The Relationship between Metal Ligation and Protein Folding. Iron Uptake by Rubredoxins

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Rubredoxins (Rds) are small (MW ~ 6,000) bacterial and archaeal proteins containing a single $[Fe(SCys)_4]$ site. Rds display a wide range of structural and metal site stability towards chemical agents, and Rds with mesophilic and hyperthermophilic stabilities are known. Incorporation of metals other than iron occurs when overexpressing Rds in *E. coli*. Non-denaturing displacement of Fe(II) by other divalent metals in Rds in vitro was also reported. Stability of the metal ligation was found to be unrelated to the thermal stability of the polypeptide. Rds is, thus, an ideal prototype for investigating protein determinants of metal-binding specificity and the relationship between metal ligation and protein folding.

Steady-state and stopped flow circular dichroism spectroscopy were used to investigate the equilibria and kinetics of iron uptake by various wild-type and mutated apoRds both in their partially structured and fully denatured forms. Whereas mesophilic apoRds could be completely unfolded at 3 M urea, hyperthermophilic apoRds became unfolded only at high concentration of guanidine-HCl. All the chaotrope-dependent structural changes in apo-Rds were fully reversible.

Iron-uptake studies carried out in the absence of denaturants indicated that apoRds having the most flexible structure (i.e., those most sensitive to denaturants) ave the highest yield of native $[Fe(SCys)_4]$ site. Formation of the native $[Fe(SCys)_4]$ site and protein structure was greatly increased when iron was added to fully denatured apoRds prior to denaturant dilution.

Using Ser variants of individual Cys ligand residues, we found Cys6, which is buried in the native structure, to be absolutely required for iron uptake by the unfolded apoRd, whereas the solvent-exposed Cys9 is not required for iron uptake or protein folding; iron addition to the C9S apoRd leads to an $[Fe(SCys)_3OSer]$ site. These results indicate that: 1) residual structure in the apoRd impairs formation of the native $[Fe(SCys)_4]$ site and protein structure.; 2) major structural rearrangements of the polypeptide occur after iron binding; 3) initial iron binding and formation of the $[Fe(SCys)_4]$ site "drive" the protein folding process; 4) buried residues are more important than solvent-exposed residues for formation of the native metal site and protein structure.

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