CATALYTIC OXIDATION OF CATECHOL BY NEW IRON COMPLEXES MODLED AFTER THE METAL BINDING SITE OF BLEOMYCIN

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Abstract
The catalytic efficiency of new models for the metal binding subunit of bleomycin in the oxidation of 3,5-di-tert-butylcatechol to its corresponding quinone was studied using UV-V is absorption spectra methods. All studied complexes exhibit catalytical activity in this reaction. Kinetics of the oxidation of 3,5-di-tert-butylcatechol were determined by the method of initial rates and a kinetic treatment on the basis of Michaelis-Menten model was then applied.

Keywords: Iron complexes; Catecholase activity; Oxidation; Bleomycin Models.

1. Introduction
The Oxidation of organic substrates with molecular oxygen under mild conditions is extremely cardinal for industrial and synthetic processes. Even though the reaction of organic substrates with dioxygen is thermodynamically favored, it is kinetically hindered due to the triplet ground state of O₂ [1-2]. In biological systems, this problem is overcome by the use of metalloenzymes, which are efficient oxidation catalysts [3]. Much effort has been devoted to mimic these enzyme active sites in order to develop new functional model complexes, which are efficient catalysts for the oxidation reactions.

In our attempt to develop new catalysts for the oxidation of organic substrates, we have synthesized new iron complexes modeled after the metal complexation subunit of Bleomycin (BLM). Bleomycins are a class of glycopeptide antitumor antibiotics isolated from streptomyces verticillus by Umezama in 1966 [4]. BLM are successfully used in chemotherapy against several type of carcinoma [5]. Their activities are attributed to their ability to mediate DNA-strand scission by means of an oxidative mechanism [6-7]. The cleavage process requires the formation of a complex with a metal ion and subsequent activation with oxygen [8-10]. The activity of BLM is also exploited for the oxidation of organic substrates [11].

The purpose of this paper is to explore the reactivity of new iron complexes model of the metal binding site of the bleomycin in the oxidation of catechol. The ligands, figure 1 below shows, were prepared by adding two moles of L-methyl histidine ester or histamine to 2,2'-biphenylcarboxylic acid dichloride.

2. Experimental
2.1 Complex synthesis
CAUTION: In general, perchlorate salts of metal complexes with organic ligands are potentially explosive. Care is recommended.

Complex 1 (His2Fe(ClO₄)₃): A mixture of ligand L1 (160mg, 0.29mmol) and NaH (27.5mg, 1.17mmol) in dry methanol (15ml) was stirred for 1h at room temperature. Then Fe(ClO₄)₃ (104mg,
0.29mmol) was added. The solution was stirred for two additional hours at room temperature. After evaporation of methanol, the brown powder was washed twice with 15ml of diethyl ether and dried under vacuum. Yield 77%. R.M.N. $^{13}$C (CD$_3$OD) 200MHz: $\delta$ 178.3 (CO ester), 172.7 (CO amide), 55.9 (CH$_3$), 53.3 (CH), 29.8 (CH$_2$). Selected IR data (KBr), cm$^{-1}$: 3414, 1739, 1644, 1554, 1438, 1146, 760, 637, 626. UV-Vis [Methanol, $\lambda_{\text{max}}$, nm ($\varepsilon$, M$^{-1}$cm$^{-1}$)]: 207 (5350), 335 (938).

Complex 2 (His2Fe(Cl)$_3$). A mixture of ligand L$_1$ (160mg, 0.29mmol) and NaH (27.5mg, 1.17mmol) in dry methanol (15ml) was stirred for 1h at room temperature. Then FeCl$_3$ (48mg, 0.29mmol) was added. The solution was stirred for one additional hour at room temperature. After evaporation of methanol, the precipitate was washed two times with diethyl ether and dried under vacuum. Yield 96%. Selected IR data (KBr), cm$^{-1}$: 3414, 1739, 1644, 1640, 1554, 1438, 760. UV-Vis [Methanol, $\lambda_{\text{max}}$, nm ($\varepsilon$, M$^{-1}$cm$^{-1}$)]: 207 (5350), 335 (938).

