Reaction between Superoxide Anion and Several Oxovanadium(IV) Compounds

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SOD-Like Function, Oxovanadium(IV) Compounds, Superoxide Anion

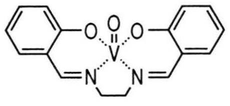
We have observed that the superoxide anion reacts with VO(salen), but not with VO(saldpt), where H_2(salen) and H_2(saldpt) are Schiff base ligands derived from salicylaldehyde and ethylenediamine or dipropylenetriamine, respectively. Based on the electrochemical, ESR, and UV spectroscopic studies of the reaction mixture, it was concluded that the superoxide anion reacts with VO(salen) to oxidize V(IV) to V(V) through the formation of a vanadium(V)-peroxide adduct with side-on configuration. The SOD-like function of VO(salen) was elucidated in terms of the above assumption.

Introduction

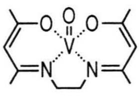
Superoxide dismutases (SOD) [1] which catalyze the dismutation of superoxide anion (O_2^-) to molecular oxygen and hydrogen peroxide [2] are found in all free-living organisms except for some oxygen-sensitive anaerobes. O_2^- is the one-electron reducing product of O_2, and is generated as a by-product of oxidative metabolism. Fridovich *et al.* found that a family of superoxide dismutases having Cu–Zn, Fe or Mn at the active site are important for protection against the toxic effect of this free radical [2]. The structures of Cu, Zn–SOD [3] Fe–SOD [4] and Mn–SOD [5] have been determined, and much attention has been focused on the reaction mechanism of these enzymes [6–8]. We have been interested in the elucidation of the reaction mechanism of SOD in terms of the model compounds and have initiated an investigating of the reaction between superoxide anion and various metal complexes [9, 10]. In the course of this work, we have found that [VO(salen)], an oxovanadium(IV) compound, exhibits a high catalytic activity for decomposition of O_2^-, whereas the activity of [VO(saldpt)] is negligibly low. In this study we have measured the cyclic voltammograms of several oxovanadium(IV) compounds including the two above complexes in the presence of O_2, and we have determined the crystal structure of [VO(saldpt)], in order to elucidate the difference observed for the reactivity of [VO(salen)] and [VO(saldpt)] complexes towards the superoxide anion. Based on these results, the SOD-like function of [VO(salen)] is discussed.

Materials and Methods

Materials. The oxovandadium(IV) compounds used in this study are [VO(salen)], [VO(acen)] [11], [VO(saldpt)] [12], and [VO(TPP)] [13]; in the former three compounds the Schiff bases are used, and H_2(TPP) represents $\alpha,\beta,\gamma,\delta$-tetra($p$-tolyl)porphyrin.



[VO (salen)]



[VO (acen)]

Electrochemical measurements. The cyclic voltammograms (CV) of these compounds were measured in the usual manner; in N,N-dimethylsulphoxide (dmso), 0.1 M tetra-(n-butyl)ammoniumtetrafluoroborate as supporting electrolyte, 0.001 M metal complex, 25 °C, glassy carbon electrode, with the potential referenced to saturated sodium chloride calomel electrode (SSCE). For the CV measurement in the presence of dioxygen, dry air was bubbled through cell for 15 min before the measurement. The concentration of dioxygen in the reaction mixture was estimated to be 0.47 mM based on the result reported by Sawyer *et al.* [14].

Crystal structure determination. A brown prismatic crystal of [VO(saldpt)] ($C_{20}H_{23}N_3O_3V$) having the approximate dimension 0.3 × 0.4 × 0.3 mm was mounted on a glass fiber. All measurements were made on a Rigaku 55 diffractometer with

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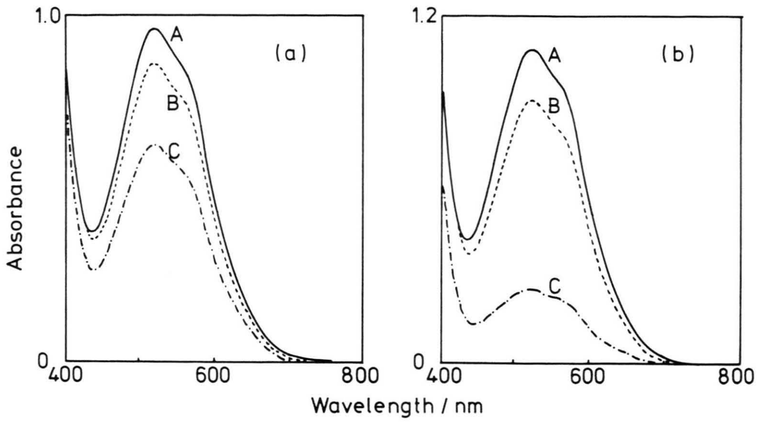


