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The Role of a Spin-Coupled Intermediate in a System Consisting of Tetraminecobalt(III) Complexes and Catechols —A Model Intermediate of Dioxygenase Reaction—

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The reactions of two Co^{III}-tetramine (N_4) complexes, *cis*-α-Dichloro(triethylenetetramine)cobalt(III) chloride $=[Co^{III}Cl_2(trien)]Cl$ (**1**) and aquabromo[(2*R*, 5*R*, 8*R*, 11*R*)-2,5,8,11-tetraethyl-1,4,7,10-tetraazacyclododecane] cobalt(III) bromide $=[Co^{III}Br(Et_4[12]\text{-}aneN_4)(H_2O)]Br_2$ (**2**) with 3,5-di-*t*-butylcatechol (H_2dbc) have been examined by visible, NMR and ESR spectroscopies to elucidate the mechanism of the activation and the oxygenation of catechol by the complex formation. The reactions proceeded in a mixed solvent (H_2O : MeOH=1 : 1) at 50 °C in the presence and absence of dioxygen. NMR showed that the complexes react with H_2dbc and dioxygen, and yield oxidatively cleavaged products. ESR measurements detected a Co^{III}-semiquinone (dbsq) radical and a high-spin Co^{II} complex, regardless of the presence of dioxygen. Especially in the case of **1**, the magnetic moment of the complex isolated from the anaerobic reaction solution, exhibited that there exists an antiferromagnetic spin-coupling between the high-spin ($S=3/2$) Co^{II} ion and the semiquinone radical in the complex. These results show that two species detected by ESR are in equilibrium with a high-spin Co^{II}-dbsq binary complex formed by intramolecular electron transfer. It is conclusively clarified that the one-electron-transferred intermediate $[Co^{II}(N_4)dbsq]^+$ is the activated intermediate, which introduces dioxygen into the semiquinone moiety.

The reactions of molecular oxygen with organic substrates by metallo proteins are crucial for metabolism, so that there have been growing interests in the mechanisms of these reactions. Especially a number of reports on non-heme enzymes have appeared in recent years, because of our rudimental understanding compared to the knowledge on heme-enzymes. Catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase (3,4-PCD) are the examples of such non-heme enzymes that serve the oxygenation of aromatic compounds.[1-3] They catalyze the intradiol ring cleavage of catechols with the incorporation of dioxygen. Recently the structure of the active site of 3,4-PCD has been revealed by X-ray structure determination[4] and other spectroscopic techniques.[5] It consists of a high-spin iron(III) ion ligated by four amino residues, two tyrosines and two histidines, and a labile water, as shown in Fig. 1. Though the detail of the reaction has not been clarified yet, an outline of the reaction sequence was suggested[6-8] by using a stopped-flow technique, as shown in Scheme 1. In this scheme there are three steps: First catecholate substrate (S) binds to the enzyme (E) to form an enzyme-substrate binary complex (ES). Second, molecular oxygen binds to it to form a ternary complex (ESO_2). The reaction proceeds further via another intermediate (ESO_2*) in which the SO_2* moiety is similar to the product, to the original enzyme and a ring-cleavaged product (P). In this scheme, the mechanism of the activation of the substrate and of the incorporation of dioxygen remains to be solved. For instance, it is controversial whether catechol binds to the iron ion in unidentate fashion or chelates bidentately. Orville et al.[9] investigated the binding of isotopically labeled substrates to the iron ion in the active site by using ESR technique, and suggested that an iron chelalte can be formed. On the contrary, the unidentate ligation was proposed by Que et al.[10] Moreover it has not been clarified whether the activation is made on the substrate

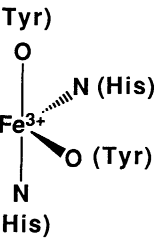
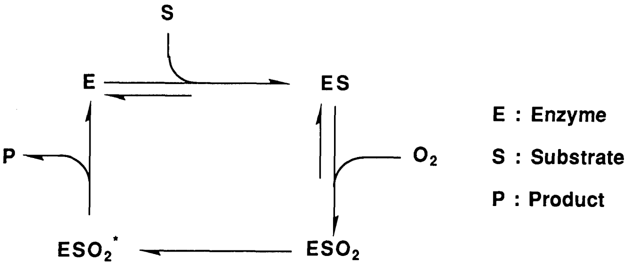


Fig. 1. Schematic drawing of the active site of dioxygenase. The high-spin iron(III) ion in the enzyme is ligated by two tyrosines and two histidines residues, and a labile water.



Scheme 1. Reaction scheme of dioxygenase proposed from the results of stopped-flow experiment.

or on the dioxygen as a first step.

Model systems that mimic the enzyme reactions have been used to elucidate these problems. The effects of the ligands around the central iron ion to the reactivity have been investigated with various ligands.[11-16] Que and his co-workers investigated the ligand effects by changing the ligand systematically.[17,18] They reported the properties of a series of ternary complexes, $[FeL(dbc)]^{2-}$, where L is a tripodal ligand, and pointed out that the Lewis acidity of the iron(III) center played an important role. They suggested also that semiquinone character of the coordinated catecholate anion arising from ligand-to-metal charge transfer is important for the substrate activation. This tendency is enhanced by replacing the ligand of oxianionic group by neutral nitrogen ligands. However, no example was reported where the intramolecular electron transfer occurred and the Fe(II)-semiquinone complex was formed as the intermediate.

