Inorganica Chimica Acta, 138 (1987) 121–126

Further Studies of a Synthetic Dicopper(I) Oxygen Carrier and Comparison with Hemocyanin

NORMAN C. LI*, JI R. TZOU, SHU W. CHEN, SUNG M. WANG**,
Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan
Y. C. CHOU**, N. T. LIANG and H. J. LIN
Department of Physics, National Tsing Hua University, Hsinchu, Taiwan

(Received March 19, 1987)

Abstract

A synthetic dicopper oxygen carrier, $Cu_2(EDTB)(ClO_4)_2$, where EDTB is *N,N,N',N'*-tetrakis(2-benzimidazolylmethyl)-1,2-ethanediamine), serves as an active-site model of hemocyanine (Hc). Its SERS (surface-enhanced Raman scattering) spectra and cyclic voltammograms have been obtained. When this colorless compound is suspended in a small amount of absolute ethanol and oxygen is bubbled through, the color changes to green. Elemental analyses of the green solid give the formula $Cu_2(EDTB)(ClO_4)_2 \cdot (O_2) \cdot 2H_2O$ for the oxygenated compound. This compound gives a Raman peak of dioxygen at 1007 cm^{-1}. Oxyhemocyanin from Taiwan snails shows a SERS peak at 745 cm^{-1}, indicating peroxo dioxygen. Cyclic voltammetry studies are consistent with the oxygenation product of $Cu_2(EDTB)(ClO_4)_2$ being Cu(II)–O_2–Cu(II) in the initial stages of oxygenation. The carbon monoxide adducts of $Cu_2(EDTB)(ClO_4)_2$ and of hemocyanin have also been studied. The affinity and effect of CO binding to the model compound and the protein are smaller than those of dioxygen binding. Cu can be removed from Hc and reincorporated to form reconstituted Hc. The oxygen affinity and fluorescence intensities of the reconstituted Hc and native Hc are compared.

Introduction

Reedijk and coworkers [1] have discussed the importance of $Cu_2(EDTB)^{2+}$ (EDTB = *N,N,N',N'*-tetrakis(2-benzimidazolylmethyl)-1,2-ethanediamine) as a model for the active site of hemocyanin (Hc). With oxyhemocyanin it is generally accepted that the coppers are Cu(II), with the bound dioxygen as peroxide, whereas with deoxyhemocyanin, the coppers are Cu(I). The purpose of the research was to investigate the reactions of $Cu_2(EDTB)(ClO_4)_2$ with dioxygen by the method of SERS (surface-enhanced Raman scattering) and compare these with a SERS study of hemocyanin from Taiwan snails (*Achatina fulica*). Cyclic voltammetry was used as a complement in probing the oxygenation process of $Cu_2(EDTB)^{2+}$. Reactions of carbon monoxide with $Cu_2(EDTB)(ClO_4)_2$ and with hemocyanin were also studied.

By treatment with KCN, Cu can be removed from hemocyanin and Cu can be reconstituted into the protein. Some properties of the reconstituted Hc and native Hc are compared.

The large enhancing effect ($\times 10^6$) of the Raman scattering intensity of molecules adsorbed on rough noble metal surfaces was first reported in 1974 [2]. Among the SERS active noble metals, Ag exhibits the highest enhancement, and has been used frequently in SERS experiments. Although the detailed mechanisms are not yet clear, it is generally accepted that the enhancement results from both the electromagnetic effects (where the electromagnetic field of the incident and scattered radiation is greatly enhanced near the rough metal surface) and the chemical effects (where the Raman cross-section is resonantly enhanced due to the charge-transfer interaction between the adsorbed molecules and the metal surface). However, the controversy over the origins of the surface-enhanced Raman scattering (SERS) does not prevent SERS from becoming an analytical tool, due to its enormous sensitivity. It is relatively easy to detect the SERS signal from the molecules with a coverage of less than one monolayer. Most biological molecules are easily adsorbed on the metal surface via the lone pair electrons of nitrogen atoms or via the π-electrons in aromatic rings. Hence, one of the potential applications of SERS is to monitor biological reactions.

