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De Novo Design and Synthesis of Polypeptides Based on Photosystem II

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Introduction

The photosystem II reaction center efficiently converts solar energy into a stable charge separation that is coupled to the oxidation of water. The charge separation begins as the special chlorophyll *a* dimer of PSII, P_{680}, gets photooxidised and rapidly transfers an electron to a plastoquinone molecule named Q_A. The charge separation is stabilized by the further transfer of the electron from Q_A to another plastoquinone molecule, Q_B. The oxidized P680 is the strongest biological oxidant and generates the required oxidising equivalents to split water molecules into molecular oxygen. The charges from P_{680}^{+} are transferred and accumulated in the oxygen evolving complex via a tyrosine residue (Tyr_z).

To fully understand the molecular mechanisms involved in the charge separation in photosystem II, a minimalist peptide that can incorporate chlorophyll and redox active molecules must be extremely useful. Unlike the linear covalently linked donor-acceptor systems (Gust & Moore, 1991; Magnuson *et al*., 1997), a peptide provides a tool to obtain not only a functional unit with electron transfer properties but also to determine the protein structural elements controlling the function. A peptide scaffold can readily be modified to change the orientation of cofactors toward each other as well as their distances. This type of approach has successfully been employed to study the function of redox proteins in particular artificial heme proteins (Gibney & Dutton, 2001). It was shown that synthetic peptides provided a suitable environment for heme but interestingly with redox potentials that are more negative than that in a natural heme protein. This is because in the natural system the protein environment not only provides the ligation sites for redox cofactors but also provides a local environment that is very different from the bulk solvent. The low dielectric hydrophobic properties of a protein, and the hydrogen bonds formed between redox cofactors and amino acid side chains are among factors that affect the redox potential of cofactors in the natural system. In the case of heme peptides, it was shown that the midpoint redox potential was altered by mutating residues in the binding pocket. As the side chain hdyrophobicity of amino acids in contact with the heme increases, the reduction potential of heme drops. The reduction potential is therefore peptide sequence dependent and can vary from –300 to –50 (Huffman *et al*., 1998; Rau & Haehnel, 1998; Shifman *et al*., 2000). We have therefore begun attempts toward the synthesis of a structurally defined peptide with a binding pocket for a chlorophyll, an electron acceptor, and an electron donor.

The primary electron donor of PSII is ligated to the protein backbone through histidines from the magnesium metal center in the porphyrin head of chlorophyll a. Here based on the designed heme proteins, we are presenting the preliminary data on binding a Cu(II) chlorophyllin to a synthetic peptide through histidine ligation sites. We have chosen

chlorophyllin to avoid the potential complications of hydrophobic interactions with the phytyl chain of chlorophyll a.

Methods

The synthetic peptide that we have used is composed of 63 residues with a binary pattern of polar and nonpolar amino acids that adopt a helical structure with a partial symmetry (Sharp *et al*., 1998). The peptide sequence is MLKKLREE ALKLLEE FKKLLEE HLKWLE GGGGGGGG ELLKLHEE LLKKCEE LLKLAEE RLKKL. The helical structure is formed as a result of projecting polar residues toward the aqueous surface and packing the hydrophobic residues inside. We synthetised the gene and cloned it into pet32b plasmid (Novagen, Inc.). The peptide was overexpressed in *E. coli* as a fusion protein with thioredoxin. The fusion protein was purified using affinity chromatography through a his-tag that is encoded in a linker between the peptide and thioredoxin genes. The peptide was efficiently cleaved from thioredoxin by overnight incubation of the purified fusion protein with the site-specific protease factor Xa. It was then purified from the thioredoxin by organic solvent precipitation to homogeneity shown by SDS-page electrophoresis and HPLC. The cleavage specificity was confirmed by N-terminus sequencing of the peptide. The chlorophyllin is a water-soluble pigment and for the binding experiments it was added to the peptide from a 20 mM stock solution. The EPR experiments were carried out at room temperature on a Bruker ESP 300E spectrometer at 100 kHz modulation frequency, 2 G modulation amplitude, 200 mW microwave power and 1.3 s time constant. The NMR experiments were performed on a Varian 500 MHz spectrometer with peptide samples made in 50 mM phosphate (pH 7.0) at 2 mM concentration in 90%H_2O/10%D_2O.

