Type I photosynthetic reaction centres: structure and function

Peter Heathcote^{1*}, Michael R. Jones² and Paul K. Fyfe²

¹*School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK*

²*Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK*

We review recent advances in the study of the photosystem I reaction centre, following the determination of a spectacular 2.5 Å resolution crystal structure for this complex of *Synechococcus elongatus*. Photosystem I is proving different to type II reaction centres in structure and organization, and the mechanism of transmembrane electron transfer, and is providing insights into the control of function in reaction centres that operate at very low redox potentials. The photosystem I complex of oxygenic organisms has a counterpart in non-oxygenic bacteria, the strictly anaerobic phototrophic green sulphur bacteria and heliobacteria. The most distinctive feature of these type I reaction centres is that they contain two copies of a large core polypeptide (i.e. a homodimer), rather than a heterodimeric arrangement of two related, but different, polypeptides as in the photosystem I complex. To compare the structural organization of the two forms of type I reaction centre, we have modelled the structure of the central region of the reaction centre from green sulphur bacteria, using sequence alignments and the structural coordinates of the *S. elongatus* Photosystem I complex. The outcome of these modelling studies is described, concentrating on regions of the type I reaction centre where important structure–function relationships have been demonstrated or inferred.

Keywords: photosynthesis; reaction centres; type I; photosystem I; green sulphur bacteria; homology modelling

1. INTRODUCTION

Chlorophyll-based photosynthesis is found in both eukaryotes and prokaryotes. The pigment–protein complexes that constitute the photosystems of these organisms use light energy to drive a series of electron transfer reactions that are coupled to the translocation of protons across a charge-impermeable membrane. The electrochemical gradient of protons established by this process is used to drive the synthesis of ATP via a membrane-bound ATP synthase. The photosynthetic apparatus consists of protein–cofactor complexes that have different roles. Antenna proteins are responsible for the capture of light energy, and consist of a mixture of protein and pigments such as chlorophylls, carotenoids and bilins that have strong absorbance properties. These complexes are either embedded in the membrane or are attached to the membrane surface. Light energy is captured by absorption, and the energy is temporarily stored in the form of a pigment excited electronic state. The pigments of the antenna complexes then transfer their energy to specialist chlorophyll cofactors in a second type of photosynthetic pigment–protein complex called the reaction centre (reviewed by Heathcote *et al.* 2002), where a photochemical reaction traps the energy.

Reaction centres are integral to the photosynthetic membrane, and consist of a mixture of protein, chloro-

phyll and other redox-active cofactors. They are classed as type I or type II, depending on the identity of the terminal electron acceptor (Blankenship 1992). In type I complexes these are four-iron/four-sulphur clusters (Fe₄S₄), whereas in the type II complexes they are (bacterio)pheophytins and quinones.

The structures of the type II reaction centre from two species of purple photosynthetic bacteria, *Rhodospseudomonas viridis* and *Rhodobacter (Rb.) sphaeroides*, were determined by X-ray crystallography in the mid-1980s (Deisenhofer *et al.* 1985; Allen *et al.* 1987). For the past 15 years these structures have provided a blueprint that has guided a great deal of research on all of the photosynthetic reaction centres. The purple bacterial reaction centre has been subjected to a detailed structural and functional analysis, using a combination of spectroscopy, mutagenesis, computational studies and crystallography (Hoff & Deisenhofer 1997). The purple bacterial reaction centre exhibits several design features that appear to be common to all reaction centres, and our understanding of the details of this complex has guided much of the thinking on the structure and mechanism of other types of reaction centre. The type II reaction centre of oxygenic photosynthesis, photosystem II, has an electron transfer chain and heterodimeric core structure that is similar to that seen in purple photosynthetic bacteria, but with the addition of the oxygen-evolving complex. The structure of this complex from the thermophilic cyanobacterium *Synechococcus elongatus*, at a resolution of 3.8 Å, was published recently by the groups of Witt and Saenger (Zouni *et al.* 2001) (see also Shen & Kamiya 2000).

* Author for correspondence (p.heathcote@qmul.ac.uk).

One contribution of 21 to a Discussion Meeting Issue 'Chloroplasts and mitochondria: functional genomics and evolution'.

Figure 1. Structural organization of the photosystem I reaction centre from *Synechococcus elongatus*, and a representation of the equivalent complex from *Chlorobium limicola*. (a) The electron transfer cofactors of the *S. elongatus* photosystem I complex. The cofactors are arranged around an axis of pseudo twofold symmetry that runs from the P₇₀₀ dimer to the F_X iron–sulphur centre. (b) The protein component of the *S. elongatus* photosystem I complex. The PsaA and PsaB polypeptides are shown in maroon and green, respectively. The PsaC subunit (cyan ribbons at the top of the structure) at the stromal surface of the complex binds the F_A and F_B iron sulphur centres. The binding and function of PsaC is assisted by two other small subunits (PsaD and PsaE) located on the stromal surface of the complex (orange and red ribbons, respectively, at the top of the structure). (c) Schematic of the type I reaction centre from *C. limicola*. This complex consists of a homodimer of two copies of the PscA gene (contrasted in green and maroon). The complex also has equivalents of the PsaC and PsaD subunits of the cyanobacterial photosystem I (cyan and orange ribbons, respectively, at the top of the structure).

Figure 2. Organization of the cofactors of the cyanobacterial photosystem I complex, and the proposed organization of their counterparts in the *Chlorobium limicola* complex. (a) The electron-transferring chlorophylls (cyan) and iron–sulphur centres (green and orange spheres) are surrounded by antenna chlorophylls (cpk colours) and carotenoids (not shown). Linker chlorophylls A40 and B39 are highlighted in dark blue, and candidates for red-shifted chlorophylls are highlighted in red. (b) The same view of the modelled organization of the antenna bacteriochlorophylls in *C. limicola*.

The energy-transducing apparatus of the type II reaction centres consists of two membrane-spanning branches of cofactors (see Heathcote *et al.* (2002) for a review). These are bound to two related but separate membrane-spanning polypeptides, each of which contains five trans-membrane α -helices. These are named L and M in purple bacteria, and D1 and D2 in photosystem II. Each branch of cofactors consists of two (bacterio)chlorophylls, one (bacterio)pheophytin and one quinone. The branches are arranged around an axis of pseudo twofold symmetry that runs perpendicular to the plane of the membrane.

Transmembrane electron transfer is a strongly asymmetric process in the type II reaction centres, with only the branch of cofactors most closely associated with the L- or D1-polypeptide (the so-called ‘A-branch’) being active in catalysing this process. This reflects the absolute requirement to deliver the electron to the quinone on the A-branch, which acts as the first component of a two electron ‘gate’. This gate converts the one electron charge sep-

aration driven by each quantum of light energy into the two electron reduction of quinone to quinol. The reaction centre is thus able to deliver a source of reducing power to secondary electron transfer, as the quinol can dissociate from the reaction centre and diffuse to the cytochrome *bc*₁ or *b₆f* complex. To achieve this the two quinone cofactors located at the cytoplasmic side of the membrane have very different electrochemical properties. The Q_A quinone operates as a one-electron redox centre, receiving an electron from the reduced A-branch (bacterio)pheophytin and passing it to the Q_B quinone. The Q_B quinone undergoes a two-electron and two-proton redox chemistry to form quinol, and this is free to exchange with other quinones in the intramembrane pool. Because the bound quinones in the *Rb. sphaeroides* reaction centre are chemically identical, these different properties are clearly conferred by the polypeptide binding pockets for the Q_A and Q_B quinones.