Fig. 1. Absorption spectra of solutions containing oxovanadium(IV) compound, O_2^-, and NBT (*cf.* text).
a) 2 min. after mixing;
b) 30 min. after mixing;
A: no complex;
B: [VO(saldpt)];
C: [VO(salen)].

graphite monochromated CuKα radiation and a 12 kV rotating anode generator at Saga University. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angle of 25 carefully centered reflections in the range $74.12° < 2\theta < 79.28°$ to a monoclinic cell with dimensions; a = 10.896(6), b = 14.048(8), c = 13.267(4) Å, β = 109.85(3)°, V = 1910(1) Å^3, space group $P2_1/a$, Z = 4, F_w = 404.363. The calculated density is 1.406 gcm^{-3} at 23 ± 1 °C. The data were collected using the ω-2θ scan technique. Of the 3138 reflections which were collected, 2966 were unique. The linear absorption coefficient for CuKα is 45.9 cm^{-1}. An empirical absorption correction based on azimuthal scan of several reflections was applied which resulted in transmission factor ranging from 0.51 to 1.00. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods, and the non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 2012 observed reflections ($I > 3.00\sigma(I)$) and 244 variable parameters and converged with unweighted and weighted agreement factor of:

$$R = 0.043,\ R_w = 0.051\ (w = 4F_0^2/\sigma^2(F_0^2)).$$

The standard deviation of an observation of unit weight was 1.70. Neutral atom scattering factors were taken from Cromer and Weber [15]. Anomalous dispersion effects were included in F_{calc}, [16] the values for $\Delta f'$ and $\Delta f''$ were those of Cromer [15]. All the calculations were performed using the TEXSAN crystallographic software package of Molecular Structure Corporation [17, 18].

Evaluation of SOD-like function. Very recently we have observed [19] that nitrobluetetrazolium (NBT) [20] reacts with superoxide anion to give a violet species which exhibits an absorption maximum at 520 nm in dmso-methanol (1:1) solution (*cf.* Fig. 1) and that this absorbance is linearly proportional to the concentration of the superoxide anion, determined by the electrochemical method reported by Sawyer *et al.* [21] (*cf.* Fig. 2). By using this method, we can evaluate the SOD-like function of the oxovanadium(IV) compounds as follows. The dmso solution of O_2^- (1.0 ml, 0.8 mM) and complex (0.5 ml, 0.4 mM) were mixed, and at an appropriate timer after mixing, the content of superoxide anion in the solution was determined by the NBT method in terms of the results shown in Fig. 2.

ESR spectra. The ESR spectra of the compounds were measured with a JEOL ESR apparatus model JES RE-2X using X-band at 77 K.

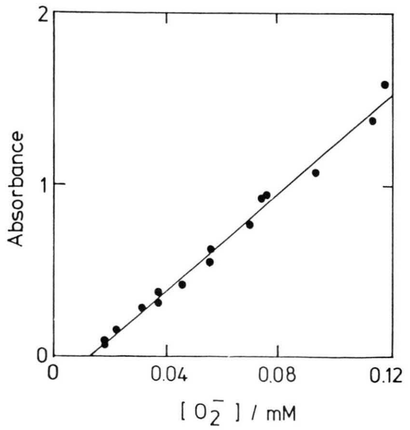


Fig. 2. The plot of the absorbance at 520 nm of the reaction mixture (O_2^- and NBT) against concentration of the superoxide anion determined by the electrochemical method [19].

