Mechanism of Iron Release from the Ferric Binding Protein of *Neisseria gonorrhoeae*

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The ferric binding protein (FBP), a periplasmic iron-binding protein in several gram-negative bacteria such as *Haemophilus influenzae* and *Neisseria gonorrhoea*, functions in mediating iron uptake from their host transferrin into the bacterial cytoplasm. FBP is structurally homologous to one lobe of transferrin, and both proteins bind iron via two tyrosines, a histidine and a carboxylate amino acid side chain. However, the bidentate synergistic carbonate anion of transferrin is replaced by a monodentate phosphate and a coordinated water molecule in FBP.

Several ligands have been used to investigate iron release from FBP, and two types of kinetic behavior have been observed. Iron release by acetohydroxamic acid (AHA), phosphonoacetic acid (PAA), ethylenediaminetetraacetic acid (EDTA) and oxalate follows first-order kinetics with respect to the ligand concentration, while iron release by nitrilotriacetic acid follows saturation kinetics. The addition of AHA to Fe-PO_4-FBP results in the rapid formation of a spectroscopically distinct intermediate, followed by the release of iron from the protein. The current hypothesis is that an AHA molecule rapidly replaces the phosphate and water ligands to form an Fe-AHA-FBP intermediate, which reacts with a second molecule of AHA to release the iron. This second, slower step is responsible for the first-order dependence on the ligand concentration. It is proposed that PAA, edta, and oxalate follow this mechanism.

In the case of iron release by NTA, it is proposed that the initial Fe-NTA(bidentate)-FBP intermediate rapidly rearranges to the Fe-NTA(tetradentate)-FBP complex that has been structurally characterized by Zhu *et al.* (*Biochem. J.* 2003, 376:35). Iron release from this complex appears to be associated with a slow conformational change in the protein, which gives rise to the saturation kinetics with respect to the NTA concentration. It also appears that the initial Fe-PO_4-FBP complex may be in equilibrium with a phosphate-free, binary Fe-FBP species. The rapid reaction of this binary species with added ligands complicates the analysis of iron release from FBP.