On the other hand, existence of such interactions between paramagnetic ions and organic radicals is a general phenomenon in bioinorganic chemistry. In recent years a number of heme and non-heme enzymes that exhibit such kind of spin-coupling phenomena have been explored and characterized by using model compounds.[19,20] Especially the interactions between semiquinone radical and metal ions have been investigated extensively in various metal complexes.[21-23]

To isolate the activated intermediate stably and to investigate its electronic structure in detail, we have chosen Co^{III} tetramine complexes as the model of the enzyme and tried to trace the reaction of the complexes with catechol and dioxygen. The reaction of cobalt complexes and catechols have been studied well.[24-26] The tetramine (N_4) ligand is considered to be the simplest model for amino acid residues, and we can easily control the ligated environment. It is expected that the substitution of the cobalt ion makes it possible to isolate the intermediate species which has not been found in the iron complexes. As a result the complex in which one electron transfers from catecholate ligand to the cobalt ion has been found as the stable intermediate species. In this paper we wish to demonstrate that this intermediate complex plays an essential role in the reaction process. Furthermore, we will clarify its electronic structure by isolating this intermediate species, and show that in this intermediate complex the Co^{II} ion and semiquinone radical are coupled antiferromagnetically. As mentioned above the related intermediates where the central metal ion and its radical ligand are spin coupled have been found in native enzymes.[27,28] In this regard such a spin-coupled intermediate is also suggested to be important in the reaction of native dioxyganase itself.

Experimental

Materials. The complexes *cis*-α-$[Co^{III}Cl_2(trien)]Cl$ (**1**),[29) $[Co^{III}(trien)\ dbc]Cl$,[30] and $[Co^{III}(trien)dbsq]Cl_2$[31] were prepared according to published procedures. $[Co^{III}Br(Et_4[12]aneN_4)(H_2O)]Br_2$ (**2**) was synthesized as described previously.[32] 3,5-di-*t*-butylcatechol (H_2dbc) was recrystallized from hexane twice and air dried. Although various catechols were used as model compounds of the substrate, H_2dbc was most suitable to obtain stable intermediates. Then we mainly used dbc as a model of the substrate to detect the intermediates.

$[Co^{II}(trien)dbsq]Cl$ was synthesized by heating **1** with an equimolecular amount of H_2dbc in MeOH : H_2O (1 : 1) solution under inert atmosphere for 24 h. The solution was evapolated to dryness and the remained powder was washed with hot hexane and recrystallized from ethanol. Found: C, 54.94; H, 8.60; N, 10.92; Cl, 8.15%. Calcd for $C_{20}H_{38}N_4O_2CoCl$: C, 52.11; H, 8.31; N, 12.15; Cl, 7.67%. The disagreement of the values is caused by $[Co^{III}(trien)dbsq]Cl_2$, which emerged in the reaction. We will discuss the reason later.

Measurements. The ESR measurements were performed using a JEOL FE-3X X-band spectrometer, whose microwave frequency was measured with a Takeda Riken TR5211A frequency counter. The temperature was controlled by a JEOL VT3A variable-temperature cryosystem (50 °C) and by an Air Products Model LTR-3-110 cryostat (4.2 K). Simultaneous ESR and optical measurements were carried out by using a JEOL ES-ESRET2 system in conjunction with an MCPD-1000 spectrometer, Ohtsuka Electronic Co., Ltd. Proton NMR spectra were recorded on a JEOL GX-500 (500 MHz) spectrometer with sodium 3-trimethylsilylpropionic acid as an internal reference. Magnetic susceptibility measurements were carried out by using a microcomputer-controlled magnetic tortion balance in which the Faraday method was employed to measure the susceptibility from 1.6 K to room temperature. The microcrystalline powder samples of 30 mg were used for the susceptibility measurements. The temperature of the sample was measured with an Au(Co)–Cu thermocouple and a Ge resister which was calibrated against the magnetic susceptibility of Mn Tutton salt.[33] The applied field gradient was estimated from the susceptibility of $HgCo(SCN)_4$.[34]

Procedures. The experimental procedures are as follows: First, $Co^{III}(N_4)$ complex (**1**,**2**) and H_2dbc were mixed in H_2O/MeOH (1 : 1) to react with dioxygen. We performed this reaction at 50 °C, as the reaction did not proceed below 30 °C. The time profile of this reaction at 50 °C was traced by NMR, ESR, and absorption spectra. In the case of **1**, prepared $[Co^{III}(trien)dbc]^+$ and $[Co^{III}(trien)dbsq]^{2+}$ were also reacted with dioxygen in H_2O/ MeOH (1 : 1) at 50 °C and traced by ESR and absorption spectra. Since the acidic conditions accelerate the reaction of $[Co^{III}(trien)dbc]^+$, we performed this by adding hydrochloric acid dropwise. On each reaction, to detect the intermediate state, parts of the reaction mixture were taken subsequently during the reaction and ESR spectra of the solution were measured at 4.2 K. Second, all the same reactions were performed in the absence of dioxygen. Finally we isolated the intermediate species and measured the magnetic moment to clarify the electronic structure of the intermediate.

