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Production of Hydroxyl Radicals from the Reactions of Some Copper(II) Complexes with Hydrogen Peroxide: Ligand Exchange Effects of Copper(II) Complexes on the Activation of Hydrogen Peroxide

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The reactions of $Cu^{II}(en)_2$ (en: ethylenediamine) with hydrogen peroxide (H_2O_2) during the successive addition of a polyamine-*N*-polycarboxylate such as ida (iminodiacetic acid) and edta (ethylenediaminetetraacetic acid) were investigated by the thiobarbituric acid (TBA) method and by the electron spin resonance (ESR) titration method. A relatively good relation between TBA method and ESR titration method was observed.

The ligand, en, in $Cu^{II}(en)_2$ was easily replaced by ida and edta. In the case of edta, which is a tetradentate ligand, Cu^{II}(edta) was formed when the concentration ratio of edta to $Cu^{II}(en)_2$ was 1. On the other hand, in the case of ida which is a bidentate ligand, the reactions between $Cu^{II}(en)_2$ and ida proceeded stepwise. That is, one is the formation of a mixed ligand complex of Cu(II), Cu^{II}(en)(ida), when the concentration ratio of ida to $Cu^{II}(en)_2$ was 1. The other is the formation of $Cu^{II}(ida)_2$ when the concentration ratio of ida to $Cu^{II}(en)_2$ was 2.

A mixed ligand complex, Cu^{II}(en)(ida), exhibited activity towards H_2O_2, whereas $Cu^{II}(ida)_2$ and Cu^{II}(edta) did not show any activity towards H_2O_2. The different reactivities of these Cu(II) complexes towards H_2O_2 were discussed.

Keywords thiobarbituric acid method; ESR titration method; hydroxyl radical; polyamine-*N*-polycarboxylate; copper(II) complex; ligand exchange; hydrogen peroxide

In the previous paper, we reported that $Cu^{II}(en)_2$ (en: ethylenediamine) has, among the Cu(II) complexes examined, the highest acivity towards hydrogen peroxide (H_2O_2) to give a hydroxyl radical (·OH) as a reactive intermediate.[1)] Further, the formation of a OH radical from the reaction of $Cu^{II}(en)_2$ with H_2O_2 was determined by the electron spin resonance (ESR) spin-trapping method.[2)] However, Cu(II) complexes with polyamine-*N*-polycarboxylates such as ethylenediaminetetraacetic acid (edta) and diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (dtpa) did not react with H_2O_2.[1)]

From the facts mentioned above, it is suggested that the difference of the activities of Cu(II) complexes towards H_2O_2 may be dependent on the ligands ligated with a Cu(II) ion. Thus, we examined the change of activities of $Cu^{II}(en)_2$ towards H_2O_2 during the successive addition of edta and iminodiacetic acid (ida) which can ligate with a Cu(II) ion with one and two molecules, respectively. In this paper, we report on these results.

Experimental

Materials $Cu^{II}(en)_2$ was prepared from $CuSO_4$ and ethylenediamine as described previously.[1)] Cu^{II}(edta) was obtained from Wako Pure Chemical Co., Ltd. and used without further purification. H_2O_2 (30%) was purchased from Mitsubishi Gas Chemical Co., Ltd. Other ligands are all commercially available and used as received. Ligands used here are edta, ethylenediamine-*N*,*N*′-diacetic acid (edda), ida, ethylenediamine-*N*,*N*′-dipropionic acid (eddp), ethylenediamine-*N*,*N*′-diacetic acid-*N*,*N*′-dipropionic acid (edapda), *N*-hydroxyethylethylenediamine-*N*,*N*′,*N*′-triacetic acid (edta-OH), 1,3-diaminopropane-2-ol-*N*,*N*,*N*′,*N*′-tetraacetic acid (dtpa-OH) and dtpa.

Other reagents were commercially available.

Preparation of Solutions of Cu(II) Complexes Copper(II) complexes, except $Cu^{II}(en)_2$ and Cu^{II}(edta), were prepared by the addition of a 2.5 times concentration of ligands to the Cu(II) ion. The exact concentration of H_2O_2 was determined by titration with 1/10 N $KMnO_4$. Reactions were done at pH 7.4 in 50 mmol of a phosphate buffer. Deionized and triply distilled water was used throughout.

