

Structural analysis of Mavicyanin from *Zucchini*

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Mavicyanin isolated from zucchini peeling is a member of the family known as cupredoxins or blue copper proteins. Proteins in this family are characterized by an intense electronic absorption band near 600 nm which gives them their characteristic blue to green-blue color, and an unusual small hyperfine coupling constants in EPR spectra. The Cu(II) sites in cupredoxins have similar distorted trigonal-pyramidal or tetrahedral geometry in which the Cu atom forms normal bonds to the N atoms of two His residues, and a long bond to the S atom of a Met residue; in the case of a trigonal pyramidal geometry, the fifth ligand is the O atom of a main chain carbonyl group. On the basis of sequence similarity, cupredoxins may be categorized into four main structural groups: 1) plastocyanin-related proteins involving amicyanin and pseudoazurin, 2) azurins, 3) soluble CuA domains derived from cytochrome oxidases, 4) phytocyanins, small blue copper proteins from the nonphotosynthetic part of plants. The amino acid sequence of mavicyanin has recently reported by Scinina et al. The sequence similarities indicate that mavicyanin belongs to the phytocyanins. together with a

cucumber basic protein (CBP), stellacyanin, umecyanin, a cucumber peeling cupredoxin, a putative blue copper protein in pea pods, and a blue copper protein from *Arabidopsis thaliana*. From the crystal structures of CBP and cucumber stellacyanin, the distinctive features of phytocyanins are the presence of a disulfide bridge close to the Cu center. However, the roles of all these phytocyanins are still unknown.

X-ray diffraction data was collected using X-ray radiation at station BL44XU of the SPring-8 synchrotron radiation source. The crystals of mavicyanin diffracted beyond 1.4 Å resolution at the synchrotron radiation source, while diffraction data beyond 1.8 Å resolution could not be collected on a laboratory X-ray source. Because the crystals were oxidized by synchrotron radiation, we tried to use the oxidized crystals. The oxidized crystals obtained under cryo protectants were succeeded to flash-freeze, but less stable in the X-ray beam and showed higher mosaicity in their diffraction. Therefore, we couldn't obtain the diffraction data beyond 1.8 Å resolution.