Results and Discussion

^1H NMR Spectra. (a) 1. The NMR spectrum of **1** is shown in Fig. 2 (a). In the spectrum of **1**, the peaks around δ=2.4—3.5 are due to protons in the ligand

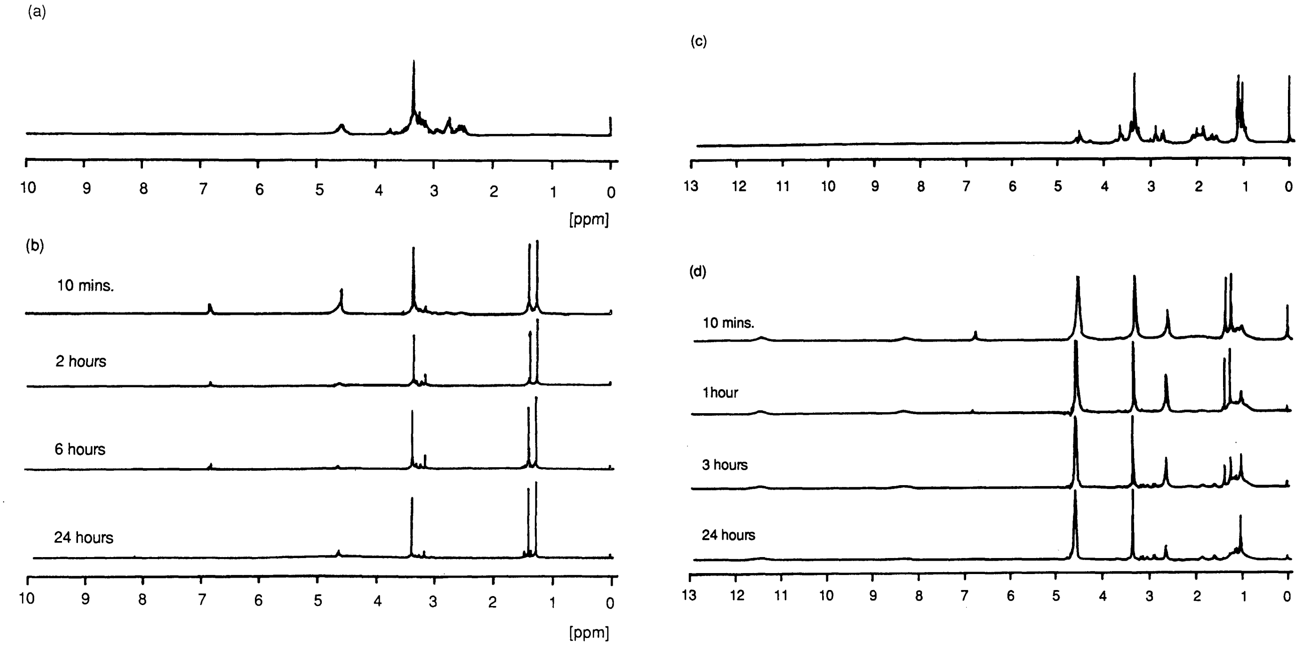


Fig. 2. ^{1}H NMR spectra of (a) [Co^{III}Cl$_2$(trien)]Cl (**1**) (b) The time profile of the reaction of **1** with H_2dbc at 50 °C in aerobic conditions (c) [Co^{III}Br(Et$_4$[12]-aneN$_4$)(H_2O)]Br$_2$ (**2**) (d) The time profile of the reaction of **2** with H_2dbc at 50 °C in aerobic conditions. Every spectra were taken in D_2O : CD_3OD (1 : 1) solution. The peak at δ=3.4 and 4.7 are due to the solvents.

tetramine moiety of the complex. H_2dbc exhibits the peaks at δ=1.25, 1.38 and δ=6.82 due to the protons in the two *t*-butyl groups and ring protons, respectively. The time dependence of the NMR spectral change in the reaction of **1** with H_2dbc in the presence of dioxygen is also shown in Fig. 2 (b). The peaks due to the protons of H_2dbc decreased and some new peaks appeared at δ=1.27, 1.43 in the case of **1**. These new peaks shows the existence of new products derived from H_2dbc.

(b) 2. The NMR spectrum of **2** is shown in Fig. 2 (c). The peaks of the ligand tetramine moiety of the complex spread over from 0.9 to 3.7 ppm. The time dependence of the reaction is shown in Fig. 2 (d). The new peaks at δ_1=8.35, 11.25 and δ_2=2.65 emerged soon after the reaction began, and gradually decreased. As the peaks at δ_1 paramagnetically shifted and broadened, they are suggested to be due to *t*-butyl part of semiquinone, and δ_2 is to an unknown intermediate. A new peak at δ_3=1.02 is ascribed to the products derived from H_2dbc. The other various peaks at 0.9 to 3.7 mainly due to the starting complex **2** emerged as the reaction went on. This indicates that **2** was reproduced after the reaction.

