ESR Spectra and Reactivity towards Catechol of Iron(III) Complexes with 2-(2-Benzimidazolyl)phenol and its Benzoxazole Derivative

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Anisotropic ESR Signal of Iron(III), Oxidative Cleavage of Catechol

The iron(III) complex with 2-(2-benzimidazolyl)phenol (abbreviated as H(L 2 )), Fe(L 2 ) 2 (NO 3 ), and its benzoxazole derivative, exhibit highly anisotropic ESR signals in the range g = 3.6–7.2; these spectral properties differ from those of regular six-coordinate iron(III) species, but are very similar to those observed for the enzyme-competitive inhibitor complexes in protocatechuate 3,4-dioxygenase. These iron(III) compounds show negligible activity for oxidative cleavage of 3,5-di-tert-butylcatechol.

1. Introduction

Catechol 1,2-dioxygenase is a non-heme iron enzyme that catalyzes the oxidative cleavage of catechols to cis,cis-muconic acids [1]. The enzyme serves as part of Nature’s mechanism for degrading aromatic molecules in the biosphere. Many spectroscopic techniques have been applied to the study of non-heme iron sites [2] and have provided insights into the coordination chemistry of the iron center. Although catechols are somewhat air-sensitive, non-enzymatic dioxygen incorporation with ring cleavage is unusual, and the catalytic mechanism of these reactions is still unknown [3]. The most extensively studied enzyme of this class is protocatechuate 3,4-dioxygenase from Pseudomonas aeruginosa (hereafter abbreviated as 3,4-PCD) [4]. Very recently the structure of this 3,4-PCD has been solved by Weber et al. at 2.8 Å resolution [5]. According to their result, the catalytic ferric center is coordinated by two tyrosines and two histidines; the iron coordination geometry forms an approximate trigonal bipyramid with one tyrosine and one histidine located in axial coordination positions, and with a bound solvent molecule completing the trigonal iron coordination in the equatorial plane.

When the substrate, protocatechuic acid, is added to the enzyme under an anaerobic condition, the electron spin resonance (ESR) signal at g = 4.3 decreases instantaneously [6]. The signal is restored to the original level by the introduction of air. The decrease of the ESR signal is also observed when various substrate analogues or competitive inhibitors are used. However, changes in the ESR signal caused by substrate analogues or competitive inhibitors are somewhat different from those caused by the substrate in that they show a marked anisotropy [6–8]. In order to elucidate the reaction mechanism of 3,4-PCD, it seems to be very important to clarify the origin of the differences between the ESR spectra observed, and relate them with the coordination geometry around the iron(III) ion.

In this study we have observed that the iron(III) compound with 2-(2-benzoxazolyl)phenol (abbreviated as H(L')), Fe(L' 2 )(NO 3 ), and its benzimidazole derivative (see below), exhibit highly anisotropic ESR signals;

\[
\text{H(L'), } X = \text{O} \quad \text{H(L'), } X = \text{NH}
\]

this ESR property is different from those of regular six-coordinate iron(III) species, but is very similar to that observed for the enzyme-competitive inhibitor complexes in 3,4-PCD. These iron(III) complexes...
compounds show negligible activity for the oxidative cleavage of 3,5-di-tert-butylcatechol. Based on these facts and the crystal structure of the Fe(L)₂(NO₃), we have discussed a plausible structure of enzyme-competitive inhibitor and enzyme-substrate complex in the 3,4-PCD.

2. Materials and Methods
The iron(III) compound, Fe(L)₃(NO₃), was prepared according to the literature [9]. The ligand, 2-(2-benzimidazolyl)phenol (abbreviated as H(L²)) was obtained according to the method of Lane [10]. The iron(III) compound, Fe(L²)₂(NO₃), was obtained as dark violet prisms by a method similar to that for H(L¹), and was recrystallized from an acetonitrile solution.

\[ \text{FeC}_{26}\text{H}_{18}\text{N}_{50} \cdot \frac{3}{2}\text{H}_{2}\text{O} \]
Calcd C 55.44 H 3.76 N 12.43 Fe 9.91.
Found C 55.37 H 3.86 N 12.69 Fe 9.8.

