Abstract for Masha G. Savelieff

Experimental evidence for a link among cupredoxins: red, blue and purple copper transformations in nitrous oxide reductase

Abstract: The cupredoxin fold is an important motif in numerous proteins which are central to several critical cellular processes ranging from aerobic and anaerobic respiration to catalysis and iron homeostasis. Three types of copper sites have been found to date within cupredoxin folds: blue type 1 (T1) copper, red type 2 (T2) copper, and purple Cu_A. Even though as much as 90 % sequence difference has been observed among some members of this superfamily of proteins that span several kingdoms, sequence alignment and phylogenic trees strongly suggest an evolutionary link and common ancestry. However, experimental evidence for such a link is lacking. We report herein the observation of pH dependent transformation between blue T1 copper, red T2 copper center and the *native* purple Cu_A center of N_2OR from *Paracoccus denitrificans* (Pd). The blue and red copper centers form initially before they are transformed into purple Cu_A center. This transformation process is pH dependent, with lower pH resulting in less trapped T1 and T2 coppers and faster transition to purple Cu_A. These observations suggest that the purple Cu_A site contains the essential elements of a T1 and T2 copper centers and that the Cu_A center is preferentially formed at low pH. Therefore this work provides an underlying link between the various cupredoxin copper sites, and possible experimental evidence *in vitro* for the evolutionary relationship between the cupredoxin proteins. The findings also lend physiological relevance to cupredoxin site biosynthesis.