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

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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 glycyl-glycyl-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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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,

activation and hydrocarbon hydroxylation chemistry associated with hemoglobin, peroxidase, and cytochrome P450. To these ends we have synthesized and characterized a novel 'bis-pocket' porphyrin, meso-tetrakis(2,4,6-triphenyl)phenylporphyrin. This 'bis-pocket' porphyrin offers rigid steric protection on *both* faces of the porphyrin preventing both oxidative degradation and μ-oxo dimerization. The O_2 complexes of our model compound show remarkable thermal stability; reversible oxygenation is observed at temperatures as high as 60 °C. The effects of solvent polarity on CO and O_2 binding show that high polarity favors O_2 binding, but *disfavors* CO binding. Good correlations are found between ΔG° (O_2), ΔG° (CO), and M with empirical solvent polarity scales (*e.g.*, ET-30 or π^*). Thus, a new means of producing O_2/CO discrimination in heme systems is uncovered. These results help explain the discrepancies between other synthetic analogs and the variety of relative CO and O_2 affinities of heme proteins.

In addition, the steric hindrance offered by the 'bis-pocket' porphyrin dramatically increases the oxidative robustness of the iron complex to the presence of a wide variety of oxidants including peracids, hydroperoxides, and iodosoarenes. This provides for the first stable analogs of the high oxidation intermediates of the peroxidases and cytochrome P450. The results of the characterization of the highly oxidized intermediate observed suggest a compound I type intermediate. Studies on the shape selective hydroxylation of hydrocarbon substrates with iron and manganese 'bis-pocket' porphyrin will also be presented.

Other studies on the Mössbauer spectra of iron porphyrin complexes oxidized beyond the Fe(III) state will be related. Specifically, we have investigated the electronic structure of a series of single atom bridged dimers, $(FeTPP)_2X^{n+}$ where X = O, N, or C and TPP = meso-tetraphenylporphyrinato. In the series of complexes which are *two* oxidizing equivalents above Fe(III)–Fe(III) dimers, we find that changing the bridging atom from O to N or C shifts the site of oxidation from the porphyrin *pi* system (as in $(FeTPP)_2O^{2+}$) to the metal, yielding complexes which can be formally viewed as true Fe(IV) dimers [as in $(FeTPP)_2N^+$ and $(FeTPP)_2C$].

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Models for the Photosynthetic Water Oxidizing Enzyme II. Synthesis of Covalently Linked β-Cyclodextrin-Hemin

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The active center of the enzyme responsible for the water splitting reaction of photosystem II is believed to be a binuclear, or possibly a tetranuclear cluster of Mn(III) and Mn(IV) ions [1, 2]. In addition to the manganese cluster, a cytochrome-*b* is believed to play an important role in the oxidation of water by the native enzyme. According to a scheme proposed by Cramer, high-potential cytochrome-*b* 559 may function in a cycle around PSII contributing to water splitting [3]. Moreover, a heme-protein which binds two manganese ions has been isolated from spinach chloroplasts [4, 5].

We reported earlier the synthesis of a Mn(III) dimer of β-cyclodextrin [6]. In this communication we report on our efforts to synthesize a binuclear Mn(III)–β-cyclodextrin complex which is covalently attached to hemin via an ester linkage to the propionic acid side chains.

Experimental

Preparation of the Acid Chloride of Hemin(I). Hemin (1 m*M*, 0.65 g) was reacted with oxalyl chloride (2.5 m*M*, 0.31 g) under Ar at 0 °C. The reaction mixture was stirred for 6 hr and then any excess of oxalyl chloride was removed under vacuum.

Preparation of Hemin derivatized with β-Cyclodextrin(II). The crude product obtained above was dissolved in freshly distilled pyridine (30 mL). β-cyclodextrin (1 m*M*, 1.3 g) was added to this solution and the reaction mixture stirred at room temperature for 10 hr.

Precipitation of product was induced by solvent stripping and cooling. It was washed with chloroform and isolated by column chromatography on CM cellulose. On elution with DMF: $CHCl_3$ (9:1 by volume), two bands are obtained. After solvent stripping the separated products were precipitated by the addition of acetone, and analysis for Fe was carried out. The slow moving fraction gave satisfactory Fe analysis, whereas the fast moving fraction did not show the presence of Fe.

Results and Discussion

The synthetic approach was to convert the propionic acid side chains of hemin to the acid chloride followed by reaction with the primary hydroxyls of β-CD (Scheme I). Oxalyl chloride is an