ESR Evidence of the Formation of a New Superoxide Complex of Tetra-*p*-tolylporphyrinatocobalt(II) in Aprotic Solvents

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Abstract

The reactions of the superoxide ion (O_2^-) with tetra-*p*-tolylporphyrinatocobalt(II) [Co(II)TTP] in dimethyl sulfoxide (DMSO) have been investigated by use of electron spin resonance (ESR) spectroscopy. In the absence of oxygen, Co(II)TTP in DMSO gives the DMSO adduct, Co(II)(TPP)(DMSO). When this DMSO adduct is exposed to air, an oxygen complex, Co(II)(TTP)(DMSO)(O_2), is formed in which the binding state between Co(II) and O_2 has been considered formally as Co(III)–O_2^-. When the superoxide ion (O_2^-) is added to this oxygen complex, a new superoxide complex, Co(II)(TTP)(O_2^-)$_2$, is formed. The same superoxide adduct is formed by the reaction of O_2^- with Co(II)TTP in the absence of oxygen.

Introduction

The nature of dioxygen binding to myoglobin and hemoglobin has been intensively investigated [1–3] during the past decade, and many model compounds that reversibly bind dioxygen have been synthesized in an effort to understand the steric and electronic factors which control oxygen binding in native proteins. These model systems include the 'picket-fence' [4, 5], 'chelated' [6], 'capped' [7, 8], 'strapped' [9] and 'basket-handle' [10] metalloporphyrins. Ideally, the spectroscopic and functional response in the model systems should be related to the corresponding properties of the native systems. In order to use such model compounds effectively, it is important to establish reliable spectroscopic probes of the key structural elements in the models and native systems, especially the metal–oxygen groups.

In these model compounds, cobalt was chosen as the central metal ion rather than iron for two reasons [11]: (i) more extensive thermodynamic data are available for related cobalt systems [12–19]; and (ii) studies of the cobalt systems include further results of differing hypotheses [20, 21] concerning the oxygen binding in cobalt-substituted and natural hemoglobins.

Ibers *et al.* have shown that cobalt(II) porphyrins bind oxygen reversibly [15]. Hoffman and co-workers [12, 13] have shown that cobalt-substituted hemoglobin and myoglobin (CoHb and CoMb) are reversible oxygen carriers and that CoHb exhibits the same allosteric linkages as normal hemoglobin. Maxwell *et al.* [22] have shown that infrared stretching frequencies of bound dioxygen are essentially the same in both HbO_2 and $CoHbO_2$. Collman *et al.* [23] have documented the same fact for simple metalloporphyrins.

It is now clear that both the Co and Fe dioxygen complexes are best formulated as M(III)–O_2^- [24, 25].

Electron spin resonance (ESR) studies on the dioxygen complex of Co(II) porphyrins indicate that Co(III)–O_2^- (superoxo complex) bonding is involved in these complexes [3, 26]. In order to examine the binding state of these complexes, we intended to study the reaction of O_2^- with the Co(III) complex of tetra-*p*-tolylporphyrin (TTP)**, [Co(III)TTP$^+$], the structure of which is shown in Fig. 1, in order to detect the Co(III)–O_2^- adduct directly. As a result,

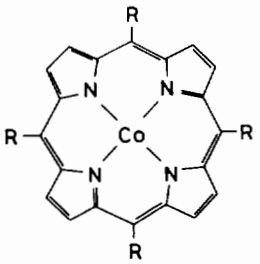

Fig. 1. Structure of Co(III)TTP (R = (*p*-CH_3)phenyl) and Co(III)TPP (R = phenyl).

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**Abbreviations used: TTP, tetra-*p*-tolylporphyrin; TPP, tetraphenylporphyrin; ESR, electron spin resonance; DMSO, dimethyl sulfoxide; CH_2Cl_2, dichloromethane; O_2^-, superoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; L, ligand; B, base; py, pyridine; CoP-450, cobalt-substituted P-450; cam, camphor; TMeOPP, tetra-(*p*-methoxyphenyl)porphyrin; CoHb, cobalt-substituted hemoglobin; CoMb, cobalt-substituted myoglobin.

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unexpectedly, Co(III)TTP$^+$ was stoichiometrically reduced to Co(II)TTP by superoxide (O_2^-) in dimethyl sulfoxide (DMSO) solution [27] and the Co(II)TTP thus formed subsequently reacted with O_2^- present in excess to form the O_2^- adduct, undergoing consecutively intramolecular electron transfer to give Co(III)TTP$^+$ and HO_2^- [28]. Using an ESR method, we have further investigated the reaction of O_2^- with Co(II)TTP in detail.

