High Activity of Binuclear Cobalt(II) Complex for Ethylene Evolution from 1-Aminocyclopropane-1-carboxylic Acid in the Presence of Hydrogen Peroxide

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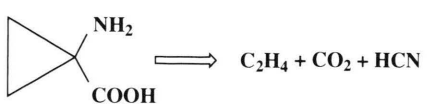
Ethylene Evolution, Binuclear Metal Complex, ACC, Hydrogen Peroxide

The binuclear Co(II) and Mn(II) complexes with H_5(HXTA), where H_5(HXTA) represents N,N′-(2-hydroxy-5-methyl-1,3-xylylene)bis(N-carboxymethylglycine), induced a strong ethylene evolution from 1-aminocyclopropane-1-carboxylic acid (ACC) in the presence of hydrogen peroxide, whereas activities of the corresponding Fe(III), Ni(II), and V(III) complexes were found negligible. Based on spectroscopic results and mass-spectral data it is proposed that a peroxide adduct of binuclear Co(II) (and Mn(II)) complex with η^1-coordination mode interacts with ACC, which is chelated to a binuclear cobalt complex leading to facile oxidative degradation of ACC and to evolution of ethylene.

Ethylene is a natural plant growth regulator involved in the control of a wide range of developmental responses; growth, abscission, senescence and fruit ripening to name but a few. Through the work of Adams and Yang (Adams and Yang, 1979; Lürssen *et al.,* 1979) and the route for its formation from methionine *via* S-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid (ACC) seems well established (see the Scheme 1) (Miyazaki and Yang, 1987). However the mechanism of the last step, namely oxidation of ACC to ethylene catalyzed by 1-aminocyclopropane-1-carboxylic acid oxidase (ACCO), remains obscure. The participation of a non-heme iron ion has been suggested in ACCO (Pirrung *et al.,* 1993) and it is well known that ACCO shows significant sequence similarity to isopenicillinN synthase (Roach *et al.,* 1997). In order to elucidate the process in ethylene evolution, suitable synthetic model systems to produce ethylene from ACC are necessary, but the models are scarce at present.



Scheme 1.

In our previous paper (Nishida *et al.,* 1992a) we have reported that binuclear iron(III) complex with H(HPTP), where H(HPTP) denotes N,N,N′,N′-tetrakis(2-pyridylmethyl)-1,3-diamino-2-propanol (Nishida *et al.,* 1992b), exhibits activity for ethylene evolution from ACC in the presence of hydrogen peroxide, and proposed that the presence of an activated oxygen species is necessary for ethylene evolution. After this report, we have observed that binuclear Co(II) and Mn(III) compounds with H_5(HXTA), N,N′-(2-hydroxy-5-methyl-1,3-xylylene)bis(N-carboxymethylglycine), (Murch *et al.,* 1987) exhibits much higher activity for ethylene evolution than that of the Fe(III) complex with H(HPTP). In this article, we will report the preparation, ethylene evolution, and spectroscopic data of these binuclear Co(II) and Mn(II) compounds with H_5(HXTA).

Experimental

Metal compounds

Binuclear Fe(III) (Murch *et al.,* 1987) Co(II) and Ni(II) with H_5(HXTA), Na_3M_2(HXTA)

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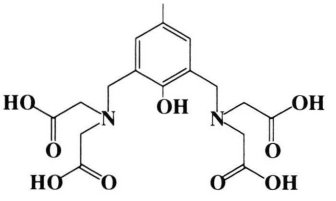
$(CH_3COO)_2$ (M = Co(II) and Ni(II)) (Nishida *et al.*, 1990) were prepared according to the literature methods, and the crystal structure of the binuclear iron(III) complex, $Fe_2(HXTA)(CH_3COO)_2^-$ was already reported (Murch *et al.*, 1987).



