Chapter 3
Plastocyanin

3.1 Introduction

Plastocyanin (Pc), a small protein containing a single Cu atom, occurs in all higher plants, green algae and some blue-green algae. It has an essential role in photosynthesis, functioning as the last electron carrier in a chain between photosystems II and I (Sykes, 1985). Pc receives an electron from the cytochrome $b_6/f$ complex and donates the electron to the P700$^+$ photoreaction centre. The electron is carried on the Cu atom which is reduced to Cu$^{+}$ on its addition and oxidised to Cu$^{II}$ on its removal. The role of Pc is illustrated in figure 3.1.

Pc is a member of a family of proteins called the "blue" copper proteins because in the oxidised (Cu$^{II}$) state, an extremely strong absorption band near 600 nm gives the proteins an intense blue colour. Other distinctive properties of these proteins include abnormally low EPR hyperfine splitting constants in the $g_{||}$ region and relatively high redox potentials (Adman, 1991). In an effort to determine the basis of these unusual properties, the "blue" copper proteins have been the subject of intensive spectroscopic, electrochemical and structural studies (Sykes, 1990).

Figure 3.1  Schematic representation of photosynthetic electron transport at the thylakoid membrane. Abbreviations: Mn, H$_2$O-splitting complex; PQH$_2$, plastoquinol; $b_6/f$, cytochrome complex; Pc, plastocyanin; Fd, ferredoxin; FNR, ferredoxin NADP$^+$ reductase; ADP, adenosine diphosphate; ATP adenosine triphosphate. Adapted from Sykes (1985).
Where the biological function of the "blue" copper site has been established, it is invariably electron transfer. As a result, the mononuclear "blue" copper proteins are known as cupredoxins (Adman, 1991).

3.2 X-ray diffraction studies of Plastocyanin

The Pc used in this study was obtained from poplar trees, *Populus nigra* var. *italica*. The protein consists of a single polypeptide of 99 amino acid residues and a single Cu atom.

X-ray diffraction (XRD) crystal structures have been determined for several forms of poplar Pc. The room-temperature structure has been determined for oxidised Pc (Cu\textsuperscript{II}Pc) at pH 4.2 and pH 6.0 (Guss & Freeman, 1983; Guss *et al.*, 1992), for reduced (Cu\textsuperscript{I}) Pc at 6 pH values from 3.8 to 7.8 (Guss *et al.*, 1986), for apo- (Cu-removed) Pc (Garrett *et al.*, 1984), and for Hg-substituted Pc (Church *et al.*, 1986). The structure of Cu\textsuperscript{II}Pc has also been determined at a temperature of 173 K (Fields *et al.*, 1994).

The protein has the form of a slightly flattened cylinder approximately 40×32×28 Å\textsuperscript{3} (figure 3.2). As is generally the case with electron-transport proteins (Sykes, 1990), the active site is located beneath but near the surface. The Cu atom is located at one end of the long axis 6 Å below the surface.

![Figure 3.2](Image)

*Figure 3.2* Stereo α-carbon diagram of Pc. The Cu-binding sidechains (filled bonds) and the Cu atom have also been included.
In CuIIPc, the Cu atom is coordinated by the Nδ(imidazole) atoms of His37 and His87, the Sγ(thiolate) atom of Cys84, and the Sδ(thioether) atom of Met92 in a distorted tetrahedral geometry. The Cu site in the 1.33 Å structure is shown in figure 3.3. Based on the Cu-ligand distances in small molecules (Bouwman et al., 1990), the two Cu-Nδ(imidazole) bonds (1.91 Å and 2.06 Å) are in the normal range, the Cu-Sγ(thiolate) bond (2.07 Å) is unusually short and the Cu-Sδ(thioether) bond (2.82 Å) is unusually long.

![Figure 3.3](image)

**Figure 3.3** The Cu site of CuIIPc as determined by XRD at 1.33 Å.

Although the structure of CuIIPc is essentially unchanged between pH 4.2 and 6.0 (Guss & Freeman, 1983), reduced Pc has two forms. At high pH (≈8), the Cu-site geometry of CuIIPc is retained with some increase in the Cu-ligand bond lengths (Guss et al., 1986). At low pH (≈4), Nδ(imidazole) of His87 is protonated and the bond to the Cu atom is broken. The imidazole ring is rotated 180° about the Cβ-Cγ bond and makes only a van der Waals contact with the Cu atom. In this state, the Cu atom is trigonally coordinated by the remaining ligands. As determined by X-ray diffraction (figure 3.4), the Cu-Sγ (thioether) bond becomes sufficiently short (2.51 Å) to be a plausible binding interaction.

![Figure 3.4](image)

**Figure 3.4** The Cu site of HCuI Pc at pH 3.8 as determined by XRD at 1.9 Å.
The high-pH form of reduced Pc is labelled CuI_Pc. The low-pH form is labelled HCuI_Pc.

The current XRD structures of poplar Pc are summarised in table 3.1. The variation of the XRD Cu-ligand distances with pH and oxidation state is graphed in figure 3.5.

**Table 3.1** Summary of the Cu-site dimensions determined by X-ray crystal structure analysis of reduced (CuI) and oxidised (CuII) poplar Pc.