A major part of the research carried out on reaction centres over the past 30 years has focused on the type II

Figure 3. Space-filling model of the cyanobacterial photosystem I, with the parts of the complex included in the model of the *Chlorobium limicola* reaction centre highlighted. Colours are as for figure 1.

complexes. There are several reasons for this, including the experimental tractability of the purple non-sulphur bacteria, and the ground-breaking determination of the X-ray crystal structure of the *R. viridis* reaction centre in the mid-1980s. In addition, the photosystem II reaction centre carries out a reaction of enormous importance to aerobes such as ourselves: the photogeneration of molecular oxygen from water. By comparison, much less attention has been focused on the type I reaction centres, despite the fact that some of these complexes carry out another globally important reaction, the photogeneration of the reducing power (NADPH) used during carbon fixation.

Type I reaction centres present several experimental challenges: for example, some bacterial type I complexes are very sensitive to oxygen, being found in strictly anaerobic organisms. However, perhaps the most challenging aspect of these complexes arises from the covalent link between the electron-transferring reaction centre and the surrounding antenna. In type II photosystems, the reaction centre proteins encase between six and eight (bacterio)chlorin-type molecules, and are attached to the extensive system of antenna pigment-protein complexes through non-covalent protein-protein interactions. During the preparation of experimental material for spectroscopic analysis it is possible to isolate the reaction centre from the antenna using biochemical procedures or, in the purple bacteria, through genetic engineering. The type I complexes are organized in a different way, with the same polypeptide binding both antenna and 'electron-transferring' (bacterio)chlorins. As a result, purified type I reaction centres contain up to 100 (bacterio)chlorin molecules, only a few of which are involved in the transmembrane electron transfer reaction. Spectroscopic studies of electron transfer in these complexes are therefore hugely complicated by the presence of a large amount of antenna (bacterio)chlorophyll.

Recently, a spectacular 2.5 Å resolution X-ray crystal structure has been determined for the photosystem I complex of *S. elongatus* (Fromme *et al.* 2001; Jordan *et al.*

2001). This new and detailed structure of a type I reaction centre from an oxygenic organism is producing a resurgence of interest in type I complexes. In addition to variations in structure and organization, differences in the mechanism of transmembrane electron transfer between photosystem I and the type II reaction centres are already becoming apparent. Furthermore, the new structural information has provided insights into the control of function in reaction centres that operate at very low redox potentials, and has prompted new queries about the evolution of photosynthesis and photosynthetic reaction centres.

Just as with the type II reaction centres, the photosystem I complex of oxygenic organisms has a counterpart found in non-oxygenic bacteria, in this case strictly anaerobic phototrophic green sulphur bacteria and heliobacteria. The most distinctive feature of these type I reaction centres is that they contain two copies of a large core polypeptide (i.e. a homodimer), rather than a heterodimeric arrangement of two related, but different, polypeptides as in the photosystem I complex (and in all type II complexes: see Heathcote *et al.* (2002) for a recent review). They also lack several low molecular weight polypeptides found in the photosystem I complex, and contain a much reduced number of antenna chlorophylls. It has been suggested that these somewhat simpler type I reaction centres are the extant descendants of the ancestors of all photosynthetic reaction centres (Baymann *et al.* 2001).

To contribute to this debate, and to compare the structural organization of the two representative forms of type I reaction centre, we have modelled the structure of the central region of the reaction centre from green sulphur bacteria, using sequence alignments and the structural coordinates of the *S. elongatus* photosystem I complex (Jordan *et al.* 2001). This central region is common to all photosynthetic reaction centres, binding the two transmembrane branches of redox cofactors that are also found in all reaction centres. We have also constructed a schematic diagram of the organization of the core antenna in green sulphur bacteria, again using information from sequence alignments and the structure of the photosystem I complex. In this report, we first review the structure and mechanism of the *S. elongatus* photosystem I complex, and compare this with the simpler complex from green sulphur bacteria such as *Chlorobium limicola*. We then describe the outcome of our modelling studies, concentrating on regions of the type I reaction centre where important structure/function relationships have been demonstrated or inferred.

2. MATERIAL AND METHODS

(a) Sequence alignment

The modelling done in this report was based on a protein sequence alignment constructed by Baymann *et al.* (2001). This alignment was derived from computer-based secondary structure predictions and alignment of structural elements (Baymann *et al.* 2001). Final assignments were made manually, taking into account knowledge relating to important residues within the complex (Baymann *et al.* 2001). In this work, we used the sequences for the PsaA and PsaB polypeptides from *Synechocystis* PCC6803 and the PscA polypeptide from *C. limicola*, aligned as in Baymann *et al.* (2001), and added the sequences

for the PsaA and PsaB polypeptides from *S. elongatus* (i.e. the species for which there is an X-ray crystal structure). The equivalent sequences from *Synechocystis* and *Synechococcus* are highly homologous, but not identical. Part of the resulting alignment is shown in figure 4.

(b) *Antenna modelling*

Modelling of the antenna (bacterio)chlorophyll molecules that are likely to be conserved between cyanobacteria and green sulphur bacteria was performed by searching the alignment and noting the (bacterio)chlorophyll cofactors for which there was conservation of the amino acid that donates the axial ligand. A schematic model of the possible organization of the antenna in green sulphur bacteria was then produced by selecting the relevant chlorophyll cofactors from the X-ray crystal structure of photosystem I from *S. elongatus* (RCSB databank, deposition code 1JB0).

(c) *Reaction centre modelling and minimization*

Homology modelling of the central 10-helix motif of the *C. limicola* reaction centre was performed using the program XFIT, part of the XTALVIEW suite of programs (McRee 1992). The 1JB0 coordinate set of the *S. elongatus* photosystem I was cropped to leave only the regions encompassing residues A431–A755 and B411–B739. The reaction centre from *C. limicola* is homodimeric; however for comparison the residue numbering of the final model reflects the initial heterodimeric 1JB0 model. This central core was then extensively rebuilt to conform to the sequence of the *C. limicola* complex. The sections remodelled include all 10 transmembrane α -helices of the reaction centre core, along with the connecting cd- and de-helices and residues within important loop regions (e.g. the F_x -binding loop). Residues were individually replaced and the favoured rotamers of each new side chain were investigated to limit the number of steric clashes within the starting model. The reaction centre of *C. limicola* has a primary electron donor that is thought to consist of two C13² epimers of bacteriochlorophyll *a* (Kobayashi *et al.* 2000). The primary donor chlorophylls from the 1JB0 structure were transformed into this different structure and stereochemistry using the program INSIGHT II, v. 2.3.0. (Molecular Simulations, San Diego, CA.). The final pre-minimization model was given the code COREMOD1.

The coordinates of COREMOD1 were prepared for energy minimization in the following manner. The addition of hydrogens and force-field parameterization was performed using INSIGHT II, and the complex was soaked in a 5 Å shell of water. Energy minimization was then performed using the program DISCOVER, v. 2.9.7. (Molecular Simulations) with the protein backbone tethered to its crystallographic coordinates. Initial energy minimization was done for 500 iterations of steepest descents with a backbone tethering force constant of 100 kcal Å⁻². This was then followed by 500 iterations of conjugate gradient minimization, with a force constant of 100 kcal Å⁻². Further rounds of conjugate gradient minimization were performed, relaxing the backbone tethering force constant each round; 500 iterations each at 50, 20, 10, 5 and 1 kcal Å⁻². Finally, 1000 iterations of conjugate gradient minimization were performed with no applied backbone tethering, to give a final average absolute derivative of energy with respect to the coordinates of 0.012 kcal Å⁻². The resulting minimized model was named COREMOD2. The figures were prepared using MOLSCRIPT (Kraulis 1991) and RASTER3D (Merritt & Bacon 1997).

