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Kinetic Study of Interaction of *ortho*-Aminophenol with Fe(III) Schiff Base Complex in Aqueous Surfactant Medium

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The interaction of phenolate substrate like *ortho*-aminophenol with [Fe(salen)]Cl has been studied in aqueous surfactant medium. The oxygenation reaction of *o*-aminophenol bound $[Fe(salen)]^+$ adduct has found to be pseudo first order. The rate constant of the oxygenation reaction has characterized in cationic, anionic and neutral surfactant medium. These rate constant values are quite encouraging. Enzyme kinetics of the oxygenation reaction has been thoroughly examined. k_{cat}/K_m value of the reaction in different surfactant medium are ranges from 7.1×10^3 to 7.9×10^3 $M^{-1}s^{-1}$ and these values support the efficiency of the enzyme. Therefore the reaction has provided a better option as model of dioxygenase enzyme.

Keywords: *ortho*-Aminophenol, Schiff base, Surfactant, Oxygenation reaction, Enzyme kinetics.

INTRODUCTION

The oxidative cleavage of catechol and other aromatic vicinal diols is a key step in the biodegradation by soil bacteria. The catechol dioxygenases are a class of non-heme iron enzymes that catalyze the oxidative cleavage of catechols. The dioxygenases are classified on the basis of regiospecificity: the intradiol dioxygenases utilize non heme Fe(III) centre by surface activation mechanism (Fig. 1) whereas extradiol dioxygenase generally utilize non-heme Fe(II) centre *via* oxygen activation mechanism. The three-dimensional crystal structure of intradiol-cleaving protocatechuate 3.4-dioxygenase from *Pseudomonas aeruginosa* and catechol 1,2-dioxygenase from *Acinetobacter calcoacetius* is ligated by two histidine ligands and two tyrosine ligands[1].

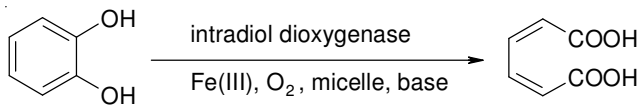

Fig. 1. Enzyme catalyzed reactions of intradiol dioxygenases

Many attempts to model the catechol dioxygenases have been reported. In all these cases the ligands are used to prepare the iron(III) complexes are tetradentate, thus resembling the coordination environment of iron(III) centre in the active site of intradiol dioxygenase. Que and co-workers[2] have proposed a surface activation mechanism for the cleaving reaction. They proposed a novel substrate for oxygenation reaction where the substrate deprotonated twice to coordinate with the Fe(III) ion. The electron transfer from iron centre to ligand give semi-quinone character to the bound substrate. The attack of dioxygen on the activated substrate yields a peroxy radical which generate alkylperoxyiron(III) species. The peroxy adduct decomposes by a Criegee-type rearrangement to muconic anhydride[1-3].

We report here, the enzyme kinetics of the oxygenation reaction of *ortho*-aminophenol by Fe(III) Schiff base complex in aqueous micellar medium. Schiff base ligand salen is utilized in this effort as it resemble the active site environment of catechol 1,2- dioxygenase. The main objective of this work is to support the projection of $[Fe(salen)]^+$ as a better model of catechol dioxygenase enzyme. To mimic the reaction medium with biomembrane system, aqueous surfactant medium is used in the analysis.

EXPERIMENTAL

Synthesis of ligand *i.e.*, 1,2-bis(2-hydroxybenzylidine-amino)ethane or SalenH_2: The Schiff base ligand, salen, has synthesized according to the published procedures[4-6] salicyl-aldehyde, ethylenediamine, *etc*. are purchased from Merck chemicals. The synthetic procedure of the ferric Schiff base complex [Fe(salen)]Cl is previously reported.

We used cationic micelle CTAB (cetyltrimethyl-ammonium bromide), anionic micelle SDS (sodium dodecylsulphate)

and neutral micelles TritonX-100 in our experiments and all micelles are purchased from sigma chemicals Co. USA and used without further purifications. Tetramethyl ammonium bromide (TMAB) are obtained from Spectrochem. Pvt. Ltd. Mumbai, India. Tetramethyl ammonium bromide is used as counter ion in micelles. UV-visible spectra are recorded in a Hitachi U-3210 model as well as in Perkin Elmer Lambda 40 UV/visibile spectrometer. The oxygenation kinetics experiments are performed in a 10 mm path length Thunberg quartz cuvette. pH dependent measurements are made by Adair Dupp. and Co.(India) model number DPH-77 pH meter. The pH electrode is SCE glass combined electrode which is standardized with known buffer solution. Throughout the work 2 %, 4 % and 3 % (w/v) solution of SDS, CTAB and TritonX-100 are used. These concentrations are adjusted above the level of critical micellar concentration (cmc). It favours the complete micellization of the complex and also reduce the chance of any change in mid point redox potential value due to any minor fluctuation in the micelle concentration.

