CAPSULES notes and applications from BASi

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Variation of the Axial Ligand with Oxidation State for the Phenylalanine-82-Histidine Mutant of Yeast Iso-1-cytochrome c

Purpose

Specific site mutagenesis is widely used to determine the effect of a given amino acid residue on the properties of proteins (e.g., redox potential, rate of electron transfer). In this study, substitution of phenylalanine by histidine at position 82 of yeast iso-1-cytochrome c provided an alternative ligand for axial coordination to the iron center of the cytochrome. It was shown using cyclic voltammetry that the axial coordination varied with the iron oxidation state.

Reference

Direct Voltammetric Observation of Redox Driven Changes in Axial Coordination and Intramolecular Rearrangement of the Phenyl-82-Histidine Variant of Yeast Iso-1-cytochrome c, B.A. Feinberg, X. Liu, M.D. Ryan, A. Schejter, C. Zhang, and E. Margoliash, Biochemistry 37 (1998) 13091-13101.

Method

Cyclic voltammetry is a very useful method for the qualitative characterization of chemical reactions coupled with electron transfer reaction, since the reactive intermediate can be generated on the forward scan, and its fate can be examined on the reverse and subsequent scans. Extraction of quantitative data typically requires simulation software (e.g., DigiSim®).

Results

Axial coordination of the iron center in cytochrome *c* is provided by histidine-18 (His-18) and methionine-80 (Met-80). Substitution of phenylalanine by histidine at position 82 (Phe82His) provides an alternative axial ligand. Since the iron (III) oxidation state favors histidine, and the iron (II) oxidation state favors methionine, changing between the two oxidation states should be accompanied by a change in the axial ligand (His-82 ↔ Met-80).

Square wave voltammograms of the wild type protein and the Phe82His mutant were recorded using a gold disk electrode (3 mm diameter) modified with bis(4-pyridyl) disulfide (in order to facilitate the electron transfer reaction). The redox potential of the wild type protein was +269 mV (vs. NHE). However, two peaks (at +229 mV and -148 mV) were observed for the Phe82His mutant (**F1**), which is consistent with two possible axial coordination ligands (Met-80 and His-82). The relative magnitudes of the peak currents depended upon the starting potential (note that the potential was held at the starting value before the potential scan). If the initial potential was -400 mV (i.e., a net oxidation during the potential scan), the peak at +229 mV was larger, whereas the peak at -148 mV was larger if the initial potential was +500 mV. Considering the known axial ligand preferences of the two iron oxidation states (iron(II) favors methionine, and iron(III) favors histidine), the peak at +229 mV is associated with Met-80 coordination, and the peak at -148 mV is associated with His-80 coordination. Similar behavior was seen for the wild type protein in the presence of imidazole (due to displacement of Met-80 by imidazole in the oxidized form).

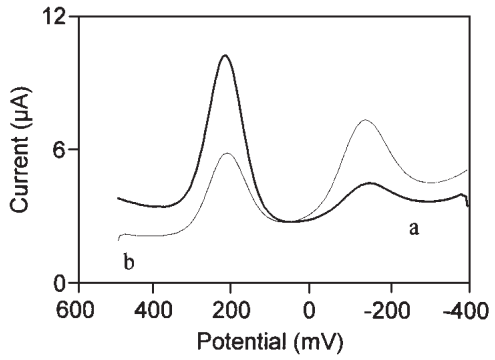

Figure 1. *Square-wave voltammograms of Phe82His mutant (1 mM) in pH 7.0 buffer, 100 mM NaCl. a) Positive scan from 400 mV to +500 mV, b) negative scan from +500 mV to 400 mV. Square wave amplitude = 25 mV, square wave frequency = 15 Hz, step potential = 4 mV, 2 s holding time before starting scan. All potentials relative to the NHE. Reprinted with permission from primary Reference. Copyright 1998 American Chemical Society.*

The variation in axial coordination with oxidation state was also shown using cyclic voltammetry (**F2**). At the slowest scan rate (10 mV s^{-1}, **F2a**), two reduction

