Yeast iso-1-cytochrome c met80X mutants: The pK_a of the spin state transition as a probe for haem pocket flexibility

Gary Silkstone, Glyn Stanway and Michael T. Wilson

Dept of Biological Sciences, University of Essex, Colchester CO4 3SQ

We are interested in developing a novel and powerful method for light induced electron transfer from c-type cytochromes to redox partner proteins. Initial experiments using a chemically modified form of cytochrome c, Cm-cyc, in which the ligand to the haem iron, met80, is carboxymethylated, have shown that laser photolysis of the CO complex of this protein yields high spin, low redox potential, ferrous haem which can act as a powerful reductant [1]. In order to better control this process and to ensure that chemical modification does not alter the surface "docking" region of the protein, we have constructed met80 mutants of yeast-iso-1-cytochrome c which possess a vacant co-ordination site for CO binding but are otherwise chemically unchanged.

Here we report the first results on the characterisation of such mutants and relate the hydrophobic/hydrophilic nature of the residue substituted for met80 to the pH induced spin state transition of the ferrous haem iron.

The met80 mutants were expressed efficiently in E.coli, following site directed mutagenesis using a single step PCR approach on the gene which encodes the yeast cytochrome (CYC1) of the plasmid construct pBPCYCl(wt)/3. The mutants and relate the hydrophobic/hydrophilic nature of the chemically unchanged.

Met80 mutants were expressed efficiently in E.coli, following site directed mutagenesis using a single step PCR approach on the gene which encodes the yeast cytochrome (CYC1) of the plasmid construct pBPCYCl(wt)/3. The residues X were selected such that they were not capable of binding to the ferrous haem iron, and that some, in part, mimicked the carboxymethylated residue of CM-cyc (i.e. acidic). Mutants prepared were asp, glu, ser and ala80, and isolation techniques following lysis of the bacterial cells included cation exchange and hydrophobic interaction chromatography.

Ferrous CM-cyc undergoes a simple reversible pH-dependent transition with pH=7.1 [2] (pK_a designates the apparent pK value). This transition can be observed spectrally, as the protein changes spin state going from high spin at acid pH to low spin at alkaline pH. This transition has been attributed to the deprotonation of a lysine residue, probably lys79, which subsequently binds to the ferrous iron rendering this low spin.

The met80 X mutants all behave in an essentially similar manner. Figure 1 shows a pH titration of ferrous met80glu cytochrome c monitored optically.

Figure 1, pH titration of the met 80 glu mutant of yeast iso-1-cytochrome c. Protein concentration 2μM temperature 20°C.

This spectral change is characterised by an increase in absorbance in the Soret region and the development of the α and β bands at 520 and 550 nm as the pH was increased. This optical transition conforms to a simple acid-base titration (n=1), with pK_a=6.8. The other mutants behaved in a generally similar fashion, yielding the following pK_a values: met80asp, 6.3; met80ser, 8.0; met80ala, 9.4.

A model to describe this spin state transition may be formulated [2] as follows;

\[ \text{HP} \leftrightarrow \text{H}^+ \text{+ P} \quad \text{P} \leftrightarrow \text{P}^* \]

where HP is the pentacoordinated form of the protein with protonated lys79; P a pentacoordinated form of the protein with lys79 deprotonated and P* is hexacoordinated low spin form in which the sixth coordination site is occupied by a deprotonated amino group of lysine. The spectral change accompanies the transition from P to P*. The simple titration curve may be described as follows;

\[ Y = K_{\text{papp}} / (K_a H^+ + K_{\text{papp}}) \]

where \( K_{\text{papp}} = K_{12} (K_{23} + 1) \)

The parameter Y is the fraction of the protein in the alkaline form. Taking the experimentally determined values of pK_app for the mutants and the pK of lys79 as 10.8 we have calculated the value of K_{23} for each mutant. These may be listed as follows

<table>
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<tr>
<th>Mutant</th>
<th>K_{23}</th>
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<tbody>
<tr>
<td>ASP-80</td>
<td>31600</td>
</tr>
<tr>
<td>GLU-80</td>
<td>10000</td>
</tr>
<tr>
<td>CM-Cyc</td>
<td>4800</td>
</tr>
<tr>
<td>SER-80</td>
<td>500</td>
</tr>
<tr>
<td>ALA-80</td>
<td>25</td>
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</tbody>
</table>

The values of K_{23} show a distinct trend from large value for hydrophilic residues to a much smaller value for alanine. As in all cases the spectroscopic evidence suggests that an amino nitrogen is binding to the iron the differences in the K_{23} values probably reflect the difficulty in rearranging the haem pocket to allow the approach of the ligand. This rearrangement requires the cavity to open and the peptide backbone to rotate to allow lys79 to occupy the position normally filled by met80. For negatively charged, hydrophilic residues such as asp and glu the folding pattern is disrupted resulting in an open flexible crevice while for alanine the protein retains its native packing and rearrangement is difficult.

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References: