Kinetic studies on the oxidation of thiourea by methylene blue induced by glutathione in aqueous medium

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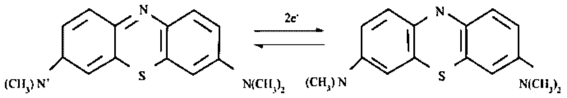
***Abstract:** The kinetics of oxidation of thiourea by methylene blue induced by glutathione in aqueous medium in presence of hydrochloric acid have been described. The order is unity in thiourea but in between half to one in methylene blue. The rate dependency on glutathione is half. The total concentration of substrate gives a straight line with a slope of 1.2. The rate increases on increasing the hydrogen ion concentration. The increase in ionic strength rate remains constant, but by increase in dielectric constant rate is increases. The stoichiometry is found to be 2:1 between thiourea and methylene blue and formamidine disulphide as a oxidation product. Addition of oxidised product does not affect the rate but by addition of leuco-base rate slightly retarded. Activation parameters have been evaluated and plausible reaction scheme has been proposed)*

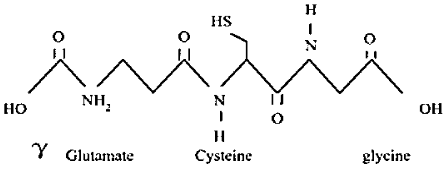
***Index Terms:** Glutathione, oxidation by methylene blue, oxidation of Thiourea, Thiourea, Thiol*

I. INTRODUCTION

Methylene blue is a well known thiazine dye[1]. It is used as a redox indicator[2]. In biological system it is used as an artificial electron accepter to capture the electron from flavin-linked enzymes for the reaction occurring in mitochondria [3,4]. Methylene blue is also known to induce the release of serotonin in human platelets [5].



The biochemical significance of this oxidant has initiated kinetic studies on the oxidation of some nucleophiles in recent years[6] including the oxidation of sulphydryl substrates in this AIlaboratory[7,8,9]. It is already reported earlier[10] thiourea and its derivatives an important class of organo-sulphur compounds. All living organism require sulphur in some suitable form and in higher animals this need is met by the amino acid L cysteine, L cystine and L methionine. Glutathione is tripeptide comprised of three amino acid-cysteine, glutamic acid and glycine [11].



Glutathione play a important role in protein synthesis[12,13,14]. Glutathione (GSH) is a most abundant antioxidant found in living organism and has multiple function most of which maintain a cellular redox homeostasis. GSH preserves sufficient levels of cystiene and detoxifies xenobiotics while also conferring therapeutic resistance to cancer cell. Glutathione act as a free radical scavenger and detoxifying agent in cells. It is useful in multitude of process, cellular proliferation, cell division and differentiation.

Recently Daryoush Hamidi Alamdari et.alreported that methylene blue even used in covid-19 treatment (Arch Bone Jt surg 2020;supplement-1:291-194 Doi10.22038/abjs 2020:47745.2349).

II. MANUSCRIPT ORGANIZATION

Thiourea supplied by reanal Budapest Hungary and later abbreviated as TU, glutathione(GSH) and methylene blue was exactly weighed and dissolved in double distilled water. The reactivity of thiourea change with time and it attains a maximum after 3 hours, because of this reason thiourea equilibrated with hydrochloric acid in thermostat for about 3 hours at 35°C. The rate is almost similar in presence and absence of nitrogen atmosphere. All other reagents used in these investigations were either BDH, AnalR or Emerck’s GR grade samples.(the purity of the reagents used was 99%)

The progress of the reaction was followed colorimetrically by using Klett-summerson photoelectric colorimeter by measuring the depletion in the absorbance of methylene blue at 660 nm. IR spectra of pre equilibrated thiourea also measured in KBr by using Perkin-Elmer IR spectro photometer model no 1430. The thiourea and methylene blue interact in acidic medium in molar ratio of 2:1 forming formamidine disulphide as oxidation product as reported by Mishra and Bhargava[15].