RESULTS AND DISCUSSION

Aromatic compounds are often carcinogenic and phenols are the waste products released in the environment by various industries. A simple method for converting toxic phenol into less toxic aliphatic compounds may be of great significance[7]. In this work we examined the interaction of *ortho*-aminophenol with Fe(III) Schiff base complex [Fe(III)(salen)(OH)] at high pH level (*i.e.*, pH > pK_a). On addition of 1 equivalent of *ortho*-aminophenol (109 mg, 1 mM) to an aqueous surfactant solution of ferric Schiff base complex $[Fe(III)(salen)]^+$ (0.357 g, 1 mM) at high pH value (pH 10.5 -10), new bands in the range 422-440 and 280-285 nm are observed (Fig. 2). Comparing these spectral positions with the spectra of related phenolate complexes of iron, the band around 422-440 nm (ε, 1300-1282 M^{-1} cm^{-1}) is assigned to phenolate $p_\pi \rightarrow FeIII(d_\pi^*)$ LMCT transition and band around 282 nm (ε, 2010 M^{-1} cm^{-1}) is a ligand based band[4,8-11]. The colour of the solution changed from light violet to red. The band at 438 nm in the visible region is an indication of binding of *o*-aminophenol to iron in [Fe(III)(salen)(OH)] complex. The no affinity of -NH_2 group of *ortho*-aminophenol to coordinate with metal centre is exemplified by adding excess aniline to [Fe(III)salen(OH)] and not observing any new band in the visible region. Therefore it is understandable that *ortho*-aminophenol binds through the oxygen atom of the phenolic group.

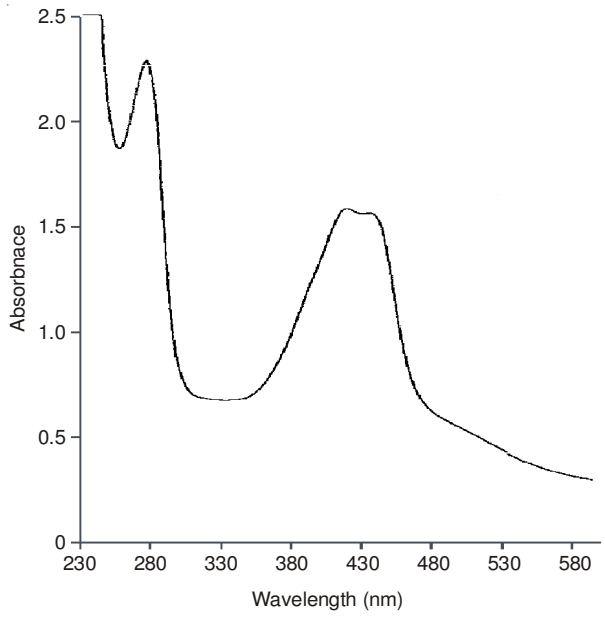

Fig. 2. Interaction of $[Fe(salen)]^+$ complex ion encapsulated in SDS micelles with *ortho*-aminophenol. pH = 10.5, temperature 25 °C

The CT transition is quite sensitive to the nature of the environment around Fe and to the nature of the solvent. The electronic band position undergo changes during gradual addition of *ortho*-aminophenol to [Fe(III)(salen)(OH)] in aqueous surfactant micelle (SDS) at pH ≈ 9.5-10.5. The intensity of band at 436 nm gradually grows and simultaneously the intensity of the high energy band at 283 nm decreases. These spectral changes form an isosbestic point at 325 nm indicates monodentate binding mode of *ortho*-aminophenol with Schiff base complex.

$$\underset{\lambda_{max}\ 385 - 391\ nm}{[Fe(III)(salen)(OH)].\ micelle} \rightleftharpoons \underset{\lambda_{max}\ 422 - 450\ nm}{[\ Fe(III)(salen)(o\text{-}aminophenol)].\ micelle}$$