Results and Discussion

In Fig. 1, the absorption spectra of the reaction mixtures containing the oxovanadium(IV) compound, O_2^-, and NBT (*cf.* experimental section) are shown; nitrobluetetrazolium reacts with superoxide anion to yield a violet solution in dmso/methanol (1:1), the absorption maximum being at 520 nm (see trace A in Fig. 1, (a)) [19]. This absorbance decreased slightly in the solution of [VO(saldpt)] and O_2^-, indicating that the SOD-like function of [VO(saldpt)] is negligible. On the other hand, a notable decrease of the absorbance at 520 nm was observed for the reaction mixture of [VO(salen)] and O_2^- (see also Fig. 3), demonstrating a higher SOD-like function of the latter compound. We have measured the ESR spectra of the reaction mixtures, as shown in Fig. 4. The ESR spectrum of O_2^- is of an axial symmetry [21]. The ESR study was already reported for oxovanadium(IV) compounds [22] and the spectra observed in this study is consistent with the reported ones (see traces B and C in Fig. 4). We have found that the addition of a dmso solution of O_2^- to the [VO(salen)] solution caused the disappearance of the signals of the [VO(salen)], but the ESR signal due to the metal ion is still observed in the reaction mixture of O_2^- (excess) and [VO(saldpt)] (see trace E in Fig. 4). The spectral studies are also agreement with the above facts. As shown in Fig. 5, the addition of O_2^- to the dmso solution of [VO(salen)] leads to notable changes in the absorption spectrum, however no change was observed in the case of [VO(saldpt)] in the d−d band region.

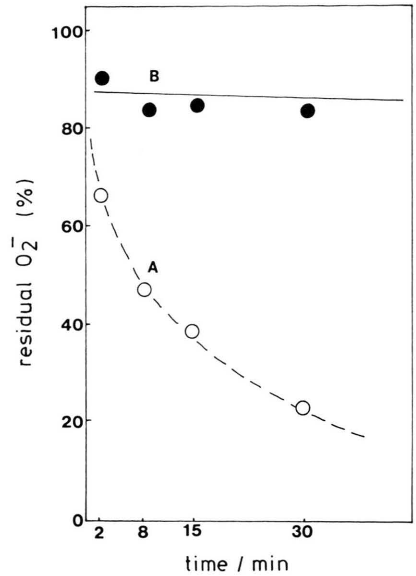


Fig. 3. Time dependence of the content of O_2^- ion in the reaction mixture, determined by the NBT method (see text) A: [VO(salen)]; B: [VO (saldpt)].

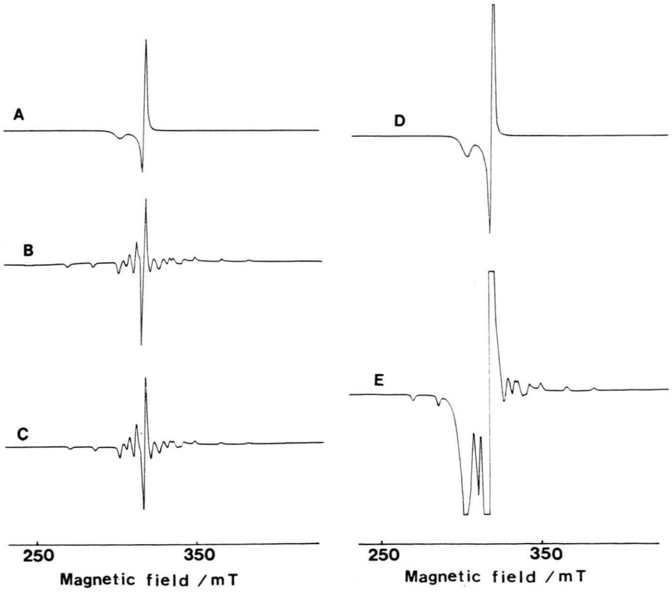


Fig. 4. ESR spectra (in dmso, 77 K, X-band).
A: O_2^- (1 mM); B: [VO(salen)] (1 mM);
C: [VO(saldpt)] (1 mM);
D: [VO(salen)] (1 mM, 1 ml)+O_2^- (2.6 mM, 1 ml);
E: [VO(saldpt)] (1 mM, 1 ml)+O_2^- (2.6 mM, 1 ml).

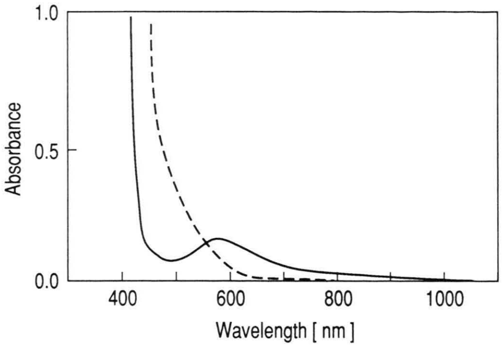


Fig. 5. Absorption spectra fo [VO(salen)] (in dmso, 25 °C);
A: [VO(salen)] (1 mM);
B: [VO(salen)] (1 mM, 1 ml)+O_2^- (2.6 mM, 1 ml).