In both cases, the broad signal around δ=2 showed the existence of paramagnetic species in the system during the reaction. Funabiki et al.[11] chemically synthesized various kinds of organic compounds which were possibly obtained in the model reactions of dioxygenase. They measured the NMR spectrum of each compound to identify the final products of the model reactions. Using their data we can suggest the products which were obtained in our reactions. The possible products are

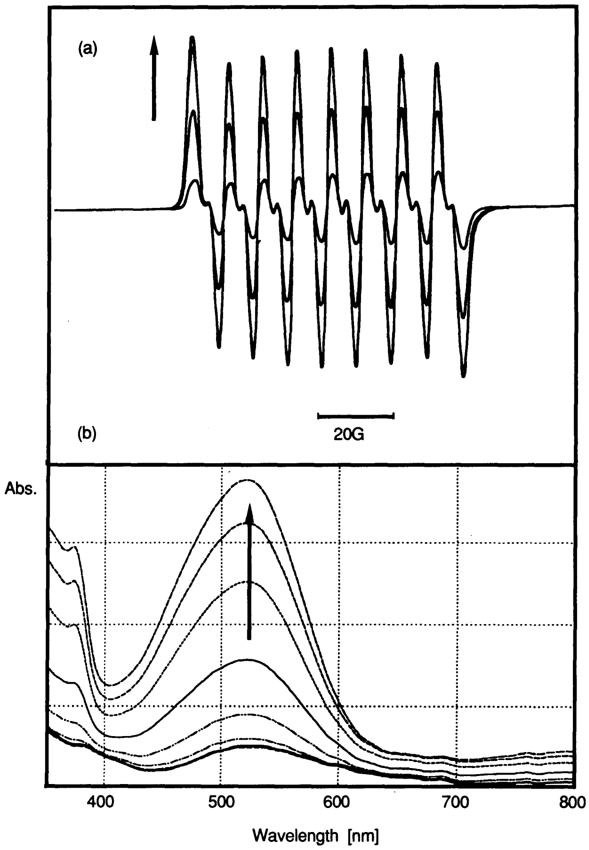


Fig. 3. The time-dependent ESR and absorption spectral change during the reaction at 50 °C. In absorption spectrum, successive scans are 60 seconds apart and ESR spectrum 90 seconds.

lactones in the reaction using **1**, and pyrogallol in that of **2**. This fact shows that ring cleavage occurred and the dioxygen was introduced into the substrate in the reaction using **1**.

ESR and Absorption Spectrum Measurements. The time-dependent change in the ESR and absorption spectra during the reaction at 50 °C are shown in Fig. 3. In both cases of **1** and **2**, almost the same ESR spectra were observed, so that we show the spectrum of **1** in the figure. Since this ESR spectrum consists of eight lines due to the nucleus of Co^{III} (I=7/2) and each line is further split into two lines by one proton on the catechol ring, it is identified as a signal of the semiquinone radical bound to the diamagnetic Co^{III} ion. An increasing band around λ_{max}=520 nm of the absorption spectrum corresponds to a π–π* transition of semiquinone radical. The correlation between the intensity of ESR and that of λ_{max} is linear, indicating that this radical is one of the intermediate of the reaction. The quantity of this radical was saturated in 30 min. In the case of **1**, these two spectra are consistent with the data previously reported as $[Co^{III}(trien)dbsq]^{2+}$.31) In the case of **2**, this semiquinone radical was also detected by NMR. These results indicate that one-electron oxidation of catechol to semiquinone radical occurred and Co^{III}-semiquinone gradually emerged in this reaction. In both cases, after the reaction for 24 h, the quantity of this radical complex gradually decreased.

Since the same reaction was expected in the case of $[Co^{III}(trien)dbc]^+$ with dioxygen, we traced this reaction for 24 h at 50 °C by ESR and absorption spectra. However, semiquinone radical could scarcely be detected, which means that the reaction did not proceed in this case. On the contrary, this reaction easily proceeded in acidic conditions by a dropwise addition of HCl into the solution of $[Co^{III}(trien)dbc]^+$. The color of the solution turned from light green to deep red and the ESR signal of the Co^{III}-semiquinone radical was detected instantaneously. This finding indicates clearly that the bidentate chelating of catechol stabilizes the substrate and protects the reaction to proceed further. In the acidic conditions the equilibrium between bidentate and unidentate ligation of catechol shifts to the unidentate form, in which the oxidation is easy to occur. Thus we can conclude from these results that the unidentate ligation of catechol develops the one-electron oxidation of catechol. Synthesized $[Co^{III}(trien)dbsq]^{2+}$ was also made to react with dioxygen. However, this complex is so stable that no further reaction occurred.

