Metal Substitution of *Neurospora* Tyrosinase

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The antiferromagnetically spin coupled copper pair of *Neurospora* tyrosinase [1] was substituted with cobalt and cadmium. Cobalt is bound specifically at the copper-binding site with a stoichiometry of 2 Co(II) per mole protein. The visible and near infrared absorption spectrum of this derivative (ϵ_{526} = 465, ϵ_{564} = 630, ϵ_{607} = 670, ϵ_{635} = 460 (sh), ϵ_{960} = 15 and ϵ_{1180} = 30 M^{-1} cm^{-1}) reveals tetrahedral coordination of the cobalt chromophore. An estimation of the ligand field strength (~5300 cm^{-1}) indicates nitrogen ligation (presumably imidazole side chains) which is in accordance with protein chemical modification data [2]. The low intensity of the Co EPR signal (high spin Co(II)) suggests similar antiferromagnetic coupling of the metal ions as in the native enzyme. Contrary to low molecular weight, binuclear Co-complexes, Co-tyrosinase does not bind molecular oxygen. This finding can be explained by the apparent constraint of the Co-chromophore to tetrahedral coordination in the protein, whereas all binuclear cobalt oxygen complexes are octahedral [3]. Competitive and noncompetitive tyrosinase inhibitors (benzoic acid, L-mimosine and KCN) give rise to characteristic perturbations of the absorption spectrum of Co-tyrosinase. Thse spectral changes are taken as evidence for a direct interaction of the inhibitors with the metal site.

Cadmium substitution of native tyrosinase leads to an enzymatically inactive derivative containing only one Cd(II) per mole protein. A Cu/Cd hybrid form is obtained upon addition of stoichiometric amounts of Cu(II); excess copper expells the cadmium from the hybrid yielding native tyrosinase.

References

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Iron and Copper in Cytochrome Oxidase: Spectral and Kinetic Studies

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Cytochrome oxidase from eukariots is a metalloprotein, integral to the mitochondrial membrane, which contains copper and iron. The basic functional unit of cytochrome oxidase contains four metals, two copper and two iron atoms, which are bound to the seven polypeptide chains in an unknown manner. Structural, spectroscopic and kinetic studies have revealed complex relationships between the metals, on the basis of which they have been assigned distinct functional roles.

The protein(s)–metal(s) complex can be looked upon as an asymmetric unit, which serves the function of coupling a one-electron donor, cytochrome c, with a four electron acceptor, dioxygen. The iron-porphyrin complex of cytochrome *a* is low-spin heme which serves the purpose of 'electron-gate' into oxidase. Electron flow involves as a second site one of the copper atoms (Cu_A), which has been characterized by optical absorption, EPR and kinetics. The other two metals (cytochrome a_3 and Cu_B) form a coupled pair, which in the reduced ($Fe^{+2}_{a_3}Cu^{+}_{B}$) state binds dioxygen, to which electrons are being transferred through a series of spectrally characterized intermediates. The whole system can be looked upon as a condenser, in which the four metals experience different environments and perform different functions, with remarkable specialization and efficiency.

Structural and Functional Aspects of Copper Transporting Proteins

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The biogenic nature and esssentiality of copper in biological systems is well known. Due to both its oxidation–reduction activity and the intriguing coordination chemistry, copper is most suited to play a vital role in cellular biochemistry. Hydrated copper ions themselves are highly reactive and would be in a position to interfere in many a biochemical pathway. For example, the Fenton type generation of •OH radicals would destroy the cellular architecture in an uncontrolled manner. Unwanted and non-specific binding of ionic copper to functional and/or structurally important biopolymers must be considered hazardous for living systems. In electron transport and oxygen reduction the specific reaction of the bound copper is dictated by the macromolecular protein portion [1, 2]. While some progress has been made on the structure-function correlation of some of these copper proteins little is known how the metal is implanted into these functionally important copper proteins.