In order to get more detailed informations on this problem, we have measured the CV of these compounds in the presence of O_2. The electrochemical properties of [VO(salen)] and [VO(acen)] under inert atmosphere have already been reported [9, 23−24]. The redox couple observed at +0.4 V (*vs.* SSCE) for [VO(acen)] (see trace A in Fig. 6) corresponds to the reaction $V(V) \rightleftharpoons V(IV)$ [11], and this potential is almost the same for other oxovanadium(IV) compounds. In this study we have investigated the change of CV behaviour of dioxygen due to the presence of oxovanadium(IV) compounds. In dmso solution containing O_2 (saturated by dry air), the reversible wave due to the $O_2 \rightleftharpoons O_2^-$ couple is observed at −0.71 V (*vs.* SSCE), as shown in trace B in Fig. 6. The presence of [VO(salen)] and [VO(acen)] causes this reversible wave to become an irreversible one, that is, the reoxidation wave ($O_2^- \rightarrow O_2$) has disappeared, as seen in traces C and D in Fig. 6. On the other hand, no change was observed for the CV behaviour of O_2 even in the presence of [VO(saldpt)] and [VO(TPP)] (*cf.* traces E and F in Fig. 6). This can be understood by the assumption that O_2^- formed at −0.71 V (*vs.* SSCE) is reacting with [VO(salen)] and [VO(acen)], but not with [VO(saldpt)] and [VO(TPP)].

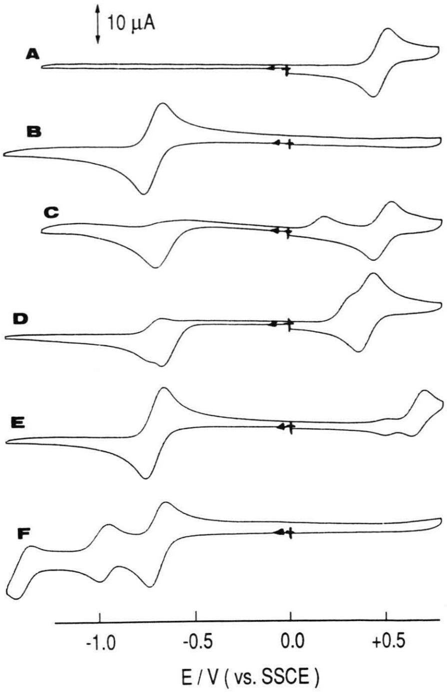
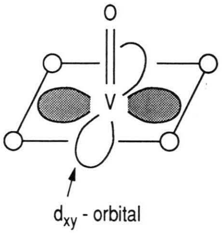


Fig. 6. Cyclic voltammograms of compounds (in dmso, 25 °C, scan speed 100 mV/sec).
A: [VO(salen)] (1 mM);
B: O_2 (saturated by air, 0.47 mM);
C: [VO(salen)] (1 mM)+O_2 (0.47 mM);
D: [VO(acen)] (1 mM)+O_2 (0.47 mM);
E: [VO(saldpt)] (1 mM)+O_2 (0.47 mM);
F: [VO(TPP)] (1 mM)+O_2 (0.47 mM).

It is well known that in the oxovanadium(IV) compounds one unpaired electron lies in the d_{xy} orbital [25], whose lobes are not directed towards the ligand atoms as shown below. If we assume that the superoxide anion can interact with the vanadium ion through the interaction with this orbital, we can rationalize the results obtained above as follows; in the case of [VO(salen)] and [VO(acen)], one of the lobes of d_{xy} is not screened by the ligand system, and this lobe may interact with O_2^-, but this interaction is impossible in the case of [VO(TPP)], because all the lobes of d_{xy} orbital are screened by the ligand system. In order to clarify the origin of the unexpected reactivity of [VO(saldpt)], we have determined the crystal structure of this complex, and the result is illustrated in Fig. 7, the structural parameters being summarized in Table I. As the structural features around