To know the role of dioxygen and the process of activation of the substrate, the same reactions were carried out in degassed conditions. Also in these reactions the solutions turned deep red and the Co^{III}-semiquinone radical was detected at 50 °C both by ESR and absorption spectrum. ESR spectra of the solutions were measured at 4.2 K, which are shown in Fig. 4. The peak near g=2.0 in the figure corresponds to the Co^{III}-semiquinone radical detected at 50 °C. The signal

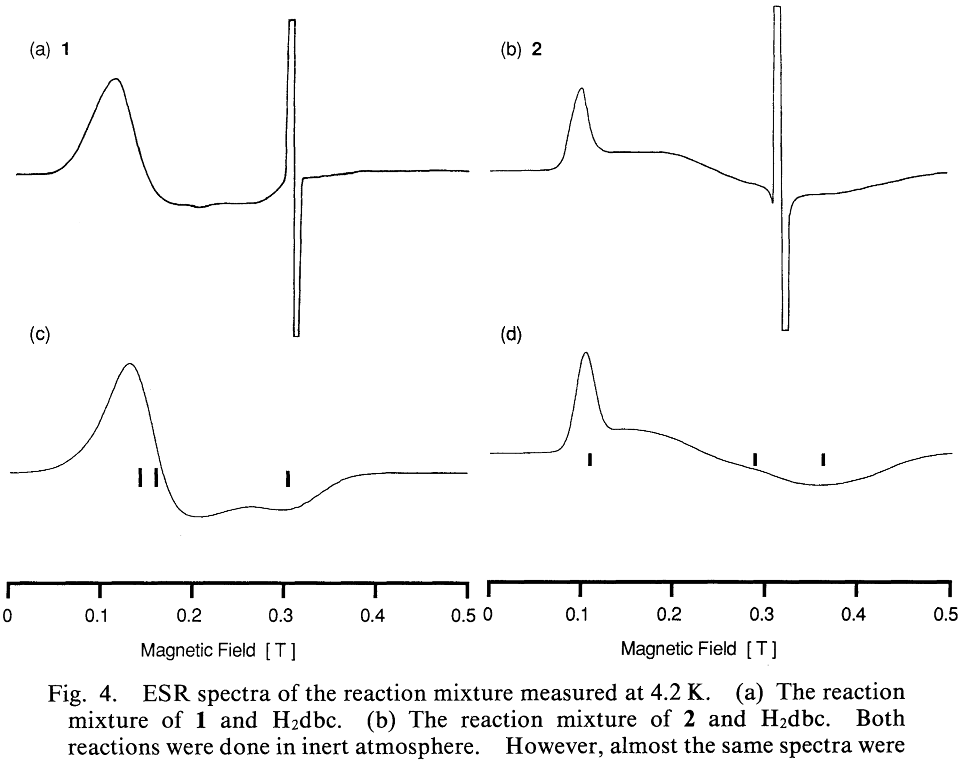


Fig. 4. ESR spectra of the reaction mixture measured at 4.2 K. (a) The reaction mixture of **1** and H_2dbc. (b) The reaction mixture of **2** and H_2dbc. Both reactions were done in inert atmosphere. However, almost the same spectra were observed in aerobic conditions. (c) Simulation spectrum of high-spin (S=3/2) Co^{II} ion for (a). The g-values used for this simulation are 4.5, 4.0, 2.2. (d) Simulation spectrum for (b). The g-values used for this smulation are 6.5, 2.5, 1.8.

around g=4 clearly indicates that this is due to an high-spin (S=3/2) Co^{II} ion. This is consistent with the paramagnetic species detected by NMR around δ=2. Simulated spectra of the Co^{II} ion are also shown in the figure. The g-values derived from the simulation procedure are g_1=4.5, g_2=4.0, g_3=2.2 for **1** and g_1=6.2, g_2=2.5, g_3=1.8 for **2**, respectively. As this reaction went on even in degassed conditions, it can be concluded that the oxidation of catechol to semiquinone is not caused by dioxygen. Thus the essence of oxidation is considered to be an electron-transfer reaction from catechol to the Co^{III} ion.

Usually the analysis of the ESR spectrum of high-spin Co^{II} ion under O_h symmetry (6-coordinate) is complicated, because of the 3-fold degeneracy of the orbital part of the ground state.[35] Nevertheless simple spin-Hamiltonian can be used unless the complex has high symmetry.[36] We used spin-Hamiltonian approximation, because these complexes are assumed to have rhombic symmetry and the g-values derived from the simulated spectrum are well characterized by the spin-Hamiltonian. So we analyze them by the following spin-Hamiltonian for an S=3/2 system:

$$\tilde{H} = D\left(S_z^2 - \frac{5}{4}\right) + E(S_x^2 - S_y^2) + g\beta \boldsymbol{H}\cdot\boldsymbol{S} \qquad (1)$$

where D and E are the zero-field splitting parameter. The E/D value exhibits the rhombicity of the complex and its relation with observed g-value can be calculated by using the perturbation theory. The relationship between E/D and g-value is plotted in Fig. 5 with the observed g-values. The E/D values were estimated to be 0.05 for **1**, and 0.28 for **2** from this analysis. In this case effective g-value is assumed to be 2.2. As the E/D value of **2** is greater than that of **1**, high-spin Co^{II} complex of **2** is more distorted than that of **1**.

