Isotopic Determination of Copper Exchange in the Preparation of Laccase Isotopomers using ICP-MS

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A variety of metal derivatives of selective blue copper proteins has been utilized in elucidating structure and function relationships in the active sites of these proteins. One such isotopic derivative, (1) of laccase, has been important in understanding the role of the tree copper atoms in the dioxygen reduction (DOR) site. There is evidence in the flexibility of the DOR site from temperature dependent and substrate binding studies that suggest a change in the pattern of antiferromagnetic coupling within the trinuclear cluster (2). Due to the importance of these derivates, particularly the mixed isotope and the type 2-depleted derivatives, an isotope counting method via EPR spectroscopy was used to determine semi-quantitatively the amount of metal exchange occurring from the protein derivative incubated with excess free Cu (I). (3)

The primary objective of this present study is to verify the viability of the laccase derivatives by monitoring the isotopic ratios during their preparation and subsequent modification. The copper ratios were measured both in protein aliquots and the eluents collected during dialyses of the preparations. Any exchange of copper in the protein should show a complementary isotopic exchange in the solution dialyzed from the protein. The 63Cu/65Cu ratios show that exchange with exogenous copper during the preparation of isotopomeric laccase does not compromise the preparation of the derivatives. Studies of the enzyme in the presence of excess Cu(I) as well as time dependent investigations of the incubation times used in the preparations will be discussed.

Also a new spectroscopic copper assay using di-2-pyridyl ketone benzoylhydrazone (dPKBH) developed by Pinto et al. (4) was adapted to the determination of the copper in the laccase derivatives. The adapted method is more sensitive and gives more consistent results than the traditionally used biquinoline assay (5). Another advantage of the assay is that it requires much smaller aliquots of protein sample to analyze for copper.

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