Supporting Information

Human Ferredoxin-2 Displays a Unique Conformational Change

Wenbin Qi \textsuperscript{a}, Jingwei Li \textsuperscript{b} and J. A. Cowan\textsuperscript{a,b,*}

\textsuperscript{a} Ohio State Biochemistry Program, the Ohio State University, Columbus, OH 43210
\textsuperscript{b} Department of Chemistry and Biochemistry, the Ohio State University, Columbus, OH 43210
\textsuperscript{*}Corresponding author; email: cowan@chemistry.ohio-state.edu

Experimental Methods

Cloning and Expression of human ferredoxin-2

A 100 ng sample of DNA, 100 ng of each primer, 2.5 units of cloned \textit{Pfu} Turbo DNA polymerase, \textit{Pfu}Turbo buffer, and 0.2 mM of each dNTP were used to amplify \textit{Hs} Ferredoxin-2 (Q6P4F2 residues 53-183) via PCR. Primers were designed as follows: 5’-ttatcatatatggctggagaggacgcg-3’ and 5’-taagctcagtcattggggtgggac-3’, where the underlined regions denote the NdeI and XhoI restriction sites, respectively. The thermocycle used was identical to that described in the \textit{Pfu}Turbo DNA polymerase manual (Stratagene). PCR products were digested with NdeI and XhoI and ligated to double-digested vector pET28-b(+) by T4 DNA ligase. Cloning into pET28-b(+) resulted in the production of the gene for N-terminal His-tagged \textit{Hs} Fd2. Cloning results were confirmed by nucleotide sequencing, and \textit{E. coli} BL21(DE3) was used for protein expression. A 10 ml Luria-Bertani (LB) culture with 30 mg/L kanamycin was grown
overnight as a starter culture, which was then used to inoculate 1 L LB medium and
grown to an OD$_{600}$ of ~ 0.6 prior to induction with 0.3 mM isopropyl-1-thio-α-D-
galactopyranoside (IPTG). Cells were pelleted 5 hours after induction and stored at –80
°C for future use.

**Purification of hFd2**

Cell pellets were resuspended in 20 mM Tris, 500 mM NaCl, 5 mM imidazole, pH = 8.0
and lysed by sonication. Cell debris was removed by centrifugation at 4 C at 15,000 rpm
for 30 min. The supernatant was then loaded onto a Ni-NTA column and eluted by
standard methods, and further purified by gel filtration chromatography using a
Superose12 column equilibrated with 50 mM Hepes, 100 mM NaCl, pH = 7.5. Eluted
proteins were analyzed by SDS-PAGE and concentrated by Amicon ultrafiltration.

**Purification of hFd1**

The expression vector for *Hs* Fd1 was kindly provided by J. Markley and protein was
expressed and purified according to literature procedures (Xia, B., Cheng, H., Bandarian,

**hFd1 and hFd2 cluster absorbance change monitored by UV/Vis spectroscopy**

A UV/Vis spectrum of 100 µL 80 µM hF1 or hFd2 was taken using a Cary-50 UV/Vis
Spectrometer (Varian). In the heating cycle experiments, the sample temperature was
raised to 60 C and cooled to 25 C at a rate of 1 C/minute. UV/Vis spectra were taken at 5
degree intervals. In the melting experiments the sample temperature was raised to 75 C at
a rate of 1 C/minute. In the kinetics experiments the sample temperature was raised to 40,
45, 47.5, 50, 52.5, 55, 57.5, and 60 C, respectively, in 2 min and then the spectrum taken
every 2 min. Absorbance measurements at 414 nm were plotted against time and fitted to
a first order exponential equation to obtain the rate constant k (Table S1).
**hFd2 cluster CD signal change at 50°C**

An 80 µM solution of hFd2 in 50 mM Heps, 100 mM NaCl, pH=7.5 was heated and maintained at 50°C. CD spectra in the range of 300 nm to 600 nm were taken at 5 min intervals using a JASCO J-815 Circular Dichroism (CD) Spectrometer. The same experiment was performed with and without the addition of 1 mM TCEP in the buffer.