<table>
<thead>
<tr>
<th></th>
<th>Reduced (CuI) Pc</th>
<th>Oxidised (CuII) Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured pH:</td>
<td>3.8 4.4 5.1 5.9 7.0 7.8</td>
<td>4.2 6.0 6.0 (173K)</td>
</tr>
<tr>
<td>Resolution (d_{min}, Å)</td>
<td>1.9 1.9 2.05 1.7 1.8 2.15</td>
<td>1.9 1.33 1.6</td>
</tr>
<tr>
<td>Final residual R (%)</td>
<td>15.2 15.4 15.4 16.7 16.0 15.0</td>
<td>15.7 16.5 13.2</td>
</tr>
<tr>
<td>Cu-ligand bond lengths (Å)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu-Nδ(His37)</td>
<td>2.12 2.08 2.21 2.11 2.13 2.12</td>
<td>2.07 1.91 1.98</td>
</tr>
<tr>
<td>Cu-Nδ(His87)</td>
<td>3.12 3.20 3.05 2.77 2.39 2.25</td>
<td>2.17 2.06 1.95</td>
</tr>
<tr>
<td>Cu-Sγ(Cys84)</td>
<td>2.13 2.15 2.13 2.13 2.17 2.11</td>
<td>2.11 2.07 2.14</td>
</tr>
<tr>
<td>Cu-Sδ(Met92)</td>
<td>2.51 2.51 2.51 2.68 2.88 2.90</td>
<td>2.87 2.82 2.78</td>
</tr>
<tr>
<td>e.s.d.?</td>
<td>0.08 0.08 0.09 0.06 0.07 0.10</td>
<td>0.08 0.04 0.05</td>
</tr>
</tbody>
</table>

1 At pH's between 5.1 and 7.0, this distance represents a mixture of the Cu–Nδ(His87) distance in CuI_Pc and the Cu···Cδ(His87) distance in HCuI_Pc. The present analysis suggests that there may be significant HCuI_Pc even at pH 7.8. The 2 lowest pH crystals appear to be close to 100% HCuI_Pc.

2 E.s.d.'s according to Guss & Freeman (1992). The e.s.d.'s for resolutions of 1.3 Å, 1.6 Å and 1.8 Å resolution were given values of 0.04 Å, 0.05 Å and 0.07 Å. The e.s.d.'s given for other resolutions were estimated from these values.

### 3.3 Implications for oxidation and reduction

The small changes between the oxidised and reduced forms of the Cu site at high pH imply that the reorganisation energy on oxidation or reduction is small. This facilitates
Figure 3.5  Cu-ligand distances in Pc as determined by X-ray diffraction plotted as functions of pH and oxidation state. The Cu-ligand distances in CuII Pc have been plotted next to the distances in reduced Pc at high pH to illustrate the decrease in bond distances on oxidation.

the conversion between the two states and contributes to efficient electron transfer via an outer-sphere mechanism.

At low pH, the reduced form is redox inactive as the trigonal S2N geometry of the Cu site strongly favours CuI (Guss et al., 1986).

3.4 Variation with pH of the ratio of the low- and high-pH forms of reduced Pc

At any pH, reduced Pc is an equilibrium mixture of the low- and high-pH forms. The equilibrium ratio of the low- to the high-pH form is given by the equation:

$$\frac{[\text{low pH}]}{[\text{high pH}]} = 10^{pK_a - \text{pH}}$$  \hspace{1cm} (3.1)

where pK_a is the pK_a for the protonation of His87. pK_a has been determined to be 4.7 at room temperature in low ionic-strength solutions (Jackman et al., 1987).
Although a changing ratio with pH of the two forms is also observed in the crystal structures as shown by the smooth change in the Cu-ligand bond distances determined at pH's from 3.8 to 7.8 (table 3.1, figure 3.5), the equilibrium is displaced to higher pH's compared to solution. The apparent $pK_a$ in the crystals is ≈6.1 (compared to 4.7 in dilute solutions) and the transition seemed to take place over an unusually large pH range.

These differences may arise from:

1. Systematic errors in the measured pH due to the high ionic strength of the crystal medium (3 M (NH$_4$)$_2$SO$_4$).
2. Actual differences due to the high ionic strength or crystal packing forces.

### 3.5 Other "blue" copper proteins: why do EXAFS?

It has become apparent from the determination of the X-ray crystal structures of the "blue" copper proteins azurin (*Alcaligenes denitrificans*: Baker, 1988; *Pseudomonas aeruginosa*: Nar et al., 1991), pseudoazurin (*Methylobacterium extorquens* AM1: Inoue et al., 1994; *Alcaligenes faecalis* S-6: Vakoufari et al., 1994), cucumber basic protein (Guss et al., 1988), amicyanin (*Paracoccus denitrificans*: Durley et al., 1993; *Thiobacillus versutus*: Romero et al., 1994), and plastocyanin (*Populus nigra*: see above; *Enteromorpha Prolifera*: Collyer et al., 1990; *Oleander nerium*: Tong, 1991; *Chlamydomonas reinhardtii*: Redinbo et al., 1993), that the Cu sites exhibit both strong overall similarities and subtle structural differences. In order to produce statistically significant estimates of these differences and to correlate these with the different properties of the members of this protein family it is necessary to have individual structure determinations of the highest precision (Guss et al., 1992).

The precision of the XRD Cu-site models is difficult to estimate. Guss & Freeman (1992) analysed the several refinements of Cu$^{II}$Pc and estimated that the Cu-ligand distances have e.s.d.'s of 0.04 Å at 1.3 Å resolution, 0.05 Å at 1.6 Å and 0.07 Å at 1.8 Å.

In principle, EXAFS analysis offers the opportunity to obtain Cu-ligand distances with e.s.d.'s of 0.02 Å or better (Gurman, 1995). This could represent a substantial improvement in the precision of the Cu-ligand distances even for the well-determined Cu$^{II}$Pc structure.