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ENERGETICS OF CU (II)-CATALYSED OXIDATION OF MERCAPTOSUCCINIC ACID BY METHYLENE BLUE IN ABSENCE AND IN PRESENCE OF PYRAZOLONE IN ACIDIC MEDIUM

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Abstract- The reaction between mercaptosuccinic acid (TMA) and methylene blue (MB) was investigated in acidic medium using Cu (II) as catalyst. Two moles of substrate are oxidized by one mole of MB forming the corresponding disulphide and dihydromethylene blue. The order in MB is zero while order in TMA is unity. The rate shows a maximum on varying [H^+] whereas it increases linearly on increasing [Cu (II)]. Electrolytes exert a diverse influence, which is attributed to environmental effect. The rate increases on increasing the dielectric constant of the medium but it remains unaffected on adding externally the disulphide and leucobase to the system. It is known that Zn (II)-pyrazole complex acts as a model for the enzyme, liver alcohol dehydrogenase (LADH) which regulates the function of liver[22].3-Methyl-1 phenyl-2-pryazoline-5-one (Pyrazolone) was also added to the system in a bid to investigate the effect of simultaneous ligation of catalyst on the rate and pyrazolone was found to retard the reaction.
Keywords:- acidic medium ,

Text

Introduction

Investigation of the chemistry of model systems at a molecular level is an important first step towards understanding the chemistry of the active sites of enzymes[1]. Of Late, the interest in relating electron transfer reaction rates to spectroscopic measurements has been an area of growing interest as a consequence of the recent progress in understanding electron transfer processes and the development of new spectroscopic techniques[2]. The tendency of transition metal thiolates, specifically those of zinc, to form oligomeric and polymeric species is well-established[3-5]. Copper is an essential trace element, which forms an integral component of many enzymes[6]. While trace amounts of copper are needed to sustain life, excess copper is extremely toxic Although various aspects of copper transport and metabolism have been investigated in the past [7-11], very little is known about the species of intracellular copper transport[12]. Copper (I) – thiolate clusters are of significant interest because of their biological relevance to the cysteine-rich copper (I) proteins such as metallothionines [13-16]. Looking to such a wide and varied involvement of metal thiolate ligation involving copper (II), investigations have been taken up using Cu(II) as catalyst [17] and the present investigation is a part thereof. The environmental factors considerably affect the reactivity of transition metal-thiolates [18] and thus, the reaction of mercaptosuccinic acid with methylene blue (I) has also been investigated in presence of biologically active coligand 3-methyl-1-phenyl-2-pyrazolone-5-one (abbreviated subsequently as pyrazolone) in acidic medium.

Experimental

The solution of mercaptosuccinic acid (later abbreviated as TMA) was prepared by dissolving an exactly weighed amount of the compound obtained from M/S Evans Chemetics, Inc. USA, in acetone (E.Merck, GR). A fresh solution was always prepared to avoid its aerial oxidation. The solution of methylene blue (MB) was prepared by dissolving an exactly weighed quantity of the sample obtained from E.Merck, Germany, in double distilled water. The solution of the oxidant was stored in a dark place in order to preclude the possibility of a photochemical interference. The solution of pyrazolone prepared by dissolving an exactly weighed amount of the sample in 10% v/v methanol (E.Merck, GR). All other reagents such as hydrochloric acid, potassium chloride, copper sulphate etc. used in these investigations were either E. Merck GR or BDH Anala R grade samples.

The disulphide of TMA i.e. dithiodimalic acid was prepared by oxidizing TMA with equivalent amounts of hydrogen peroxide [19-21]. Dihydromethylene blue was prepared by passing hydrogen gas liberated from Sn-HCl couple[22] and was stored in a nitrogen atmosphere.

The kinetics of the reaction were followed spectrophotometrically by measuring the depletion in

[MB] with time (ε_{max}=6.921x10^4 cm^2 mol^{-1} at 664 nm) by ATI Unicam UV2-100 and Thermospectronic Unicam UV 530 spectrophotometer.

Reaction vessels made of pyrex glass and coated black from outside Black Japan were used. These were themostated for a sufficient length of time (Julabo, Germany, variation ±0.1^{o}C) and the aliquots were analyzed at different time intervals with the help of Beer's law plots. The law is found to be obeyed by methylene blue in the concentration range employed presently.

Results

The run with an excess concentration of mercaptosuccinic acid (TMA) over the oxidant (ca. 2.0 x 10^{-5} M) were made in presence of hydrochloric acid (ca. 2.0 x 10^{-2} M) under anaerobic conditions. The time order in MB is zero while the concentration order is unity because the rate constant increases linearly on increasing [MB] (Table I)

The order in TMA is unity as revealed by the linear plots of log k_o against log [TMA] with a slope of 1.1. This conclusion was again verified by the initial rate measurement method.

