etc.). All mammalian forms characterized are single chain proteins of 61 amino acid residues with binding sites for 7 metal ions [1]. The large metal-binding capacity is conditioned by the presence of 20 cysteine residues which provide thiolate ligands for the formation of two adamantane-like metal–thiolate cluster structures containing 3 and 4 metal ions, respectively [2, 3]. The unique occurrence of these clusters in metallothionein is believed to be related to the presence of 7 –Cys–X–Cys– sequences in the polypeptide chain where X stands for an amino acid residue other than Cys. To explore the metal complexing features of such dithiol sequences, we have now chemically synthesized by the Merrifield procedure the hexapeptides Ser–Cys–Val–Cys–Ala–Ala, Ala–Cys–Lys–Cys–Ala–Ala and Ala–Cys–Ser–Cys–Ala–Ala and have examined the spectroscopic features of their complexes with Cd(II) and related metal ions. Stepwise addition of Cd(II) to aqueous solutions of these peptides yields complexes of 1:2, 2:3 and 1:1 metal-to-peptide stoichiometry (Fig. 1(a)). The first two complexes display features

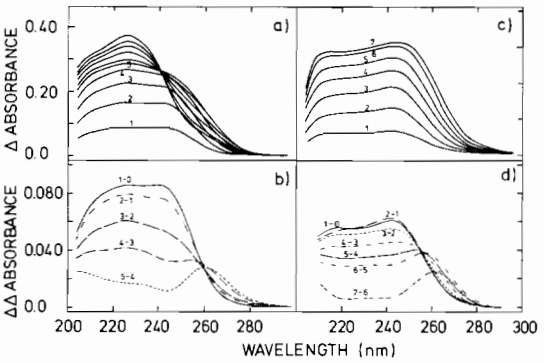

Fig. 1. Complex formation of Cd(II) with Ser–Cys–Val–Cys–Ala–Ala and with apometallothionein (=metal-free form of metallothionein). (a) Difference absorption spectra (peptide absorption subtracted) of a 30 μM solution of the peptide following successive additions of Cd(II) in 5 mM sodium phosphate, pH 7.0. At each titration step the concentration of Cd is increased by about 3.5 μM. (b) Difference–difference spectra showing absorption increments at each titration step (same data as in a)). (c) Difference absorption spectra (absorption of apometallothionein subtracted) of a 3.5 mM solution of apometallothionein following successive additions of Cd(II), in 5 mM Tris chloride buffer, pH 8. At each titration step the concentration of Cd is increased by about 3.5 μM. (d) Difference–difference spectra showing absorption increments at each titration step (same data as in c)).

typical of tetrahedral tetrathiolate coordination also seen in Cd(II)–metallothionein [4] (Fig. 1(c)). The 2:3 complex is thought to be a binuclear cluster complex composed of two tetrahedral cadmium–thiolate units connected via two bridging thiolate ligands. It differs from the mononuclear 1:2 complex by a slight red shift of the absorption envelope which can be attributed to the greater polarization of the bridging sulfur ligands by the metal. In addition, its formation is accompanied by a nearly complete loss of the strong circular dichroism denoting the greater symmetry of its structure. The spectral shift signalling the formation of the ligand-bridged binuclear complex is most clearly indicated by the emergence of a maximum near 260 nm in the difference–difference absorption spectrum (Fig. 1(b)).

Spectral changes entirely analogous to those accompanying the successive formation of the mononuclear and binuclear tetrahedral Cd(II)–peptide complex also occur in the course of reconstituting Cd(II)–metallothionein from Cd(II) and apometallothionein (Figs. 1(c) and (d)). Under the conditions employed (pH 8), Cd(II) binds at first to separate tetrathiolate sites. However, with all thiolate ligands becoming occupied, a red shift develops signifying the change-over to the clustered structure containing 40% bridging ligands. Interestingly, these spectral changes are much less pronounced when Cd(II) is incorporated at lower pH. Below pH 4, the binding of successive equivalents of Cd(II) is, in fact, accompanied by a blue shift of the Cd(II)–thiolate absorption envelope indicating an initial preferential formation of thiolate-bridged structures. Hence, it would appear that depending on pH the building-up of the metal–thiolate clusters in metallothionein proceeds through different pathways.

