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Detection of Superoxide Anion and Singlet Oxygen in the Decomposition of Several Peroxovanadium(V) Complexes

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Several peroxovanadium(V) complexes with an organic chelate ligand decompose spontaneously, depending on the nature of the chelate ligand. The self-decomposition reactions of the dinuclear peroxovanadium(V) complex with 2-oxo-1,3-diaminopropane-*N,N,N',N'*-tetraacetate (dpot) and the peroxovanadium(V) complexes with *N*-carboxymethylhistidinate (cmhist) and histamine-*N,N*-diacetate (histada) accompany the reduction of vanadium(V) to vanadium(IV). This implies that the peroxide anion acts as a reducing agent and thus the peroxide is oxidized in the decomposition process of the peroxovanadium(V) complexes. The oxidized dioxygen species have been characterized spectrophotometrically. Superoxide anion has been detected in 2-3 % yields using the reduction of cytochrome *c* method and chemiluminescence method utilized MCLA as a fluorescer. Singlet oxygen has also been detected in higher yields on the basis of chemiluminescence of tryptophan.

Key words: peroxovanadium(V) complexes, superoxide, singlet oxygen, MCLA(*Cypridina* luciferin derivative), cytochrome *c*, chemiluminescence

INTRODUCTION

Recently peroxovanadium(V) complexes (pVs) attract the interest of the chemists as well as biologists with regard to their insulin mimetic activities and as an important intermediate species of the enzymatic process of vanadium-dependent haloperoxidase (VHPO) [1]. The relation between the insulin mimetic activities and the chemical properties of pVs is, however, quite complex. This complexity comes from, in part, the redox reaction of the pVs. We have shown that the stability of pVs depends on the coexisiting ligand and vanadium(V) has been reduced to vanadium(IV) in the decomposition process of some peroxovanadium(V) complexes [2]. The reduction of vanadium(V) implies that the peroxide acts as a reducing agent. Thus, we have tried to characterize the oxidized species of the peroxide.

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MATERIALS AND METHODS

Preparation of Peroxovanadium Complexes. $Na[VO(O_2)(histada)]\cdot 4H_2O$, $K[VO(O_2)(D,L\text{-}cmhist)]\cdot H_2O$, and $Cs_3[(VO)_2(O_2)_2(dpot)]\cdot 3H_2O$ were synthesized according to the method previously reported [2].

Spectroscopic Measurement. UV/VIS spectra were recorded using a PERKIN ELMER Lambda Bio 20. Fluorescence spectra were measured using JASCO FP-750.

Detection of Superoxide Anion. *MCLA Fluorescence Measurement*: $K[VO(O_2)(D,L\text{-}cmhist)]$ (3.7 mg) was dissolved in 2 ml of 50 mM sodium acetate buffer (pH 4.5) to give a final concentration of 50μM. The concentration of MCLA solution was spectrophotometrically determined. Each solution was mixed with a 50 mM sodium acetate buffer (pH 4.5). The final concentration of $K[VO(O_2)(D,L\text{-}cmhist)]$ and MCLA were 1mM and 2mM, respectively. The fluorescence spectra were measured from 400 to 600 nm (exited at 350 nm) using JASCO FP-750 fluorescence spectrophotometer during the interval time (0 to 360 min). The fluorescent intensity (450 nm) of the product produced by the reaction of superoxide with MCLA was increased. The reaction mixture of vanadium(V) oxide (V_2O_5) and MCLA was used as a control. The amounts of superoxide anion produced in the process of the decomposition of $K[VO(O_2)(D,L\text{-}cmhist)]$ in the aqueous solution was determined by subtracting the fluorescence intensity of the control sample from that of the reaction solution.

The Reduction of NBT Method: The 1 mM solutions of each vanadium complexes were prepared in 50 mM sodium phosphate buffer (pH7.8). A 1 mM solution of vanadyl sulfate ($VOSO_4$) was prepared in distilled water. A 3 mM solution of NBT was prepared in 50 mM sodium phosphate buffer (pH7.8). Each solution was mixed with 50 mM sodium phosphate buffer (pH7.8). The final concentration of the vanadium complex, vanadyl sulfate and NBT were 20 μM, 20 μM and 400 μM, respectively. Blue formazan was generated by the reaction of superoxide with NBT in these reaction mixtures. The absorption spectra were measured in the region of 400 to 700 nm every 60 min for 180 min using PERKIN ELMER Lambda Bio 20 spectrophotometer. The reaction mixtures containing vanadyl sulfate and NBT was used as a control. The amounts of the reduced NBT on the decomposition of each peroxovanadium complex in the aqueous solution were determined by subtracting the absorbance of the control solution from that of the reaction mixtures containing the vanadium complex, vanadyl sulfate and NBT.