Another advantage for SERS in observing the behavior of large biological molecules is that the fluorescence is less enhanced by at least one order

*On leave from Duquesne University and Catholic University of America.
**Authors to whom correspondence should be addressed.

0020-1693/87/$3.50

of magnitude than the Raman scattering [3]. Hence, the interference between the two effects will not cause as much of a problem for SERS experiments as for resonance Raman scattering. There are several methods of preparing the SERS active rough metal surfaces [4]; for example, oxidizing and reducing the surface of metal electrodes several times, evaporating metal island film on a dielectric substrate, and generating colloidal metal by rapid reduction of the metal salt. In this research we used the first two methods in order to produce the SERS active metal surface.

Experimental

Preparations

EDTB, $Cu_2(EDTB)(ClO_4)_2$ and $Cu_2(EDTB)Cl_4$ were prepared and analysed as described previously [5, 6]. The dicopper(I) compound, in the form of a dry white powder, is not air-sensitive. When this was suspended in a small amount of absolute ethanol and oxygen was bubbled through, the color changed to green. After filtration, the green solid was dried at 70 °C for 6 h. *Anal.* Found (Calc.): Cu, 13.17 (13.03); C, 41.94 (41.86); H, 3.54 (3.69); N, 14.17 (14.37); O, 19.91 (19.70)%. The values in parenthesis are those calculated for $Cu_2(EDTB)(ClO_4)_2 \cdot (O_2) \cdot 2H_2O$. Elemental analysis of the dioxygen adduct of any hemocyanin model compound has not been published previously.

Hemocyanin from Taiwan snails was prepared and purified in the manner previously described [7]. Cu can be removed from Hc by KCN treatment [8]. The reconstitution of Hc, by treating the Cu-free solution with $Cu(CH_3CN)_4ClO_4$, was carried out in accordance with the method of Wang *et al.* [6]. The native Hc has a Cu content of 0.24%. After reconstitution, a Cu content of 0.21% was found, corresponding to a reconstitution factor of 87.5%.

Physical Measurements

Island silver films were used for EDTB mainly because of its insolubility in water. The silver films were evaporated in a vacuum of 4×10^{-7} torr on a microscope slide. The thickness of the film was monitored by a quartz microbalance. Typical evaporation rate was 1 Å/s.

SERS spectra of the synthetic model compounds were obtained by depositing these compounds on rough silver films by dipping the films into the corresponding Me_2SO solution and spinning off the solution after 2 min. SERS experiments with hemocyanin solutions were carried out in an electrochemical cell containing an Ag working electrode (roughened surface after several oxidation–reduction cycles, ORC), a Pt counter electrode and a saturated Calomel electrode as reference. The polycrystalline electrode was first mechanically polished by 0.05 μm abrasive Al_2O_3 and then etched in a 1:1 volume mixture of H_2O_2 and NH_4OH and rinsed with doubly distilled water. 0.1 M KCl solution was used as the supporting electrolyte. The potential was driven by a cyclic voltammograph (CV-1B, BAS). The potential sweeping rate was 5 mV/s. The scattered light was dispersed by a triple-stage spectrograph and detected by an optical multichannel analyser. The coverage of the spectra was about 1200 cm^{-1} with a resolution of 10 cm^{-1}. An Ar^+ laser line (514.5 nm) was used for excitation [9, 10]. Cyclic voltammograms were obtained with a potentiostat, programmer and a recorder. Pt electrodes were used as working and counter electrodes, and a saturated calomel electrode as reference. The supporting electrolyte was 0.1 M $[(C_2H_5)_4N]BF_4$. UV–Vis spectra were obtained on a Perkin-Elmer Lambda 5 spectrophotometer. The metal contents were determined by neutron activation analysis, using the university reactor. ESR experiments were carried out using a Bruker ER 200D spectrometer operating at 9.1 GHz at room temperature. Fluorescence spectra were obtained with a Perkin-Elmer Model LS-5 luminescence spectrometer. Oxygen equilibria were performed by a spectrophotometric method [11]. Indirect measurements of carbon monoxide equilibria were obtained by measuring oxygen binding in the presence of carbon monoxide.