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1 Y. Kojima and J. H. R. Kägi, *Trends Biochem. Sci., 3*, 90 (1978).
2 J. D. Otvos and I. M. Armitage, *Proc. Natl. Acad. Sci. USA, 77*, 7094 (1980).
3 M. Vašák and J. H. R. Kägi, *Proc. Natl. Acad. Sci. USA, 78*, 6709 (1981).
4 M. Vašák, J. H. R. Kägi and H. A. O. Hill, *Biochemistry, 20*, 2852 (1981).

N12

Modeling Studies of the Iron/Copper Binuclear Active Site of Bovine Cytochrome *c* Oxidase

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In attempts to model possible μ-imidazolato [1], μ-oxo [2, 3], and μ-mercapto [4] active site structures for resting (beef heart) cytochrome *c* oxidase

where $-J_{(Fe^{III}-Cu^{II})} \geqslant 200$ cm^{-1} [1], a number of [Fe^{III}(B)Cu^{II}] heterobinuclear iron porphyrin complexes with B = imid$^-$, O^{2-}, and RS^- have been synthesized, isolated as analytically pure solids, and examined by magnetochemical, electrochemical, and various spectroscopic methodologies. The three idealized active site structures which have been targeted for synthesis and study are shown in Fig. 1.

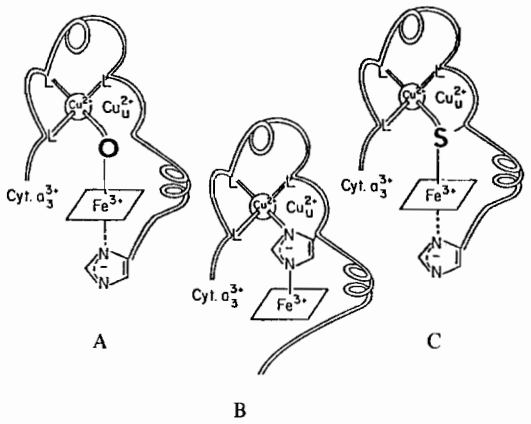

Fig. 1. The three proposed ligand-bridged models for the active site structure of resting cytochrome *c* oxidase: A) the μ-oxo model; B) the μ-imidazolato model; C) the μ-mercapto model.

The μ-imidazolato model species have been prepared from either [Fe^{III}_{hs}(TPP)X] (X = Cl^- or $SO_3CF_3^-$) and [Cu^{II}(imidH)$_2$DAP]$^{2+}$ [5], or from [Fe^{III}_{hs}(UroTPP)Cl] and [Cu^{II}(acac)$_2$] where $UroTPP^{2-}$ is the dianion of a new appendaged-tail porphyrin derived from urocanic acid chloride and *mono*-NH_2TPP [6]. Additionally, new mixed-metal binuclear complexes with [Co^{II}(imid)Cu^{II}]$^+$ and [Mn^{II}(imid)Cu^{II}]$^+$ cores have also been prepared from [Co^{II}(TPP)] and [Mn^{II}(TPP)] precursors. In most cases, $-J$ in the μ-imidazolato species is $\leqslant 15$ cm^{-1}; however, in *two* cases the imidazolate-bridged binuclear centers exhibit epr and full-temperature (4.2–300 K) magnetochemical behavior consistent with a 'strong' *anti*ferromagnetic exchange interaction possibly as large as the 200 cm^{-1} value postulated for the [Fe^{III}_{hs}/Cu^{II}] active site of the aerobically oxidized (resting) enzyme itself [1]. New magnetochemical data for the cyanide and formate derivatized enzyme and for an *an*aerobically oxidized ('resting') enzyme form will also be reported and discussed in light of the μ-imidazolato (and μ-oxo and μ-mercapto) structural option.