The oxygenation reactions of [Fe(salen)(*ortho*-aminophenol)] adduct prepared *in situ* (using base) are investigated in various solvents like CH_3CN and aqueous surfactant medium. The oxygenation reaction of [Fe(III)salen(*ortho*-aminophenol)] adduct is studied by monitoring the slow change of the absorbance of the *phenolate*(π) → iron(III)(dπ*) LMCT band (λ_{max} 436 nm). Gradual decrease of absorbance, at band position 436 nm (λ_{max}), with time indicates the decomposition of the substrate. The reactivity of the oxygenation reaction in aqueous surfactant medium can be exemplified by comparing the change of absorbance (λ_{max} 436 nm) both in presence or absence[10,12-15] of O_2.

The oxygenation reaction is carried out in all the three micellar mediums using the following procedure. 2×10^{-4} M of $[Fe(III)(salen)]^+$ is encapsulated in aqueous anionic surfactant (SDS). The pH of the solution is increased to 10.5 and equivalent molar amount of substrate (*i.e.*, *ortho*-aminophenol) is made to react with the complex. The interaction of substrate with Fe(III) complex is evident from the change of colour of solution to red. The solution of substrate-Fe(III) complex adduct is exposed to O_2 (air) at room temperature. The oxygenation kinetics is measured by monitoring the slow disappearance of the lower energy charge transfer band at λ_{max} 436 nm as shown in the Fig. 3.

The process is considered as pseudo first order kinetics due to an excess of dioxygen[15-17]. The electronic spectrum of the reaction mixture after 48 h show the disappearance of the 436 nm band and appearance of the band around 300 nm indicating the completion of the oxygenation reaction. The rate constant (k_{obs}) is calculated by fitting the decrease in intensity of the band into the following equation (Fig. 4) applicable for relatively slow reaction

$$A = A_\alpha + (A_0 - A_\alpha) \exp(-k_{obs}\, t) \quad (1)$$

where t is the time, A, A_0 and A_a are the absorbance at the time t, 0 and α, respectively and k_{obs} is the pseudo first order rate constant. The second order rate constant is then calculated from the equation[11,13,16,17].

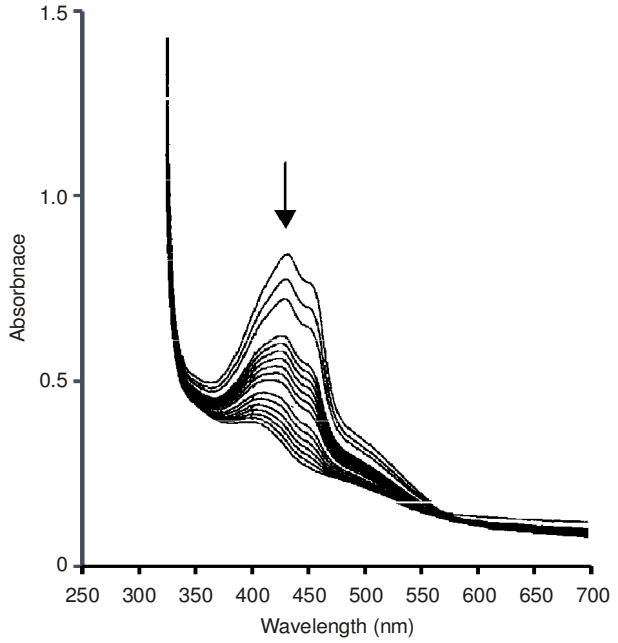

Fig. 3. Progress of the reaction of [Fe(salen)(*ortho*-amino phenol)] (generated *in situ*) encapsulated in SDS micelle with air at 27 °C monitored by UV-visible spectroscopy. The spectra are shown at 1 min interval

