CAPSULES notes and applications from BASi

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The Effect of P80 Mutants on the Redox Potential of Pseudoazurin

Purpose

Although the copper sites of blue copper proteins are similar, the redox potentials of these proteins span a range of several hundred millivolts. The redox potential is sensitive to many factors, including the degree of interaction between the copper and the axial ligand, electrostatic interactions between non-coordinated amino acids and the copper center, the hydrophobicity and solvent accessibility of the copper site, and the geometry imposed on the copper by the protein. In this study, the effect of changing the proline-80 residue of pseudoazurin to alanine and isoleucine on the redox potential was investigated and rationalized using molecular structures (from X-ray crystallography) and electrostatic calculations.

Reference

Site-Directed Mutants of Pseudoazurin: Explanation of Increased Redox Potentials from X-ray Structures and from Calculation of Redox Potential Differences, C.A.P. Libeu, M. Kukimoto, M. Nishiyama, S. Horinouchi, and E.T. Adman, Biochemistry 36 (1997) 13160-13179.

Method

Redox potentials were determined by redox titration. Once equilibration at a given potential had been attained, the concentrations of the oxidized and reduced species were measured using UV/vis spectroscopy. The redox potential was extracted from the Nernst plot of the log of the concentration ratio vs. potential (see Capsule 349 for more details).

Results

It has been shown that the geometry of the copper site of a given blue copper protein is essentially the same, both in the absence and presence (in both oxidation states) of the coordinated copper (1, 2); that is, the coordination geometry is imposed on the copper center by the protein (3). Since the protein structure is determined by electrostatic and hydrogen bonding interactions between the residues, mutations of amino acids that are involved in these interactions can alter the redox potential by changing the protein geometry in the active site.

One difference between pseudoazurin and azurin is the presence in pseudoazurin of a proline residue adjacent to a coordinating histidine residue. The bulky proline side chain prevents the formation of a hydrogen bond between the amide group of this residue and the cysteine sulfur that is found in azurin. In this study, the influence of the proline residue on the redox potential was examined by changing this residue to alanine or isoleucine, based on the premise that formation of this hydrogen bond should be feasible in these P80A and P80I mutants. In addition, the molecular structures of the oxidized and reduced forms of both mutants and the wild type protein were determined using X-ray crystallography in order to correlate structural changes in the mutants with the variations in the redox potentials.

The redox potentials of the two mutants were +409 mV (vs. NHE) (for P80A) and +450 mV (for P80I), both of which show a considerable increase relative to the wild type protein (+330 mV). These changes were attributed to two factors. First, the vacancy in the P80A mutant due to the substitution of proline by the less bulky alanine allowed the incorporation of an additional water molecule at a position where it could affect the solvation of the copper center, and hence alter the redox potential. This water molecule was not present in the P80I mutant, due to the larger size of the isoleucine side chain. Second, the geometry of the copper site was more flexible in the mutants than in the wild type proteins. This allowed a more trigonal copper coordination geometry to be adopted in the reduced form, which increased the favorability of this oxidation state.

References:

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2. *E. Vakoufari, K.S. Wilson, and K. Petratos, FEBS Lett. 347 (1994) 203-206.*
3. *G. Malmstrom, Eur. J. Biochem. 23 (1994) 711-718.*