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### Structure and Function of CueO, a Multicopper Oxidase Concerned in Homeostasis of Copper

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#### Abstract:

CueO is a multicopper oxidase involved in Cu-homeostasis of *E. coli*. CueO has the four catalytic copper binding sites, a type 1 Cu, a type 2 Cu and a pair of type 3 Cu's, in a single chain protein molecule consisting of 484 amino acids. CueO has the fifth Cu-binding site constructed by Met355, Asp360, Met439, Met439 as the substrate-binding site, which is isolated from bulk waters by the Met-rich segment comprised of amino acids 355-400. The high cuprous oxidase activity of CueO is realized by the presence of this fifth Cu-binding site as revealed by the  $K_m$  value and the point mutations for the ligating amino acids. CueO showed enhanced oxidizing activities for organic substrates in the presence of Cu(II) ion at the fifth Cu-binding site because this extra Cu(II) ion functioned as the mediator of electron-transfer between exogenous substrate and type 1 Cu. Further, mutations at Asp112 adjacent to the trinuclear Cu center formed by type 2 Cu and type 3 Cu's indicated that this acidic amino acid functioned not only as a proton donor for dioxygen but also as a modulator for the binding of dioxygen.

**Key words:** Cu-homeostasis, CueO, multicopper oxidase, four-electron reduction of dioxygen, point mutation

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