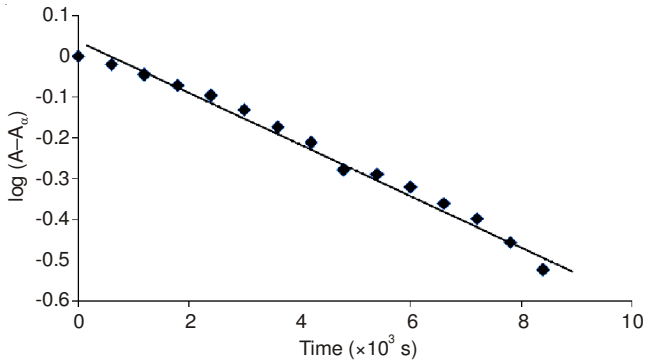

Fig. 4. Plot of log (A-A_α) *vs*. time observed at 436 nm of [Fe(salen)(*ortho*-amino phenol)] (generated *in situ*) with O_2 at 27 °C in SDS micelle

$$k_{O_2} = \frac{k_{obs}}{[O_2]} \quad (2)$$

The oxygenation reaction of *ortho*-aminophenol-[Fe(salen)] adduct is also studied in cationic (CTAB) and neutral aqueous micellar solution (TritonX-100) (Table-1). The rate of the oxygenation reaction indicates that rate of the reaction depends on the nature of the surfactants and Lewis acidity of the ferric complex[18]. For [Fe(salen)(*o*-aminophenol)] adduct rate of the oxygenation reaction in surfactant micelles decreases in the order SDS > CTAB > Triton X-100.

TABLE-1
KINETIC DATA FOR THE OXIDATIVE CLEAVAGE OF PHENOLATE SUBSTRATE (*i.e.*, *ortho*-AMINOPHENOL) IN DIFFERENT AQUEOUS SURFACTANT MICELLES AT 27 °C (ANALYZED AFTER 48 h)

Complex	Solvent	K_{obs} (10^{-5} s^{-1})	k_{O_2} (10^{-2} $M^{-1}s^{-1}$)
[Fe(salen)(*o*-amph)]	CTAB	11.21 ± 0.21	9.58 ± 0.01
	SDS	19.30 ± 0.11	16.5 ± 0.11
	Triton X-100	7.31 ± 0.10	6.2 ± 0.01

(*o*-amph = *ortho*-aminophenol)

The activity of the ferric complexes can be compared with catechol-1,2 dioxygenase and it is exemplified with *in situ* prepared complexes. The reactivity and position of the M→L CT band is influenced by the amount of base added to the reaction mixture of *o*-aminophenol and ferric complex. The addition of base assists deprotonation of the substrate. Highly favourable reaction condition is provided by the addition of approximately 1 to 2 equivalent bases and consequently UV-visible bands are shifted to a minimum energy. However, at high concentration of base retards the reaction rate due to competitive coordination of base to ferric centre. Experiments are carried out for each complex at the highest possible reaction rate. For comparable kinetic results the exact amount of base was determined before reaction[19-24].

The addition of the reagents and solvents are always carried out in the order: micelle, $[Fe(L)]^+$, *o*-aminophenol and base. The rate of product formation at 436 nm is linear over time up to 10 min when final concentration of $[Fe(L)]^+$ and *o*-aminophenol are each 0.3 mM. The effect of changing the relative concentration of the four reagents led to varied behaviour. Saturation kinetics is examined by plotting initial rates (V_0) *vs*. concentration of *ortho*-aminophenol ([S]) (Fig. 5).

According to best known model of enzyme kinetics (Michaelis-Menten kinetics) K_m (Michaelis constant), V_{max} (maximum rate achieved by the system) and k_{cat} (turn over number) are calculated by applying Lineweaver-Burk or Eadie-Hofstee plots (Table-2). From the initial velocity measurements (V_0) the values of K_m and V_{max} can be obtained in several ways, like, Substrate saturation curve (Fig. 5a), Double reciprocal plot (Fig. 5b) and Eadie-Hofstee plot (Fig. 5c). The K_m, V_{max} and $\frac{k_{cat}}{K_m}$ values obtained from these different experimental plots are in well agreement with each other.

These values strongly suggest that the complex of $[Fe(L)]^+$, the "active site" of the model reaction, is formed before combining with the substrate. The constant $\frac{k_{cat}}{K_m}$ indicate the efficiency of the concern enzyme. These values encourage the utility of the proposed enzyme[1,24].