Results and Discussion

Of the SERS spectra of EDTB and dicopper EDTB complexes, only the green compound $Cu_2(EDTB)(ClO_4)_2 \cdot (O_2) \cdot 2H_2O$ shows a peak at 1007 cm^{-1} (Fig. 1a). This Raman peak compares well with the resonance Raman peak of 1011 cm^{-1} (νO_2) for the dioxygen adduct of [*N,N'*-ethylenebis(salicylideniminato)]cobalt(II), $[Co(salen)_2]_2O_2$ [12]. From the Raman shifts listed for peroxo (740–840 cm^{-1}) and for superoxo (1100–1140 cm^{-1}) dioxygen stretching frequencies [12], we suggest that the SERS peak at 1007 cm^{-1} may be ascribed to the dioxygen peak in a mixture of Cu(I)-Cu(II)(EDTB)$(ClO_4)_2(O_2^-)$ and Cu(II)Cu(II)(EDTB)-$(ClO_4)_2(O_2^{2-})$.

Figure 1b shows the SERS spectrum of the white compound, $Cu_2(EDTB)(ClO_4)_2$. The large reduction of the dioxygen peak at 1007 cm^{-1} is evident.

In this study, our attention was focussed on the oxygen-related peaks. The remaining Raman peaks at 1060, 1150, 1280, 1430, 1490, 1530 and 1580 cm^{-1} are believed to originate from the benzimidazole ring modes of EDTB [13–15]. The relative intensities, as well as the peak positions of the benzimidazole ring modes, are different for the green and white compounds. The origin of this difference may be that the

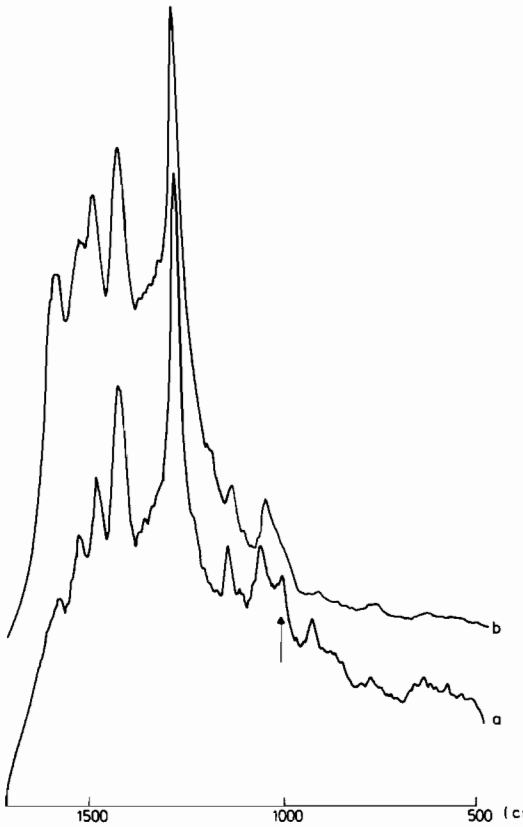

Fig. 1. SERS spectra of: (a) the green compound, $Cu_2(EDTB)(ClO_4)_2(O_2)\cdot 2H_2O$ in Me_2SO (arrow points to the 1007 cm^{-1} peak), and (b) the white compound, $Cu_2(EDTB)(ClO_4)_2$, in Me_2SO.