In this paper, we report ESR evidence of a new superoxide complex of Co(II)TTP, Co(II)(TTP)-$(O_2^-)_2$, which is produced from the reaction of O_2^- with Co(II)TTP in the presence of oxygen in DMSO.

Experimental

Materials

Co(II)TTP was synthesized by a similar method to that described in the literature for the tetraphenylporphyrin (TPP) complex [29]. Potassium superoxide (KO_2) was purchased from Alfa Products. Dicyclohexyl-18-crown-6 (Nippon Soda Co.) was used without further purification. Other reagents used were commercially available. DMSO was distilled at reduced pressure from CaH_2 and stored over freshly activated 4A molecular sieves under argon gas. Dichloromethane (CH_2Cl_2), which had been distilled, was passed through an alumina column to remove the stabilizer and impurities. Other solvents were distilled immediately prior to use.

Preparation of Solutions of Superoxide

DMSO and acetonitrile solutions of O_2^- were prepared as described previously [30, 31] in order to examine the solvent effects. The concentration of O_2^- was determined by the titration method with ferricytochrome *c*, as described by Bielski *et al.* [32].

Spectral Measurements

ESR spectra were measured at room temperature or at 77 K with a JEOL-PE-1X (X-band) spectrometer with 100 kHz field modulation. ESR parameters were calibrated by comparison with the standard sample of Mn^{2+} doped on MgO and 2,2-diphenyl-1-picrylhydrazyl (DPPH, $g = 2.0036$).

Electronic absorption spectra were recorded at room temperature with a Union Giken SM-401 spectrophotometer.

ESR Experiments

Aliquots of 10^{-3}–10^{-2} mol dm^{-3} KO_2/dicyclohexyl-18-crown-6 were added to 1 ml of 10^{-3} mol dm^{-3} Co(II)TTP dissolved in CH_2Cl_2–DMSO (1:4). This reaction mixture was tranferred to the ESR quartz tube and was rapidly frozen at 77 K. The ESR spectrum was recorded at 77 K. In anaerobic experiments, a two-armed quartz tube (one-arm was 25 cm long and the other 5 cm) was used. Aliquots of Co(II)TTP and superoxide solutions were transferred into the separate arms. Each solution was frozen, then evacuated repeatedly by the freeze–pump–thaw method. After being warmed to room temperature, the reactants were mixed completely and the ESR spectra were recorded at 77 K.

Results and Discussion

The DMSO–CH_2Cl_2 (4:1) solution of Co(II)TTP, which was evacuated to remove dissolved oxygen, gave an ESR spectrum at 77 K with $g_\parallel = 2.017$, $g_\perp = 2.277$, $A_\parallel = 93.8$ G and $A_\perp = 16.4$ G, as shown in Fig. 2a. The ESR spectra of the unoxygenated cobaltous porphyrins are characteristic of a low-spin d^7 configuration with the unpaired electron in the d_{z^2} orbital [3]. The spectra exhibit two major sets of features, assigned as $g_\parallel$ and $g_\perp$, which are characteristic of a species with essentially axial symmetry. These regions can further split into an octet by hyperfine splittings (hfs) from the ^{59}Co ($I = 7/2$) nucleus. The ESR spectrum of a five-coordinate Co(II) porphyrin also gives evidence as to the nature of the axial ligand. When the axial ligand is a strong nitrogenous base [e.g. pyridine (py)], the eight hfs lines in the $g_\parallel$ region each exhibit an additional splitting into a triplet resulting from superhyperfine coupling (shfs) due to the axial ^{14}N ($I = 1$) nucleus. On the other hand, neither sulfur nor oxygen donor ligands have a naturally abundant isotope with nuclear spin, and therefore there are no shfs in the $g_\parallel$ region. Furthermore, when a weak Lewis base (such as alcohol, ketone, ether or thiol) coordinates to Co(II) porphyrins, the most noticeable feature is partial resolution of the cobalt hfs in the perpendicular region. Since the ESR spectrum in Fig. 2a does not show any shfs in the $g_\parallel$ region but shows the partial resolution of the cobalt hfs in the perpendicular region, it is suggested that DMSO coordinates to Co(II)TTP to give the five-coordinated complex Co(II)(TTP)-(DMSO). This suggestion is supported by the UV–Vis spectral changes accompanied by the stepwise addition of DMSO to Co(II)TTP dissolved in CH_2Cl_2, which is a non-coordinating solvent. That is, the visible absorption spectrum of Co(II)TTP in CH_2Cl_2 (Soret band at 414 nm and another at 528 nm) shifted to that of Co(II)(TTP)(DMSO) (Soret band at 418 nm and another at 533 nm) [28].

