

## Synthesis and Characteristics of Novel Transition Metal Complexes with High Superoxide Dismutase Activity

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Several N,N,N',N'-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) analogues were synthesized and the superoxide dismutase (SOD) activities of their Fe(II), Co(III), Mn(II), and Ni(II) complexes were investigated. The results indicated that N,N,N',N'-tetrakis(2-pyridylmethyl)-(±)-trans-1',2'-cyclohexanediamine (TPCN) and Fe(II) formed  $[\text{Fe(II)}_3(\text{TPCN})_2]$  complex. The  $[\text{Fe(II)}_3(\text{TPCN})_2]$  complex was found to increase the SOD activity and exhibited longer stability than that of  $[\text{Fe(II)}\text{TPEN}]^{2+}$  in aqueous solution.

Superoxide ( $\text{O}_2^-$ , superoxide anion radical) is a highly reactive radical correlated not only with a cause of several diseases such as inflammation<sup>1)</sup> or carcinogenesis<sup>2)</sup>, etc., but also with a significant role in the biological defense system such as the sterilization of neutrophilic leukocyte.<sup>3)</sup> Superoxide dismutase (SOD) catalyzes the conversion of superoxide into hydrogen peroxide and molecular oxygen<sup>4)</sup> as shown in the following reaction;



SOD mimics of copper,<sup>5-7)</sup> iron,<sup>8)</sup> nickel,<sup>9)</sup> and manganese<sup>10)</sup> complexes have been extensively studied. These investigations gave some suggestive results for the elucidation of the active site and the reaction mechanism of SOD. Recently it has been reported by Hirobe and his coworkers that  $[\text{Fe(II)}\text{TPEN}]^{2+}$  exhibits high SOD activity,<sup>11)</sup> which is not attenuated by chelating agents in living cells. Therefore, several TPEN analogues were reported to be synthesized and the SOD activities of their iron(II), cobalt(III), manganese(II), and nickel(II) complexes were studied.

Several TPEN analogues as the ligands were synthesized by two general procedures;<sup>12)</sup> (a)

diaminoalkane was added to 2-pyridylmethyl chloride hydrochloride (4 mol) under  $N_2$  atmosphere and was stirred in NaOH solution containing a small amount of cetyltrimethylammonium chloride (CTMAC) as a phase transfer catalyst. The mixture was stirred at 30 °C for 24 h. The product was obtained by alumina chromatography and recrystallization, yielding about 70%; (b) reductive amination products of the imines, prepared by the reaction between 2-pyridylmethyl aldehyde analogues (2 mol) and diaminoalkane (1 mol), were added to 2-pyridylmethyl chloride (2 mol), and the mixture was stirred under the same conditions as (a). The product was yielded about 60%. The structure of the ligands was identified by means of chemical analysis,  $^1H$ -NMR and Mass spectra. The synthesized ligands are shown in Fig.1.

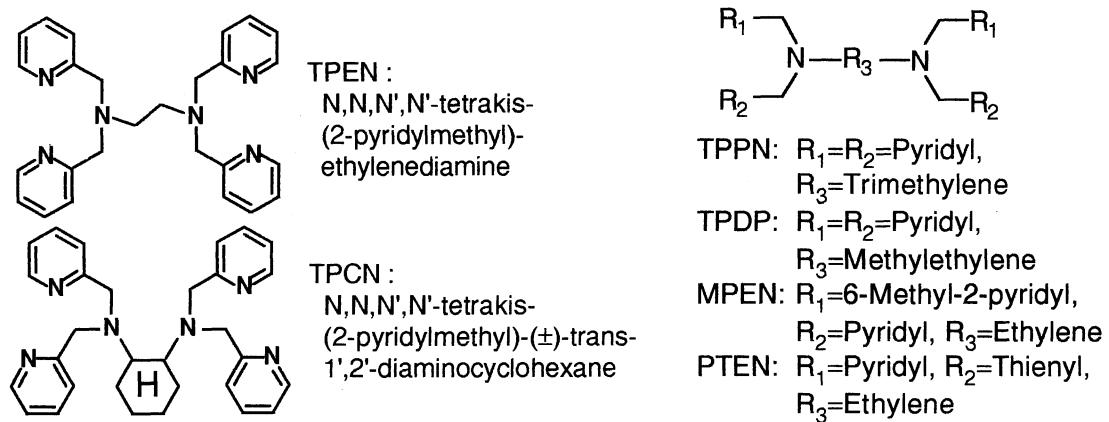


Fig.1. Structure of the ligands.