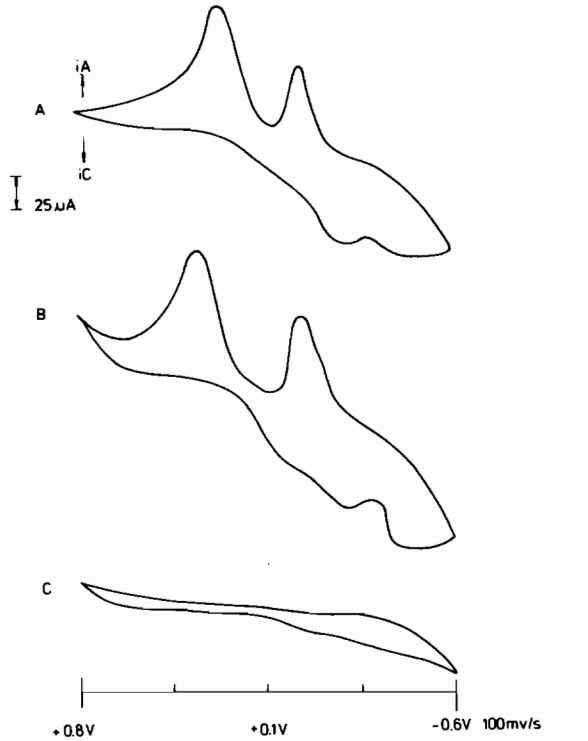

Fig. 2. Cyclic voltammograms of 0.005 M $Cu_2(EDTB)(ClO_4)_2$ + 0.1 M $[C_2H_5)_4N]BF_4$ in Me_2SO: (A) colorless, under nitrogen; (B) colorless under nitrogen, with passage of CO for 2 h; (C) under oxygen, green solution.

orientations of adsorption of these two compounds are different on the silver films. It is noted that near 1000 cm^{-1} there is a Raman peak from the benzene in-plane ring bending mode [14] which may interfere with the peroxy mode studied here. However, the observed reduction of the intensity of the 1007 cm^{-1} peak is highly reproducible when the dioxygen is removed. We believe that the contribution of the benzene ring bending mode to the SERS spectra is probably small.

The SERS spectra of oxyhemocyanin from Taiwan snails show a peak at 745 cm^{-1} in the potential range −1.0 to −1.3 V (SCE). The peak intensity increases with an increase in negative potential. Beyond −1.3 V, the peak size starts to decrease because of competition with dioxygen from the hydrogen produced at the Ag cathode. The intensity of the 745 cm^{-1} peak is drastically reduced when deoxyhemocyanin is formed by adding sodium sulfite under nitrogen. The 745 cm^{-1} SERS peak is from the stretching vibration of peroxo dioxygen, and thus complements the findings of Chen *et al.* [7] that a resonance Raman spectrum of the oxyhemocyanin from Taiwan snails shows the O_2^{2-} vibration at 752 cm^{-1}. Both coppers at the active site of *A. fulica* hemocyanin are therefore Cu(II). Previously, Freedman *et al.* [16] had reported the frequency of the O_2^{2-} vibration at 744 cm^{-1} in *Cancer magister* (arthropod) and at 749 cm^{-1} in *Busycon canaliculatum* (mollusc hemocyanin).

Cyclic voltammograms show cathodic and anodic peaks for both $Cu_2(EDTB)(ClO_4)_2$ (Fig. 2) and $Cu_2(EDTB)Cl_4$ (Fig. 3) in Me_2SO. The cathodic process is the reduction of $Cu_2(II)$ to Cu(I)Cu(II) and then to $Cu_2(I)$, and the anodic process is the oxidation of $Cu_2(I)$ to Cu(I)Cu(II) and then to $Cu_2(II)$. When dioxygen is introduced into a solution of $Cu_2(EDTB)(ClO_4)_2$, the cathodic and anodic peaks become smaller and finally disappear, as shown in Fig. 2C. The data suggest the formation of an O_2 adduct in the early stages of oxygenation. There are no longer simple anodic reactions of Cu(I) to Cu(II) and cathodic reactions of Cu(II) to Cu(I). The oxygenated solution displayed irreversible reaction with dioxygen, changing color. This involved possibly ligand oxidation by dioxygen, catalyzed by Cu. On the other hand, $Cu_2(EDTB)Cl_4$ does not react with dioxygen, since the voltammograms are identical in nitrogen and oxygen atmospheres (Fig. 3).