Complex 3 (Ht2Fe(ClO$_4$)$_3$). A mixture of ligand L$_2$ (100mg, 0.23mmol) and NaH (22mg, 0.92mmol) in dry methanol (15ml) was stirred for 1h at room temperature. Then Fe(ClO$_4$)$_3$ (81mg, 0.23mmol) was added. The solution was stirred for two additional hours at room temperature. After evaporation of methanol, the precipitate was washed two times with 15ml of diethyl ether and dried under vacuum. Yield 50%. R.M.N. $^{13}$C (CD$_3$OD) 200MHz: $\delta$ 178.3 (CO ester), 172.7 (CO amide), 55.9 (CH$_3$), 53.3 (CH), 29.8 (CH$_2$). Selected IR data (KBr), cm$^{-1}$: 3426, 1635, 1633, 1549, 1437, 840, 758, 637, 626. UV-Vis [Methanol, $\lambda_{\text{max}}$, nm ($\varepsilon$, M$^{-1}$cm$^{-1}$)]: 212 (23745), 374 (5096).

Complex 4 (Ht2Fe(Cl)$_3$). A mixture of ligand L$_2$ (100mg, 0.23mmol) and NaH (22mg, 0.92mmol) in dry methanol (15ml) was stirred for 1h at room temperature. Then FeCl$_3$ (37.5mg, 0.23mmol) was added. The solution was stirred for one additional hour at room temperature. The precipitate was washed two times with 15ml of diethyl ether and dried under vacuum. Yield 54%. Selected IR data (KBr), cm$^{-1}$: 3426, 1635, 1633, 1549, 1437, 840, 758. UV-Vis [Methanol, $\lambda_{\text{max}}$, nm ($\varepsilon$, M$^{-1}$cm$^{-1}$)]: 212 (23745), 374 (5096).

### 2.2 Catecholase activity study

The oxidation reactions were followed spectrophotometrically by monitoring the formation of O-quinone, using a magnetically stirred, thermostated 1cm path-length cell.

Solutions of complexes 1-4 in methanol were treated with 50 equivalents of 3,5-DTBC in the presence of air. The UV-Vis spectra of the solutions were recorded every ten minutes.

### Kinetics of 3,5 di-t-ButylCatechol Oxidation

Experiments were carried out at 30°C. The concentration of the iron complex was kept at 10$^{-4}$M while that of the substrate was varied from 10$^{-3}$M to 10$^{-2}$M.

The formation of the quinone was followed by observing the increase of the absorption band at 400 nm ($\varepsilon$=1900 M$^{-1}$cm$^{-1}$, in methanol).

### 3. Results and discussion

#### 3.1 Catecholase activity

In most of the catecholase activity studies of model complexes, 3,5-di-tert-butylcatechol (3,5-DTBC) has been employed as the substrate. The product 3,5-di-tert-butylquinone (3,5-DTBO) is considerably stable and has a strong absorption at $\lambda_{\text{max}}$=400nm ($\varepsilon$=1900 M$^{-1}$cm$^{-1}$, in methanol). Therefore, the activities can be determined, using electronic spectroscopy, by following the appearance of the absorption maximum of the quinone.

![Figure 2: Oxidation of 3,5-DTBC with mononuclear iron(III) complexes.](image)

In order to get an estimation of the ability of the complexes to oxidize catechol, 10$^{-4}$M solutions of complexes 1-4 were treated with 50 equivalents of 3,5-DTBC in the presence of air. The reactions were performed in methanol, as it is the only efficient solvent for the iron complexes 1-4.

The course of the reaction was followed by UV-Visible spectroscopy over 80 min. Figure 3 shows the increase of absorption versus wavelength spectra of the oxidation of 3,5-DTBC by complex 3.