Various metal complexes were prepared from the reaction of the ligands (1 mol) with corresponding metal salts in EtOH/H<sub>2</sub>O (1:1),<sup>13</sup> and the perchlorate salts of these complexes were purified by recrystallization from H<sub>2</sub>O. The Job's method of continuous variation and the elemental analysis indicated that these TPEN analogues were found to form the 1:1 metal:ligand complexes except for TPCN and Fe(II) ion. The continuous variation plot for TPCN and Fe(II) ion is shown in Fig.2. The point of intersection was observed to be 0.4 mole fraction of TPCN. The results of the elemental analysis and two-dimensional NMR spectrum suggested that

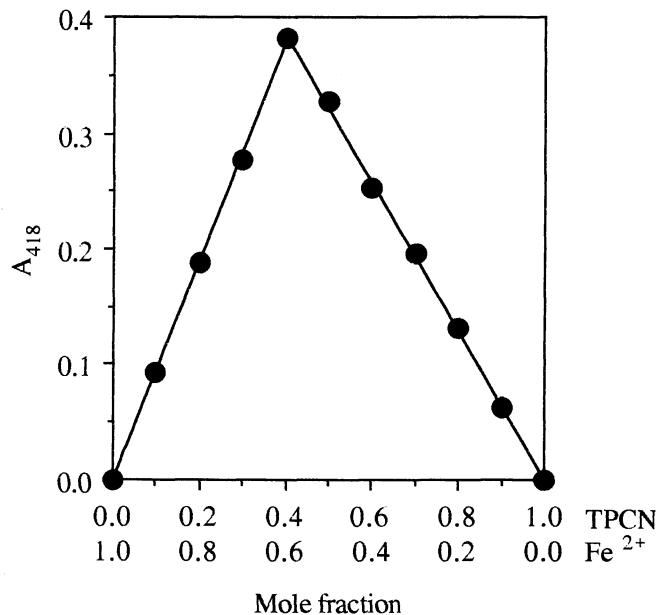


Fig.2. Continuous variation plot for  $[Fe(II)_3(TPCN)_2]$  complex. The plot demonstrates the increase and decrease of the absorbance at  $\lambda_{max}$ : 418 nm.

Table 1.  $IC_{50}$  values for various transition metal complexes

SOD mimic	$IC_{50}/\mu M$
[Fe(II)TPEN]	0.82
[Fe(II) <sub>3</sub> (TPCN) <sub>2</sub> ]	0.20
[Fe(II)TPDP]	0.86
[Fe(II)TPPN]	>100
[Fe(II)MPEN]	3.8
[Fe(II)PTEN]	31
[Fe(II)(terpy) <sub>2</sub> ] <sup>a)</sup>	>100
[Co(III)TPEN]	12
[Co(III)TPCN]	80
[Co(III)TPPN]	95
[Mn(II)TPEN]	>200
[Ni(II)TPEN]	>400

a)terpy; terpyridine.

[Fe(II)TPPN] revealed much higher  $IC_{50}$  value than that of [Fe(II)TPEN], indicating that substitution of the ethylenediamine moiety of the ligand to the trimethylenediamine moiety decreased the SOD activity of the complex. The  $IC_{50}$  values for the Co(III) complexes were fairly high, and [Co(III)TPEN] exhibited the smallest  $IC_{50}$  value among the Co(III) complexes. [Mn(II)TPEN] and [Ni(II)TPEN] exhibited extremely high  $IC_{50}$  values, exhibiting almost no SOD activity.

The temperature dependence of the  $IC_{50}$  values for [Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] and [Fe(II)TPEN] is shown in Fig.3. These data demonstrate that the SOD activity of [Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] decreased with an increase in temperature. However, the SOD activity of [Fe(II)TPEN] slightly increased with

[Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] complex was formed.

Apparent SOD activity was monitored according to the xanthine oxidase-cytochrome c assay,<sup>4)</sup> and 1 unit of the apparent SOD activity was evaluated by the measurement of the 50% inhibition concentration ( $IC_{50}$ ). The  $IC_{50}$  values for various transition metal complexes were summarized in Table 1. It was found that [Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] exhibited the lowest  $IC_{50}$  value of 0.20  $\mu M$  among the Fe(II) complexes.