Figure 2B shows the voltammogram obtained for $Cu_2(EDTB)(ClO_4)_2$ under CO. Comparison with

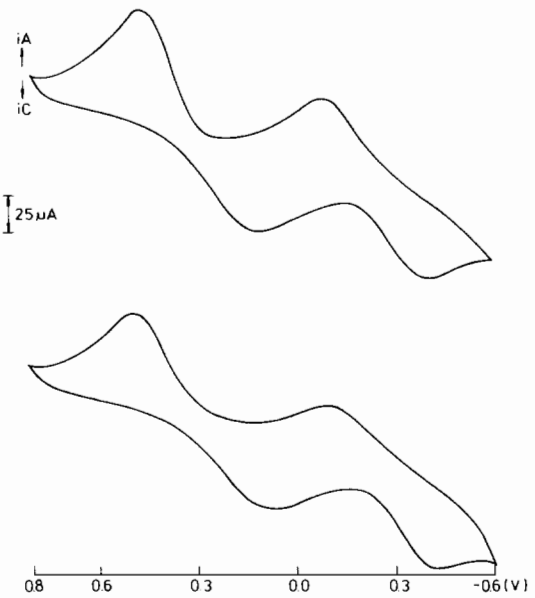

Fig. 3. Cyclic voltammograms of 0.005 M $Cu_2(EDTB)Cl_4$ + 0.1 M $[(C_2H_5)_4N]BF_4$ in Me_2SO: bottom, under oxygen; top, under nitrogen.

Fig. 2A shows that only the more positive anodic peak moves to a more positive potential (from +0.25 V under N_2 to +0.36 V under CO). This indicates CO binding to the dicopper(I) compound, in the formation of $Cu_2(EDTB)(ClO_4)_2(CO)$, with CO bound to only one of the two coppers. From Fourier-transform IR spectroscopy, Fager and Alben [17] have previously proposed a model for the hemocyanin carbonyl complex which includes a trigonal oxygen of CO coordinated to one copper atom (C–O–Cu angle near to 120°), with the second copper atom of the active site coordinated only to protein.

Oxygen and Carbon Monoxide Equilibria

The CO affinity of hemocyanin can be measured by direct or indirect spectrophotometric methods. The direct method makes use of small changes in the hemocyanin absorption band which accompany CO binding. Since the absorption changes are very small, we have determined CO binding indirectly by measuring the effect of known concentrations of CO on subsequent oxygen binding. This replacement method has been reported by Bonaventura *et al.* [18]. $p_{50}(O_2)$ values were determined in the presence and absence of CO. The partition coefficients, M, were calculated as a function of the percentage saturation with oxygen (Y) according to

$$M_Y = (1 - Y)p_{O_2}/(Y)p_{CO} \quad (1)$$

where p_{O_2} and p_{CO} are the partial pressures of these gases. The partial pressure of CO required to produce a given degree of saturation (Y) with CO in the absence of oxygen was calculated according to

$$(p_{CO})_Y = (p_{O_2})_Y/M_Y \quad (2)$$

where $(p_{O_2})_Y$ is the partial pressure of O_2 required to produce the degree of saturation denoted by Y, in the absence of CO.

The values of $p_{50}(O_2)$, $p_{50}(CO)$, $n_{50}(O_2)$ and $n_{50}(CO)$ with *A. fulica* hemocyanin and reconstituted hemocyanin are listed in Table I; $n_{50}(O_2)$ and $n_{50}(CO)$ are the Hill coefficients for the oxygen and carbon monoxide equilibria, respectively.

The partial pressures required to give half-saturation with CO at pH 8.0 and 9.0 are 10.6 and 9.0 mm, respectively, from Table I. These values show that the CO affinity for *A. fulica* hemocyanin is closer to that for *Busycon* hemocyanin (p_{50} = 3.6 mm at pH 8–9) than for *Limulus* hemocyanin (p_{50} = 100 mm at pH 8–9) [18]. The CO affinity (with negative cooperativity) is smaller than the oxygen affinity (with slight positive cooperativity).