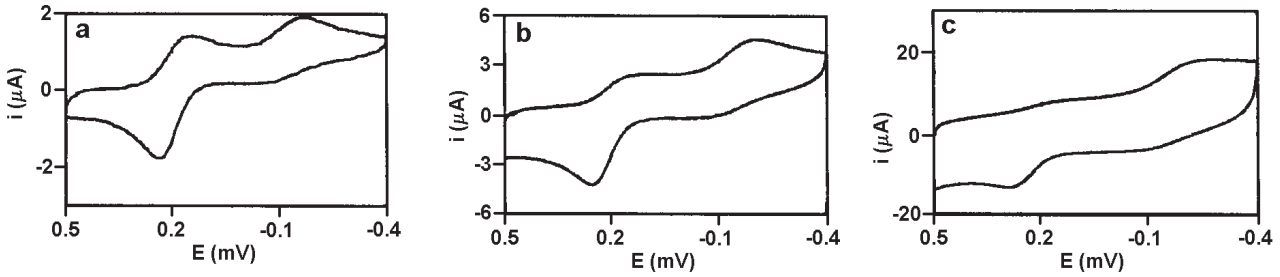

Figure 2. *Cyclic voltammograms of Phe82His mutant (1 mM) in pH 7.0 buffer, 100 mM NaCl. Scan rate = 10 (a), 100 (b), and 500 mV s^{-1} (c). All potentials relative to the NHE. Reprinted with permission from primary Reference. Copyright 1998 American Chemical Society.*

peaks are present on the forward scan, and there is one oxidation peak on the reverse scan. As the scan rate is increased, the current of the reduction peak at the more positive potential decreases. This behavior is consistent with an equilibrium between two species, with the thermodynamically favored species having the more negative reduction potential. In this case, the two species are iron(III) cytochrome with axial Met-80 (Met_{80}-Fe^{3+}) and iron(III) cytochrome with axial His-82 (His_{82}-Fe^{3+}), with the latter being the more thermodynamically stable of the two. The relative magnitudes of the two peak currents depends upon the rate of the isomerization reaction relative to the scan rate. If the scan rate is fast relative to the rate of the His_{82}-Fe^{3+} $\rightarrow$ Met_{80}-Fe^{3+} reaction, the isomerization reaction has little effect, and the peak currents are determined by the equilibrium constant (i.e., the His_{82}-Fe^{3+} reduction peak is larger, as is shown at a scan rate of 500 mV s^{-1} in **F2c**). However, at slower scan rates (**F2a** and **F2b**), the His_{82}-Fe^{3+} $\rightarrow$ Met_{80}-Fe^{3+} reaction can occur during the potential scan. Since Met_{80}-Fe^{3+} is reduced first, the perturbation of the equilibrium caused by the reduction leads to the net conversion of His_{82}-Fe^{3+} to Met_{80}-Fe^{3+}, which is reflected in an increase of the current of Met_{80}-Fe^{3+} reduction peak with decreasing scan rate.

Since Met-80 coordination is favored in the iron(II) oxidation state, reduction of His_{82}-Fe^{3+} to His_{82}-Fe^{2+} is followed conversion of the latter to Met_{80}-Fe^{2+}. The rate of this reaction is fast relative to the scan rate, even at a scan rate of 500 mV s^{-1}, since there is no peak on the reverse scan due to the reoxidation of Met_{80}-Fe^{2+}. The variation of the peak currents on both the forward and reverse scans is consistent with a square scheme, which would be expected for a system with two oxidation states, and two isomers.

In order to extract quantitative data from these voltammograms using DigiSim, approximations were made for the redox potentials of the two isomers. The redox potential of the Met_{80}-Fe isomer was approximated using the redox potential reported for the Phe82Ser mutant (1) (+247 mV), and the redox potential of the His_{82}-Fe isomer was approximated using the redox potential of the Met80His mutant (2) (+47 mV). Based on these approximations, it was possible to calculate the equilibrium constant and rate constants for the interconversion of the iron(III) isomers, as well as the rate constant for the electron transfer reaction of the Met-80 species. However, since there was no peak on the reverse scan for the oxidation of His_{82}-Fe^{2+}, it was not possible to calculate values for the rate constants for the interconversion of the iron (II) isomers. Therefore, since the peak potential for the reduction of His_{82}-Fe^{3+} is affected by these rate constants, unique values for other parameters that also affect this peak (the equilibrium constant for the interconversion of the iron (II) isomers, and the rate constant for the electron transfer reaction of the His-80 species) could not be calculated.

DigiSim is a registered trademark of Bioanalytical Systems, Inc.

References

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2. *A.L. Raphael and H.B. Gray, J. Am. Chem. Soc. 113 (1991) 1038.*