When the solution of Co(II)(TTP)(DMSO) was exposed to air, the ESR spectrum of this Co(II) complex underwent a dramatic change to give a new ESR signal (Fig. 2b) with the following parameters: $g_\parallel = 2.089$, $g_\perp = 1.998$; $A_\parallel = 25.6$ G, $A_\perp = 14.4$ G. These parameters and ESR spectral patterns closely resemble those assigned to cobalt(II)–dioxygen complexes, Co(II)(L_4)(B)(O_2) (where L = ligand; B = base) in

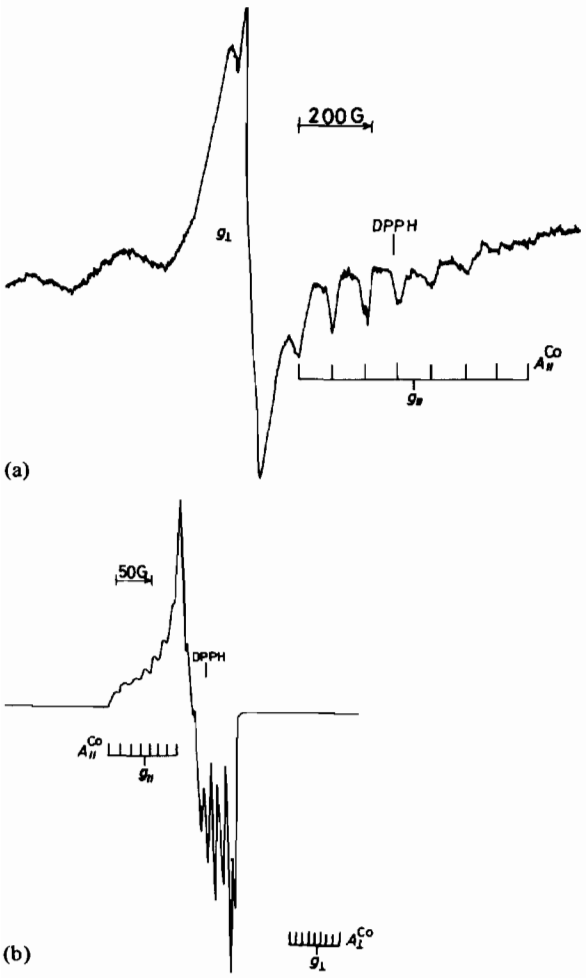

Fig. 2. ESR spectra observed for Co(II)TTP in DMSO–CH_2Cl_2 (4:1) mixed solvent at 77 K in the presence or absence of oxygen: (a) in the absence of oxygen; (b) in the presence of oxygen. Concentration of Co(II)TTP was 10^{-3} mol dm^{-3}.

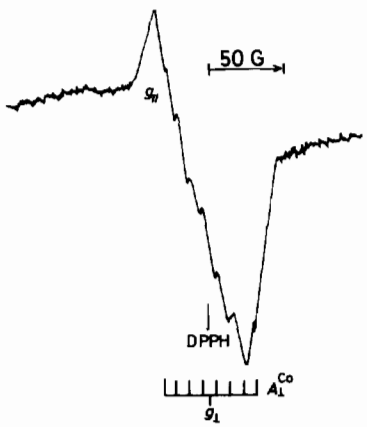

Fig. 3. ESR spectrum observed after addition of the O_2^- solution to Co(II)(TTP)(DMSO)(O_2) in DMSO–CH_2Cl_2 (4:1) solution at 77 K. Concentrations: Co(II)TTP, 10^{-3} mol dm^{-3}; O_2^-, 5×10^{-3} mol dm^{-3}.

which the binding state between Co(II) and O_2 has been considered formally as Co(III)–O_2^- [3]. Then, the ESR spectrum observed upon the reaction of Co(II)TTP with O_2 is assignable to the six-coordinated Co(II)(TTP)(DMSO)(O_2).

When Co(II)(TTP)(DMSO)(O_2) in DMSO–CH_2Cl_2 was mixed with O_2^- in DMSO, the ESR spectrum further changed to give a new signal (Fig. 3) with the following parameters: $g_\parallel = 2.070$, $g_\perp = 2.000$; $A_\perp = 8.6$ G. The $A_\parallel$ component could not be clearly observed. A similar spectrum ($g_\parallel = 2.072$, $g_\perp = 2.004$; $A_\parallel = 15.8$ G, $A_\perp = 9.3$ G) was also observed on reaction of Co(II)(TTP)(O_2) in DMSO–CH_2Cl_2 with O_2^- in acetonitrile. In this spectrum, the $A_\parallel$ component could be observed. These ESR spectral differences indicate the different solvent effects between DMSO and acetonitrile. The new ESR spectrum as shown in Fig. 3 is different from that of Co(II)(porphyrin)-