H_5(HXTA)

$Na[Mn_2(HXTA)(CH_3COO)_2]\cdot 3/2H_2O$: An aqueous solution (20 ml) containing Mn(III) acetate (1.0 g) and $Na_3H_2(HXTA)$ (Murch *et al.*, 1987) (0.95 g) was evaporated to small volume (ca. 3 ml), and the ethanol (5 ml) was added to the residue. After one day deposited needles was filtered. Found: C, 37.34; H, 3.50; N, 4.10, Mn, 16.6%. Calcd for $C_{21}H_{26}N_2O_{14.5}Mn_2Na$: C, 37.57; H, 3.90; N, 4.17; Mn, 16.9%.

$[(C_4H_9)_4N][V_2(HXTA)(CH_3COO)_2]\cdot H_2O$: The calculated amounts of VCl_3 (0.65 g) and $Na_3H_2(HXTA)$ (0.95 g) were mixed in water/methanol (1:1, v/v) (30 ml) under an anaerobic condition, and greenish-blue crystals deposited when excess of $NaCH_3COO\cdot 3H_2O$ (1.0 g) and $(C_4H_9)_4NBr$ (0.7 g) were added to the solution. Found: C, 51.06; H, 7.02; N, 4.59. Calcd. for $C_{37}H_{61}N_3O_{14}V_2$: C, 50.86; H, 7.04; N, 4.81%. By the similar way, the corresponding 5-Cl derivative of the (HXTA) , (5-Cl-HXTA), of the V(III) complex was prepared. Found: C, 49.07; H, 6.41; N, 4.65. Calcd. for $C_{36}H_{56}N_3O_{13}V_2Cl$: C, 49.35; H, 6.44; and N, 4.80%. These V(III) compounds are stable only in the solid state, and they are readily oxidized to a V(IV) species in solution under aerobic condition.

Determination of ethylene evolved

Binuclear metal complex (0.02 mmol) and ACC (30 mg) were dissolved in 2.5 ml of water, and to this solution was added 2 ml of aqueous hydrogen peroxide (0.1 mol/l); the vessel (total volume, 19 cm^3) was sealed with a butyl rubber cap. At appropriate time after mixing, 1 ml of the air in the head space was analyzed by GC. GC conditions are: glass column (diameter 3 mm; length 1.1 m); packing material, active alumina; carrier gas, nitrogen (flow rate, 60 ml min^{-1}); column temperature, 80 °C; detector temperature, 90 °C; flame ionization detector.

Spectroscopic measurements

The aqueous solutions containing cobalt(II) complex, hydrogen peroxide, and amino acid (glycine or ACC) were prepared, and absorption spectra of these solutions were measured with a Shimadzu UV-2200 at 298 K. Final concentrations are Co(II) complex, 2×10^{-3}, H_2O_2, 2×10^{-2}, and amino acid, 2×10^{-2} mol/l, respectively.

Evaluation of catalase-like function

Catalase-like function of the metal complexes was evaluated by measuring the volume of dioxygen molecule collected in a gas biuret, which evolved from the solution (total volume 6 cm^3) containing a metal complex (10 mmol) and hydrogen peroxide (1 mmol) at 25 °C in water (Okuno *et al.*, 1997). Theoretical quantity of the dioxygen molecule, which evolves via decomposition of hydrogen peroxide under our experimental conditions is ca 16 cm^3, which was exemplified by the authentic experiment by the use of Mn_2O as a catalyst in water.

Mass spectral measurements

Mass spectra of the solution were obtained with a API 300 triple quadrupole mass spectrometer (Ion-spray interface of PE-Sciex, Thomhill, ON, Canada) at room temperature. All the spectra were compared with the calculated isotope patterns, as shown in Fig. 4.

Results and Discussion

Ethylene evolution from ACC by the metal complexes

The time course of ethylene evolution catalyzed by Mn(III), Co(II) and Fe(III) complexes are shown in Fig. 1a. It is clear that the activity of the

Mn(III) and Co(II) compounds are much higher than those of Fe(III), Ni(II), and V(III) complexes, the activities of the latter two complexes being lower than that of the Fe(III) complex, and essentially the same as that without a metal complex.