Detection of Oxidation Product of Deoxyribose by OH Radical Using Thiobarbituric Acid (TBA) Method TBA reactive oxidation products of deoxyribose by a OH radical were determined according to the described method[3)] with a minor modification. The standard reaction procedure is as follows: 0.2 ml of Cu(II) complex (1 mmol) was added to 0.5 ml deoxyribose (7.5 mmol) together with 0.9 ml distilled water. Where appropriate, 0.2 ml of substrate or distilled water was added, followed by 0.2 ml of H_2O_2 (100 mmol). Samples were incubated at 37°C for 1 h, and thiobarbiturate reactivity developed following the addition of 1.0 ml TBA (1%, (w/v) in 50 mmol NaOH) and 1.0 ml glacial acetic acid. The sample tubes were heated for 15 min at 100°C, cooled and the absorbance at 532 nm was read.[1,3)]

Electron Spin Resonance (ESR) Measurements ESR spectra were measured by a JEOL JES-RE-1X ESR spectrometer (X-band) at room temperature. ESR parameters were calibrated by 1,1-diphenyl-2-picrylhydrazyl (DPPH, $g=2.0036$) and Mn^{2+} doped on MgO. For accurate calibration, a NMR field meter (JEOL ES-FC 5) and a microwave counter (Advantest) were used.

Results and Discussion

In the previous paper,[1)] we showed that when Cu(II) ion was complexed with a polyamine-*N*-polycarboxylate such as edta or dtpa, these complexes did not react with H_2O_2 at all. Thus, in order to ascertain whether or not the reactivity of Cu(II) towards H_2O_2 was lost, when the Cu(II) ion was complexed with another polyamine-*N*-polycarboxylate, the reactions of H_2O_2 with some Cu(II) complexes with polyamine-*N*-polycarboxylates were investigated using the TBA method. The results obtained are shown in Table I. It is apparent from Table I that almost no Cu(II) polyamine-*N*-polycarboxylate complexes could react with H_2O_2. This fact may be explained as follows: that is, the redox potential of the Cu(II) ion was changed by increasing the stability constants of the Cu(II) ion complexing with polyamine-*N*-polycarboxylate. In Table I, the stability constants which have been reported until now are also shown. According to the stability constants shown in Table I, the en ligand of $Cu^{II}(en)_2$ may be replaced with polyamine-*N*-polycarboxylate, then the activity of the Cu(II) ion towards H_2O_2 may be lost. Thus, in order to ascertain this assumption, the change in activity of the Cu(II) ion by adding polyamine-*N*-polycarboxylates such as ida or edta to $Cu^{II}(en)_2$ was followed by the TBA method.

Further, the change in coordination structures of Cu(II)

TABLE I. Production of a Hydroxyl Radical from the Reactions of Copper(II) Complexes with Hydrogen Peroxide at pH 7.4 and the Stability Constants of Copper(II) Complexes Examined

Copper(II) complexes	Relative activity[a]	Stability const.	
		$\log K_1$	$\log K_2$
$Cu^{II}(en)_2$	100	10.76[b]	9.37[b]
$Cu^{II}(edta)$	1	18.79[c]	
$Cu^{II}(edda)$	2	16.20[d]	
$Cu^{II}(eddp)$	2	15.2[e]	
$Cu^{II}(edapda)$	1	16.3[d]	
$Cu^{II}(edta\text{-}OH)$	1	17.35[f]	
$Cu^{II}(dtpa\text{-}OH)$	1	17.21[g]	
$Cu^{II}(ida)_2$	14	10.55[h]	5.65[h]
$Cu^{II}(dtpa)$	1	21.53[g]	

[a] These results are the mean of 3 separate experiments. The final concentration of Cu(II) ions, hydrogen peroxide and deoxyribose were 0.1, 10 and 1.9 mmol, respectively. [b] Ref. 4. [c] Ref. 5. [d] Ref. 6. [e] Ref. 7. [f] Ref. 8. [g] Ref. 9. [h] Ref. 10.