The reaction was monitored in a thermostated magnetically stirred cell. The temperature during the measurement was kept constant at 30°C. The first apparent result is a significant difference in the reactivities of the iron complexes. Compounds 1 and 3 show comparatively catecholase activity, while complex 2 shows little activity. A detailed kinetic study is thereby dispensable.
3.2 Kinetic studies

The kinetics of the oxidation of 3,5-DTBC were determined by the method of initial rates. The absorption at 400nm was measured as a function of time over the first 5 min.

The concentration of the iron complexes during the catalytic measurement was kept at 0.1mM while that of the substrate was varied from 1 to 10mM. For each complex, it was possible to obtain the reaction rate and to observe the rate dependence on the concentration of the substrate. A treatment on the basis of Michaelis-Menten model, which is originally developed for enzyme kinetics, was applied to characterize the behavior in most cases using the parameters $k_{\text{cat}}$, $K_m$ and $V_{\text{max}}$.

![Figure 3](image)

**Figure 3**: Time dependent spectral changes accompanying the oxidation of 3,5-DTBC (100 equiv) with complex 3 (0.1mM) in the presence of air. The spectra were recorded every 10min.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$V_{\text{max}}$ (µMs⁻¹)</th>
<th>$K_m$ (mM)</th>
<th>$k_{\text{cat}}$ (h⁻¹)</th>
<th>$R^2$ b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.776</td>
<td>6,152</td>
<td>28</td>
<td>0.993</td>
</tr>
<tr>
<td>2</td>
<td>0.140</td>
<td>2,188</td>
<td>5</td>
<td>0.986</td>
</tr>
<tr>
<td>3</td>
<td>0.620</td>
<td>7,796</td>
<td>22</td>
<td>0.978</td>
</tr>
<tr>
<td>4</td>
<td>0.292</td>
<td>3,463</td>
<td>10</td>
<td>0.979</td>
</tr>
</tbody>
</table>

Table 1: Kinetic parameters for the catalytic oxidation of 3,5-DTBC in methanol at 30°C.

- Standard deviations are taken from the Lineweaver-Burk plot.
- Discrepancy value of the Lineweaver-Burk plot.

Results evaluated from the lineweaver-burk plot, are summarized in table 1 above.

The investigation of the catecholase activity of compounds 1, 2, 3 and 4 revealed that these iron complexes have significant catalytic activity with respect to the aerial oxidation of 3,5-DTBC to its corresponding o-quinone.

Complexes 1 and 3 show the highest activities with a turnover number of 28h⁻¹ and 22h⁻¹ respectively which go along with higher Km values. (High affinity of the catalysis toward the substrate).

As described in previous reports [12] the catechol coordinates to the iron in a bidentate fashion and form a catecholate intermediate before electron transfer from the catechol to the metal ion. (Fig. 4) These observations indicate that the solvent-derived ligands are displaced upon formation of this intermediate complex. Therefore, if the coordinating molecule is a stronger ligand than the catechol no oxidation will be observed.

![Figure 4](image)

**Figure 4**: Catecholate intermediate.

In our case, chloride anions or perchlorate anions occupy two coordination sites. These
ligands must be released before the association of oxygen; the oxidation of catechol is then possible.

The reaction rate depends on the lability of these ligands. Since perchlorate ions are easy leaving groups than chloride ions, the complexes 1 and 3 can oxidize catechol more rapidly. Thus, we can explain the lower activity of complexes 2 and 4 for which the sixth and fifth coordination sites are occupied by chloride anions.

4. Conclusion

In this paper we studied the catecholase activity of new iron (III) complexes models of bleomycin. We demonstrated that all the complexes exhibit an efficient catalytic activity in the oxidation of catechol to its corresponding o-quinone. Complexes 1 and 3 show the highest efficiency with the turnover numbers of 28h⁻¹ and 22h⁻¹ respectively.

References