By combining these results with those of NMR shown in Fig. 2, the following scheme of the reaction can be deduced: Unidentate binding of catechol facilitates the charge transfer from catechol to $Co^{III}(N_4)$ ion to produce an intermediate, $Co^{II}(N_4)$-semiquinone binary complex. If dioxygen exists in the solution, oxygenation of catechol occurs to yield a ring-cleavaged product. Since in the case of $[Co^{III}(trien)dbc]^+$ or $[Co^{III}(trien)dbsq]^{2+}$ the reaction with dioxygen did not proceed, this $Co^{II}(N_4)$-semiquinone complex, not $Co^{III}(N_4)$-catechol complex or $Co^{III}(N_4)$-semiquinone complex, is considered to facilitate oxygen binding to the substrate. If the scheme described above is correct, the intermediate complex must be the Co^{II} ion ligated by a semiquinone radical, $Co^{II}(N_4)$dbsq. The high-spin Co^{II} ion is a paramagnetic substance with S=3/2 spin and the semiquinone radical has S=1/2 spin. If they bind together, there should be an exchange interaction between the two spins. This interaction erases or changes the ESR spectrum of pure high-spin Co^{II} ion. Nevertheless, from the ESR study of the mixed solution without dioxygen, it was shown that there were Co^{III}-semiquinone radical and high spin $Co^{II}(N_4)$ species in the reaction mixture. Therefore we postulate the following equilibrium:

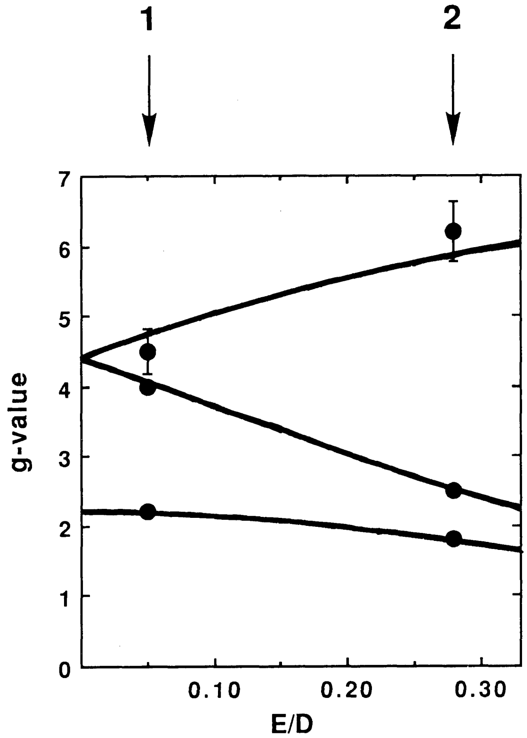


Fig. 5. Theoretical curves of g-values versus E/D accompanied by the experimental results of **1** and **2**.

$$Co^{II}(N_4)dbsq + Co^{III}(N_4) \rightleftharpoons Co^{III}(N_4)dbsq + Co^{II}(N_4) \qquad (2)$$

This scheme means that at first an electron transfer occurs and $Co^{II}(N_4)$dbsq is created. Since there still remains unreacted $Co^{III}(N_4)$ complex in the system, the exchange of the cobaltous ion with the cobaltic ion produces the right-hand-side complexes. As the reaction goes on and the concentration of unreacted $Co^{III}(N_4)$ complexes decreases, the equilibrium shifts to the left side of the scheme. Then the Co^{III}-semiquinone radical on the right side gradually decreases, as was shown in the ESR measurement, and finally we can get $Co^{II}(N_4)$dbsq quantitatively. Here we can observe the ESR signal of the compounds on the right-hand-side of this scheme. Although we could not identify the species in this scheme by NMR spectra, it was suggested the existence of paramgnetic species in the system by the broad signal around δ=2 ppm. Since the compounds on the left-hand-side can not be detected by ESR there is no direct evidence to justify this scheme. Thus it was necessary to clarify the existence of $Co^{II}(N_4)$dbsq and of some interaction between the Co^{II} ion and the semiquinone radical.

Magnetic Susceptibility Measurement. We measured the magnetic susceptibility of $[Co^{II}(trien)dbsq]^+$,

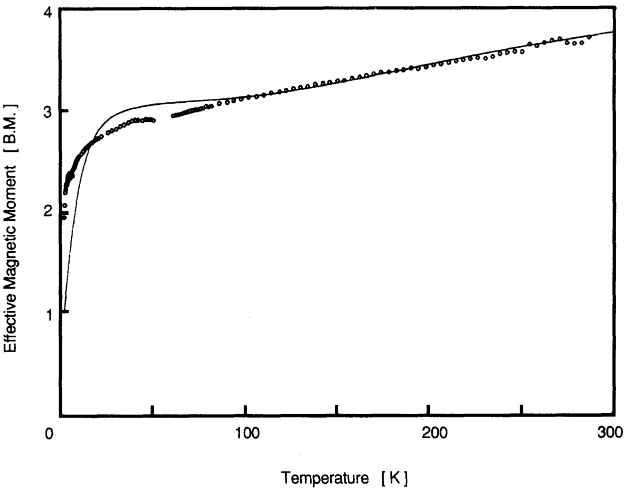


Fig. 6. Temperature dependence of the μ_{eff} of $[\mathrm{Co^{II}(trien)dbsq}]^{+}$. The solid line in the figure exhibits the calculated value by using Hamiltonian (3) and eigenfuncton (4) for $J=-100$ K and $D=25$ K.