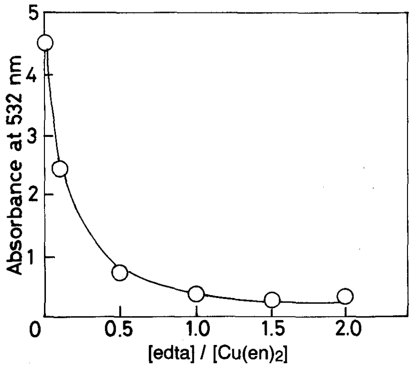

Fig. 1. Plot of the Absorbance at 532 nm Due to the TBA-Reactive Substrate against the Concentration Ratio of edta to $Cu^{II}(en)_2$

Final concentrations: $Cu^{II}(en)_2$, 0.1 mmol; H_2O_2, 5 mmol; deoxyribose, 3.8 mmol.

complexes when ida and edta were successively added to $Cu^{II}(en)_2$ solutions was investigated by the ESR titration method.

1. TBA Method Figure 1 shows the change in the activity of the Cu(II) ion towards H_2O_2 when the concentration of $Cu^{II}(en)_2$ was kept at a constant and that of edta was varied. It is apparent from Fig. 1 that when the concentration ratio of edta to $Cu^{II}(en)_2$ was 1, a OH radical was barely formed. This result suggests that the en in $Cu^{II}(en)_2$ was replaced by edta, which is a tetradentate ligand, to give $Cu^{II}(edta)$. This suggestion was supported by the values of the stability constants shown in Table I.

Since en is a bidentate ligand and edta is a tetradentate ligand, respectively, a ligand exchange reaction as shown in Eq. 1 may occur.

$$Cu^{II}(en)_2 + edta \rightarrow Cu^{II}(edta) + 2en \tag{1}$$

The same results were also obtained when edda was used in place of edta.

On the other hand, when ida was successively added to a $Cu^{II}(en)_2$ solution and the concentration ratio of ida to $Cu^{II}(en)_2$ reached about 1, the yield of the OH radical was reduced to one-half of its original value (reaction mixture without ida) (Fig. 2). Further, when the concentration ratio of ida to $Cu^{II}(en)_2$ was 2, the OH radical was barely generated (Fig. 2). Since ida is two-coordinate, ida ligates to a Cu(II) ion with two molecules. Thus, when the concentration ratio of ida to $Cu^{II}(en)_2$ is 1, a mixed ligand complex of a Cu(II) iron both with en and ida, $Cu^{II}(en)(ida)$, may be formed. This mixed ligand complex has still maintained activity towards H_2O_2. The ligand exchange reactions between $Cu^{II}(en)_2$ and ida may be represented as the following equations:

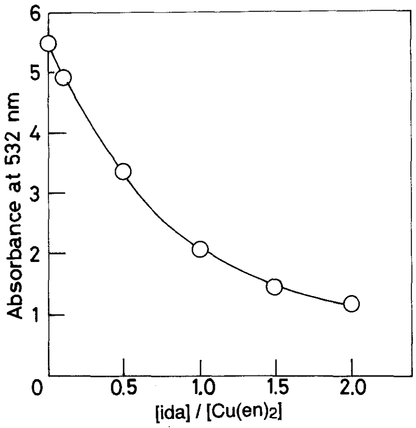

Fig. 2. Plot of the Absorbance at 532 nm Due to the TBA-Reactive Substrate against the Concentration Ratio of ida to $Cu^{II}(en)_2$

Final concentrations are the same as Fig. 1.

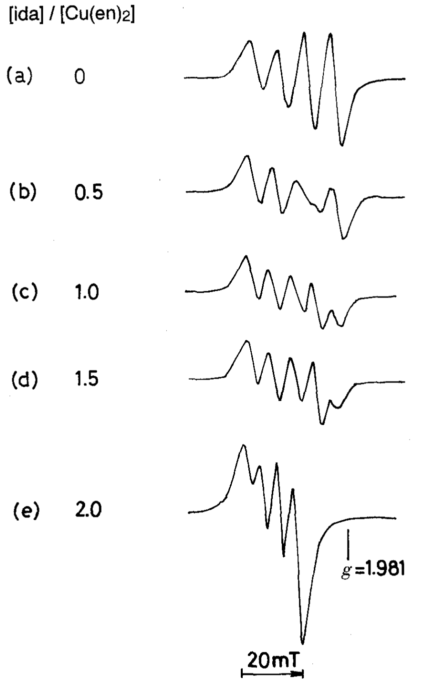

Fig. 3. ESR Spectral Changes Observed When the Concentration Ratio of ida to $Cu^{II}(en)_2$ Was Changed