Catalase-like function of the complexes

The addition of hydrogen peroxide to a binuclear metal complex usually induces the formation of a peroxide adduct in several cases (Nishida *et al.*, 1992b), and the formation of a (μ-η^1:η^1-peroxo)diiron(III) species (see below) has been confirmed by X-ray crystallography (Dong *et al.*, 1996). These discussions are suggesting that (μ-η^1:η^1-peroxo)diiron(III) is almost inactive for ethylene evolution from ACC, although some of them are known to be active for decomposition of hydrogen peroxide, catalase-like function (Akamatsu *et al.*, 1997).

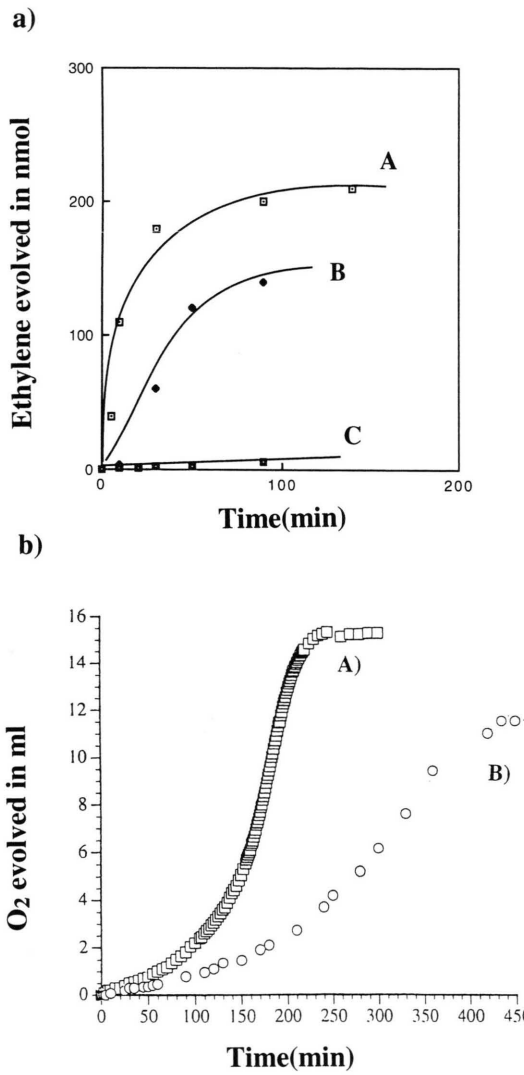
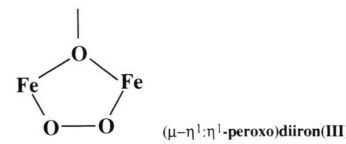


Fig. 1. a. Ethylene evolution catalyzed by metal compounds A: Mn(III), B: Co(II), C: Fe(III); b. Evolution of gas form the solution containing cobalt(II) complex and hydrogen peroxide. A. O_2 evolution from Co(II) complex and hydrogen peroxide; B. gas evolution from Co(II) complex, hydrogen peroxide, and glycine.



(μ-η^1:η^1-peroxo)diiron(III)

Slow at first, but later rapid evolution of dioxygen was observed in the solution containing the cobalt(II) complex and hydrogen peroxide (see trace A in Fig. 1b), however activities of the corresponding Mn(III), V(III), and Ni(II) compounds to evolve oxygen are almost negligible in water (data not shown). It should be noted here that addition of amino acid, such as glycine or ACC decreases the activity of the catalase-like function of the cobalt(II) complex (see trace B in Fig. 1b). In the case of Mn(III) complex, the brown color due to Mn(III) ion disappeared upon the addition of hydrogen peroxide (not shown), indicating that the Mn(III) state of the (HXTA)-complex is reduced by hydrogen peroxide, and the resulted binuclear Mn(II) species cannot be re-oxidized to a Mn(III) state by hydrogen peroxide; this is consistent with negligible activity of this complex for decomposition of hydrogen peroxide; it is clear that a Mn(II) species in the solution is the main species for evolution of ethylene from ACC. No remarkable spectral change was observed for the case of Ni(II).