3. RESULTS AND DISCUSSION

(a) *The photosystem I complex of oxygenic photosynthesis*

(i) *Structure of the protein/cofactor system*

The heterodimeric core of the photosystem I reaction centre of oxygenic photosynthetic organisms is made up of two polypeptides termed PsaA and PsaB (green and maroon, respectively, in figure 1b). Both of these related polypeptides have 11 membrane-spanning α -helices, and they form a heterodimeric structure around an axis of pseudo twofold symmetry (Fromme *et al.* 2001; Jordan *et al.* 2001). This structure accommodates around 90 light-harvesting chlorophylls, in addition to six chlorophylls that are associated with the electron transfer chain. In addition to the two core polypeptides these photosynthetic reaction centres contain several smaller polypeptides. Cyanobacteria such as *S. elongatus* contain 12 polypeptides, PsaA, B, C, D, E, F, I, J, K, L, M and X (Jordan *et al.* 2001; Xu *et al.* 2001). Three extrinsic subunits are bound to the stromal (eukaryotes) or cytoplasmic (cyanobacteria) side of the membrane. PsaC contains the two terminal iron-sulphur centres of the electron transfer chain, and PsaD and PsaE stabilize and facilitate the interaction between PsaC, PsaA/B and the soluble ferredoxin that accepts electrons from photosystem I.

The remaining minor polypeptides are integral membrane proteins and some of these serve different functions in the cyanobacterial and eukaryotic photosystems (Scheller *et al.* 2001; Xu *et al.* 2001). PsaI, PsaL and PsaM are located at the interface between the monomers of photosystem I that form a trimer in the membranes of cyanobacteria. PsaF, PsaJ, PsaK and PsaX are located at the interface between the monomers of photosystem I and the lipid membrane (or the detergent-exposed surface in the crystallized complex), and play a part in stabilizing the complex. In higher plants there are several additional polypeptides not found in cyanobacteria, namely PsaG, H, N and O (Scheller *et al.* 2001). Some of these play a part in facilitating the interaction between photosystem I and the associated light-harvesting complexes (i.e. the Lhc1–4 pigment protein complexes).

During the past 10 years, the groups of Witt and Saenger in Berlin have worked to resolve the X-ray crystal structure of the photosystem I reaction centre isolated from the thermophilic cyanobacterium *S. elongatus*. Initial reports of the structure at 4 Å resolution (Fromme *et al.* 1996; Krauss *et al.* 1996; Schubert *et al.* 1998; Klukas *et al.* 1999) showed that the complex is organized into three principal domains. The C-terminal regions of the PsaA and PsaB polypeptides were observed to each contribute five membrane-spanning α -helices to a heterodimeric central core domain that houses the electron transfer cofactors, and exhibits a two-fold symmetry. This part of the complex shows structural homology to the L/M and D1/D2 motifs in the type II reaction centres. The N-terminal regions of the PsaA and PsaB polypeptides were each observed to contribute six membrane-spanning α -helices to antenna domains located on either side of the core domain. These six helix domains have structural homologies to the core antenna CP47 and CP43 pigment proteins of photosystem II.

These groups have recently published a new structure of the cyanobacterial photosystem I complex at 2.5 Å resolution (Jordan *et al.* 2001). This is a considerable achievement, as this reaction centre crystallizes as a trimer, and each monomer contains 12 polypeptides and a total of 127 cofactors, and has a molecular mass of 340 kDa. The cofactor content of this protein is relatively high at *ca.* 30% of the dry weight of the protein. The polypeptide structure of a monomer of this reaction centre is summarized in figure 1*b* and has been described in detail (Fromme *et al.* 2001; Jordan *et al.* 2001).

(ii) *The electron transfer chain of the photosystem I reaction centre*

The electron transfer chain in type I reaction centres is more extensive than that in type II reaction centres, and is shown in figure 1*a*. On one side of the membrane an asymmetric dimer comprising a chlorophyll *a* (bound to PsaB) and a C13² chlorophyll *a* epimer (bound to PsaA) form the primary donor of electrons (termed P₇₀₀). Leading from this are two transmembrane branches of cofactors, each consisting of two molecules of chlorophyll *a* and one quinone, which converge again at the F_X iron–sulphur centre on the opposite side of the membrane to P₇₀₀ (figure 1*a*).

The first spectroscopically resolvable electron acceptor in transmembrane electron transfer is a chlorophyll termed A₀, located approximately halfway across the membrane (figure 1*a*). Between A₀ and P₇₀₀ is a second chlorophyll (termed A) that occupies a position analogous to that occupied by the so-called accessory bacteriochlorophyll in the purple bacterial reaction centre. By analogy with the purple bacterial reaction centre, the A chlorophyll probably acts as an electron transfer intermediate between P₇₀₀ and A₀. Interestingly, of the six chlorophyll *a* molecules involved in the electron transfer chain in photosystem I, only the two forming the P₇₀₀ primary donor have histidines as axial ligands to the central magnesium atom of the chlorophyll macrocycle (see below).

The secondary electron acceptor (A₁) is a phyloquinone in cyanobacteria (figure 1*a*). The electron transfer chain terminates with three bound iron–sulphur clusters (Fe₄S₄ centres) (see Vassiliev *et al.* (2001) for a review of these iron–sulphur centres). One of these, the F_X centre, is located on the axis of twofold symmetry, and accepts an electron from the phyloquinone (or A₁) redox centre(s). Its position is similar to that occupied by the non-haem iron atom in the purple bacterial reaction centre (Heathcote *et al.* 2002), and both have a structural role. The F_X centre is coordinated by four cysteine residues, two located in the PsaA protein and two in the PsaB protein, and is therefore a relatively rare example of an interpolypeptide iron–sulphur cluster. The remaining iron–sulphur centres, F_A and F_B, are located in the extrinsic polypeptide PsaC (cyan ribbons at the top of figure 1*b*).

(iii) *The kinetics of electron transfer in the photosystem I reaction centre*

Although the components of the electron transfer chain in photosystem I were identified many years ago, demonstration of the kinetic competence of all of the components, and particularly confirmation that both the quinone and F_X iron–sulphur centre are obligatorily involved, has

only been obtained relatively recently (Luneberg *et al.* 1994; Moenne-Loccoz *et al.* 1994; Van der Est *et al.* 1994). The phyloquinone to F_X electron transfer step is in fact the only reaction that is easy to study (see below). The large number of chlorophyll antenna molecules in photosystem I mask the small absorbance changes associated with oxidation and reduction of the iron–sulphur centres, and the decay of excited states in these antenna chlorophylls mask the ultrafast absorbance changes associated with electron transfer from P₇₀₀ through to the A₀ acceptor. However, it has been established that electron transfer from P₇₀₀ to the A₁ acceptor in photosystem I is analogous to the reaction seen in type II reaction centres (Brettel & Liebl 2001; Gobets & Van Grondelle 2001). Charge separation between P₇₀₀ and the primary electron acceptor A₀ occurs in less than 1 ps (10^{−12} s), and the subsequent reduction of the phyloquinone electron acceptor A₁ takes from 20–50 ps. This process is an order of magnitude faster than the analogous process in the type II reaction centre, and it is thought that this is necessary to trap excitons before they can migrate back to the antenna chlorophylls from P₇₀₀. This requirement stems from the close proximity of the reaction centre chlorophylls to the antenna chlorophylls in the type I complexes.