III. RESULTS AND DISCUSSIONS

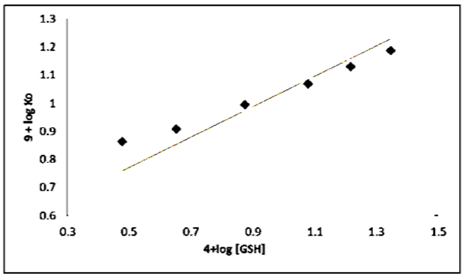
The order of reaction in methylene blue was determined by Ostwald's isolation method. The number of runs with excess concentration of thiourea and a fixed concentration of oxidant were made. The concentration of glutathione and other parameters kept unchanged in these variations (the deviations between all the rate constants in each run resulted in C.Vs. that was < 5%.). The rate constants obtained for all such runs are consolidated in table

10^3.[TU] M	10^9.k_0 .mol Lit^{-1}.Sec^{-1}	(k_0/[TU])X1 0^6	(k_0/$[TU]^2$)X 10^4
3.75	5.32	1.41	3.8
4.80	6.43	1.33	2.79
6.00	8.17	1.36	2.26
6.90	9.32	1.35	1.98
9.00	11.80	1.31	1.45
10.50	14.02	1.33	1.27

([GSH]=4.50x10^{-4}M;[MB]=3.0x10^{-5}M;[HCl]=12.0x10^{-2}M;I=12.0x10^{-2}M;Temp=35°C)
Table 1: Rate constants of reaction for different thiourea concentration

The zero order rate constants obtain at different [TU]. The relation k_0 /[TU] gives near constant values and thus the order in the substrate seems to be unity. A plot of log [TU] against log k_0 gives a straight line with a slope=0.91.

For determination of order in glutathione (inductor) a number of runs were made at different concentration of glutathione while keeping all other parameters unchanged in Figure 1



[TU]=6.0x10^{-3}M; [MB]=3.0x10^{-5}M;[HCl] = 12.0x10^{-2}M; I= 12.0x10^{-2}M; Temp= 35°C
Figure 1

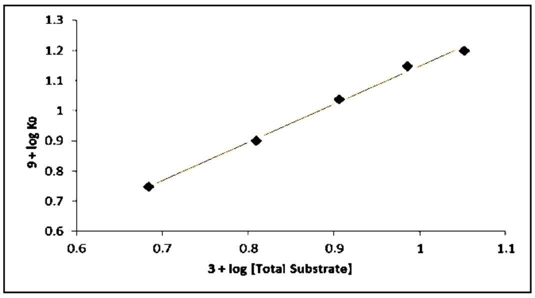
A glimpse of the figure indicates that rate increases on increasing [GSH]. A double log plot between k_0 and [GSH] gives a straight line with a slope of 0.57, which indicates the participation of this sulphydryl substrate in the reaction system in a some what complex manner.

The effect of total sulphydryl group on the reaction are monitor by changing concentration of thiourea and glutathione simultaneously by maintaining fixed concentration ratio between two sulphydryl compounds and other parameters kept unchanged.

10^3.[TU] M	10^4.[GSH] M	10^9 .k_0 .mol Lit^{-1}.Sec^{-1}
4.50	3.37	5.59
6.00	4.50	7.95
7.50	5.62	10.95
9.00	6.75	14.10
10.50	7.87	15.80

[MB]=3.0x10^{-5}M;[HCl] = 12.0x10^{-2}M; I= 12.0x10^{-2}M; Temp= 35°C
Table 2

A perusal of the above table shows that the rate increases on varying simultaneously the concentration of two susbtrate of a constant concentration ratio. A double log plot between pseudo zero order and the total concentration of the substrates gives a straight line with a slope of 1.2 (figure 2) which confirms that their interaction with the oxidant involves the participation of both the substrates in the rate determining step.



[MB]=3.0x10^{-5}M;[HCl] = 12.0x10^{-2}M; I= 12.0x10^{-2}M; Temp= 35°C
Figure 2

The rate is influenced by initial concentration of methylene blue(Table 3).

10^5 .[MB] M	10^9 .k_0 .mol Lit^{-1}.Sec^{-1}
1.50	5.25
2.00	5.85
2.50	7.10

3.00	7.87
3.50	8.74

[TU]=6.00x10^{-3}M;[GSH]=4.50x10^{-4}M;
[HCl]=12.0x10^{-2}M; I=12.0x10^{-2}M;Temp=35°C
Table 3

A glance at the above table shows that the rate increases with[MB]. A plot between log k_0 and log [MB] gives a straight line with a slope of 0.73. The rate increases on increasing the concentration of [H+] ions results indicate that rate in hydrogen ion is unity. The rate is not influenced by addition of electrolytes (maintaining ionic strength constant) but it retarded on decreasing the dielectric constant of the medium.

