COPPER(II) ION INTERACTION WITH SPERM WHALE METMYOGLOBIN AND MODEL PEPTIDE SYSTEMS

by

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The Interaction of Copper(II) with Sperm Whale Metmyoglobin, Apomyoglobin and Model Peptide Systems

1. A new and simple isolation and purification procedure for myoglobin was described. The method made use of ammonium sulfate fractionation and ion-exchange chromatography. Homogeneous component fractions of sperm whale and harbor seal metmyoglobin were obtained.

2. Phosphate ion interaction and stabilization of myoglobin was studied by denaturing the "native" myoglobin with excess copper(II) ions. One to four moles of phosphate per mole of protein were found to be strongly inhibitory toward copper(II) ion denaturation.

3. A procedure was described for alkylating metmyoglobin with iodoacetamide. The carboxamidomethyl derivative obtained had 6 unmodified histidines, 4 dicarboxamidomethyl histidines and 2 monocarboxamido histidines. No conformational changes were found with the modified metmyoglobin.

4. Previously obtained results were verified that only 7 copper(II) ions could bind to sperm whale metmyoglobin in the presence of twenty-fold excess of copper(II) ions. The carboxamidomethyl metmyoglobin which had 4 dicarboxamidomethyl histidyl residues could bind only 3.2 copper(II) ions per molecule of modified myoglobin.

5. Carboxamidomethylation of acetyl-glycyl-glycyl-L-histidine yielded acetyl-glycyl-glycyl-N^{1}, N^{3}-dicarboxamidomethyl-L-histidine which was incapable of binding copper(II) ions as tested by titration measurements.

6. The characteristic spectral change in the Soret region with binding of copper(II) ions was observed for both unmodified myoglobin and carboxamidomethyl myoglobin. However, this change was not accompanied by the characteristic optical rotatory change at 233 mμ. The modified myoglobin lost some "native" conformation but not nearly as much as the unmodified protein. Conclusions concerning the possible role of the copper(II) ions in promoting these changes are made.

7. The copper(II) coordination to β-alanyl-L-histidine, β-alanyl-L-1-methyl histidine and β-alanyl-L-3-methyl histidine was investigated. Formation and peptide proton ionization constants were determined from titration data. Absorption, optical rotatory dispersion and circular dichroism spectra were determined.

8. β-alanyl-L-histidinato copper(II) and β-alanyl-L-1-methylhistidinato copper(II) complexes gave extremely closely correlated structures. It was concluded that the structures were dimeric in solution and therefore, identical to the crystal structure of β-alanyl-L-histidinato copper(II).

9. β-alanyl-L-3-methylhistidinato copper(II) gave results that closely paralleled those previously obtained on glycyl-L-histidinato copper(II). X-ray diffraction had found the glycyl-L-histidinato copper(II) complex to be monomeric and the same structure was assigned to β-alanyl-L-3-methylhistidinato copper(II).

10. A series of tetra- and pentapeptides were compared with the N-terminal pentapeptide of sperm whale myoglobin. The titration curves in the presence and absence of 1 mole of copper(II) ion per mole of peptide were determined and the appropriate constants calculated.

11. Visible absorption spectra were obtained and conclusions made concerning the nature of the metal ion interaction. Sources of enhanced optical rotatory power were discussed with a comparison of the experimentally derived spectra from optical rotatory dispersion and circular dichroism measurements. The ligand environment around the copper(II) ion was found to be distorted square planar for all the complexes. The α-amino nitrogen and three deprotonated amide nitrogens occupied the four corners of the square plane. The conclusions were consistent with the reported crystal structures of disodium-glycyl-glycyl-glycyl-glycino copper(II) • decahydrate and disodium-glycyl-glycyl-glycyl-glycyl-glycino copper(II) • tetrahydrate.

12. The copper(II) complex of the N-terminal pentapeptide of myoglobin, valyl-leucyl-seryl-glutaminyl-glycine and the acetyl-glycyl-glycyl-histidyl-glycino copper(II) complexes were compared with a 4:1 copper(II)-apomyoglobin complex. Correlations of absorption, optical rotatory dispersion and circular dichroism spectra were made. Strict similarities were not, in general, found. A close parallel between the titration behavior of copper(II)-apomyoglobin complexes and model peptides has been obtained, however, on the assumption that 1 of the 4 bound metal ions is situated at the N-terminal portion of myoglobin. Various facets of the difference observed for the overall comparison are discussed.

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