In the case of the Co(II) complex, a drastic change in absorption spectra was seen as illustrated in Fig. 2; by the addition of hydrogen peroxide, the absorbance in the range 350–500 nm increases (0–6 min) and after 6 min the decreases of the absorbance began (see traces F to G in Fig. 2b). It should be noted that the absorption spectrum of the last trace in Fig. 2b is very similar to that of general Co(III) complexes, especially the appearance of d–d band in the range 500–650 nm, which should correspond to the so-

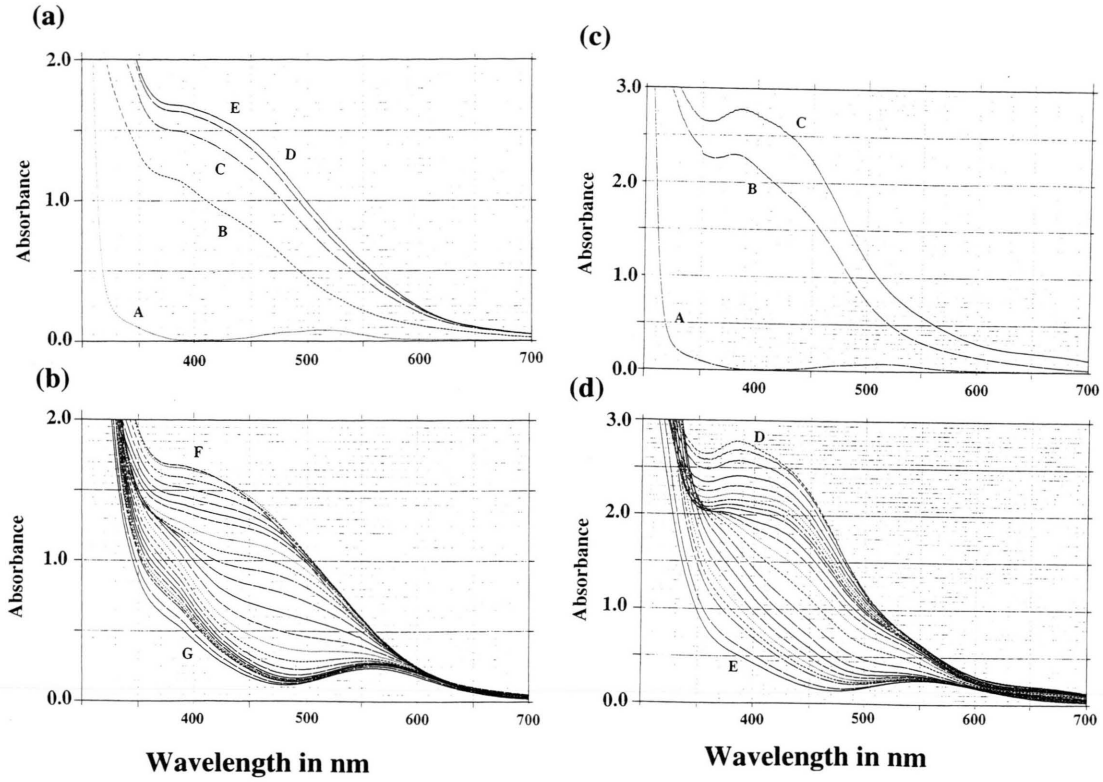
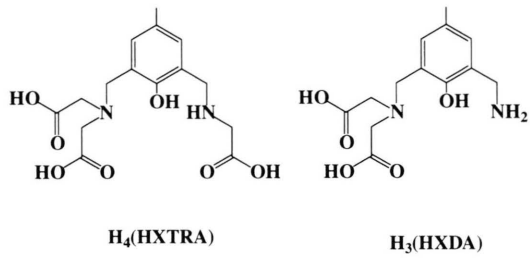