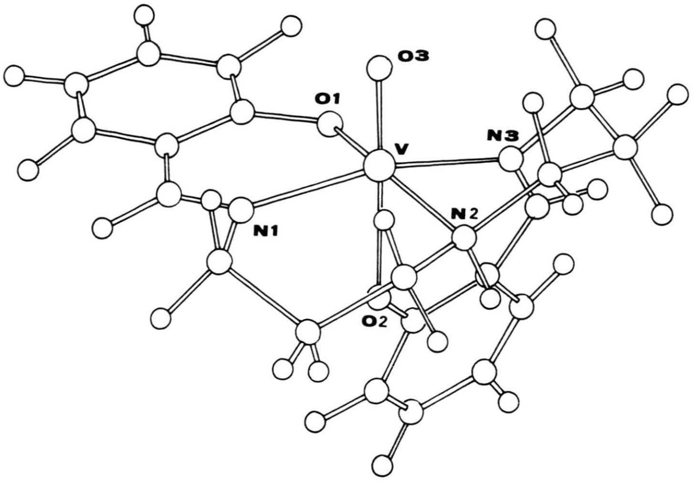
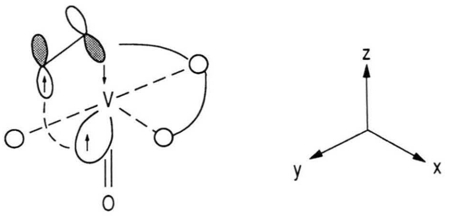


Fig. 7. Crystal structure of [VO(saldpt)].

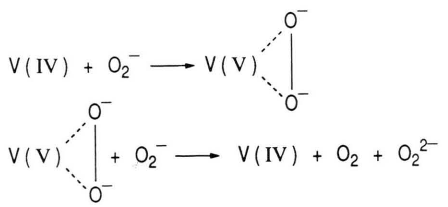
Table I. Structural parameters of [VO(saldpt)].

a) Bond distance (Å)			
V−O2	2.078(3)	V−N1	2.109(3)
V−O1	1.961(3)	V−N2	2.184(4)
V−N3	2.085(3)	V−O3	1.616(3)
b) Bond angle (°)			
O2−V−N1	81.8(1)	O2−V−O1	88.4(1)
O2−V−N2	82.8(1)	O2−V−N3	83.7(1)
O2−V−O3	170.5(1)	N1−V−O1	90.0(1)
N1−V−N2	97.7(1)	N1−V−N3	165.5(1)
N1−V−O3	93.5(1)	O1−V−N2	167.3(1)
O1−V−N3	89.1(1)	O1−V−O3	100.0(2)
N2−V−N3	80.9(1)	N2−V−O3	89.7(1)
N3−V−O3	100.9(1)		

the plane perpendicular to V=O bonding in [VO(saldpt)] are similar to those of [VO(salen)] and [VO(acen)], the superoxide anion can approach the vanadium atom of [VO(saldpt)] at the region where one lobe of d_{xy} orbital spreads, similar to the case of [VO(salen)] and [VO(acen)], but this complex does not interact with the superoxide anion. Based on these facts, we can conclude that the superoxide anion interacts with oxovanadium(IV) compound through a two-point interaction, (i) coordination to vanadium atom at the apical position trans to the oxo atom of V=O, and (ii) interaction between the superoxide anion and unpaired electron in d_{xy} orbital as depicted below, leading to a chelated vanadium(V)-peroxide adduct.



The assumption is supported by our recent observation that the vanadium(V) complex, $NH_4[V(dipic)(O)_2]$ [26] is reduced to a V(IV) species in the reaction with superoxide, and the V(IV) species thus formed is oxidized to the V(V) state by another superoxide anion, yielding a vanadium(V)-peroxide adduct where the peroxide is chelating to the vanadium(V) atom [27, 28]. The formation of a V(V)-peroxide adduct reasonably explains the ESR and absorption spectra observed for the reaction mixtures of [VO(salen)] and superoxide anion as described before. Based on the above facts, the SOD-like function of [VO(salen)] complex may be written as follows:



Several authors have postulated reaction mechanism for the action of SOD in biological systems [6–8], but the details on this problem remain unclear. The mechanism assumed in this study, *i. e.*, formation of a V(V)-peroxide adduct through oxidative chelation of a V(IV) species by superoxide anion may give a valuable key to elucidate the reaction mechanism of native SOD.

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