The re-oxidation of the phylosemiquinone A₁[−] by F_X can be monitored by optical spectroscopy in the near ultraviolet, and by time-resolved EPR detection of the decay of the ESP signal arising from the P₇₀₀⁺/A₁[−] geminate radical pair generated by a nanosecond excitation flash. Several groups have used biochemical removal of the iron–sulphur centres to show that oxidation of the semiquinone with a lifetime of 200 ns represents the rate of electron transfer from A₁ to F_X (Luneberg *et al.* 1994; Moenne-Loccoz *et al.* 1994; Van der Est *et al.* 1994).

The interaction between the bound phylosemiquinone and the surrounding protein can be studied using ENDOR (Rigby *et al.* 1996) and electron spin echo envelope modulation (ESEEM) paramagnetic resonance spectroscopy of the phylosemiquinone (Hanley *et al.* 1997). Chemical reduction of the phyloquinone is not possible given its low redox potential; however, it can be reduced by photoaccumulation in the presence of a reductant at 205 K (Heathcote *et al.* 1996). The proton ENDOR spectrum of the phylosemiquinone (Rigby *et al.* 1996) indicates an altered electronic structure compared with that of phylosemiquinone *in vitro*, with a different hydrogen bond to each of the two carbonyl oxygens of the quinone head-group. By contrast, the X-ray crystal structure (Jordan *et al.* 2001) identifies only one potential hydrogen-bond donor that is within hydrogen-bonding distance of these carbonyl oxygens. One possibility is that the hydrogen-bond interactions between the quinone head-group and the surrounding protein vary according to the redox state of the quinone, as the phyloquinone is in the unreduced state in the X-ray crystal structure, whereas ENDOR spectroscopy probes the semiquinone state.

(iv) *Transmembrane electron transfer: symmetry or asymmetry?*

The X-ray crystal structure of the photosystem I reaction centre raises an intriguing question: which of the two symmetrically arranged phyloquinones is the A₁ electron acceptor? Likewise, is the A₀ electron acceptor the chloro-

Figure 4. Parts of an alignment of protein sequences for PsaA and PsaB of the cyanobacterial photosystem I and PscA of the *Chlorobium limicola* reaction centre (see text for details).

phyll most closely associated with PsaA or PsaB? In type II reaction centres, transmembrane electron transfer is a highly asymmetric process, using only one of the two available cofactor branches. As outlined above, this asymmetry is associated with the different redox chemistries of the two quinones: the Q_A quinone operates as a tightly bound one-electron carrier whereas the Q_B quinone undergoes a two-electron/two-proton chemistry, and through dissociation provides an exit point for reducing equivalents from the complex. The situation in type I reaction centres is different, however, as the accepted mechanism is one in which the quinone passes a single electron from the A_0 acceptor to the F_X iron-sulphur centre (which is located on the axis of pseudo twofold symmetry). As a result it seems possible that both branches of cofactors could be active in transferring electrons from P_{700} to F_X , although the different amino-acid sequences of the PsaA and PsaB proteins could confer a functional asymmetry on the com-

plex in the same way that the L/M or D1/D2 proteins achieve this in the type II reaction centres.

One way to tackle this question is to use mutagenesis to disturb one of the cofactor branches and look at the effect on electron transfer. As an illustration of this approach, Purton *et al.* (2001) have recently mutated a highly conserved tryptophan residue that engages in a stacking interaction with the phyloquinone on the PsaA side of the reaction centre (on the left in figure 1a). This tryptophan (residue 693 of PsaA) was mutated to His (PsaAW693H) or Leu (PsaAW693L). Spectroscopic analysis demonstrated that both the 200 ns re-oxidation of A_1^- and the phylosemiquinone photo-accumulated at 205 K can be attributed to electron transfer on the PsaA cofactor branch, as both parameters were affected by these mutations.

During the past three years, strong evidence has been presented in favour of the involvement of both cofactor

branches in transmembrane electron transfer in the photosystem I reaction centre. The key results have come from the development of new spectroscopic assays, and the analysis of site-directed mutants of the unicellular green alga *Chlamydomonas* (*Cd.*) *reinhardtii*. First, using a new optical spectrometer that allowed measurements on whole cells of the alga *Chlorella*, Joliot & Joliot (1999) obtained evidence for two rates of oxidation of the A_1 acceptor at room temperature (20 and 140 ns, with approximately equal amplitudes). Most recently, Joliot and co-workers have investigated the two rates of reoxidation of A_1^- in whole cells of wild-type *Cd. reinhardtii*, and in mutants where the tryptophan that stacks against the phyloquinone bound by PsaA and PsaB has been replaced by phenylalanine (PsaAW693F and PsaBW673F, respectively) (Gueorgova-Kuras *et al.* 2001). In the PsaAW693F mutant the 'slow' 140 ns component of A_1^- oxidation was further slowed to 490 ns, whereas in the PsaBW673F mutant the 'fast' 13 ns component was slowed to 70 ns. The implication of these results is that both phyloquinones are capable of being photoreduced by electron transfer from P_{700} , and that they pass electrons on to the F_X iron-sulphur centre at different rates. Transmembrane electron transfer from P_{700} to F_X therefore involves both cofactor branches, with a roughly equal likelihood of electrons proceeding down the PsaA or PsaB branch after photoexcitation. However, this conclusion is still disputed, mainly because some groups are not convinced that the optical measurements are detecting electron transfer on the two branches of electron transfer in photosystem I. However, there is general agreement that the EPR measurements described above are monitoring the PsaA branch of electron transfer, and therefore that the PsaA branch of electron transfer is active.

Subsequent to this work, new paramagnetic resonance spectroscopic assays of electron transfer and protein-cofactor interactions on the PsaB branch of photosystem I have been developed, with the result that it has been possible to photo-accumulate the phylosemiquinone on the PsaB branch (Rigby *et al.* 2002). Although forward electron transfer on the PsaB side from A_1^- is too fast to measure by EPR, occurring on a time-scale of a few tens of nanoseconds, the ESP signal measured at 100 K can be used to monitor forward electron transfer from A_1^- (Muhiuddin *et al.* 2001). At this temperature the rates recorded probably reflect the influence of the two different protein environments (PsaA and PsaB) on the decay of the correlation of the P_{700}^+/A_1^- geminate radical pair, rather than the recombination between P_{700}^+ and A_1^- . This is because the rates obtained are an order of magnitude faster than the reported rate for electrons to return to P_{700}^+ from A_1^- . One decay rate can be observed in frozen photosystem I which can be attributed to electron transfer on the PsaA side of the reaction centre, whereas two rates of decay can be observed if the F_X electron acceptor is prereduced to block electron transfer from the PsaB side to F_X (Muhiuddin *et al.* 2001). These results have been interpreted as indicating that electron transfer from P_{700} can occur to either the PsaA or PsaB side phyloquinone at 100 K, in both *Cd. reinhardtii* and the cyanobacterium *S. elongatus*. In addition, a mutant has been constructed of the methionine axial ligand to the A_0 electron acceptor on the PsaA branch of electron transfer, which is generally

Figure 5. Arrangement of the P_{840} primary donor bacteriochlorophylls in the model of the *Chlorobium limicola* reaction centre (cpk colours). Also shown are the histidines that provide the axial ligands to the central magnesium atoms of the macrocycles. The P_{840} bacteriochlorophylls are overlaid on the P_{700} dimer from the cyanobacterial photosystem I (orange).

accepted to be active. This mutation (PsaA M684H) in *Cd. reinhardtii* blocks forward electron transfer on the PsaA branch of electron transfer, but not on the PsaB branch, as monitored by EPR (Fairclough *et al.* 2001). It is significant that this mutant can grow photoautotrophically, demonstrating conclusively that not only is the PsaB branch active in electron transfer, but that PsaB electron transfer on its own is sufficient for growth.