TABLE I. Oxygen and Carbon Monoxide Equilibria at 30 °C

pH	$p_{50}(O_2)$	$n_{50}(O_2)$	$p_{50}(CO)$	$n_{50}(CO)$
(a) Native *Achatina fulica* hemocyanin				
8.0	6.60	1.08	10.56	0.83
9.0	6.50	1.08	9.02	0.86
pH	Native		Reconstituted	
	$p_{50}(O_2)$	$n_{50}(O_2)$	$p_{50}(O_2)$	$n_{50}(O_2)$
(b) Native hemocyanin and reconstituted hemocyanin in the presence of 0.02 M CaCl2				
8.0	6.5	1.09	7.05	1.17
9.0	4.0	1.30	6.76	1.06

In the presence of 0.02 M $CaCl_2$, the oxygen affinity for the reconstituted hemocyanin is smaller than that for native hemocyanin. This is in the direction that we would expect, since the concentration of CuO_2Cu groups is higher in native hemocyanin than in reconstituted hemocyanin (Cu contents of 0.24% and 0.21%, respectively).

Kuiper *et al.* [19] have reported the effect of CO on fluorescence of *Helix pomatia* α-hemocyanin and *Panulirus interruptus* hemocyanin. The carbon monoxide derivatives of these hemocyanins show emission maxima between 540 and 560 nm in addition to the tryptophan resonance at 340 nm. The 540 nm emission originates from a fluorescent Cu(I)–CO complex, interpreted as charge-transfer luminescence. With hemocyanin from Taiwan snails, with excitation wavelength at 295 nm, the fluorescence spectra for the oxy- and carbon monoxy-derivatives of native (Hc) and reconstituted hemocyanins (R-Hc) are shown in Fig. 4. At 330 nm, the fluorescence intensity varies in the order: R–Hc–CO

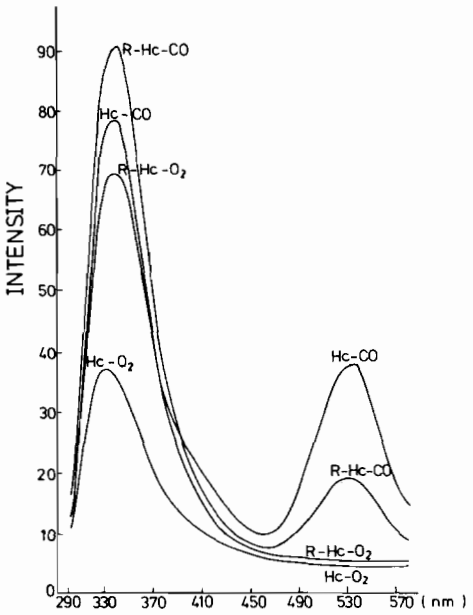

Fig. 4. Fluorescence emission spectra of native and reconstituted oxyhemocyanin (HcO_2, R-HcO_2) and of native and reconstituted carboxyhemocyanin (HcCO, R-Hc–CO), pH 9.0.