The lone [Fe^{III}–O–Cu^{II}]$^+$ species, in hand, has been prepared by the low-temperature reaction of [O=Fe^{IV}(TPP)] with [Cu^{I}(imidH)$_2$DAP]$^+$ [7]. For this μ-oxo heterobimetallic compound the ground state appears most like S = 1 in nature, as reflected by its magnetic, ^{57}Fe Mössbauer and epr (silent) behavior. Furthermore, results from a qualitative [Fe–O^{18}–Cu]$^+$ + H_2O^{16} ⇌ [Fe–O^{16}–Cu]$^+$ + H_2O^{18} isotopic exchange experiment indicate that the oxygen bridge of the μ-oxo model compound undergoes rapid scrambling with bulk water (CH_3CN/H_2O mixture). This finding is new support for the oxo-bridged postulation, since it adequately explains published Resonance Raman [8] and O_2-turnover [9] results which previously have been used to argue against the presence of an oxo-bridge originating from O_2. A complete catalytic cycle proposed for the enzymatic active site of oxidase ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$) and encompassing a μ-oxo structural model for the active site of the resting state is then shown in Fig. 2.

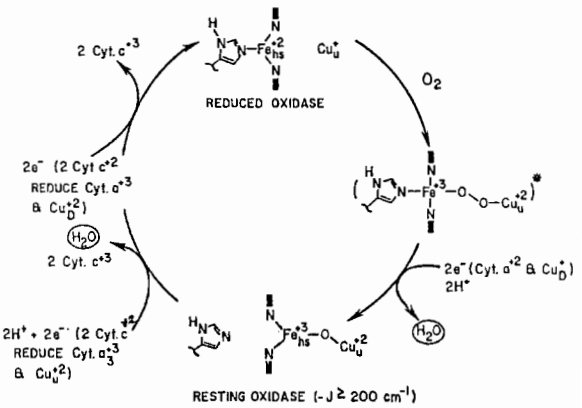

Fig. 2. A proposed catalytic mechanism incorporating the μ-oxo model as the active site structure for resting cytochrome *c* oxidase.

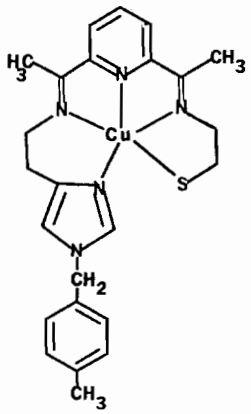

Fig. 3. Structure of the [Cu^{II}(imidR)(S)(DAP)]$^+$ cation with R = -$CH_2\phi CH_3$.

Finally, our newest model compounds, μ-mercapto species, derived from [Fe^{III}(TPP)Cl] and [Cu^{II}(imidR)(S)DAP]$^+$ (Fig. 3) or from [Cu^{I}(imidH)$_2$-DAP]$^+$ [7] and a 'thioferryl' species of TPP^{2-} will also be discussed as they comment upon a recently

proposed [Fe^{III}–$\overset{R}{\underset{S}{}}$–$Cu^{II}$] site structure suggested from the EXAFS studies of Chance and Powers [4].

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1 M. F. Tweedle, L. J. Wilson, L. Garcia-Iniguez, G. T. Babcock and G. Palmer, *J. Biol. Chem., 22*, 8065 (1978).
2 R. H. Petty, B. R. Welch, L. J. Wilson, L. A. Bottomley and K. M. Kadish, *J. Am. Chem. Soc., 102*, 611 (1980).
3 C. A. Reed and J. T. Landrum, *FEBS Lett., 106*, 265 (1979).
4 B. Chance and L. Powers, *Biophys. J., 33*, 95a (1981).
5 S. E. Dessens, C. L. Merrill, R. J. Saxton, R. L. Ilaria, Jr., J. W. Lindsey and L. J. Wilson, *J. Am. Chem. Soc., 104*, 4357 (1982).
6 R. J. Saxton, *Ph.D. Dissertation*, William Marsh Rice University, Houston, Texas, 1982.
7 R. J. Saxton, L. W. Olson and L. J. Wilson, *Chem. Commun.*, 984 (1982).
8 W. H. Woodruff, R. F. Dallinger, T. Antalio and G. Palmer, *Biochem., 20*, 1332 (1981).
9 R. W. Shaw, J. E. Rife, M. H. O'Leary and H. Beinert, *J. Biol. Chem., 256*, 1105 (1981).