The results summarized above clearly indicate that electron transfer occurs through both cofactor branches of the photosystem I reaction centre, albeit at slightly different rates. The differences in rate presumably stem from inequivalences in the two cofactor branches, arising from the different sequences of the PsaA and PsaB protein scaffolds. In one respect it is entirely logical that both cofactor branches should be active in this complex, as their role is to pass electrons from a redox centre on the symmetry axis at one side of the membrane to a redox centre on the symmetry axis at the opposite side. However, it is perhaps tempting to speculate that the two branches have different roles.

Another interesting question is why a complex that uses both branches to carry out transmembrane electron transfer would have evolved a markedly asymmetric P_{700} primary electron donor. In photosystem I, the P_{700} dimer consists of a C13² epimer of chlorophyll *a* that is extensively hydrogen bonded to the PsaA polypeptide, and a chlorophyll *a* that lacks equivalent hydrogen-bond interactions with the PsaB polypeptide. Despite this structural asymmetry, P_{700} can apparently transfer electrons to both the PsaA and PsaB branches with approximately equal probability.

(v) *Protein-cofactor interactions that allow photosystem I to function at relatively low redox potentials*

A particular feature of the photosystem I reaction centre is the requirement to carry out redox chemistry at very low redox potentials. The role of the complex is to reduce ferredoxin, at a redox potential of around -420 mV. By contrast, type II reaction centres reduce quinone to quinol, at redox potentials around 0 mV. To operate at such low redox potentials, the photosystem I reaction centre uses chlorophyll *a* as the initial electron acceptor, rather than the (bacterio)pheophytin used in type II reaction centres. Similarly, the next redox centre in the chain is naphthoquinone or phyloquinone rather than the ubiquinone or plastoquinone found in type II reaction centres. Although isolated chlorophyll *a* and naphthoquinone/phyloquinone function as single electron acceptors at relatively low redox potentials in solvent, available evidence indicates that the photosystem I protein further tunes the redox properties of these cofactors to enable them to function at even lower potentials. The primary electron acceptor (A_0) chlorophyll *a*/chlorophyll *a* anion redox couple in photosystem I functions at -1050 mV, as opposed to -900 mV for isolated chlorophyll *a* in solvent (Brettel 1997). The secondary electron acceptor (A_1) phyloquinone/phylosemiquinone redox couple functions at -800 mV in photosystem I, whereas it functions at -170 mV in water (Swallow 1982) and -570 mV in the less polar dimethylformamide (Schmid *et al.* 1999) (see Brettel & Liebl (2001) for a discussion).

Based on spectroscopic evidence, and the 2.5 Å X-ray crystal structure of the *S. elongatus* photosystem I complex, it has been suggested that the low operating redox potential of the chlorophyll A_0 is a result of having an unusual weak axial ligand to the central magnesium, donated by the sulphur of a methionine side chain (Fromme *et al.* 2001; Jordan *et al.* 2001). Certainly no other example of a sulphur ligand being used in this way can be found in the protein structure database. Not only would the methionine offer a very weak ligand, but it would also prevent water approaching to offer a stronger ligand (Fromme *et al.* 2001; Jordan *et al.* 2001).

A second proposal is that the low redox potential of the phyloquinone A_1 is a result of having only one available hydrogen bond to the oxygens of the two carbonyl groups located on opposite sides of the phyloquinone head-group (Fromme *et al.* 2001; Jordan *et al.* 2001; Kaupp 2002). Based on the X-ray crystal structure the hydrogen-bond donor is proposed to be the backbone amino group of a leucine residue, resulting in asymmetric hydrogen bonding to one of the two carbonyl oxygens. Other features in the binding pocket suggested as having a possible effect on the redox potential of A_1 are a relatively apolar environment (i.e. low dielectric constant), electrostatic effects from nearby negative charges, and a π - π interaction between the phyloquinone ring and an adjacent tryptophan residue (see above) (reviewed in Brettel & Liebl (2001)).

(vi) *The antenna chlorophylls and carotenoids of photosystem I*

The light-harvesting chlorophylls of the *S. elongatus* photosystem I complex (figure 2a) also exhibit some novel binding interactions with the surrounding protein. Of the

79 antenna chlorophylls coordinated by the PsaA and PsaB polypeptides, only 48 are bound to the transmembrane α -helices, the remainder being bound to the loop regions on either side of the membrane (Fromme *et al.* 2001; Jordan *et al.* 2001). In most of these chlorophylls, the central magnesium atom engages in a fifth (axial) bonding interaction with a histidine imidazole side chain, as is seen in the reaction centre of purple photosynthetic bacteria. However, in other chlorophylls this axial ligand is formed by an oxygen atom of a glutamine, glutamate, aspartate or tyrosine side chain, by a peptide carbonyl oxygen, or by a water molecule (Fromme *et al.* 2001; Jordan *et al.* 2001). In one surprisingly novel interaction, the axial ligand for one chlorophyll is provided by the head-group of a molecule of phosphatidylglycerol (Fromme *et al.* 2001; Jordan *et al.* 2001).

The antenna chlorophylls are organized into three domains (Jordan *et al.* 2001), with a central domain surrounding but separated from the electron transfer chain, and spanning the membrane. There are two more remote domains of antenna, organized on either side of the membrane-embedded region of the photosystem I complex. Energy transfer from the antenna to the electron transfer chain and P_{700} is rapid and extremely efficient (Gobets & Van Grondelle 2001), facilitated by the proximity of the antenna chlorophylls to each other in this highly absorbing and densely packed pigment array.

Two particular chlorophylls have been identified as possibly providing a connection between the antenna chlorophylls and the chlorophylls of the electron transfer chain (Fromme *et al.* 2001; Jordan *et al.* 2001; figure 2a). These chlorophylls (ac-A40 on the PsaA polypeptide, ac-B39 on PsaB polypeptide) are in the middle of the membrane, and close (12.8 Å and 10.9 Å, respectively) to the A_0 electron acceptor chlorophylls (Jordan *et al.* 2001). As a result it has been suggested (Fromme *et al.* 2001) that electron transfer could be initiated by charge separation from A_0 rather than P_{700} , although other approaches based upon modelling and measurements of rates of energy transfer (reviewed by Gobets & Van Grondelle (2001)) have indicated that these 'connecting' chlorophylls are not an essential component of energy transfer to the chlorophyll of the electron transfer chain.

Partly because of the different types of axial coordination, the chlorophylls in photosystem I absorb over a wide range, including some that absorb at a longer wavelength than P_{700} (the long wavelength or 'red' chlorophylls). These long wavelength chlorophylls could be responsible for capturing light energy in the red when shaded by other photosynthetic organisms and/or directing and retaining excitation energy close to the electron transfer chain (reviewed in Fleming & Van Grondelle 1997). Although the location and identity of these pigments is still debated, the structure at 2.5 Å did reveal several tightly interacting chlorophylls that form dimers and trimers (Fromme *et al.* 2001; Jordan *et al.* 2001; in red in figure 2a). These could be excitonically coupled, and are possible candidates for some of the long wavelength chlorophylls (Fromme *et al.* 2001; Jordan *et al.* 2001).