Final concentrations: $Cu^{II}(en)_2$, 5 mmol. ida; (a) 0; (b) 2.5 mmol; (c) 5 mmol; (d) 7.5 mmol; (e) 10 mmol.

$$Cu^{II}(en)_2 + ida \rightarrow Cu^{II}(en)(ida) + en \tag{2}$$

$$Cu^{II}(en)(ida) + ida \rightarrow Cu^{II}(ida)_2 + en \tag{3}$$

2. ESR Titration Method Aqueous solutions of $Cu^{II}(en)_2$ showed a quartet ESR spectrum as shown in Fig. 3a [$a^{Cu}(1) = 8.77$ mT, $g = 2.097$]. When aqueous solutions of ida were successively added to those of $Cu^{II}(en)_2$, the ESR spectrum due to $Cu^{II}(en)_2$ was changed as shown

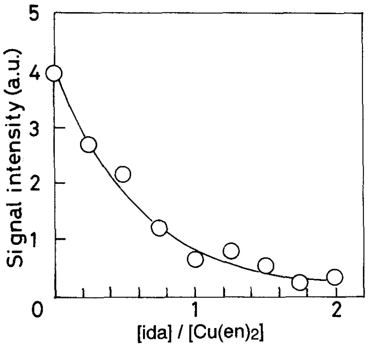

Fig. 4. Plot of the ESR Signal Intensity of the Highest Field of $Cu^{II}(en)_2$ against the Concentration Ratio of ida to $Cu^{II}(en)_2$

Concentration of $Cu^{II}(en)_2$ is 5 mmol.

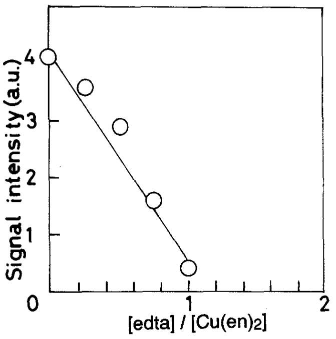

Fig. 5. Plot of the ESR Signal Intensity of the Highest Field of $Cu^{II}(en)_2$ against the Concentration Ratio of edta to $Cu^{II}(en)_2$

Concentration of $Cu^{II}(en)_2$ is 5 mmol.

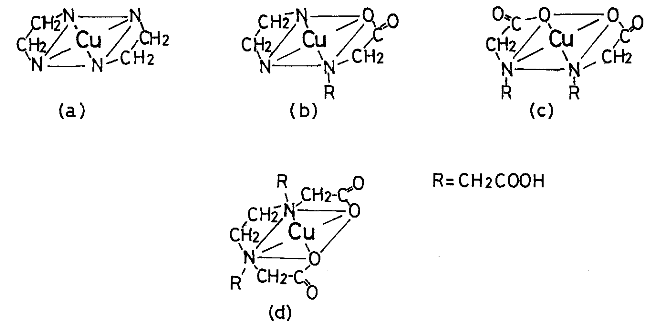

Fig. 6. Assumed Coordinate Structures of Cu(II) Complexes at pH 7.4

(a) $Cu^{II}(en)_2$; (b) $Cu^{II}(en)$ (ida); (c) $Cu^{II}(ida)_2$; (d) $Cu^{II}(edta)$. $R=CH_2COOH$.

in Fig. 3, depending on the concentration ratio of ida to $Cu^{II}(en)_2$. Finally, the ESR spectrum varied due to $Cu^{II}(ida)_2$ (Fig. 3e), when the concentration ratio of ida to $Cu^{II}(en)_2$ reached 2. Figure 4 shows a plot of the change of ESR signal intensity of the highest field ($g=1.981$) in Fig. 3 against the concentration ratio of ida to $Cu^{II}(en)_2$. It is apparent from Fig. 4 that the ESR spectrum due to $Cu^{II}(en)_2$ completely disappeared when the concentration ratio of ida to $Cu^{II}(en)_2$ reached 2. The shape of the plot shown in Fig. 4 resembles that shown in Fig. 2. Further, when the concentration ratio of ida to $Cu^{II}(en)_2$ was 1, an ESR spectrum due to the mixed ligand complex of Cu(II), $Cu^{II}(en)(ida)$, was observed (Fig. 3c).

