Models for Hemocyanin and Tyrosinase

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A problem encountered in the study of biological systems is that detailed investigations are often hampered by the lack of a high resolution crystal structure. Proteins and enzymes are often exceedingly difficult to crystallize due to their large size and complex structure. This situation is certainly the case with the oxygen-carrying protein hemocyanin (Hc) and the mono-oxygenase tyrosinase (Tyr).

Hemocyanin (1) serves as the oxygen carrier in the hemolymph of arthropods (crabs, lobsters, etc.) and molluscs (snails, clams, octopus, etc.). It is well known that the active site of Hc consists of a binuclear copper center that binds oxygen reversibly [1]. The oxygen-coordinated form of the Hc(oxyHc) and its derivatives have been studied extensively [2]. The present active site picture for oxyHc and metHc (figure 1) has arisen from the combination of resonance Raman [3], Extended X-ray Absorption Fine Structure (EXAFS) [4], magnetic susceptibility [5], and electronic absorption [6] studies. The salient features of oxyHc are the 1,2-μ-peroxo bridge and the endogenous bridge which is believed to mediate the strong antiferromagnetic coupling.

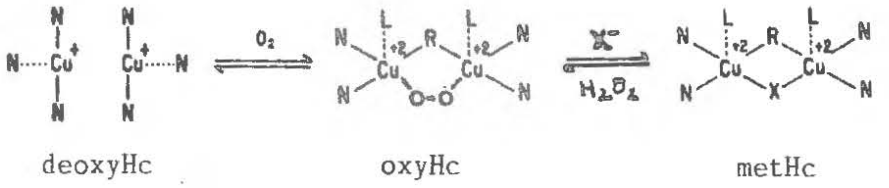

Figure 1. Possible active site picture for hemocyanin.

There is less information available on the active site of deoxyHc because the d^{10} Cu(I) center is not amenable to many spectroscopic techniques. EXAFS results suggest that each copper center is bound to two [6] or three [4b] imidazole-like ligands. A recent crystal structure of Panulirus interruptus deoxyHc shows that each Cu(I) ion is separated by a distance of 3.8 ± 0.4Å with three histidine ligands coordinated to each copper [8] (figure 1).

Tyrosinase [9] serves as both a mono-oxygenase and a two electron oxidase in various microorganisms, plants, and animals. It catalyzes the o-hydroxylation of monophenols to catechols (mono-oxygenase activity) and the subsequent oxidation of catechols to o-quinones (oxidase activity). Although Tyr has a different biological function than Hc, comparison of the spectroscopic and chemical properties of these two metalloproteins indicate that the binuclear copper active sites are very similar [10].

Some current model studies have focused on mimicking the magnetic properties of Hc and Tyr. In order to identify the endogenous bridging ligand, Reed and coworkers have utilized an alkoxytetrakis(imidazole) ligand to prepare a diamagnetic 1,3 μ-azido bridged dicopper(II) complex with a Cu-Cu separation of 3.615Å. This compound features an "endogenous" alkoxide bridge which is believed to provide the superexchange pathway [11]. Sorrell and coworkers have

synthesized an analogous 1,3 μ-azido bridged Cu(II) binuclear complex that is diamagnetic and features an "endogenous" phenoxide bridge [12]. The hydroxide ion is also a viable candidate for the "endogenous" bridge [13].

It is known that CO binds to deoxyHc in a terminal fashion with a binding ratio of 1CO/2Cu(I) [14]. Model studies indicate that two coordinate complexes are relatively inert towards CO, whereas related three coordinate complexes readily bind CO [15]. These model studies suggest a possible explanation for the CO binding ratio of deoxyHc [14].

The oxygen binding properties of Tyr and Hc can be modelled by Cu(I) complexes of the binucleating ligand m-XYLpy2 (shown below) [16]. When exposed to oxygen, the aromatic ring of the dicopper(I) complex becomes hydroxylated. A phenoxo and hydroxo Cu(II) binuclear compound forms. Labeling studies show that the phenoxo and hydroxo oxygens are derived from the labeled oxygens [16]. The Cu(II) ions can be leached out of the phenoxo bridged dicopper(II) species to give the free phenol. Reaction of the free phenol with Cu(I) results in the formation of a phenoxo bridged dicopper(I) complex [17]. This complex has been shown to reversibly bind oxygen by visible spectroscopy.

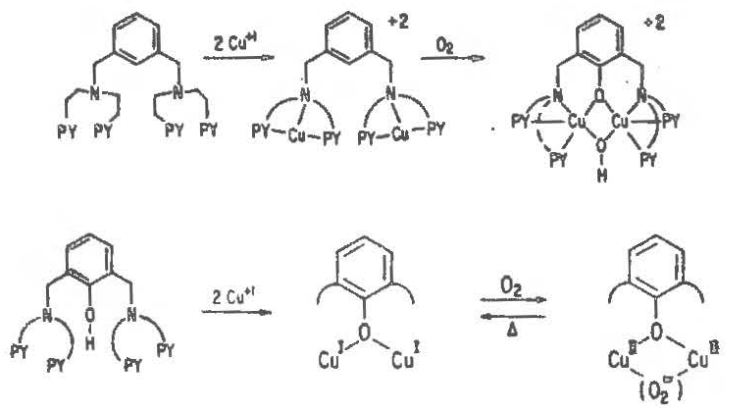

Figure 2. Reaction scheme for m-XYLpy2 as described above.

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