(b) *The reaction centres of heliobacteria*

Heliobacteria were only discovered in the early 1980s, and contain a unique bacteriochlorophyll *g*. The heliobacteria contain no light harvesting pigments or pigment-pro-

teins, and rely on a single polypeptide to form the homodimeric reaction centre. The reaction centre of heliobacteria (reviewed in Neerken & Ames 2001) contains 37 bacteriochlorophylls in addition to the six chlorins that form the two branches of electron transfer. The electron donor P_{798} is thought to be a dimer of bacteriochlorophyll *g* epimers. The primary electron acceptor A_0 in heliobacteria is thought to be 8¹-hydroxychlorophyll *a*. Because heliobacteria lack the PsaC equivalent it is hard to see how they could contain the bound iron–sulphur centres equivalent to F_A and F_B , and evidence for the presence of F_X is not conclusive.

Evidence has been presented to indicate that heliobacteria contain a naphthoquinone in the reaction centre that can be reduced by photoaccumulation (Muhiuddin *et al.* 1999), but the participation of this quinone in forward electron transfer has not been conclusively demonstrated (Neerken & Ames 2001).

Because the homology between the redox cofactors and electron transfer chain of photosystem I and heliobacteria breaks down at the level of the iron–sulphur centres, we chose to model the central electron transfer region of green sulphur bacteria.

(c) *The reaction centres of green sulphur bacteria*

(i) *Structure of the protein/cofactor system*

Photosynthetic growth of the green sulphur bacteria is supported by a type I reaction centre that has many similarities to the photosystem I reaction centre, but is much simpler in terms of polypeptide composition (see figure 1c). The green sulphur bacterial reaction centre has only four subunits, equivalent to the two core polypeptides of photosystem I and the PsaC and PsaD extrinsic proteins (reviewed in Hauska *et al.* 2001). Although the photosystem I reaction centre of oxygenic photosynthetic organisms contains a core heterodimer of related but different polypeptides (PsaA and PsaB), the reaction centre of green sulphur bacteria has a homodimeric core of two identical polypeptides termed PscA (Büttner *et al.* 1992). This is because these organisms appear to contain only one gene for the core polypeptides of the reaction centre, rather than the two genes seen in cyanobacteria, algae and higher plants.

The green sulphur bacteria also contain several novel light-harvesting pigment proteins. The Fenner–Matthews–Olsen pigment protein is unique in that it contains no carotenoids, and the chlorosome structures bound on the cytoplasmic membrane surface contain up to 10 000 bacteriochlorophylls that, uniquely, are not bound to protein.

(ii) *Redox cofactors and electron transfer in the reaction centres of green sulphur bacteria*

The reaction centres of green sulphur bacteria contain bacteriochlorophyll *a* and chlorophyll *a*. However, although the core proteins (the two copies of PscA) are of comparable size to PsaA and PsaB they bind far fewer pigments, with only eight bacteriochlorophylls and two chlorophylls per PscA (Permentier *et al.* 2000). This means there are only 20 chlorins per reaction centre, in comparison with the 96 bound by the PsaA and PsaB polypeptides in photosystem I. There are also only two carotenoids per reaction centre in contrast to the 22 carotenoids bound to the

photosystem I reaction centre, perhaps reflecting a reduced need for photoprotection in this strictly anaerobic phototrophic organism.

The primary electron donor, termed P_{840} in green sulphur bacteria, may well be composed of two C13² epimers of bacteriochlorophyll *a* (Kobayashi *et al.* 2000). The accessory chlorophylls are presumed to be present by analogy, but it is not known whether these are bacteriochlorophyll or chlorophyll. The primary electron acceptor A_0 is known to be chlorophyll *a* esterified to 2,6-phytanediol (Van de Meent *et al.* 1992; Feiler *et al.* 1994). Because there are two other such chlorophyll-like molecules in this reaction centre, in addition to the two A_0 molecules, these may well be in the positions occupied by the accessory chlorophylls (A) in photosystem I (figure 1a).

The remaining electron acceptor molecules include iron–sulphur centres, starting with centre F_X as in photosystem I. From the latter, the electron is transferred to the terminal electron acceptors F_A/F_B . In the green sulphur bacteria these are located on a membrane extrinsic protein subunit termed PscB, that resembles the PsaC subunit present in photosystem I. All three of these iron–sulphur centres have been detected in the reaction centre of green sulphur bacteria using EPR spectroscopy (Vassiliev *et al.* 2001).

Conclusive evidence for the participation of a quinone electron acceptor functioning between A_0 and F_X in these bacterial type I reaction centres is still lacking. For many years there was considerable controversy concerning the nature and role of such an electron acceptor in photosystem I. Similar controversies now apply to the reaction centres of green sulphur bacteria and heliobacteria (reviewed in Muhiuddin *et al.* 1999; Hauska *et al.* 2001). Quinone species have been identified in both heliobacteria and green sulphur bacteria that, in principle, could perform the role of an A_1 electron acceptor. Menaquinones are a family of quinone compounds that are closely related to phyloquinone, and menaquinone-7 has been identified in the green sulphur bacteria, in addition to the so-called *Chlorobium* quinone and 1'-hydroxymenaquinone-7 (Powls & Redfearn 1969). However, photochemically active reaction-centre preparations that lack menaquinone have been isolated from green sulphur bacteria, calling the involvement of menaquinone into question (reviewed in Hauska *et al.* 2001). The reoxidation of A_0 apparently occurs in *ca.* 600 ps in green sulphur bacteria (Nuijs *et al.* 1985), and this is much slower than the 20–50 ps reported for this reaction in photosystem I (reviewed in Gobets & Van Grondelle (2001)). Although an EPR signal that can be attributed to a menasemiquinone can be photoaccumulated in membranes from green sulphur bacteria (Muhiuddin *et al.* 1999), indicating that the reaction centres contain a quinone that can be reduced by photosynthetic electron transfer, there is no evidence that such a quinone is kinetically competent to function in forward electron transfer to the iron–sulphur centres.

(iii) *Alignment-based modelling of the antenna in green sulphur bacteria*

To investigate the possible structure of the reaction centre from green sulphur bacteria, we have modelled aspects of this complex using an alignment of the *C. limicola*

Figure 6. Binding of the A_0 chlorophylls in photosystem I and the model of the *Chlorobium limicola* reaction centre. (a) The A-side A_0 chlorophyll in the X-ray crystal structure of the cyanobacterial photosystem I. (b) Model of the A-side A_0 chlorophyll in *C. limicola*.

Figure 7. Binding of the A_1 quinones in photosystem I and the model of the *Chlorobium limicola* reaction centre. (a) The A-side A_1 quinone in the X-ray crystal structure of the cyanobacterial photosystem I. (b) Model of the A-side A_1 quinone in *C. limicola*. The grey ribbon shows part of the protein backbone.

PscA polypeptide with the cyanobacterial PsaA and PsaB polypeptides. The alignment is based on that constructed by Baymann *et al.* (2001), and is described in § 2.