Fig. 2. Absorption spectra of Co(II) compound in water at 298 K. a–b A: original Co(II) complex; B: H_2O_2 was added to the Co(II) complex; C, D, E: 2, 4 and 6 min after addition of H_2O_2; F → G: spectral change after 6–360 min. c–d Absorption spectra of Co(II) compound in the presence of glycine (in water at 298 K). A: Original Co(II) complex; B: H_2O_2 was added to the Co(II) complex; C: 2 min after addition of H_2O_2; D → E: spectral change after 2–300 min.

called "the first band" of the Co(III) complexes (Shibata, 1983). These indicate that a Co(III) species is formed in the solution containing $Co(II)_2(HXTA)(CH_3COO)_2^{3-}$ and hydrogen peroxide, but it is clear that a phenolic group of the original ligand (HXTA) is destroyed because the absorbance in the range 350–500 nm has disappeared. The addition of ACC (or glycine) accelerates the spectral changes, as seen in Fig. 2c–d.

Mass spectra of the metal complex solutions

Mass spectrum of the solution containing $Co_2(HXTA)(CH_3COO)_2^{3-}$ indicates that acetate groups dissociate in the aqueous solution forming a $Co_2(HXTA)^-$ (peak at m/z = 511.0 in Fig. 3a), and the same phenomena were observed for the corresponding nickel(II) and manganese(II) complexes. (see Fig. 4). When hydrogen peroxide was added to the solution of the cobalt(II) complex, Mass spectra have changed with time, as illustrated in traces b–d of Fig. 3. The peak at m/z = 452.8 and 338.8 may correspond to the cobalt(II) complexes $Co_2(HXTRA)$ and $Co(HXDA)^-$, respectively. Formation of $H_3(HXTRA)$ was confirmed by the crystal structure determination of its cobalt(III) compound (Sasaki *et al.*, 1999).



$H_4(HXTRA)$ $H_3(HXDA)$

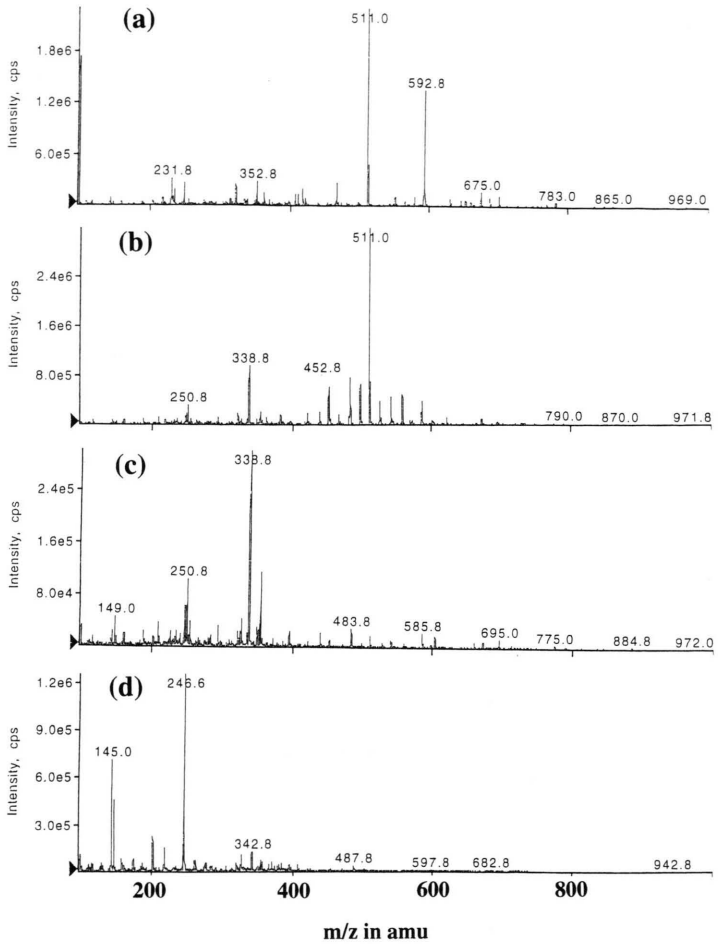
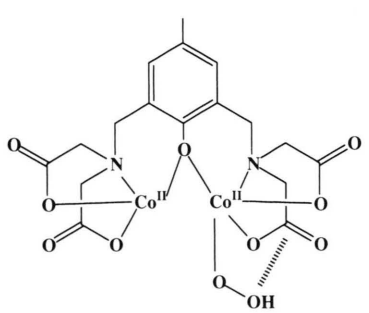