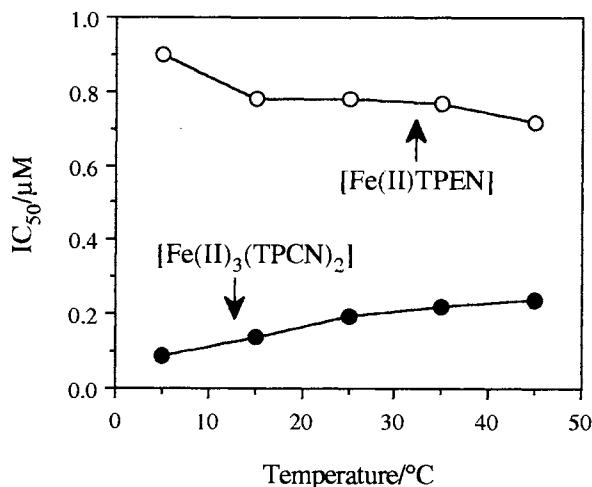


Fig.3. Temperature dependence of the  $IC_{50}$  values for [Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] and [Fe(II)TPEN] in aqueous solution containing 50 mM potassium phosphate buffer, pH 7.8.

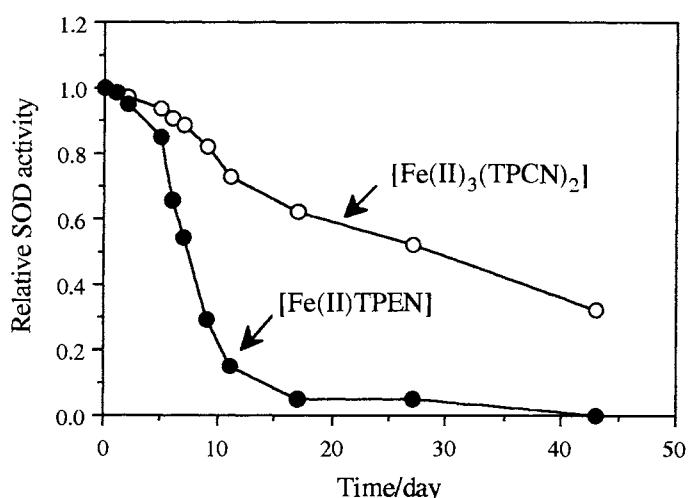


Fig.4. Variations of the relative SOD activity against the time in day for [Fe(II)<sub>3</sub>(TPCN)<sub>2</sub>] and [Fe(II)TPEN] stored in aqueous solution in air at room temperature.

an increase in temperature.

Figure 4 shows the stability of the relative SOD activities of  $[\text{Fe(II)}_3(\text{TPCN})_2]$  and  $[\text{Fe(II)}\text{TPEN}]$  in aqueous solution stored in air at room temperature. The relative SOD activity of  $[\text{Fe(II)}\text{TPEN}]$  was found to decrease to 10% within 10 days. On the other hand, the relative SOD activity of  $[\text{Fe(II)}_3(\text{TPCN})_2]$  was retained for more than a month.<sup>10</sup>

Hence, it was concluded that  $[\text{Fe(II)}_3(\text{TPCN})_2]$  exhibited fairly high SOD activity and high durability. Therefore, the application of these complexes to a superoxide sensor is now under way for the detection of superoxide produced by activated leukocytes.

#### References

- 1) Y. Oyanagui, *Biochem. Pharmacol.*, **25**, 1465(1976).
- 2) A. M. Michelson and M. F. Buckingham, *Biochem. Biophys. Res. Commun.*, **58**, 1679(1974).
- 3) B. M. Babior, R. S. Kipnes, and J. T. Curnutte, *J. Clin. Invest.*, **52**, 741(1973).
- 4) J. M. McCord and I. Fridovich, *J. Biol. Chem.*, **244**, 6049(1969).
- 5) R. Brigelius, R. Spottl, W. Bros, E. Lengfelder, M. Saran, and U. Weser, *FEBS. Letts.*, **47**, 72(1974).
- 6) M. Younes and U. Weser, *FEBS. Letts.*, **61**, 209(1976).
- 7) E. Kimura, A. Sakonaka, and M. Nakamoto, *Biochim. Biophys. Acta*, **678**, 172(1981).
- 8) R. F. Pasternack, A. Banth, J. M. Pasternack, and C. S. Johnson, *J. Inorg. Biochem.*, **15**, 261(1981).
- 9) E. Kimura, T. Koide, Y. Shimizu, and M. Kodama, *Inorg. Chem.*, **25**, 2242(1986).
- 10) D. Darr, K. A. Zarilla, and I. Fridovich, *Arch. Biochem. Biophys.*, **258**, 351(1987).
- 11) T. Nagano, T. Hirano, and M. Hirobe, *J. Biol. Chem.*, **264**, 9243(1989).
- 12) M. Sato, Y. Mori, and T. Iida, *Synthesis*, in press (1992).
- 13) H. Toftlund and S. Yde-Andersen, *Acta Chem. Scand.*, **35**, 575(1981).

(Received December 19, 1991)