CAPSULES notes and applications from BASi

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Correlation between the Redox Potentials of Anabaena Ferredoxin and Rates of Electron Transfer between Anabaena Ferredoxin and Ferredoxin:NADP+ Reductase

Purpose

Many physiological processes (e.g., respiration, photosynthesis) involve the transfer of electrons between proteins, or between different locations in the same protein. The rate of interprotein electron transfer can be affected by a variety of different factors, including the thermodynamic driving force for the transfer (which is controlled by the redox potentials of the two sites), the relative orientation of the redox centers, and the structure of the medium between the two centers (which is controlled by the structure of the two proteins, as well as the structure of the complex formed by the two proteins prior to the electron transfer). In this study, the molecular structures and redox potentials of wild type and mutant *Anabaena* vegetative cell [2Fe-2S] ferredoxins were studied to see if there was any correlation between these properties and the rate of electron transfer between these proteins and the ferredoxin:$NADP^+$ reductase (FNR) protein (the function of the FNR protein *in vivo* is to reduce $NADP^+$ to NADPH using the electrons it receives from ferrodoxins).

Reference

Structure-Function Relationships in Anabaena Ferredoxin: Correlations between X-ray Crystal Structures, Reduction Potentials, and Rate Constants of Electron Transfer to Ferredoxin:$NADP^+$ Reductase for Site-Specific Ferredoxins Mutants, J.K. Hurley, A.M. Weber-Main, M.T. Stankovich, M.W. Benning, J.B. Thoden, J.L. Vanhooke, H.M. Holden, Y.K. Chae, B. Xia, H. Cheng, J.L. Markley, M. Martinez-Julvez, C. Gomez-Moreno, J.L. Schmeits, and G. Tollin, Biochemistry 36 (1997) 11100-11117.

Method

Redox potentials were determined using potentiometric titrations. The concentrations of the oxidized and reduced species were determined at each potential using a UV/visible spectrometer, and these data were used to construct a Nernst plot. The redox potential and the number of electron transferred were calculated from the intercept and the slope, respectively (see Capsule 349 for more details).

Results

Site-specific mutagenesis is used to change individual amino acids in proteins in order to examine the effects of these amino acids on various protein properties. In this study, amino acids that were thought to be important in the formation of the complex between the ferredoxin and the FNR protein were changed. These included acidic surface residues (D62, D68, E94, E95 (1)) that could interact with basic residues on the surface of the FNR protein, as well as Q70, F65, and S47.

The redox potentials and electron transfer rate constants for the wild type ferredoxin and various mutants are shown in ***T1***. Looking first at the rate of electron transfer, it can be seen that the mutants can be divided into two groups; those with rate constants similar to those of the wild type protein, and those with rate constants that are some orders of magnitude lower. Changing the acid residues D62, D68 , and E95 to a basic residue has little effect, whereas changing E94 to either a basic or a neutral residue decreases the electron transfer activity. However, changing E94 to another acidic residue (D) has little effect. Changing F65 to another neutral aromatic residue has little effect, whereas changing it to a neutral aliphatic residue (A or I) also decreases the rate of electron transfer. Changing S47 to a different residue with a hydroxyl group (T) has little effect. In contrast, removing the hydroxyl group again decreases the rate of electron constant. Therefore, it seems that the following are required for rapid electron transfer:

—an acidic residue at 94
—an aromatic residue at 65
—a hydroxyl side chain at 47

One possible explanation for the loss of electron transfer activity is that the protein structure is distorted by the mutations, and hence cannot form the complex with FNR that is required for electron transfer.

However, no major structural changes near the active site could be observed the molecular structures derived from X-ray crystallography (although there were small changes in the E94K and S47A mutants that were attributed to the loss of the hydrogen bond between E94 and S47).

It can also be seen from ***T1*** that those mutants that have low electron transfer activity have redox potentials more positive than the wild type, whereas the mutants electron transfer activity similar to that of the wild type have redox potentials similar to that of the wild type; that is, there appears to be a correlation between redox potential and rate of electron transfer. However, this is an oversimplified view, since we have to consider the redox potential of the ferredoxin relative to that of the FNR protein. Furthermore, we have to consider the values of the two active sites within the protein complex that must be formed prior to electron transfer. Measurement of the redox potentials of the protein:protein complex showed that the redox potential of the FAD active site of the FNR protein was more positive than or equal to the redox potential of the [2Fe-2S] active site of the ferredoxin; that is, the electron transfer reaction within the complex is thermodynamically favorable. Therefore, although the mutations may affect both the redox potential and the rate of electron transfer, the decrease in the rate of electron transfer is not due to the change in the redox potential. The decreased electron transfer was attributed to subtle changes in protein (e.g, removal of the hydrogen bond between E94 and S47) led to the formation of a complex not suitable for electron transfer, or interference with the path required for electron transfer (e.g., removal of the aromatic residue at 65 can disrupt p-p interactions with the FAD active site).

1. Abbreviations: A = alanine, D = aspartate, E = glutamate, F = phenylalanine, I = isoleucine, K = lysine, Q = glutamine, S = serine, T = threonine, Y = tyrosine

Ferredoxin	k x 10^{-8} ($M^{-1}s^{-1}$)	E^o (mV, vs. NHE)
Wild type	1.2	-384
D62K	1.0	-373
D68K	1.9	-380
Q70K	1.0	-382
E94D	1.3	-367
E95K	1.2	-372
F65Y	1.3	-390
E94K	<0.0001	-304
E94Q	<0.0001	-319
F65A	<0.0001	-291
F65I	<0.0001	-328
S47A	<0.005	-337
S47T	0.9	-438

Table 1. *Rate constants for Oxidation of Reduced Vegetative Cell Ferredoxin by Oxidized FNR and Redox Potentials of Mutant and Wild Type Ferredoxin (adapted from Reference).*