TABLE-2
STEADY STATE KINETIC PARAMETERS FOR $[Fe(salen)]^+$ IN DIFFERENT AQUEOUS MICELLAR MEDDIUM

Substrate	Complex	Medium	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}s^{-1}$)
ortho-Aminophenol	$[Fe(salen)]^+$	CTAB	156	11.1×10^{-1}	7.1×10^3
		SDS	153	12.2×10^{-1}	7.9×10^3
		Triton-100	158	11.9×10^{-1}	7.5×10^3

Conclusion

We have characterized the experimental evidences to support the proposed ring cleavage of the *ortho*-amino phenol encapsulated in aqueous surfactant medium. The natures of the surfactant and ferric complex have considerable influenced on the rate of the oxygenation reaction. The rates of dioxygenase of the complex could be illustrated not on the basis of the Lewis acidity of the iron (III) centre alone but by assuming

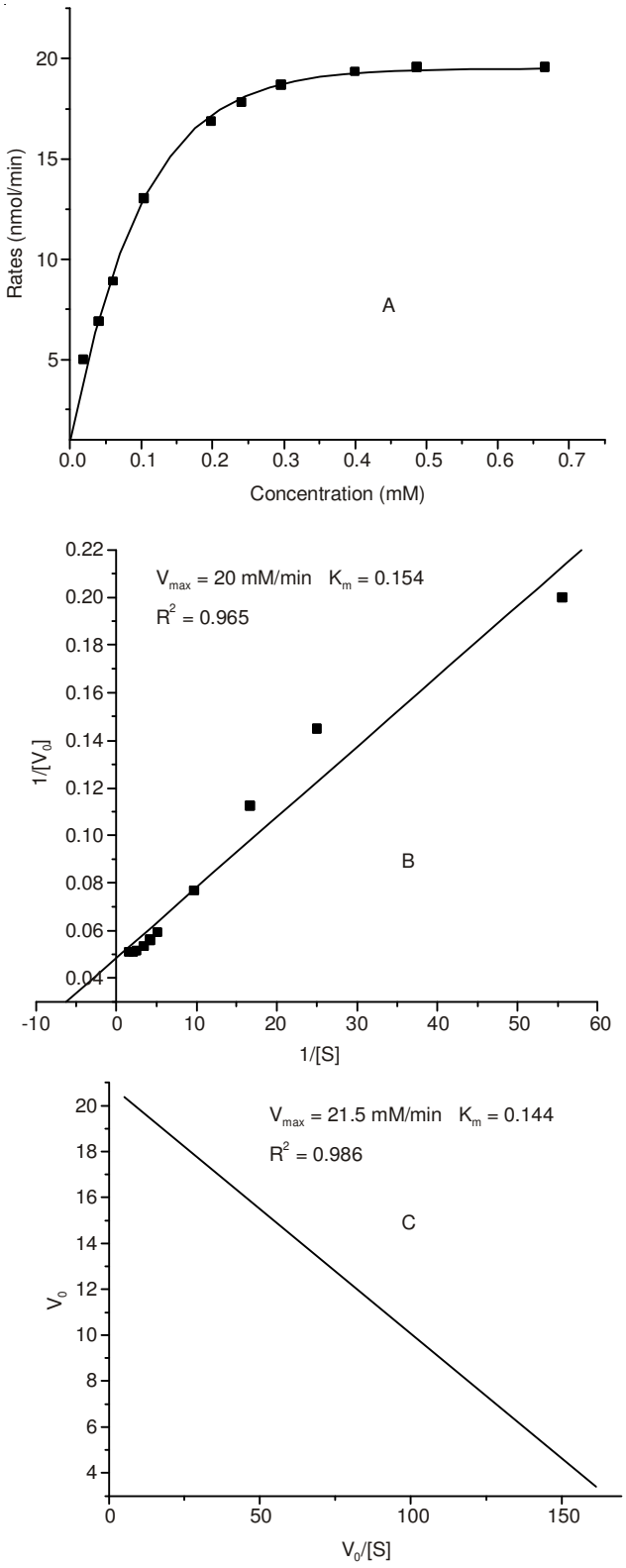

Fig. 5. Kinetic behaviour of model reaction of $[Fe(salen)]^+$ with substrate *ortho*-aminophenol in CTAB micelle

that the product release is the rate determining phase of the catalytic reaction[3,21]. Study of the enzyme kinetics of the oxygenation reaction also encourages the utility of proposed Schiff base complex as a model of catechol dioxygenase. It is remarkable that the N-based complex confers as enhanced reaction rate with efficient conversion of substrate to intradiol cleavage products.

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