As described above, the reaction centre from green sulphur bacteria such as *C. limicola* has far fewer antenna (bacterio)chlorophylls than its cyanobacterial counterpart. Accounting for the six (bacterio)chlorins of the electron transfer chain, the antenna in *C. limicola* is expected to consist of 14 bacteriochlorophyll *a* molecules (assuming that the four chlorophylls are located in the electron transfer chain rather than the antenna). To investigate the possible organization of the antenna in *C. limicola*, the sequence alignment was used to search for conservation of residues that have been shown, from the structure of the cyanobacterial photosystem I, to donate axial ligands to the antenna chlorophylls. The model shown in figure 2b was then constructed by taking the 1JBO coordinate set for the cyanobacterial photosystem I, and deleting those antenna chlorophylls whose axial ligand was not conserved in the sequence of *C. limicola* PscA.

The resulting model contained the expected number of 14 antenna bacteriochlorophylls, with equal numbers bound to the two copies of PscA. Figure 2a shows the cofactors of the electron transfer chain of the cyanobacterial reaction centre, and the disposition of the surrounding antenna chlorophylls, whereas figure 2b shows the same

view of the modelled organization of the antenna bacteriochlorophylls in *C. limicola*. All 14 of these antenna bacteriochlorophylls had a histidine as the donor of the axial ligand, and 12 of the 14 are arranged in symmetry-related pairs. Figure 2a also shows, in dark blue, the two 'linker' chlorophylls (A40 and B39) that are proposed to connect the antenna chlorophylls to the chlorophylls of the electron transfer chain (Fromme *et al.* 2001; Jordan *et al.* 2001). These chlorophylls were not conserved in our model of the *C. limicola* reaction centre. Finally, the chlorophylls highlighted in red in figure 2a are those dimers and trimers suggested to be the long wavelength chlorophylls (Fleming & Van Grondelle 1997; Fromme *et al.* 2001; Jordan *et al.* 2001). Only one of these chlorophylls (from a dimer) was conserved in our model of the *C. limicola* antenna (top right in figure 2b).

To summarize, a simple homology model based on sequence conservation predicts the correct number of antenna bacteriochlorophylls in the *C. limicola* reaction centre, all of these predicted antenna bacteriochlorophylls are coordinated to histidine axial ligands, and figure 2b shows their possible disposition relative to the electron transfer chain. However, none of the antenna (bacterio)chlorophylls proposed to have a specialist function or specialist properties in photosystem I are conserved in *C. limicola*.

(iv) *Homology modelling of the reaction centre core in green sulphur bacteria*

To investigate the possible arrangement of the electron transfer chain and surrounding protein in *C. limicola*, the central 10-helix motif that encases the electron transport chain in this bacterium was modelled by homology with the analogous region of the cyanobacterial photosystem I reaction centre. The region included in this model is highlighted in figure 3, and the details of the modelling procedure are given in § 2. The alignment used to guide this modelling process is shown in figure 4, and as described in § 2 was based on an alignment constructed by Baymann *et al.* (2001).

Figure 5 shows the possible arrangement of the P₈₄₀ primary donor in *C. limicola* (cpk colours) overlaid on the P₇₀₀ dimer from the cyanobacterial photosystem I (orange). P₈₄₀ was modelled as being a dimer of the C13² epimer of bacteriochlorophyll *a*, in line with proposals (Kobayashi *et al.* 2000). In the final model two histidine residues, A680 and B660, were in a suitable position to form the fifth axial ligands to the magnesiums of the P₈₄₀ macrocycles, in accord with their conservation in the protein sequence alignment (figure 4). A feature of P₇₀₀ in the cyanobacterial photosystem I is the asymmetric nature of the hydrogen-bond interactions between the chlorophylls and the surrounding protein. The chlorophyll half of the dimer (PsaB polypeptide) is free from such interactions, whereas the chlorophyll epimer (PsaA polypeptide) engages in three hydrogen-bond interactions. In the modelled P₈₄₀ dimer, both bacteriochlorophyll epimers were free from hydrogen-bond interactions.

Turning to the accessory or A chlorophylls, in the cyanobacterial photosystem I complex the axial ligand to the central magnesium of both A chlorophylls is donated by a water molecule that, in turn, is connected to an asparagine. In the sequence of the *C. limicola* PscA polypeptide this asparagine is replaced by a lysine, and in the structural model of the PscA dimer each of the two lysines adopted a conformation that was consistent with liganding of the central magnesium of the A chlorophyll (data not shown).

The A₀ chlorophylls in photosystem I are required to operate at a very low redox potential and, as described above, one factor that may enable this is an unusual arrangement where the sulphur of a methionine side chain provides the axial ligand to the central magnesium (figure 6a). According to the sequence alignment (figure 4) these methionines, A688 and B668, are replaced by non-bonding alanine residues. In the structural model of the *C. limicola* reaction centre the axial ligand to each of the A₀ chlorophylls appeared to be provided by glutamic acid, these being residues A692 and B672. The arrangement for the A-side A₀ chlorophyll is shown in figure 6b. In the cyanobacterial photosystem I these glutamic acid residues are both serines (see figure 6a). In addition to this, the nine-keto carbonyl of the A₀ chlorophyll of photosystem I engages in a hydrogen-bond interaction with tyrosine A696 (A-side) and tyrosine B676 (B-side). These tyrosines are serine in the *C. limicola* PscA polypeptide. In the structural model of the *C. limicola* reaction centre an alternative hydrogen-bond interaction with a glutamine (A700

or B680) appeared to be possible (figure 6b). These glutamine residues are both leucine in the cyanobacterial photosystem I.

The A₁ quinone acceptors in the cyanobacterial photosystem I reaction centre engage in hydrogen-bond interactions between the carbonyl group of the quinone head-group and the backbone amide of Leu A722 (A-side) or Leu B706 (B-side). These interactions are conserved in the model of the *C. limicola* reaction centre (figure 7). As with the photosystem I reaction centre there is no obvious interaction between the second carbonyl group and the surrounding protein for either A₁ quinone. The π -stacking interactions between the A₁ quinones and Trp A697 (A side) (figure 7a) or Trp B677 (B-side) are not conserved, as this tryptophan is replaced by an arginine in PsaC. In the model of the *C. limicola* reaction centre this arginine residue packed adjacent to the A₁ quinone (figure 7b).

As discussed above, the involvement of an A₁ menaquinone in electron transfer from A₀ to F_x in green sulphur bacteria has been called into question following the isolation of photochemically active reaction centre preparations that lack menaquinone (reviewed in Hauska *et al.* 2001). However, this observation has to be rationalized with finding that re-oxidation of A₀ apparently occurs in *ca.* 600 ps in green sulphur bacteria (Nuijs *et al.* 1985). In the model of the *C. limicola* reaction centre the shortest edge-to-edge distance between the A₀ chlorophylls and the F_x centre was 17.3 Å for the A-side and 17.1 Å for the B-side. According to the equation derived by Moser & Dutton (1996) for energetically optimized electron transfer reactions in proteins, the fastest time constant for electron transfer over such distances should be 24 μ s and 18 μ s, respectively; the observed rate is *ca.* 3×10^5 faster than the observed rate (Nuijs *et al.* 1985). Furthermore, from scrutiny of the model there is nothing unusual about the protein environment between A₀ chlorophylls and the F_x centre that could account for such a rapid rate. The distances between the A₀ molecules and F_x in the model of the *C. limicola* reaction centre are similar to the equivalent distances in the structure of the cyanobacterial photosystem I.