The zero order rate constant decreases on increasing [KCl] while in presence of NaCl, KNO_3 and K_2SO_4, the order in MB changes from zero to half and the half order rate constant ($k_{1/2}$} remains practically unaffected on varying the ionic strength. The rate shows a direct proportionality with the concentration of Cu(II). The rate constants obtained for all such runs are consolidated in Table II.

The rate decreases on increasing [H$^+$] at lower concentrations but beyond 2.0 x 10^{-2} M, the rate slightly increases on increasing [H$^+$] and attains a limiting value. The ionic strength of the system was maintained constant (ca. 0.20M) adding the requisite amount of KCl in these variations.

The rare of reaction increases on increasing the dielectric constant of the medium while it remains almost unaffected on adding externally the corresponding disulphide and dihydromethylene blue to the system.

It has already been mentioned that 3-methyl-l-phenyl-2-pyrazoline-5-one (abbreviated as pyrazolone) was added to the system in a bid to understand the role of ligation of bioactive molecules with metal ions acting as micronutrients. It is known that Zn (II) - pyrazole complex acts as a model for the enzyme, liver alcohol dehydrogenaase (LADH) which regulates the function of liver[23]. Thus runs were made in presence of Cu(II) and pyrazolone under anaerobic conditions. The kinetic features in the principal reactants viz. TMA and MB remained unaffected on adding pyrazolone to the reaction system. The reaction follows a zero order kinetics in MB, while the order in TMA is unity (Table III). The rate, however, decreases adding pyrazolone to the reaction system.

The UV-Visible absorption spectra of pyrazolone, Cu-pyrazolone complex prepared externally and those of the reaction system (recorded after completion of reaction) indicate that Cu-pyrazolone complex is not chemically active in the reaction. In fact the species formed, in situ, due to Cu-Pyrazolone interaction giving a strong peak at 547 nm is participating in the reaction system and this removes Cu(II) from the reaction arena to produce a retarding influence. It is further seen that at higher concentration of TMA (ca. 3.0 x 10^{-3}M), the zero order rate constant has a larger value when the runs are made in presence of pyrazolone (Table IV). This deviating behaviour highlights the specific mode of participation of pyrazolone in this system vis-à-vis the ligation with Cu(II). This aspect could not be investigated in detail because the runs tend to become erratic at higher [TMA]. However, this observation is typically characteristic of enzymatic reactions.

Discussion

These kinetic findings can be explained by postulating the interaction between Cu(II) and mercaptosuccinic acid (TMA) to form a coordination compound represented subsequently as C. The coordination compound is presumed to be a square planar complex and incidentally the existence of such coordination compounds of Cu(II) with sulphydryl substances and nucleic acid has been widely reported in literature [24,25]. It is also known that MB is protonated in acidic medium [26-30] and forms the protonated species MBH$^+$ as shown below.

$$Cu^{II} + RSH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

$$MB + H^+ \overset{K}{\rightleftharpoons} MBH^+$$

The square planar complex may subsequently react with protonated methylene blue (MBH$^+$) to produce a reactive intermediate C* which may dissociate to produce the thiyl radical and half reduced methylene blue radical (HM*) as shown below.

$$C + MBH^+ \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} C^* + CU^{II} + H^+$$

$$C^* \overset{K_3}{\rightleftharpoons} HM^{\bullet} + RS^{\bullet}$$

The formation of thiyl radicals has been frequently reported in literature in the oxidation of thiols [31] whereas the formation of semireduced methylene blue racical has been reported in the reaction of MB with ascorbic acid[32] as well as in several other systems [33]. This species, in turn participates in the system to give the end products as elaborated subsequently. It may be pointed out here that the structural rearrangement in the transition state leading to deprotonation has been reported in a variety of elimination reaction [34]. Thus

$$HM^{\bullet} + RSH \xrightarrow{Fast} RS^{\bullet} + H_2M$$

$$RS^{\cdot} \xrightarrow{Fast} RSSR$$

The participation of radicals in these reaction systems was qualitatively confirmed by the capability of the reaction system to initiate the polymerization of acrylamide [35].

On presuming up step (4) as the rate limiting step, the rate of reaction will be given by –

$$-\frac{d[MB]}{dt} = k_3[C^*] \quad (7)$$

It may be recorded here that Cu- TMA complex prepared externally under the prevailing experimental conditions did not show reactivity towards methylene blue and hence, it seems that the complex C formed in situ is transient. Thus on presuming steady state for the species C and C*, the rate expression is given as

$$-\frac{d[MB]}{dt} = \frac{k_1 k_2 k_3 K[MB][RSH][Cu^{II}][H^+]}{k_2 k_3 K[MB][H^+] + k_{-1} k_{-2}[Cu^{II}][H^+] + k_3} \quad (8)$$

The rate expression (8) explains a first order kinetics in TMA and a near zero order dependence in hydrogen ion. The order in MB is zero which is readily explained by the fact that trapping of radicals by molecular oxygen will be considerably suppressed under anaerobic conditions giving a larger magnitude of k_2 and k_3 and thus, in eq. (8); the term $k_2 k_3 K[MB][H^+]$ will be appreciably larger giving a zero order in MB. This contention was further verified by making the runs under aerobic conditions because under these conditions k_3 is expected to be larger which would result in a fractional order in MB as has been actually observed (Table IV).