Results & Discussion

The peptide folds into a dimer to form a four helix bundle protein as shown by CD spectroscopy and HPLC elution profile. There are two histidines engineered into the peptide to ligate the chlorophyllin. A cysteine residue is also present in the peptide to attach a nitroxide spin label for monitoring the binding of chlorophyllin. The thiol-specific spin label of 1-Oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate (Toronto Research Chemical Inc., Canada) was used to specifically label the cysteine. In Figure 1 the inset shows an EPR spectrum of free nitroxide spin label in solution. The labelling allowed us to monitor the binding of chlorophyllin to the peptide by EPR spectroscopy. Figure 1a shows EPR spectrum of the nitroxide spin label attached to the peptide that represents a very broad characteristic spectrum of a spin label immobilised on the EPR time scale due to slow motional regime of a tumbling peptide. However, when the chlorophyllin was added to the peptide, the spectrum amplitude was significantly decreased which is a clear indication that Cu(II) interacts magnetically with the nitroxide spin label (Figure 1b). The loss of the signal intensity is due to the linewidth broadening caused by the effect of bound Cu(II) on the T_2 relaxation time of the spin label and exchange interactions. From the extent of loss in signal amplitude, a distance of about 10 Å between chlorophyllin and nitroxide spin label can be estimated which is in a good agreement with the distance between histidine and cysteine residues predicted from the peptide structure. This suggests that chlorophyllin is ligated to the histidine and placed into the engineered binding pocket.

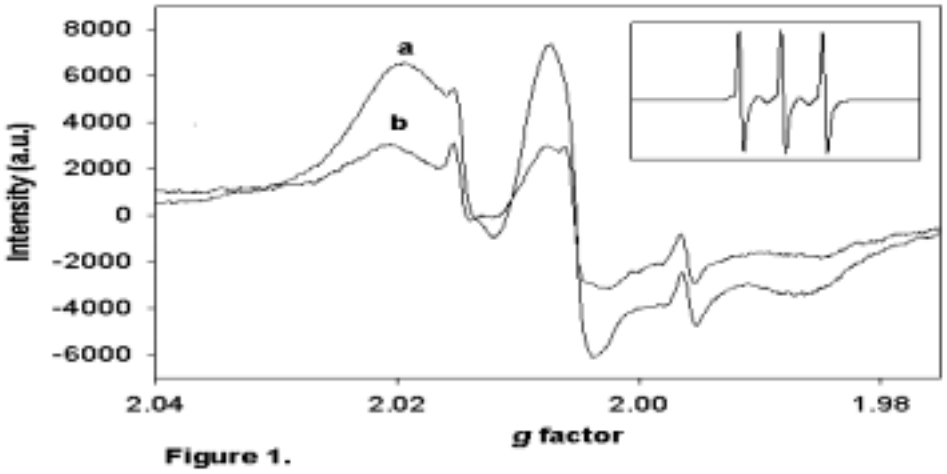

Figure 1.

Figure 2 presents the effect of ferricyanide on the optical spectrum of free chlorophyllin and chlorophyllin in the presence of peptide. As it is shown in Figure2a, the addition of ferricyanide causes a dramatic decrease in the intensity of the free chlorophyllin optical spectrum monitored at 630nm. However, this behavior was not observed when ferricyanide was added to chlorophyllin in the presence of peptide (Figure2b). This indicates that chlorophyllin is bound to the peptide and thus shielded from ferricyanide.