**Rate of ferredoxin reduction by adrenodoxin reductase and cluster transfers monitored by the cytochrome c assay**

All solutions were purged by argon prior to use. For the measurement of enzymatic parameters, cytochrome c (80 µM), adrenodoxin reductase (200 nM) and NADPH (400 µM) were combined in 10 mM sodium phosphate buffer under anaerobic condition. Ferredoxin solutions at final concentrations of 0.1-8 µM were added and the absorbance at 550 nm was monitored over 5 min to define the initial slope of the kinetic profiles. For measurement of the holo-ISU to apo ferredoxin cluster transfer rate, a solution of 20 µL apo ferredoxin (200 µM) was added to 20 µL DTT (50 mM) and incubated at R.T. for 30 min. An aliquot of this solution (7 µL) was subsequently added to 35 µL of holo ISU (200 µM) and 6 µL of this mixture was used in the cytochrome c assay. For the cytochrome c assay, the total reaction volume used was 100 µL. This solution contained 80 µM cytochrome c, 200 µM adrenodoxin reductase, 400 µM NADPH, and 10 mM sodium phosphate at pH 7.4. The absorbance was measured at 550 nm for the first minute and the resulting initial slope was used for subsequent calculations. For details see previously published work from this laboratory.16

**Nuclear magnetic resonance spectroscopy**

All NMR samples (0.45 mM) were exchanged to 90% phosphate buffer (40 mM Na₂HPO₄, 100 mM NaCl, pH 7.4)/ 10% D₂O with Illustra MicroSpin™ G-25 Column
(GE Healthcare) and data were acquired in 5 mm NMR tubes (Wilmad-Labglass). \(^{15}\text{N-}^{1}\text{H}\) Heteroquantum Single Quantum Coherence (HSQC) spectra for all samples were recorded on a Bruker DMX 600 MHz spectrometer (carrier frequency 600.13MHz) equipped with a 5 mm TXI \(^{13}\text{C}/^{15}\text{N}\) probe with x,y,z-axes gradients to a dimension of \([1024 \times 512]\) and processed with XWIN-NMR v1.1. Contour level was defined with a multiplier of 1.4 and a total of 8 positive layers. All NMR experiments were recorded at 301.1 K except for the variable temperature experiments, which were achieved with the spectrometer’s built-in calibrated temperature controller.
Figures

Fd1      ITVHFINRDGETLTKGVGDSLLDVVENNLDIDGFGAEGTLA5THLIFEDHIYEK 60
Fd2      VNVVFVDRSGQRIPVSQRGDNVLHLAQRHGVDLEGACEASLACSTCHVYVSEDHLDL 58
Fd1      LDAITDEENDMLDALYGLTDRSRLGCQICLTKSMNVRPETV 105
Fd2      LPPPEEREDDMLMAPLLQENSRLGCQLTPELEGAETLPKIT 103

Figure S1. Protein sequence alignment for hFd1 and hFd2. Cysteine residues are highlighted.

Figure S2. Crystal structure of human ferredoxin1 (PDB: 3P1M). The [2Fe-2S] cluster is ligated by four cysteine residues (shown in yellow).
Figure S3. Crystal structure of human ferredoxin2 (PDB: 2Y5C). The [2Fe-2S] cluster is ligated by four cysteine residues (shown in yellow).

Figure S4. Comparison of UV/vis spectra for holo hFd1 and holo hFd2.
Figure S5. Comparison of CD spectra for holo hFd1 and holo hFd2.

Figure S6. Differential scanning calorimetry experiments of hFd2 (left) and hFd1 (right).
Figure S7. Change in the CD spectrum for holo hFd2 (from 300 to 600 nm) following incubation from 25 C (black) to 65 C (pink).
Figure S8. Far UV absorption spectra of hFd2 obtained at various temperatures: 35 °C (black), 55 °C (red), 75 °C (blue) and 95 °C (teal). Secondary structural analyses by K2D3 are summarized in Table S2.
Figure S9. Overlay of variable temperature [\(^{15}\text{N}\)-\(^{1}\text{H}\)] HSQC spectra for hFd1 (A) and hFd2 (B) at 28 C (black) and 55 C (red).

Table S1. Dependence of rate constant on temperature.

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>40</th>
<th>45</th>
<th>47.5</th>
<th>50</th>
<th>52.5</th>
<th>55</th>
<th>57.5</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>k (min(^{-1}))</td>
<td>0.0099</td>
<td>0.0495</td>
<td>0.0676</td>
<td>0.135</td>
<td>0.204</td>
<td>0.278</td>
<td>0.455</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Table S2. Temperature dependent secondary structural prediction and analysis for hFd2 by K2D3

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>Prediction</th>
<th>35</th>
<th>45</th>
<th>55</th>
<th>65</th>
<th>75</th>
<th>85</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-helix (%)</td>
<td>17.5</td>
<td>16.4</td>
<td>19.2</td>
<td>17.3</td>
<td>18.2</td>
<td>17.4</td>
<td>9.7</td>
<td>9.7</td>
</tr>
<tr>
<td>β-strand (%)</td>
<td>21.2</td>
<td>22.6</td>
<td>21.6</td>
<td>22.6</td>
<td>22.3</td>
<td>22.3</td>
<td>26.8</td>
<td>26.9</td>
</tr>
</tbody>
</table>