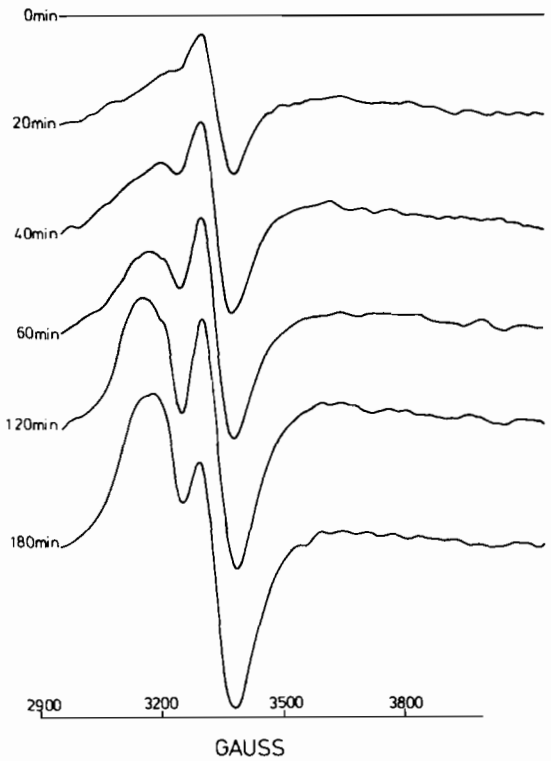

Fig. 5. ESR spectra, obtained at 9.73 GHz and room temperature, of colorless 0.006 M $Cu_2(EDTB)(ClO_4)_2$ under argon in Me_2SO (top) and at various times after exposure to air.

$>$ HcCO $>$ R-Hc–O_2 $>$ HcO_2. This order is as expected, since the concentration of CuO_2Cu groups is higher in native hemocyanin than in reconstituted hemocyanin and the concentration of Cu(I)–CO groups is also higher in native than in reconstituted hemocyanin (Cu contents of 0.24% and 0.21%, respectively). Moreover, the quenching of fluorescence is in the order Cu(II) $>$ Cu(I) and O_2 $>$ CO. At 540 nm, HcCO exhibits greater fluorescence than R-HcCO, again because of the greater concentration of Cu(I)–CO groups in native hemocyanin. The oxy derivatives show no fluorescence at 540 nm, because the Cu(I)–CO group is absent.

Deoxy forms and carbon monoxy forms of native Hc and reconstituted Hc, as well as the model compound $Cu_2(EDTB)(ClO_4)_2$ (under N_2), are all ESR-silent. The oxy forms of native Hc and reconstituted Hc are also ESR-silent, because of antiferromagnetic coupling between the two coppers. However, the distance between the two coppers in $Cu_2(EDTB)(ClO_4)_2(O_2)\cdot 2H_2O$ is too large for coupling, so that this compound is paramagnetic. The ESR spectra of the oxygenated compound definitely show the existence of two kinds of Cu. Starting with diamagnetic $Cu_2(EDTB)(ClO_4)_2$ (under argon) in Me_2SO, exposure to air causes the higher-field Cu(II) signal to appear first, followed by appearance of the lower-field Cu(II) signal (Fig. 5).

Starting with 0.006 M $Cu_2(EDTB)(ClO_4)_2$ in Me_2SO, exposure to air caused the absorbance at 690 nm to increase. A plot of $-\log(A - A_0)$ *versus* time, where A_0 is the absorbance at $t = 0$ at 690 nm, shows that the data may be discussed in terms of two consecutive reactions involving reaction of oxygen with a 'fast reacting' Cu and then a slower-reacting Cu. Beltramini *et al.* [20], in a kinetic study of the reaction between cyanide and hemocyanin, have proposed a kinetic model on the assumption that the two copper ions are removed sequentially from the active site by cyanide. They have obtained equilibrium constants for the formation of a complex between the first and second copper ion with cyanide and the rate constants of their decomposition. Our results on the absorbance study of oxygen reacting with $Cu_2(EDTB)(ClO_4)_2$ therefore show that the two coppers are also not equivalent.

Conclusions

In this paper we report for the first time the elemental analysis of the dioxygen adduct of a synthetic dicopper oxygen carrier, $Cu_2(EDTB)(ClO_4)_2\cdot(O_2)2H_2O$, which serves as an active site model of hemocyanin. In Me_2SO, the colorless solution of $Cu_2(EDTB)(ClO_4)_2$ shows an uptake of dioxygen of O_2/Cu_2, resulting in a green solution [2]. The green product, which is isolated at room tem-

perature, gives analysis which also corresponds to O_2/Cu_2. This compound gives a Raman peak of dioxygen at 1007 cm^{-1}. The SERS spectra of oxyhemocyanin from Taiwan snails show a peak at 745 cm^{-1}, ascribed to the stretching vibration of peroxo O_2. In O_2 atmosphere, the cyclic voltammograms of the dicopper(I) compound are different from those obtained in nitrogen and carbon monoxide atmospheres. The CV studies are consistent with the formation of an O_2 adduct in the initial stages of oxygenation.