On the other hand, the ESR spectrum due to $Cu^{II}(en)_2$ also changed when edta was successively added to aqueous solutions of $Cu^{II}(en)_2$. Finally, only an ESR spectrum due to $Cu^{II}(edta)$ which is a broad singlet line ($g=2.174$) was observed. The change in ESR signal intensity of $Cu^{II}(en)_2$ when edta was successively added to $Cu^{II}(en)_2$ solutions was plotted against the concentration ratio of edta to $Cu^{II}(en)_2$ (Fig. 5). The shape of the plot shown in Fig. 5 resembles that obtained by the TBA method as shown in Fig. 1. That is, one molecule of edta replaced two molecules of en in $Cu^{II}(en)_2$ to give $Cu^{II}(edta)$.

From the results mentioned above, it is indicated that ESR titration has a good relation to the TBA method. Further, polyamine-*N*-polycarboxylate complexes of Cu(II), $Cu^{II}(edta)$ and $Cu^{II}(ida)_2$, did not activate H_2O_2, whereas the mixed ligand complex of Cu(II) ion, $Cu^{II}(en)(ida)$, still exhibits activity towards H_2O_2. Although the exact coordination structures of $Cu^{II}(en)(ida)$ and $Cu^{II}(ida)_2$ at pH 7.4 were not clear, bonding atoms to the Cu(II) ion of $Cu^{II}(en)_2$, $Cu^{II}(en)(ida)$ and $Cu^{II}(ida)_2$ are 4N, 3N1O and 2N2O, respectively, as shown in Figs. 6a—c. Further, bonding atoms to the Cu(II) ion in $Cu^{II}(edta)$ are 2N2O (Fig. 6d). From these facts, it is suggested that the Cu(II) ion ligating with 2N2O type ligands cannot activate H_2O_2. Further, $Cu^{II}(en)_2$ could not form the complex with H_2O_2 at an initial reaction step.[11)]

From these results, it is concluded that the polyamine-*N*-polycarboxylate complexes of the Cu(II) ion do not activate H_2O_2 because of the change in redox potential of the Cu(II) ion by ligation with different ligands.[12)]

Further studies on the redox potential of these Cu(II) complexes are now in progress.

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References

1) T. Ozawa, H. Goto, F. Takazawa and A. Hanaki, *Nippon Kagaku Kaishi*, **1988**, 459.
2) T. Ozawa and A. Hanaki, *J. Chem. Soc., Chem. Commun.*, **1991**, 330.
3) J. M. C. Gutteridge and S. Wilkins, *Biochim. Biophys. Acta*, **759**, 38 (1983).
4) F. Basolo and R. K. Murmann, *J. Am. Chem. Soc.*, **74**, 5243 (1952).
5) G. Schwarzenbach, G. Gut and G. Anderegg, *Helv. Chim. Acta*, **37**, 937 (1954).
6) S. Chaberek and A. E. Martell, *J. Am. Chem. Soc.*, **74**, 6228 (1952).
7) H. Irving, R. Shelton and R. Evans, *J. Chem. Soc.*, **1958**, 3540.
8) L. G. Sillan and A. E. Martell, "Stability Constants," Supplement No. 1, The Chemical Society, London, 1971.
9) L. C. Thompson and S. Kundra, *J. Inorg. Nucl. Chem.*, **28**, 2945 (1966).
10) S. Chaberek and A. E. Martell, *J. Am. Chem. Soc.*, **74**, 5052 (1952).
11) T. Ozawa, A. Hanaki and K. Onodera, *Biochem. Int.*, **24**, 661 (1991).
12) T. Ozawa, A. Hanaki, H. Goto, F. Takazawa, N. Yoshida, Y. Matsushima, *J. Pharmacobio.-Dyn.*, **13**, s-2 (1990).