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

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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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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,