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# Synthesis and characterization of *de novo* designed peptides modelling the binding sites of [4Fe–4S] clusters in photosystem I

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ABSTRACT

Photosystem I (PS I) converts the energy of light into chemical energy *via* transmembrane charge separation. The terminal electron transfer cofactors in PS I are three low-potential [4Fe–4S] clusters named $F_X$, $F_A$ and $F_B$, the last two are bound by the PsaC subunit. We have modelled the $F_A$ and $F_B$ binding sites by preparing two apo-peptides (maquettes), sixteen amino acids each. These model peptides incorporate the consensus [4Fe–4S] binding motif along with amino acids from the immediate environment of the iron–sulfur clusters $F_A$ and $F_B$. The [4Fe–4S] clusters were successfully incorporated into these model peptides, as shown by optical absorbance, EPR and Mössbauer spectroscopies. The oxidation–reduction potential of the iron–sulfur cluster in the $F_A$-maquette is $-0.44 \pm 0.03$ V and in the $F_B$-maquette is $-0.47 \pm 0.03$ V. Both are close to that of $F_A$ and $F_B$ in PS I and are considerably more negative than that observed for other [4Fe–4S] model systems described earlier (Gibney, B. R., Mulholland, S. E., Rabanal, F., and Dutton, P. L. Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 15041–15046). Our optical data show that both maquettes can irreversibly bind to PS I complexes, where PsaC-bound $F_A$ and $F_B$ were removed, and possibly participate in the light-induced electron transfer reaction in PS I.



## 1. Introduction

Iron–sulfur clusters are ubiquitous in biology and play many different roles in the living cell (reviewed in [1–5]). They act as catalysts, sensors and transcription regulators and play a signalling role during DNA repair. By far most common is their participation as redox cofactors in electron transfer reactions, either bound to a small soluble protein, as in ferredoxins, or as part of an electron transfer chain in a large protein or protein complex, as in hydrogenase or photosystem I. A wide variety of different iron–sulfur clusters exists in biology. The inventory of unique protein folds for different iron–sulfur proteins was recently compiled by Meyer [6].

Owing to their biological significance, iron–sulfur clusters have been extensively modelled in the past. A large body of literature exists on the investigation of chemically synthesized model compounds, mimicking iron–sulfur clusters of different nuclearity, in organic solvents (for a recent review see [7]). While providing valuable insight into the chemistry of the iron–sulfur clusters, the majority of these models cannot account for the interactions of the iron–sulfur cluster with its protein binding site, which includes interaction with non-ligating amino acids or with surrounding water molecules. Both types of interactions are believed to strongly influence the physiologically relevant properties of iron–sulfur clusters, *e.g.*, redox potential and catalytic activity.

In the past two decades several attempts were made to prepare models containing peptide or protein ligated iron–sulfur clusters in aqueous buffers [8–17]. Initially, the entire polypeptide sequence of ferredoxin from *Clostridium pasteurianum* [14,15], and a truncated polypeptide sequence from *Desulfovibrio gigas* ferredoxin II [16] were synthesized *in vitro*. More recently two main approaches for modelling binding sites were explored: the first relies on designing

*Abbreviations:* Chl *a*, Chlorophyll *a*; CW, Continuous Wave; DCPIP, 2,6-dichlorophenolindophenol; DIPEA, N,N-diisopropylethylamine; β-DM, n-dodecyl-β-D-maltoside; ENDOR, Electron Nuclear DOuble Resonance; EPR, Electron Paramagnetic Resonance; ESEEM, Electron Spin Echo Envelope Modulation; HYSCORE, HYperfine Sublevel COrRElation; HPLC, High Performance Liquid Chromatography; NADP, nicotinamide adenine nucleotide phosphate; NMP, N-methylpyrrolidone; MALDI-TOF-MS, Matrix Assisted Laser Desorption Ionisation – Time-of-Flight – Mass Spectrometry; P700-$F_X$ core, PS I preparation where the stromal polypeptides PsaC, PsaD and PsaE, together with [4Fe–4S] clusters $F_A$ and $F_B$, have been removed and the final electron acceptor is the [4Fe–4S] cluster $F_X$; P700-$A_1$ core, P700-$F_X$ core where [4Fe–4S] cluster $F_X$ have been removed and the final electron acceptor is the quinone $A_1$; PAL-PEG-PS, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeryl polyethylene glycol-polystyrene; Pbf, 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; PS I, Photosystem I; SHE, Standard Hydrogen Electrode; TCTU, O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; Trityl, Triphenylmethyl