Fig. 3. Mass spectrum (ESI; negative pattern) of solution containing Na_3-$Co_2(HXTA)(CH_3COO)_2$ (in water, 298 K, $\sim 10^{-6}$ mol). a: The molecules at m/z = 511.0 and 592.8 correspond to $Co_2(HXTA)^-$ and $Co_2(HXTA)^- \cdot NaCH_3COO$, respectively; b: 10 min. after addition of H_2O_2 to solution A; c: 60 min after addition of H_2O_2 to solution A; d: 3 h after addition of H_2O_2 to solution A.

This indicates that the addition of hydrogen peroxide to the solution of $Co_2(HXTA)(CH_3COO)_2^-$ leads to degradation of the ligand system, especially to the decomposition of acetato-arm in (HXTA)-ligand, and an assumed intermediate for this reaction is illustrated below; a peroxide adduct of Co(II) with η^1-coordination mode is reacting with carboxylate group.



On addition of an amino acid to the solution of Co(II) and Ni(II) complexes, mass-spectra (Fig. 5) have revealed that the amino acid is chelated to the cobalt(II) species in solution. (see the Scheme 2, A). We reported in the previous section that the addition of ACC to the solution has lead to the decrease of catalase-like function by the binuclear cobalt(II) complex; it is generally accepted that catalase-like function emerges through the formation of (μ-η^1:η^1-peroxo)dimetal species (Okuno *et al.*, 1997) so it seems reasonable to assume that the chelation of ACC to the binuclear Co(II) complex prevents formation of (μ-η^1:η^1-peroxo)cobalt(II) species, decreasing the catalase-like function.

Mechanism of ethylene evolution

As the corresponding nickel(II) shows negligible activity for evolution of ethylene, it seems