The Cu(II) – catalyzed oxidation of mercaplosuccinic acid by MB has also been investigated in presence of a Schiff base.3-methyl-1-phenyl-2-pyrazoline-5-one (II, later abbreviated as pyrazolone for brevity).

The reaction is found to be inhibited by pyrazolone but other kinetic features are almost similar to those obtained for this reaction in absence of pyrazolone. It may be recorded here that the reaction of pyrazole with metal ions specifically Zn (II) has been extensively used as a model for an enzyme liver alcohol dehydrogenese.

It has been mentioned in the literature that pyrazolone mainly exists in the enol form in the solid state and the 5-OH group of the pyrazolone moiety (enol form of II) is involved in the intramolecular or intermolecular hydrogen bonding with the lone pair of nitrogen [36]. Thus, it seems that Cu(II) is preferentially coordinated to pyrazolone moiety: thereby decreasing the availability of the catalyst for coordination with mercapto succinic acid. Though sulphydryl compounds are very effective ligands but the influence of pyrazolone may be more effective due to donor sites involving nitrogen because it is more electronegative than sulphur. This may result in the inhibition of rate. A possibility may also be considered in terms of the formation of a mixed ligand complex which perhaps blocks the reactive site of Cu-TMA complex and thus, the rate decreases. Unfortunately it has not been possible to ascertain the correct alternative but certainly the participation of pyrazolone ring in these reaction system is highlighted by the present investigations. It is, therefore obvious that the reaction scheme discussed earlier for the reaction studied in absence of pyrazolone is also applicable in the present case.

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Table 1- Rate constants at different [TMA] and [MB]

[TMA] x 10^3 M	10^8 k_o mol l^{-1} s^{-1}	[MB] x 10^5 M	10^8 k_o mol l^{-1} s^{-1}
0.6	0.80	0.5	0.20
0.8	1.26	1.0	0.50
1.0	1.05	1.5	0.98
1.5	1.43	2.0	1.05
2.0	1.82	2.5	1.35
2.5	2.32	3.0	1.74
3.0	2.40		

[MB]= $2.0x10^{-5}$ M [TMA]= $1.0x10^{-3}$ M, [HCl]= $2.0x10^{-2}$ M, [KCl]= 0.1M, [Cu(II)]= $2.0x10^{-5}$ M, Acetone = 34% v/v, μ= 0.12M, Temperature = 35°C

Table II - Rate constants at different [Cu(II)].

10^5 [$CuSo_4$] M	10^8 k_o moll^{-1} s^{-1}
1.00	0.50
1.50	0.98
2.00	1.05
3.00	1.90
4.00	2.40
5.00	2.70

[TMA]= $1.0x10^{-3}$ M, [MB]= $2.0x10^{-5}$ M, [HCl]= $2.0x10^{-2}$ M, [KCl]= 0.1M, Acetone = 34% v/v, μ= 0.12 M, Temperature = 35°C

Table III- Rate constants at different [TMA] in presence of pyrazolone.

[TMA].10^3 M	10^8 k_o moll^{-1} s^{-1}
0.60	0.60
1.0	0.89
1.5	1.38
2.0	1.45
2.5	2.10
3.0	3.98

[Pyr]= $5.0x10^{-4}$M, [MB]= $2.0x10^{-5}$M, [HCl]= $2.0x10^{-2}$M, [Cu(II)]= $2.0x10^{-5}$M, [KCl]= 0.1M, Acetone = 34% v/v, μ= 0.12 M, Temperature = 35°C

Table V- Variation of TMA under aerobic conditions.

[TMA]. 10^3 M	$K_{1/2}.10^6$ k_o $mol^{1/2}$ l^{-1} s^{-1}
0.6	1.69
0.8	2.30
1.0	2.60
1.5	3.51
2.0	4.85
2.5	6.27
3.0	8.55

[Pyr]= $5.0x10^{-4}$M, [MB]= $2.0x10^{-5}$M, [HCl]= $2.0x10^{-2}$M, [Cu(II)]= $2.0x10^{-5}$M, [KCl]= 0.1M, Acetone = 34% v/v, μ= 0.12 M, Temperature = 35°C