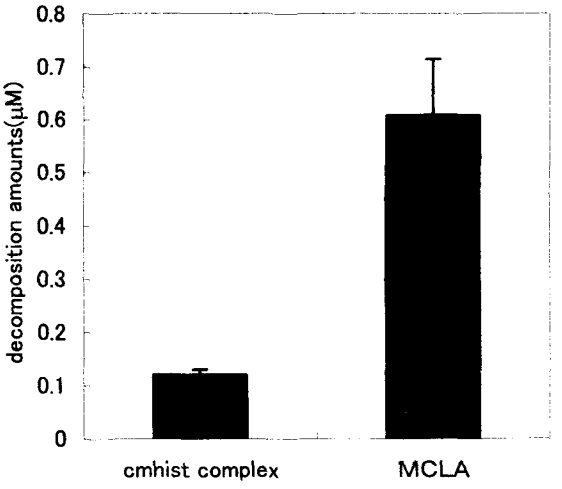

Fig.1 The amounts of MCLA and $K[VO(O_2)(D,L\text{-}cmhist)]$ decomposed in 50 mM sodium acetate buffer (pH 4.5) during 360 min. $[cmhist]_0 = 1$ μM, and $[MCLA]_0 = 2$ μM

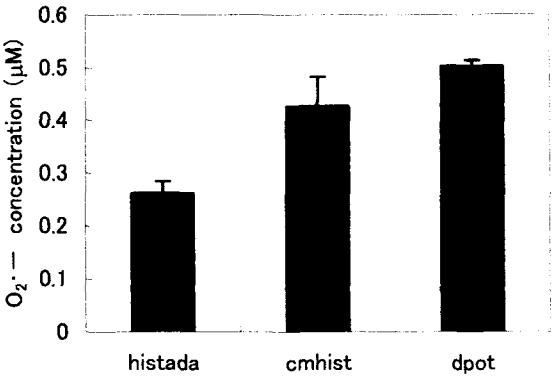

Fig.2 The amounts of superoxide anion produced by the decomposition of each peroxovanadium complex in 50 mM Sodium Phosphate buffer (pH7.8) during 180 min. $[complex]_0 = 20$ μM, $[VOSO_4]_0 = 20$ μM, and $[NBT]_0 = 400$ μM

Detection of Singlet Oxygen. *Measurement of Tryptophan Fluorescence*: The 0.1 mM solutions of each vanadium complex were prepared in 100 mM sodium phosphate buffer (pH7.4). A 0.1 mM solution of L-tryptophan was prepared in 100 mM sodium phosphate buffer (pH7.4). A 0.1 mM solution of vanadyl sulfate($VOSO_4$) was prepared in distilled water. Aliquots of each

solution were added to 100 mM sodium phosphate buffer (pH7.4) and the resulting solution was gently mixed. The final concentrations of the vanadium complex, vanadyl sulfate and L-tryptophan were 5μM. *N*-formyl-kynurenine was produced by the reaction of the singlet oxygen with tryptophan. The decrease of tryptophan in the reaction mixtures were determined spectrophotometrically based on the fluorescence in the region of 300 to 450 nm (exited at 278 nm) every 60 min for 240 min using JASCO FP-750 fluorescence spectrophotometer. The reaction mixture of vanadyl sulfate and tryptophan was used as a control. The amounts of the singlet oxygen produced on the decomposition of the peroxovanadium complexes in the aqueous solution were determined by subtracting the fluorescence intensity of the control solution from that of the reaction mixtures containing the vanadium complex, vanadyl sulfate and tryptophan,.

RESULTS

As shown in Fig. 1, the amounts of MCLA decomposed in the reaction mixtures were more than three times compared with the decomposition amount of K[VO(O$_2$)(D,L-cmhist)] itself. Thus, it seems that a MCLA fluorescence method is unsuitable as a method for quantitation of the superoxide anion on the decomposition process of K[VO(O$_2$)(D,L-cmhist)]. This result suggests that other oxidant is generated in addition to the superoxide anion in the decomposition of K[VO(O$_2$)(D,L-cmhist)].

In the NBT assay, the amounts of the superoxide anion produced relative to the decomposition amounts of K[VO(O$_2$)(D,L-cmhist)], Na[VO(O$_2$)(histada)] and Cs$_3$[(VO)$_2$(O$_2$)$_2$(dpot)] complexes were calculated to be about 2.1, 1.3 and 2.5%, respectively (Fig.2).

The amounts of the singlet oxygen produced relative to the decomposition amount of K[VO(O$_2$)(D,L-cmhist)] and Cs$_3$[(VO)$_2$(O$_2$)$_2$(dpot)] complexes were estimated to be about 6.9 and 11.7%, respectively (Fig.3). However, the tryptophan assay is not a specific method to detect singlelet oxygen solely. Therefore, further studies are now proceeding to determine the produced singlet oxygen.

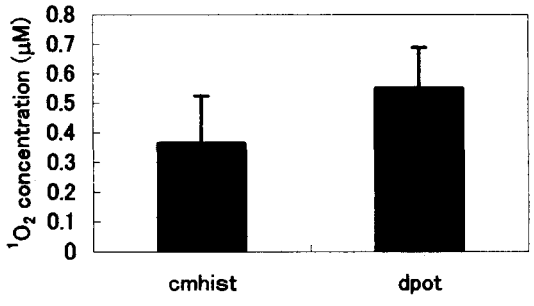

Fig.3 The amounts of the singlet oxygen produced by the decomposition of the peroxovanadium complexes in 100 mM sodium phosphate buffer (pH7.4) during 240 min. $[complex]_0$ = 5 μM, $[VOSO_4]_0$ = 5 μM, and $[tryptophan]_0$ = 5 μM

DISCUSSION

Production of superoxide anion and singlet oxygen in the decomposition process of pVs can be explained based on the reaction scheme proposed by Bonchio *et al*, though they did not mentioned whether the produced dioxygen is in the singlet state or in the triplet state[3]. The present dpot complex must be a good model for the putative dimeric species proposed by them.

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