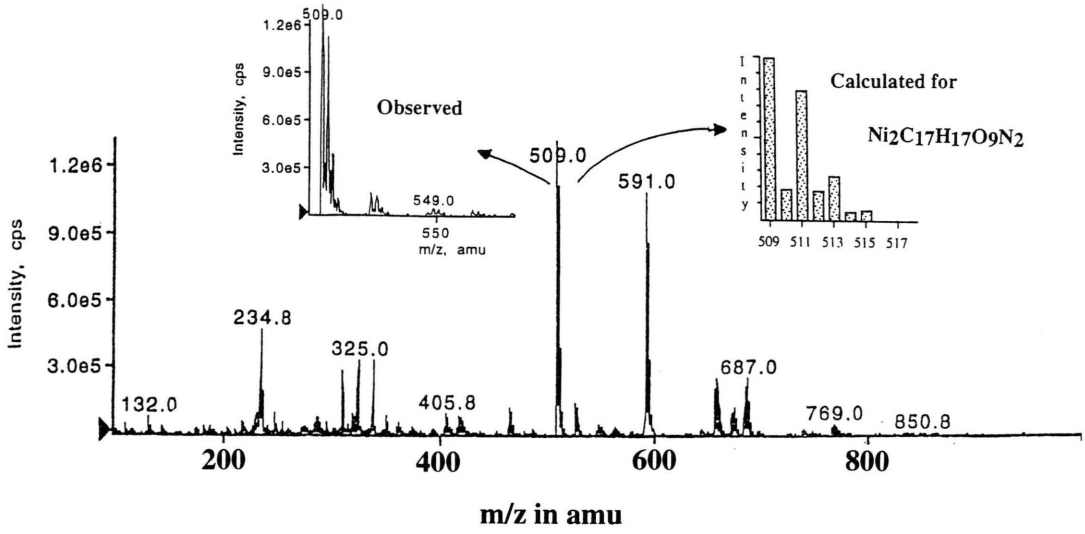
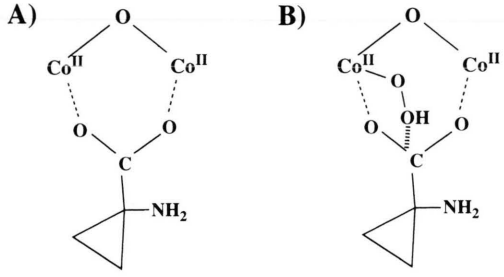


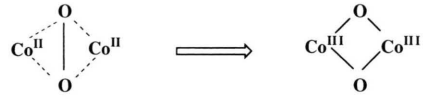
Fig. 4. Mass spectrum (ESI; negative pattern) of solution containing $Na_3Ni_2(HXTA)(CH_3COO)_2$ (in water, 298 K, $\sim 10^{-6}$ mol). The molecules at m/z = 509.0 and 591.0 correspond to $Ni_2(HXTA)^-$ and $Ni_2(HXTA)^- \cdot NaCH_3COO$, respectively. Isotope pattern calculated for $Ni_2(HXTA)^-$ was also included.



Scheme 2.

quite likely that more one electron is necessary to activate the peroxide ion, to lead to oxidative degradation of ACC, which is similar to the degradation observed in the ligand (HXTA)-system. Based on the facts and discussion described above it can be concluded that a peroxide adduct of the binuclear cobalt(II) complex with η^1-coordination mode (see Scheme 2, B) attacks the chelated ACC at the acetato group, leading to oxidative degradation of ACC associated with the oxidation of Co(II) ion to Co(III) state, and to evolution of ethylene. Similar reactivity by a metal-peroxide adduct has been observed in Cytochrome P-450 (Robichaud *et al.*, 1995). Since Ni(III) oxidation state is unfavorable, and a chelated structure of (μ-η^1:η^1-peroxo) core is more favorable for the binuclear $Fe_2(HXTA)(CH_3COO)_2^-$, negligible activity of these Ni(II) and Fe(III) compounds can be rationalized based on the above discussion. Above discussion may suggest that a monomeric Fe(II) ion in the active site of ACCO (Roach *et al.*, 1997) may act as one-electron donor, as observed for Co(II) and Mn(II) ions in this paper.

According to the recent results by Hikichi *et al.* (1998) a binuclear cobalt(II) complex with (μ-η^2:η^2-peroxo) mode is homolytically oxidized to give the corresponding Co(III)-oxo species (see below).



μ-η^2:η^2-peroxodicobalt(II) di-μ-oxodicobalt(III)

In our present case, however homolytic oxidation of the cobalt(II) ion by the peroxide ion is less likely due to the steric requirements by the ligand $(HXTA)^{5-}$ system.