ESR spectra were obtained with a JEOL ESR apparatus model JES-FE-3X by using the X-band, MnO (in MgO) being used as a standard marker.

3. Results and Discussion
The ligand, H(L²), can function as a bidentate chelate via one phenol and one imidazole as coordinating groups, and thus the iron(III) atom in Fe(L²)₂(NO₃) is probably coordinated by two phenolic and two imidazole groups, similar to the native 3,4-PCD. In Fig. 1, the absorption spectrum of Fe(L²)₂(NO₃) in methanol is shown; a strong band is observed at 545 nm (ε/Fe = 3710 M⁻¹cm⁻¹), yielding a violet solution. This should be due to a phenolate-to-Fe(III) charge transfer transition [11]. This spectral property is almost the same as that observed for the L¹ derivative [9], suggesting that the structure of the L² compound is very similar to that of the L¹ derivative [9]. When 3,5-di-tert-butylcatechol was added to this violet solution, it turned dark green, due to the increase of absorbance in the range 600–850 nm as illustrated in Fig. 1. From the acetonitrile solution of the iron(III) compound (0.001 M) and 3,5-di-tert-butylcatechol (0.1 M), we could isolate the corresponding quinone and muconic acid anhydride [12, 13] after one week, but the yield of the muconic acid anhydride was extremely low (<3%) compared with those of oxovanadium(IV) compounds (40% yield after one day under the same experimental condition) [14]. This may be due to a lower reactivity of the green species towards the dioxygen molecule. In Fig. 2, the ESR spectrum of the L² compound is shown, the spectral property of the L¹ compound being almost the same. It should
be noted here that this ESR spectral property is different from those of regular six-coordinate iron(III) compounds [15], but rather similar to that observed for the enzyme-competitive inhibitor complexes in 3,4-PCD.

Since the solvent molecule is located at the equatorial plane of the trigonal bipyramidal structure of Fe(III) in 3,4-PCD [4], two structures, chelated (B-type) and monodentate (A-type), are possible for the approach of catechol to the iron(III) atom, as shown below. It should be noted here that the species, produced by the addition of 3,5-di-ter/-butylcatechol to the violet solution of Fe(L^2)_{2}(NO_3), is almost the same as that of the original one, suggesting that the green species is also of a B-type coordination geometry. The very low reactivity of this green species towards the dioxygen molecule is consistent with the result by Fujisawa et al. and with the fact that the chelated catechol is a poor reductant for dioxygen molecule [16, 17]. These facts lead to the conclusion that the structure of the intermediate of enzyme substrate in 3,4-PCD should be different from that proposed for the competitive inhibitors (A-type coordination).

The possible presence of a monodentate structure (A-type coordination) has been pointed out by Que et al. [3, 18]. They proposed the following mechanism, and have conceived that the reaction of the ES complex with O_2 as an attack of O_2 on the substrate, which would be activated by the coordination to the iron and the loss of both its protons. But this mechanism cannot explain the fact that chelated catecholate is a poor reductant
for $O_2$ [16, 17]. In the previous papers, we have showed that the distorted tetrahedral copper(II) complexes exhibit quite different reactivity as compared to that of square planar complexes, and pointed out that the unique reactivity of distorted tetrahedral compounds should be due to a d-orbital having the following specific properties: (i) it contains one unpaired electron, and (ii) the lobes of the orbital are not completely screened by the ligand atoms [19]. In the A-type coordination mode, such a d-orbital is present as illustrated below. Based on the above discussion, it seems very likely that in the A-type coordination model, the dioxygen may react with the unscreened lobe of the d-orbital which is interacting with the substrate through σ-bonding (see above figure), making the formation of intermediate (C) of Que's model, substrate$^{2-}$-Fe(III)-$O_2^-$, more facile. The dioxygen molecule in such an intermediate may be of some singlet oxygen character ($^1A_g$) [20], and this activated dioxygen molecule [21] will attack the substrate.

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