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synthetic peptides containing the conserved iron–sulfur binding site [8–11]; the second relies on the introduction of the iron–sulfur cluster binding site into a naturally occurring or synthetic protein that was previously incapable of binding an iron–sulfur cluster [8,12,13,17]. It should be noted, however, that up to date only peptide-ligated models of [4Fe–4S] clusters were reported in the literature.

Incorporation of low-potential $[4Fe–4S]^{2+/1+}$ clusters into peptides of different sizes was investigated with the aim of determining the minimal requirements for successful binding [8–10]. Mulholland et al. investigated the influence of the amino acid composition of model peptides on the binding efficiency of iron–sulfur clusters [9,10]. In this study several model peptides with lengths between 4 and 16 amino acids were investigated. It was found that aside from the presence of a consensus iron–sulfur binding motif, containing at least three cysteines, which are appropriately spaced (CxxCxxC), the choice of non-liganding amino acids plays a decisive role in the efficiency of [4Fe–4S] cluster incorporation. By analysis of the amino acid sequence of 510 naturally occurring ferredoxins the prevalence of specific non-ligand amino acids in certain sequence positions within the consensus binding motif was established [10]. It was shown that β-branched amino acids like Ile or Val are dominant in the second position and Gly in the third and the fifth position. There seems to be less restriction for the choice of the sixth non-ligand amino acid in the consensus iron–sulfur cluster binding motif. While the apolar Ala is prevalent in this position (18%), a positively charged Arg is the second most prominent (12%) and Gln is also relatively common (8%).

Despite the variation of the amino acid composition, all previously studied maquettes containing a ferredoxin binding site show similar biophysical and biochemical properties, namely identical EPR spectra and a redox potential of about −0.350 V [8–10], or even higher, up to −0.289 V [11].

We consider that the next logical step is to model [4Fe–4S] cluster(s) which function within a large protein complex and whose biological role is well-known. Therefore, our attention turned to photosystem I (PS I), where the crystal structure of the overall complex is known [18], and the function of the [4Fe–4S] clusters in the electron transfer chain have been studied in detail for over 30 years and is well-established (see recent reviews collected in [19]).

PS I is a membrane-bound, multi-cofactor, energy-transforming protein complex, that is an indispensable part of the photosynthetic electron transfer chain in plants and cyanobacteria. PS I is a Type I reaction center, where the terminal electron acceptor is a [4Fe–4S] cluster. Three low-potential [4Fe–4S] clusters are bound on the reducing (stromal) side of PS I, usually referred as $F_X$, $F_A$ and $F_B$ (reviewed in [20]).

The [4Fe–4S] cluster $F_X$ is an unusual case of an interpolypeptide iron–sulfur cluster, with two cysteine ligands provided by the PsaA subunit and two by the PsaB subunit of PS I. The binding site of $F_X$ is identical on both subunits. Interestingly, $F_X$ has one of the lowest midpoint reduction potentials known for a [4Fe–4S] cluster, values ranging from −0.730 V [21] to −0.705 V [22] (all potentials *versus* SHE). Scott et al. incorporated a binding motif of PS I [4Fe–4S] cluster $F_X$ into the 4-α-helix bundle designed by the group of DeGrado [13]. This is the first, and so far the only model of both an iron–sulfur cluster involved in photosynthesis and of a interpolypeptide iron–sulfur cluster. It has an EPR spectrum nearly identical to maquettes containing the ferredoxin binding site, which is quite different from the EPR spectrum of the interpolypeptide [4Fe–4S] cluster $F_X$ in PS I. This model, however, exhibits the lowest reduction potential found for a [4Fe–4S] cluster bound to a peptide maquette up to now (−0.422 V).