and $[\mathrm{Co^{III}(trien)dbsq}]^{2+}$. The effective magnetic moment (μ_{eff}) is more convenient than the magnetic susceptibility itself in order to investigate the spin state of the complex. The μ_{eff} of the complex was derived from the measured value of χ by $\mu_{\mathrm{eff}}{}^2=3kT\chi/\beta^2$. Figure 6 shows the temperature dependence of the μ_{eff} of synthesized $[\mathrm{Co^{II}(trien)dbsq}]\mathrm{Cl}$. This figure shows that the μ_{eff} gradually increases as temperature rises. This tendency exhibits the existence of antiferromagnetic coupling between the two spins: That is, at low temperature region, the $S=1$ ground state ($\mu_{\mathrm{eff}}=2.83$) is occupied, and as temperature rises, an $S=2$ excited state is partially occupied, which increases the value of μ_{eff}.

This result was analyzed by the following Hamiltonian,

$$\tilde{H} = -J\boldsymbol{S}\cdot\boldsymbol{s} + D\left(S_z^2 - \frac{5}{4}\right) + \mathrm{E}\left(S_x^2 - S_y^2\right) + g\beta\boldsymbol{H}\cdot(\boldsymbol{S}+\boldsymbol{s}), \quad (3)$$

where $S=3/2$ is the cobalt(II) spin, $s=1/2$ the radical spin, and J is the exchange interaction between the two spins. The eigenfunctions of the system can be obtained by,

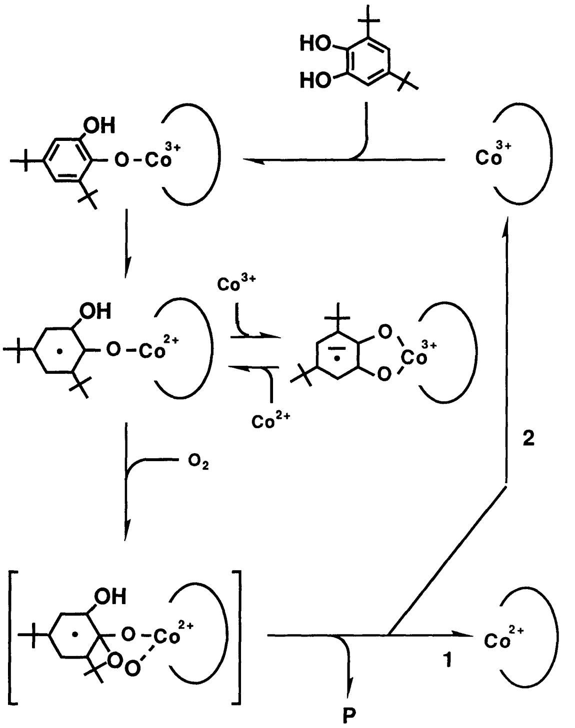
$$|SM_s\rangle = \sum_{M,m} \left\langle \frac{3}{2}M\frac{1}{2}m \,\middle|\, SM_s\right\rangle \left|\, \frac{3}{2}M\right\rangle \left|\, \frac{1}{2}m\right\rangle. \quad (4)$$

Using these Hamiltonian and eigenfunctions, numerical calculation by a computer was done to derive the magnetic moment. The solid line in Fig. 6 was obtained by the model that the high-spin $\mathrm{Co^{II}}$ ion and the semiquinone radical form a binary complex and antiferromagnetically couple each other by the energies $J=-100$ K and $D=25$ K. The calculated values fit well to the experimental value in the high-temperature region, which means that the spin-coupled $\mathrm{Co^{II}(trien)dbsq}$ was formed almost quantitatively. In the low-temperature region the experimental data slightly deviate from theoretical curve. This is probably due to the paramagnetic species formed according to Eq. 2, which caused the disagreement of the elemental analysis values. On the contrary, the μ_{eff} of $[\mathrm{Co^{III}(trien)dbsq}]^{2+}$ was temperature independent from 1.6 K to room temperature and the value was 1.70 B.M. This result indicates that $[\mathrm{Co^{III}(trien)dbsq}]^{2+}$ has pure radical spin ($S=1/2$) and its electronic structure is completely different from that of $[\mathrm{Co^{II}(trien)dbsq}]^{+}$.