Experiments on oxygen and carbon monoxide equilibria demonstrate that the CO affinity to hemocyanin is smaller than the oxygen affinity. Moreover, the oxygen affinity for the reconstituted hemocyanin is smaller than that for native hemocyanin. The latter is as expected, since the native hemocyanin contains more CuO_2Cu groups than does the reconstituted hemocyanin. The relative fluorescence intensities of native and reconstituted hemocyanins are also in line with the higher Cu content in native hemocyanin (0.24%), compared to 0.21% in reconstituted hemocyanin.

ESR and absorbance studies of the synthetic dicopper compound have shown that the two coppers, like the two coppers in hemocyanin, are not equivalent. However, unlike the diamagnetic character of oxyhemocyanin, the compound $Cu_2(EDTB)(ClO_4)_2 \cdot 2H_2O$ is paramagnetic, presumably because the two coppers are too far apart to experience antiferromagnetic coupling between them.

Acknowledgement

This work was supported by the National Science Council, Taiwan.

References

1 H. M. J. Hendriks, P. M. W. L. Birker, J. Van Rijn, G. C. Verschoor and J. Reedijk, *J. Am. Chem. Soc., 104,* 3607 (1982).
2 M. Fleishmann, P. J. Hendra and J. A. McQuellan, *Chem. Phys. Lett., 26,* 163 (1974).
3 D. A. Weitz, S. Garoff, J. I. Gersten and A. Nitzan, *J. Chem. Phys., 78,* 5324 (1983).
4 R. K. Chang and T. E. Furtak (eds.), 'Surface Enhanced Raman Scattering', Plenum Press, New York, 1982.
5 P. J. M. W. L. Birker, H. M. J. Hendriks, J. Reedijk and G. C. Verschoor, *Inorg. Chem., 20,* 2408 (1981).
6 S. M. Wang, J. R. Tzou, M. N. Chen and N. C. Li, *Inorg. Chim. Acta, 125,* 229 (1986).
7 J. T. Chen, S. T. Chen, C. S. Chung, H. Chang, S. M. Wang and N. C. Li, *Biochemistry, 18,* 3097 (1979).
8 R. Lontie, V. Blaton, M. Albert and B. Peters, *Arch. Intern. Physiol. Biochim., 73*(1), 150 (1965).
9 Y. C. Chou, N. T. Liang and W. S. Tse, *J. Raman Spectrosc., 17,* 481 (1986).
10 H. P. Kung and T. T. Chen, *Chem. Phys. Lett., 130,* 311 (1986).
11 A. F. Riggs and R. A. Wolbach, *J. Gen. Physiol., 39,* 585 (1956).
12 M. Suzuki, T. Ishiguro, M. Kozuka and K. Nakamoto, *Inorg. Chem., 20,* 1993 (1981).
13 S. Salama and T. G. Spiro, *J. Am. Chem. Soc., 100,* 105 (1978).
14 D. Thierry and C. Leygraf, *J. Electrochem. Soc., 132,* 1009 (1985).
15 B. Borah and J. L. Wood, *Can. J. Chem., 54,* 2470 (1976).
16 T. B. Freedman, J. S. Loehr and T. M. Loehr, *J. Am. Chem. Soc., 98,* 2809 (1976).
17 L. Y. Fager and J. O. Allen, *Biochemistry, 11,* 4786 (1972).
18 C. Bonaventura, B. Sullivan, J. Bonaventura and S. Bourne, *Biochemistry, 13,* 4784 (1974).
19 H. A. Kuiper, F. A. Alessandro, E. Antonini and M. Brunori, *FEBS Lett., 99,* 317 (1979).
20 M. Beltramini, F. Ricchelli, L. Tallandini and B. Salvato, *Inorg. Chim. Acta, 92,* 219 (1984).