The iron–sulfur clusters, $F_A$ and $F_B$, follow $F_X$ in the electron transfer chain of PS I. They are bound to the PsaC subunit of PS I, which is located on the stromal side of the thylakoid membrane (Fig. 1A, for a recent review on PsaC structure and its binding to PS I see [23]). It was shown spectroscopically that $F_B$ is the terminal electron acceptor in PS I [24–28] (reviewed in [20]). The midpoint reduction potentials of $F_A$ and $F_B$ in fully assembled PS I were measured at cryogenic

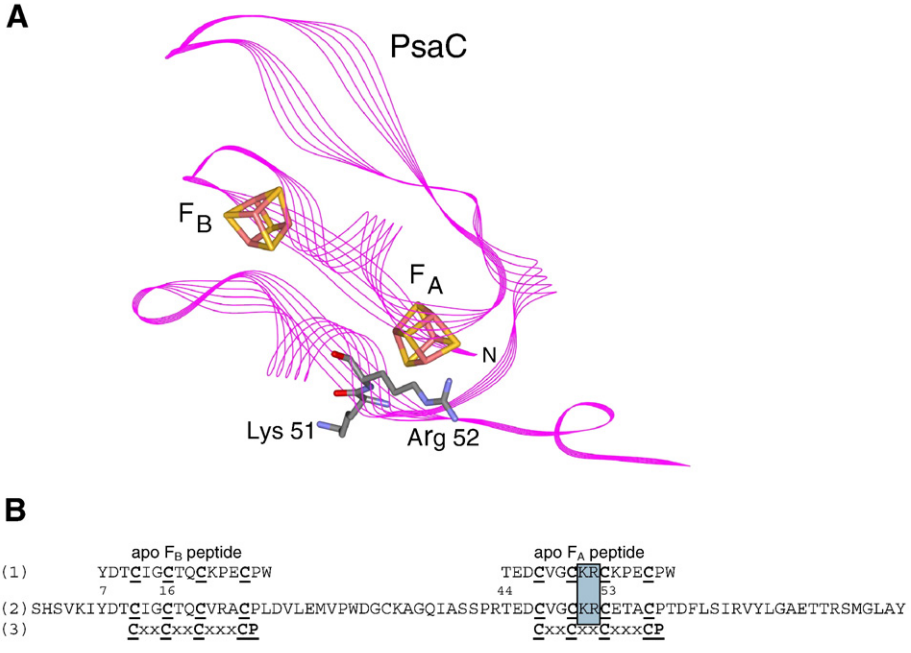


**Fig. 1.** (A) Three-dimensional structure of PsaC subunit taken from the X-ray structure of PS I at 2.5 Å resolution (PDB entry 1JB0) [18]. Detail of the structural model of the PS I monomer showing the backbone of the PsaC subunit and the [4Fe–4S] clusters $F_A$ and $F_B$ bound by it. The iron–sulfur clusters are shown as cubes, in which the yellow corners indicate position of sulfur atoms and light-brown corners the position of iron atoms. (B) Amino acid sequence of the designed peptides $F_A$ and $F_B$ (1) compared to the amino acid sequence of the PsaC subunit of PS I from *Synechococcus* sp. PCC 7002 (2) and the consensus low-potential [4Fe–4S] cluster binding motif (3). Numbering refers to the PsaC sequence. The Lys 51 and Arg 52 are crucial for binding of PsaC within PS I *via* the formation of salt bridges with amino acids on the PsaA and PsaB subunits [23,36]. They are shown as "stick" models in (A) and identified by the light blue box in (B).