene blue in acidic medium.[9] In the enzyme-catalysed reaction, Laidler and Hoare have postulated the Langmuir adsorption of urea on the enzyme in the homogeneous phase. In the TS–MB reaction, the Langmuir adsorption was attributed to the participation of colloidal sulfur formed due to auto-decomposition of thiosulfate.[9] In the present case, such a molecular network is absent and thus, an alternative explanation for the negative order in MB has been invoked.

Table 3 Rate data at different [TMA]

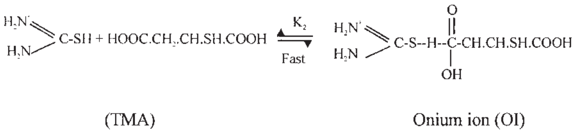
[TMA] x 10^4/M	$k_0 \times 10^9$/M s^{-1}
0.1	1.6
0.5	2.7
1.0	2.8
2.0	3.1
3.0	3.2
4.0	3.1
5.0	3.2
6.0	3.1

Solution condition
[TU] = 4.0 × 10^{-3}M; [MB] = 2.0 × 10^{-5}M; [HCl] = 0.25 M; ionic strength = 0.252M; temperature = 35°C.

The reactivity of protonated thiourea molecule (RSH_2^+) increases on interaction with TMA perhaps due to a synergistic effect, producing a reactive specie analogous to an onium ion (OI)[10], in a fast equilibrium (Eqn (2)) which facilitates the formation of HM$^•$ and RS$^•$. Thus,



(1)



(2)

It may be admitted that a complete explanation for the cooperativity effect of TMA could not be given but an interaction postulated above seems probable on the grounds that the bending vibrational modes of the sulfydryl group[11] in the TU and TMA molecules undergo changes when they are taken together, while the stretching modes recorded for the TU, TMA mixture resemble closely those of TMA in the FTIR spectra. It is shown by the FTIR spectra of protonated TU, TMA and the mixture of TU and TMA molecules, that the interaction between TU and TMA involves the stretching modes of the C–N, – SH and – NH groups and the bending modes of –NH[12] group. Further, the – COOH and N–C = S groups also participate in the formation of the onium ion (OI).

The intermediate OI may subsequently lose a TMA molecule and facilitate the formation of a thiourea analogue X' as shown below. Such a presumption is similar to the postulate made by Martin and Spence in the oxidation of sulfydryl substrates by Mo (VI)[13,14] especially in reference to glutathione. In the oxidation of glutathione (GSH) by Mo (VI), these authors have explained the kinetics of the reaction by postulating a rearrangement of the GSH molecule to give a more reactive cyclic species (G'SH) that subsequently reacts with the oxidant. In the present case, it has been surmised that the protonated TU molecule also exhibits the same structural features and perhaps such a change is facilitated by TMA *inter alia* together with a cooperativity effect. It thus seems probable that the onium ion paves the way for the formation of X' which is comparatively more reactive, like G'SH.

The transient species X' is presumed to react with MB to produce RS$^•$ and HM$^•$ (half-reduced MB) radicals. Thus,

$$\mathrm{OI} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{X' + TMA} \tag{3}$$

$$\mathrm{X'+MB} \xrightarrow{k_2} \mathrm{RS^{\bullet}{}'+HM^{\bullet}+H^+} \tag{4}$$

The radical HM$^•$ may subsequently react with the substrate molecule to produce the leuco-base (H_2M) and the disulfide as shown below.

$$\mathrm{HM\ + X'} \xrightarrow{k_3} \mathrm{RS^{\bullet}+H_2M+H^+} \tag{5}$$

$$\mathrm{2RS} \xrightarrow[\text{fast}]{k_4} \mathrm{RSSR} \tag{6}$$

It may be emphasised that the formation of such radicals in these reaction systems has been frequently reported[15-17] and, in the present case, the participation of free radicals during the course of reaction was qualitatively confirmed by the initiation of polymerisation of acrylamide by the reaction system.[18]

In the case of some dyes, it is known that semiquinone radicals (HD$^•$) disproportionate to give the leuco-base and the parent dye.[19] Thus,

$$\mathrm{HM^{\bullet}+HM} \xrightarrow{k_5} \mathrm{H_2M+MB} \tag{7}$$

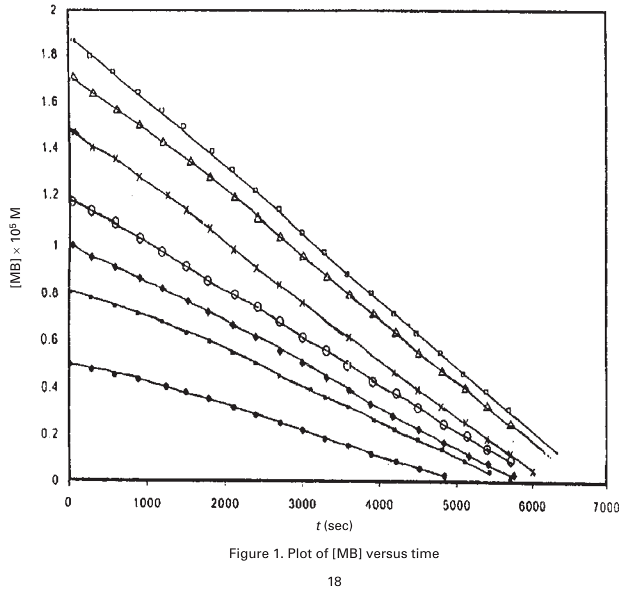


Fig. 1 Plot of [MB] versus time: [TU] = 4.0 × 10^{-3}M; [TMA] = 4.0 × 10^{-4}M; [MB] = 0.5 × 10^{-5}M[●]; [MB] = 0.8 × 10^{-5}M[■]; [MB] = 1.0 × 10^{-5}M [◆]; [MB] = 1.2 × 10^{-5}M[O]; [MB] = 1.5 × 10^{-5}M[x]; [MB] = 1.8 × 10^{-5} M[Δ]; [MB] = 2.0 × 10^{-5}M[▲]; ionic strength = 0.252M; temperature = 35°C.