TABLE I. ESR Parameters of Cobalt Porphyrins and Cobalt-substituted Hemoproteins

Complex	ESR parameters				Solvent	Reference
	$g_\parallel$	$g_\perp$	$A_\parallel$	$A_\perp$		
O_2^-	2.104	2.007			DMSO	This work
O_2^-	2.083	2.008			CH_3CN	[a]
Co(II)TTP(DMSO)	2.017	2.277	93.8	16.4	[b]	This work
Co(II)TTP(py)	2.025	2.326	79.5	12.0	[b]	This work
Co(II)TTP(DMSO)(O_2)	2.089	1.998	25.6	14.4	[b]	This work
Co(II)TTP(O_2^-)$_2$	2.070	2.000		8.6	[b]	This work
Co(II)TTP(O_2^-)$_2$	2.072	2.004	15.8	9.3	[c]	This work
Co(II)TPP(CO)(O_2)	2.070	2.014	12.4	9.6	toluene	[d]
Co(II)TPP(Bu_3P)(O_2)	2.070	2.014	12.0	9.0	toluene	[d]
Co(II)TTP(py)(O_2)[e]	2.074	2.002	17.1	10.8	pyridine	[f]
Co(II)(TMeOPP)(py)(O_2)[g]	2.077	2.002	16.0	10.7	toluene	[h]
CoP-450$_{cam}$(O_2)[i]	2.079	2.008	15.5		H_2O	[j]

[a]Ref. 37. [b]CH_2Cl_2–DMSO (1:4). [c]CH_3CN–CH_2Cl_2–DMSO (5:1:4). [d]Ref. 2. [e]py = pyridine. [f]Ref. 33. [g]TMeOPP = tetra-(*p*-methoxyphenyl)porphyrin. [h]Ref. 38. [i]CoP-450 = cobalt-substituted P-450. [j]Ref. 37.

(Lewis base)(O_2) (py was used as a Lewis base) [33] and has smaller $A_{\parallel}$ and $A_{\perp}$ components than those of Co(II)(TTP)(Lewis base)(O_2). It is known that an increase of the negative charge in the axial ligand tends to reduce the cobalt $d\pi$–oxygen π^* bonding, from which the ^{59}Co ($I = 7/2$) hyperfine splitting in the oxygen complexes arises [2]. Then, the axial coordination of the negatively charged O_2^- to Co(II)-(TTP) may reduce the ^{59}Co hyperfine coupling compared to Co(II)(TTP)(DMSO)(O_2). The possibility of the formation of Co(II)(TTP)(O_2^-)(O_2) is excluded because this compound, even if formed, has either 0, 2 or 4 unpaired electrons. Furthermore, the ESR spectrum shown in Fig. 3 resembles those of oxygen complexes of Co(II)(TPP)(B) (B = $(Bu)_3P$ and CO) [2] (Table I). Therefore, this paramagnetic species is assigned to a new Co(II) complex, Co(II)-(TTP)(O_2^-)$_2$. The formation of the superoxide adduct of Co(II)TTP is also supported by the fact that when hydrogen peroxide, which can destroy O_2^- [34, 35], was added to the radical-containing solutions, the ESR signal shown in Fig. 3 disappeared completely. Furthermore, it is noteworthy that the small hyperfine values observed here are also obtained in the oxygenated Co(II)-substituted P-450 complex [36]. The g-values of Co(II)(TTP)(O_2^-)$_2$ approximate to those of the free superoxide ion ($g_{\parallel} = 2.083$ and $g_{\perp} = 2.008$) [37]. The life-time of this new superoxide adduct is more than 2 h at 77 K. In Fig. 3 the concentration of O_2^- is five times higher than that of Co(II)TTP. Nevertheless, the signal of excess, free superoxide did not appear in Fig. 3. Perhaps the redox reaction between O_2^- with Co(II)TTP may occur [28], although the exact reaction mechanism is not clear. Then, almost all the O_2^- should be consumed by the reaction of O_2^- with Co(II)TTP. Co(II)-(TTP)(O_2^-)$_2$ is also formed by the reaction of Co(II)-TTP with O_2^- in the absence of oxygen.

Conclusions

In the absence of oxygen, Co(II)TTP in DMSO gives Co(II)(TTP)(DMSO). When this adduct is exposed to air, an oxygen complex, Co(II)(TTP)(O_2), in which the binding state is formally Co(III)–O_2^-, is formed. If O_2^- solution is added to this oxygen complex, a new superoxide adduct, Co(II)(TTP)-(O_2^-)$_2$, is formed.

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