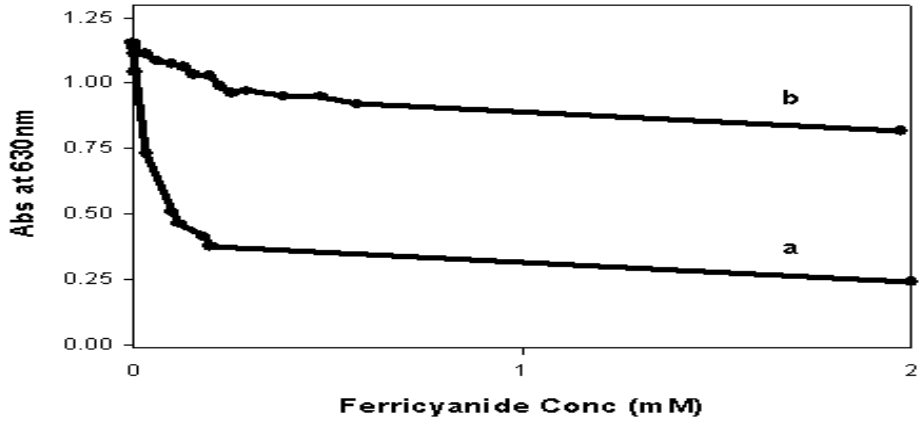

Figure 2.

We have also performed NMR measurements on the peptide in the presence (Figure 3a) and absence of chlorophyllin (Figure 3b). The NMR spectrum of peptide in the presence of chlorophyllin shows more resolved peaks in consistency with the other data supporting that chlorophyllin binds to the peptide and causes structural changes. These are rather conformational changes since the chlorophyllin binding did not change the alpha helical content of the peptide as shown by CD measurements.

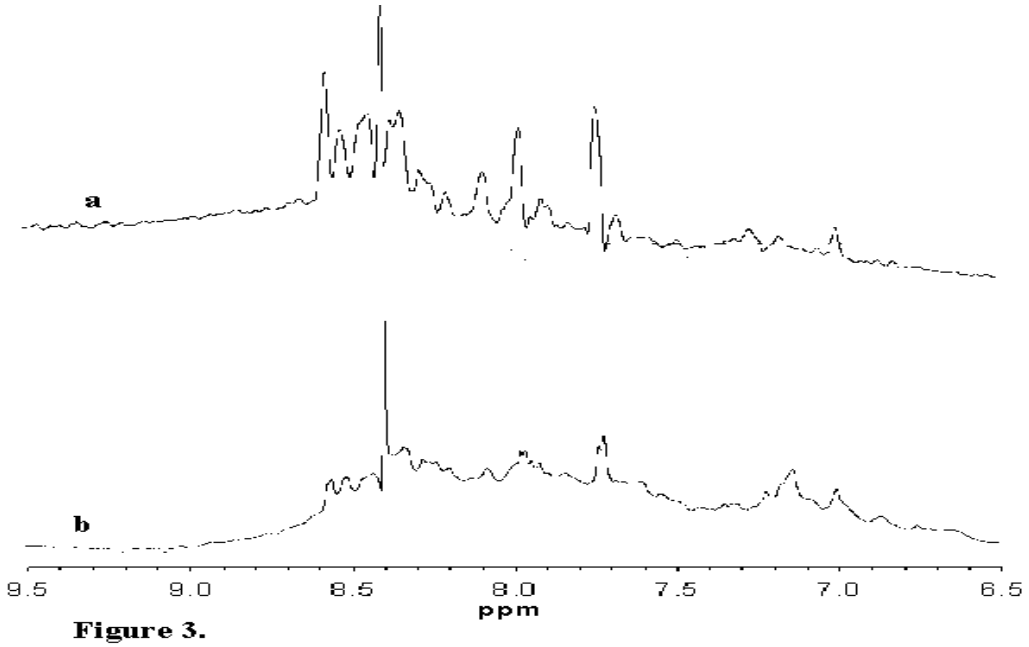

Figure 3.

Taken together, we have shown that *de novo* synthesis can be used to make constructs that can ligate chlorophyll analogues through histidine residues. Since the *de novo* design is a constructive approach, we will try to incorporate an electron acceptor such as quinone and an electron donor like a metal center into the peptide through cysteine residues. By optimising the design for the redox potential of pigment and the spatial geometry of cofactors, we then hope to stabilise a charge separation.

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