CAPSULES notes and applications from BASi

354

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The Effects of Mutation on the Redox Potential of Clostridium pasteurianum Rubredoxin

Purpose

The redox potential of electron transfer metalloproteins can be influenced by a number of factors, including electrostatic and hydrogen bonding interactions between the active site and the protein, as well as the hydrophobicity and solvent accessibility of the active site. In this study, the effect of mutations (changing amino acids at one or more sites) on the redox potential of *C. pasteurianum* rubredoxin was study using cyclic voltammetry.

Reference

Protein Determinants of Metal Site Reduction Potentials: Site-Directed Mutagenesis Studies of Clostridium pasteurianum Rubredoxin, Q. Zeng, E.T. Smith, D.M. Kurtz, and R.A. Scott, Inorg. Chim. Acta 242 (1996) 245-251.

Method

Direct electrochemistry of proteins using cyclic voltammetry typically requires the use of modified electrodes in order to bind the protein to the electrode surface in an orientation suitable for electron transfer. In this example, an edge-plane pyrolytic graphite electrode was used. The surface of this electrode has negatively charged functional groups, which can form a ternary complex with the negatively charged proteins in the presence of multivalent cations (e.g., Mg^{2+}).

Results

The active site of rubredoxins is an Fe(III) center coordinated to four cysteine residues in a pseudo-tetrahedral arrangement (at positions 6, 9, 39, and 42). The following amino acids were selected for mutation based on their proximity to the active site: T5, T7, V8, and L41 (1), and the mutations involved substitution of these neutral amino acids with charged amino acids, D (negatively charged) and R (positively charged). None of the mutations at these sites caused any major changes in the protein conformation or the structure of the active site (as shown by the invariance of various spectroscopic parameters).

The redox potentials of the wild type protein and the various mutations are shown in ***T1***. From simple electrostatic considerations, it would be predicted that the incorporation of a positively charged residue would stabilize the lower oxidation state (iron(II)), since the active site has a larger negative charge in this state (-2 vs. -1 for the iron(III) state), thereby leading to a positive shift in the redox potential. Examination of the potential shifts caused by introducing R are consistent with this argument. The V8R and L41R mutants show significant positive shifts in the redox potential, with essentially no shift detected for the T5R, T7R mutant (T5 and T7 are further from the active site (10-11 Å) than V8 and L41 (8.5 and 7.4 Å, respectively), so any electrostatic effects due to changes at T5 and T7 would be expected to be smaller).

Using the same argument, incorporation of negatively charged residue would be expected to stabilize the higher oxidation state, leading to a negative shift in the redox potential. However, the redox potentials of V8D and L41D mutants are again more positive than that of the wild type protein. These values show that there are factors other than simple electrostatic interactions that affect the redox potential.

One factor that is commonly used to rationalize variations in the redox potentials of electron transfer proteins is solvent accessibility to the active site. Increased solvent accessibility (and the concomitant increase in the polarity of the environment of the active site) should stabilize the more highly charged oxidation state, which is the reduced state in this example. The stabilization of the reduced species by the mutations at positions 8 and 41 is therefore consistent with increased solvent accessibility in these mutants. Since T5 and T7 are further from the active site, mutations at these positions should have less effect on solvent accessibility.

Rubredoxin	E^o (mV, vs. NHE)	Distance of charged group from iron center (Å)
Wild type	-55	
T5R, T7R	-50	10.0, 10.9
V8R	-15	8.5
L41R	+3	7.4
V8R, L41R	+30	8.5, 7.4
V8D	-28	4.7, 6.0
L41D	-23	5.5, 6.5

Table 1. *Redox Potentials of Wild Type and Mutant C. pasteurianum Rubredoxins and Distance of Mutated Charged Groups from Iron Center.*

1. Abbreviations: D = aspartate, L = leucine, R = arginine, T = threonine, V = valine.