It is noticed that the rate is considerably retarded when acidified thiourea is kept with glutathione for a few minutes. It is seen that the rate continuously decreases when pre equilibrated thiourea is kept with glutathione up to 30 minutes and after that rate attains an limiting value (Table 4)

Time in Minutes	10^9 .k_0 .mol Lit^{-1}.Sec^{-1}
0.0	7.57
10.0	7.86
30.0	7.17
60.0	7.03
120.0	7.04

[TU]=6.0x10^{-3}M; [GSH]=4.5x10^{-4}M;[MB]=3.0x10^{-5}M; [HCl]=12.0x10^{-2}M; I=12.0x10^{-2}M;Temp=35°C

The external addition of disulphide of thiourea and leuco base slightly retarded the rate of reaction. The Arrhenius equation was found to be applicable and was used for the calculation of activation parameters. The enthalpy, entropy and free energy of activation were found to be 40.4kJ mole^{-1}, -269.9 J deg^{-1} mole^{-1} and 122.18 kJ mole^{-1}.

The kinetic features recorded for the induced oxidation of thiourea by methylene blue indicate that thiourea is the principal substrate and glutathione act as a inductor. The IR spectroscopic evidence suggests that TU and other-SH mutually interact to influence the bending vibrational mode of-SH group of thiourea molecule and it seems that the resulting intermediate has distorted symmetry of p-orbital of sulphur atom of thiourea. This strained configuration seems to be time dependent and this is actually notice in observation.

Thus it seems that the interaction of thiourea with GSH produces a configuration resembling a dithiol which in turn, increases the reactivity of thiourea.

Methylene Blue is supposed to be protonated to give the species MBH^+

$$MB + H^+ \overset{K}{\rightleftharpoons} MBH^+ \qquad \dots\dots\dots\dots\dots(i)$$

Thiourea partially present in thiol form(RSH) may be protonated and react with glutathione(R'SH) to give a reactive configuration C

$$RSH + H^+ \overset{K_1}{\rightleftharpoons} RSH_2^+ \qquad \dots\dots\dots\dots\dots(ii)$$

$$RSH_2^+ + R'SH \overset{K_2}{\rightleftharpoons} C \qquad \dots\dots\dots\dots\dots(iii)$$

The pronated MB may react with thiourea molecule to produce the radicals $RS^\cdot$ and $HM^\cdot$. The half reduced methylene blue is presumed to react in the rate determining step with intermediate C which has a close resemblance with a dithiol as shown in step (iii).

$$RSH + MBH^+ \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RS^\cdot + HM^\cdot + H^+ \qquad ----------(iv)$$

$$HM^\cdot + C \xrightarrow[\text{rate determining step}]{k_2} RS^\cdot + H_2M + R'S\text{ı} + H^+ \qquad \dots\dots\dots\dots\dots(v)$$

The rate of reaction for the above scheme is given by

$$-\frac{d[MB]}{dt} = k_2\,[HM^\cdot][C] \qquad \dots\dots\dots\dots\dots(vi)$$

$$-\frac{d[MB]}{dt} = \frac{a[RSH][GSH]^{1/2}[MB][H^+]}{k_{-1}[H^+] + (b[MB]^{\frac{1}{2}} + c[GSH]^{1/2})} \qquad \dots\dots\dots(viii)$$

CONCLUSION

The rate expression (viii) explains a first order in thiourea and a fractional order in hydrogen ions. The order in GSH is expected to lie between half to zero has been observed. The order in methylene blue is also expected to have a value between half and one. It however seems that the concentration of $HM^\cdot$ radical increases gradually in a reaction system and this may interact with the intermediate C relatively rapidly in subsequent stages of the chemical reaction to cause a transition towards zero order kinetics in methylene blue. The present investigation symbolise a model kinetic studies involving thiol enzyme and the work can be profitably exploited to understand the chemistry of SH group in living systems specifically keeping in view the steric influence of the substrate

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