

EXAFS Studies of Mixed-Metal Hybrid Hemoglobins

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Mixed-metal hybrid hemoglobins (Hbs), in which one or more of the heme irons has been replaced by another metal, have been used extensively as models of partially oxygenated intermediates of the native all-iron tetrameric Hb, and are instrumental in the identification of the third quaternary state in the cooperative oxygen binding pathway of Hb (Ackers *et al.* 1992. *Science* 255:54-63). However, the observation that the energetics of association of (metal 1-containing) and (metal 2-containing) dimers into tetramers vary widely among different metals (Huang *et al.* 1996. *Proc Natl Acad Sci USA* 93:4425-4430) has led to the suggestion that metal-substitution can alter interactions at the subunit interface where quaternary structural changes are triggered that eventually modify the ligand affinity of the metals (cooperativity); as a result, mixed-metal hybrid Hbs follow qualitatively, but not quantitatively, the cooperative ligand binding pathway of the native all-iron tetramer. To provide structural evidence for the effect of metal-substitution on tetramer assembly and function, EXAFS studies were carried out on four oxygenated tetrameric Hbs: It was found that metal-axial ligand bond lengths are shorter in the a Fe subunits in (a Fe-O₂)₂(bCo-O₂)₂ Hb than the Fe subunits in (aCo-O₂)₂(bFe-O₂)₂ Hb, the subunit differences are analogous but their magnitude smaller than those revealed by the x-ray. These results are the first structural demonstration that metal-substitution and hybrid formation alter metal-ligand bond lengths and provide an explanation for the differences in free energies of ligand binding and cooperativity between mixed-metal hybrids and the homometal as well as all-iron tetramers.