We also measured the magnetic susceptibility of $[\mathrm{Co^{II}(Et_4[12]aneN_4)dbsq}]^{+}$. This complex was synthesizd in the same way as $[\mathrm{Co^{II}(trien)dbsq}]^{+}$ described in the experimental section. Nevertheless, the μ_{eff} was about 2.5 B.M. (20—300 K), so that it is smaller than what is expected. This correspond to the fact that in the case of **2**, $[\mathrm{Co^{II}(Et_4[12]\text{-}aneN_4)dbsq}]^{+}$ reproduces the starting complex **2** as is mentioned in the NMR section. Furthermore it reflects the difference of the electronic structure of $[\mathrm{Co^{II}(N_4)dbsq}]^{+}$, especially the difference of magnetic interaction. In fact δ_1 peaks were not observed in the case of **1**.

Makinen et al.[36] theoretically proposed that the zero-field splitting of $\mathrm{Co^{II}}$ high-spin complex is a good parameter to estimate the coordination number of the complexes. According to their estimate, D of 25 K for **1** is a value of a pentacoordinate complex, so that this complex is considered to be five-coordinate which means the unidentate ligation of the semiquinone radical. From these results, it is concluded that the intermediate is a $\mathrm{Co^{II}}$-semiquinone radical complex in which the semiquinone binds to the $\mathrm{Co^{II}}$ ion unidentately and coupled antiferromagnetically.

Overall Reaction Scheme. Based on the results described in the preceding sections, we wish to propose the overall reaction scheme, as is shown in Scheme 2. First catechol binds to the $\mathrm{Co^{III}(N_4)}$ complex in a unidentate fashion and one electron oxidation of catechol takes place by an electron-transfer from catechol to the cobalt ion. Here is the equilibrium between $\mathrm{Co^{II}(N_4)dbsq + Co^{III}(N_4)}$ and $\mathrm{Co^{II}(N_4) + Co^{III}(N_4)dbsq}$, where the latter species are ESR detectable. When there is dioxygen in the system, dioxygen is introduced into $\mathrm{Co^{II}(N_4)dbsq}$. In the case of **1**, the ring cleavaging reaction goes on and the complex remains divalent. In the case of **2**, addition of dioxygen occurs and $\mathrm{Co^{II}}$ returns to $\mathrm{Co^{III}}$. This difference between the two complexes is brought about by the difference of the redox potential of the complexes. As is shown in the ESR spectra of $\mathrm{Co^{II}}$ complexes, divalent complex of **2** is more distorted than that of **1**, so that the valence of **2** is easier to change compared with **1**. In the five-coordinate intermediate $\mathrm{Co^{II}(trien)dbsq}$, the cobalt spin and radical spin interact antiferromagnetically. The spin coupled intermediate is considered to play an important role in the reaction, which activates the substrate and facilitates the introduction of dioxygen into the substrate. On the



Scheme 2. Overall reaction scheme of Co(N_4) with H_2dbc.

contrary, $[Co^{III}(trien)dbsq]^{2+}$ which can be synthesized chemically, is stable in the air and the reaction does not go on further. $[Co^{III}(trien)dbc]^{+}$ is also stable with the coexistence of dioxygen in alkaline condition, where catechol chelates to the Co^{III} ion in a bidentate fashion to prevent the charge transfer from catechol to the cobalt center. These facts also support that the formation of the charge-transfered complex, $Co^{II}(N_4)$dbsq, is the essential process, which can introduce dioxygen to the substrate.

Our result that the charge-transferred complex is essential, is consistent with what Cox et al. proposed.[18] They reported an investigation of dioxygen reactivity of $[Fe^{III}(L)dbc]^{2-}$ complexes, where L represented a series of tripodal ligands. They indicated that the more Lewis acidic the metal center becomes, the more increases the reactivity. Still more, from the results on NMR properties of the complexes, the coordinate dbc ligand is proposed to have semiquinone character, and greater the semiquinone character in the complex, the faster it react with dioxygen. They remarked that the semiquinone character is a result of covalency of the Fe^{III}-catechol bond, which is enhanced as the Lewis acidity of the metal center increases. The radical character facilitates the attack of O_2 on substrate, generating a transient peroxyl radical, which is reduced by the nascent Fe^{II} center, forming the intermediate Fe^{III}-peroxide complex. However they could not detect the Fe^{II}-semiquinone species directly.

We have used the Co^{III} ion instead of the Fe^{III} ion as the central metal to get the intermediate state stably and detect it directly. This aim was achieved by isolating $Co^{II}(N_4)$dbsq. We postulate the reason of this stability as follows: When the charge transfer occurs, dioxygen binds to the semiquinone moiety in the binary complex. In the case of iron enzyme or iron complexes, the back charge transfer from the iron ion to dioxygen immediately occurs soon after the binding of dioxygen, and this superoxide-semiquinone radical is led to yield the ring-cleavaged products; while in the case of our cobalt complex, the charge transfer from Co^{II} ion to dioxygen does not occur in spite of the coexistence of dioxygen. Then the further reaction hardly goes on compared with the iron complexes. Such difference may be caused mainly by the difference of the redox potential between the iron ion and the cobalt ion.

In conclusion we have succeeded to obtain the charge-transferred intermediate in the model reaction of dioxygenase by substituting the central iron ion to the cobalt ion. This charge-transferred Co^{II}-semiquinone radical complex is essential in the dioxygenase reaction.

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