N13

Studies of Peptide Analogues of the Copper(II)-Transport Site of Dog Serum Albumin

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The transport protein for Cu(II) in serum is albumin. Unlike human serum albumin (HSA), dog serum albumin (DSA) does not possess the characteristics of the specific first binding site for Cu(II) [1]. Results with DSA in the presence of 1 Cu(II) strongly suggest the partitioning of the first Cu(II) between two sites. However, the NH_2-terminal site of DSA still seems to be the preferred site. Copper(II) bound to this site appears to be the transport form of Cu(II) in dog serum. The amino acid sequence analysis at the NH_2-terminal region of DSA showed that the important histidine residue in the third position, responsible for the Cu(II)-binding specificity in HSA, is replaced by a tyrosine residue in DSA [2]. In order to study the influence of the tyrosine residue in the third position of DSA, Cu(II)-binding studies are carried out with glycylglycyl-L-tyrosine-N-methyl amide (GGTNMA) using CD and ^{13}C-NMR spectroscopy. Furthermore, the 24-residue peptide fragment from the NH_2-terminal (P_{24}) of DSA has been obtained in pure form to study the nature of the Cu(II)-transport site of DSA.

Experimental

The peptide glycylglycyl-L-tyrosine-N-methyl amide was synthesized according to the previously published procedure [3]. The CD spectra were recorded on a JASCO JV1A spectrometer using a Cu(II) concentration of 5×10^{-3} *M* (Cu(II):Peptide = 1:2). The ^{13}C-NMR spectra were obtained on a Nicolet 360 at 90.54 MHz and on a Bruker WP80SY at 20.15 MHz using a Cu(II):peptide ratio of 1:500 and Cu(II) concentration of 10^{-1} *M*. The P_{24} was obtained by controlled peptic digestion and purified by Sephadex G-25 fractionation and Celex D-ion exchange chromatography.

Results and Discussion

A variation was observed in the d–d transition band energy as the pH of the Cu(II)–GGTNMA solution was raised indicating the progressive involvement of the nitrogens around the Cu(II) nucleus. No charge transfer transition O^-–Cu(II) was observed suggesting that no phenolic oxygen is involved in the binding. In the ^{13}C-NMR investigation of Cu(II)-binding to GGTNMA at pH 7.9, the temperature variation and corresponding T_2 measurements established that the fast exchange limit was obtained. At pH 7.9, broadening of the first C=O and CH_2 of both glycine residues was observed. This would imply that Cu(II) coordinated to the α-NH_2 and the first peptide nitrogen. No line broadening of the tyrosine ring carbons was observed which is consistent with our earlier observation that tyrosine group is not involved in the Cu(II) binding to DSA. The chemical shifts of the side chains of the amino acid residues of P_{24} have been assigned by ^{13}C- and ^{1}H-NMR experiments and the Cu(II)-binding studies are currently underway.

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1 D. W. Appleton and B. Sarkar, *J. Biol. Chem., 246*, 5040 (1971).
2 J. W. Dixon and B. Sarkar, *J. Biol. Chem., 249*, 5872 (1974).
3 J. D. Glennon and B. Sarkar, *Biochem. J., 203*, 25 (1982).

N14

New Synthetic Analogs of Heme Proteins

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We have been exploring the chemistry of iron porphyrin complexes as they mimic the O_2 binding,