Acknowledgements

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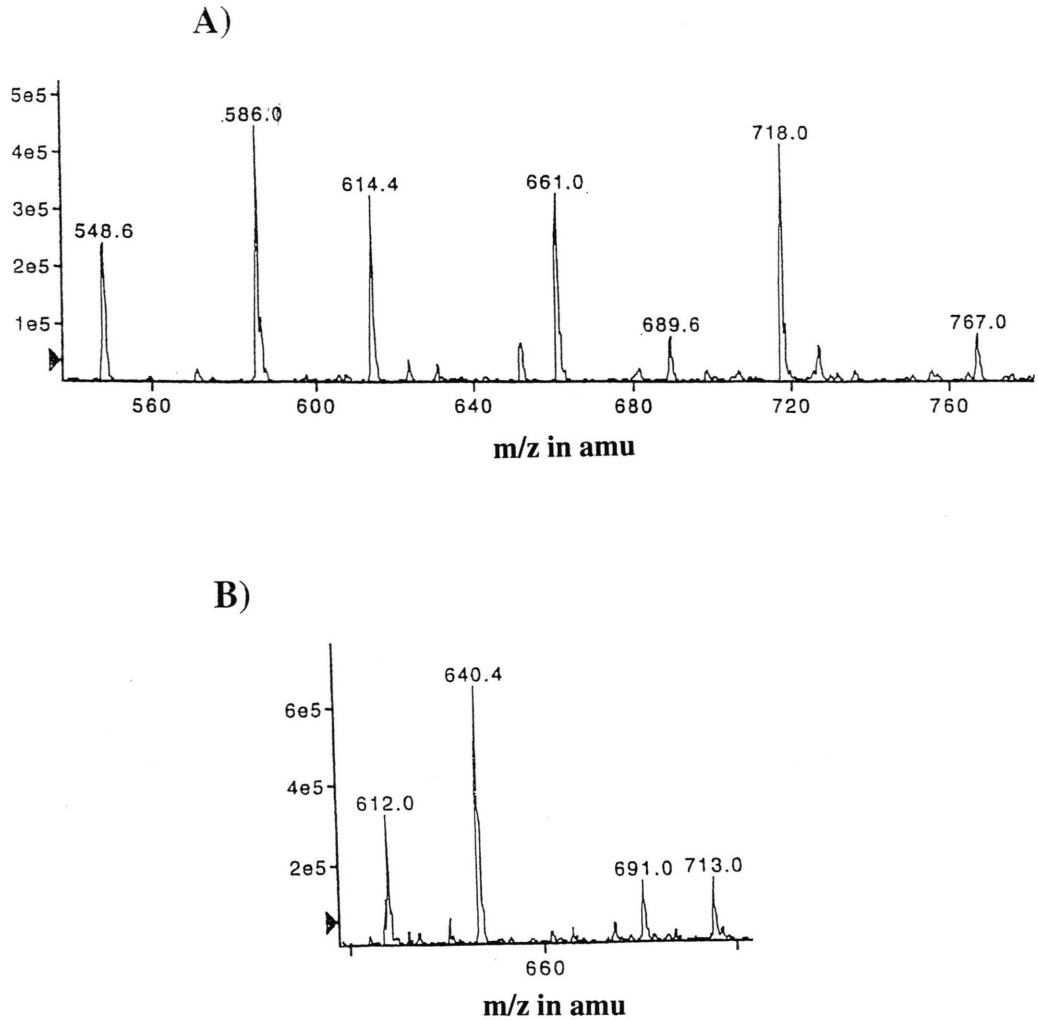

Fig. 5. Mass spectrum (ESI; negative pattern) of solution containing $Na_3Co_2(HXTA)(CH_3COO)_2$ and amino acid (in water, 298 K, $\sim 10^{-6}$ mol). A: The molecules at m/z = 586.0 and 661.0 correspond to $Co_2(HXTA)(glycine)^-$ and $Co_2(HXTA)(glycine)_2^-$, respectively. B: The molecules at m/z = 612.0 and 713.0 correspond to $Co_2(HXTA)(ACC)^-$ and $Co_2(HXTA)(ACC)_2^-$, respectively.

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