Step (7) has been considered as a direct step for the sake of simplicity because the equilibrium constant for the reaction

$$H_2M + MB \rightleftharpoons HM^{\bullet} + HM^{\bullet}$$

is reported to be 3.6 × 10^{-6}, which makes the disproportionation reaction more favourable.[17]

The concentration–time curves for the runs with different [MB] were plotted (Fig.1) and it is seen that at lower [MB], there is a deviation in the locus of these curves. Incidentally, a transition in order in MB from zero to –½ has been noticed in these runs (Table 4).

On presuming step (4) as the rate limiting step, the rate of reaction is given as:

$$-\frac{d[MB]}{dt} = k_2[X'][MB] \qquad (8)$$

On presuming a steady state of X', the rate expression is given as

$$-\frac{d[MB]}{dt} = \frac{k_1k_2[OI][MB]}{k_{-1}[TMA]+k_2[MB]} \qquad (9)$$

On substituting [OI] and [RSH_2^+] from equilibria (1) and (2), we obtain

$$-\frac{d[MB]}{dt} = \frac{k_1k_2K_1K_2[RSH][TMA][MB][MB][H^+]}{k_{-1}[TMA]+k_2[MB]} \qquad (10)$$

Table 4 Rate data at different [MB]

[MB] × 10^5/M	$k_0 \times 10^9$/Ms^{-1}	$k_{-½} \times 10^{12}$/M$^{3/2}$s^{-1}
0.5	0.9 (10.7%)*	1.6 (4.7%)*
0.8	1.3 (9.9%)	3.0 (4.8%)
1.0	8.6 (8.6%)	4.3 (4.3%)
1.2	1.9 (2.8%)	5.6 (9.1%)
1.5	2.4 (0%)	7.9 (9.0%)
1.8	2.8 (3.8%)	10.2 (12%)
2.0	3.1 (4.1%)	12.4 (14.7%)

Solution condition
[TU] = 4.0 × 10^{-3}M; [TMA] = 4.0 × 10^{-4}M; [HCl] = 0.25M; ionic strength = 0.252M; temperature = 35°C.
*The coefficient of variance c.v.

Table 5 Rate data in presence of EDTA

[MB]x10^5/M	k_0x10^9/Ms^{-1}
0.5	1.2
1.0	2.3
1.5	2.9
1.8	3.3
2.0	3.6

Solution condition
[TU] = 4.0 × 10^{-3}M: [TMA] = 4.0 × 10^{-4}M: [EDTA] = 2.0 × 10^{-5}M: [HCl] = 0.25M; ionic strength = 0.252M; temperature = 35°C.

Equation (10) explains a first order kinetics in TU and a near zero order kinetics in TMA and MB.

At lower concentrations of MB, the concentration of $HM^{\bullet}$ is expected to be smaller and thus, step (5) may become the rate determining step. This will lead to

$$-\frac{d[MB]}{dt} = k_3[HM^{\bullet}][X'] \qquad (11)$$

On presuming a steady state for $HM^{\bullet}$, X' and substituting [OI] and [RSH_2^+]; we obtain

$$-\frac{d[MB]}{dt} = \frac{k_3K_1K_2[RSH][TMA][H^+]}{k_{-1}[TMA]+k_2[MB]} \bullet \left[\frac{k_1k_2K_1K_2[RSH][TMA][MB][H^+]}{k_5(k_{-1}[TMA]+k_2[MB])}\right]^{\frac{1}{2}} \qquad (12)$$

The rate expression (Eqn (12)) predicts a near first order kinetics in TU and H^+ as well as a near zero order kinetics in TMA. It also explains a negative order in MB, although the complex nature of the chemical system has deprived us from obtaining a quantitative interpretation of –½ order in MB.

A scrutiny of the proposed reaction scheme shows that at lower concentrations of MB, the disproportionation reaction (Eqn (7)) becomes prominent and there is a competitive reaction of $HM^{\bullet}$ with X' (TU analogue; Eqn (5)). This postulate was verified by adding EDTA (disodium salt) to the reaction system. EDTA is known to react with MB *inter alia* $HM^{\bullet}$ producing a photocatalytic effect[20] and thus, in the present case, its addition may counteract the competing reaction between $HM^{\bullet}$ and TU. This, in turn, may cause a reversal of order in MB from –½ to zero as has been actually observed (Table 5). Plots were drawn between log k_0 and log [MB] for the runs summarised in Tables 4 and 5 which give straight lines with slopes of 0.98 and 1.3 for the runs made in absence and in presence of EDTA respectively. These results suggest that the zero order rate constant increases almost linearly on increasing [MB] under these conditions. Thus, it may be concluded that the kinetics of the reaction are appreciably influenced by the participation of reaction intermediates as has been proposed presently.

The effect of EDTA could also be interpreted in terms of the influence of metal ion(s), present in the reaction system as a trace impurity. This possibility was, however, ruled out on the grounds that the individual reactions of TU and TMA with MB in acidic medium do not show a negative order in MB.[3,21] Further, EPR studies reveal that no paramagnetic metal ion is present in the reaction system. In the light of this, the proposed reaction scheme seems justified.

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