Finally, as stated above, the re-oxidation of A₀ in green sulphur bacteria is much slower than in photosystem I, occurring with time constants of 600 ps and 20–50 ps, respectively. On comparing the model of the *C. limicola* reaction centre with the structure of photosystem I, the shortest edge-to-edge distance between the A₀ centres and the A₁ quinones was the same, at *ca.* 6.4 Å. This distance gives a minimum time constant for electron transfer of 7 ps for both *C. limicola* and the cyanobacterial photosystem I (Moser & Dutton 1996), and this time constant would be longer if the reaction was not energetically optimized. The most obvious source of the difference in rate for this reaction between the two complexes is the nature of the medium between A₀ and A₁. In the cyanobacterial photosystem I a tryptophan residue is located between A₀ and A₁ (A697 on the A side and B677 on the B side in cyanobacteria). Both tryptophans are in Van der Waals contact with the redox-active head-groups of A₀ and A₁ on their respective branches. In *C. limicola* these tryptophans are replaced by arginines, changing both the size and aromaticity of the intervening residue.

4. CONCLUSION

In summary, the changes in protein primary sequence required to convert the PsaA/PsaB polypeptides into two copies of the PsaA polypeptide did not lead to any large changes in the structure and relative geometry of the electron transfer components of the reaction centre. The conclusion, therefore, is that it is likely that the structure of the electron transfer chains in the *C. limicola* reaction centre is similar that in the cyanobacterial complex. The model depicted several changes in protein-cofactor interactions that could be predicted from the sequence alignments, such as the lysine ligand to the accessory bacteriochlorophylls for example. However, the model also highlighted several changes that are not obvious from the sequence alignment, such as the use of glutamic acid A692 or B672 as a ligand to the magnesium of the A_0 chlorophyll rather than the A688 or B668 methionine used in the cyanobacterial complex, and the replacement of tyrosine A696/B676 by glutamine A700 or B680 as possible hydrogen-bond donors to the nine-keto carbonyl of the A_0 chlorophyll. The modelling exercise also predicts the arrangement of the 14 antenna bacteriochlorophylls in *C. limicola*, and comes to the conclusion that the linker bacteriochlorophylls identified in the cyanobacterial complex are not conserved in the green sulphur bacteria.

The authors thank Dr Richard Sessions from the University of Bristol for his help and guidance with the molecular modelling. This work is funded by the Biotechnology and Biological Sciences Research Council of the UK.

REFERENCES

- Allen, J. P., Feher, G., Yeates, T. O., Komiya, H. & Rees, D. C. 1987 Structure of the reaction center from *Rhodospirillum rubrum* R-26: the cofactors. *Proc. Natl Acad. Sci. USA* **84**, 5730–5734.
- Baymann, F., Bruga, M., Muhlenhoff, U. & Nitschke, W. 2001 Daddy, where did PS(I) come from? *Biochim. Biophys. Acta Bioenergetics* **1507**, 291–310.
- Blankenship, R. E. 1992 Origin and early evolution of photosynthesis. *Photosynth. Res.* **33**, 91–111.
- Brettel, K. 1997 Electron transfer and arrangement of the redox cofactors in photosystem I. *Biochim. Biophys. Acta* **1318**, 322–373.
- Brettel, K. & Liebl, W. 2001 Electron transfer in photosystem I. *Biochim. Biophys. Acta Bioenergetics* **1507**, 100–114.
- Büttner, M., Xie, D. L., Nelson, H., Pinther, W., Hauska, G. & Nelson, N. 1992 The photosystem I-like P840-reaction centre of green-S bacteria is a homodimer. *Biochim. Biophys. Acta Bioenergetics* **1101**, 154–156.
- Deisenhofer, J., Epp, O., Miki, K., Huber, R. & Michel, H. 1985 Structure of the protein subunits in the photosynthetic reaction center of *Rhodospseudomonas viridis* at 3 Å resolution. *Nature* **318**, 618–624.
- Fairclough, W. V., Evans, M. C. W., Purton, S., Rigby, S. E. J. & Heathcote, P. 2001 Site-directed mutagenesis of PsaA:M684 in *Chlamydomonas reinhardtii*. In *PS2001 Proc. 12th Int. Congress on Photosynthesis* Melbourne, Australia: CSIRO.
- Feiler, U., Albouy, D., Pourret, C., Mattioli, T. A., Lutz, M. & Robert, B. 1994 Structure and binding site of the primary electron acceptor in the reaction center of *Chlorobium*. *Biochemistry* **33**, 7594–7599.
- Fleming, G. R. & Van Grondelle, R. 1997 Femtosecond spectroscopy of photosynthetic light harvesting systems. *Curr. Opin. Str. Biol.* **7**, 738–748.
- Fromme, P., Witt, H. T., Schubert, W. D., Klukas, O., Saenger, W. & Krauss, N. 1996 Structure of photosystem-I at 4.5 Å resolution: a short review including evolutionary aspects. *Biochim. Biophys. Acta* **1275**, 76–83.
- Fromme, P., Jordan, P. & Krauss, N. 2001 Structure of photosystem I. *Biochim. Biophys. Acta Bioenergetics* **1507**, 5–31.
- Gobets, B. & Van Grondelle, V. 2001 Energy transfer and trapping in photosystem I. *Biochim. Biophys. Acta Bioenergetics* **1507**, 80–99.
- Guergova-Kuras, M., Boudreaux, B., Joliot, A., Joliot, P. & Redding, K. 2001 Evidence for two active branches for electron transfer in photosystem I. *Proc. Natl Acad. Sci. USA* **98**, 4437–4442.
- Hanley, J., Deligiannakis, Y., MacMillan, F., Bottin, H. & Rutherford, W. 1997 ESEEM study of phylloquinone radical A_1^- in ^{14}N and ^{15}N -labeled photosystem I. *Biochemistry* **36**, 11 543–11 549.
- Hauska, G., Schoedl, T., Remigy, H. & Tsotis, G. 2001 The reaction centre of green sulphur bacteria. *Biochim. Biophys. Acta Bioenergetics* **1507**, 260–277.
- Heathcote, P., Moenne-Loccoz, P., Rigby, S. E. J. & Evans, M. C. W. 1996 Photoaccumulation in photosystem I does produce a phylloquinone (A_1^-) radical. *Biochemistry* **35**, 6644–6650.
- Heathcote, P., Fyfe, P. K. & Jones, M. R. 2002 Reaction centres: structure and mechanism in biological solar power. *Trends Biochem. Sci.* **27**, 79–87.
- Hoff, A. J. & Deisenhofer, J. 1997 Photophysics of photosynthesis. Structure and spectroscopy of reaction centers of purple bacteria. *Physics Rep.* **287**, 1–247.
- Joliot, P. & Joliot, A. 1999 *In vivo* analysis of the electron transfer within photosystem I: are the two phylloquinones involved? *Biochemistry* **38**, 11 130–11 136.
- Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W. & Krauss, N. 2001 Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**, 909–917.
- Kaupp, M. 2002 The function of photosystem I. Quantum chemical insight into the role of tryptophan–quinone interactions. *Biochemistry* **41**, 2895–2900.
- Klukas, O., Schubert, W. D., Jordan, P., Krauss, N., Fromme, P., Witt, H. T. & Saenger, W. 1999 Localisation of phylloquinones, Q_K and Q'_K in an improved electron density map of photosystem-I at 4 Å resolution. *J. Biol. Chem.* **274**, 7351–7360.
- Kobayashi, M. (and 11 others) 2000 The primary electron acceptor of green sulfur bacteria, bacteriochlorophyll 663, is chlorophyll a esterified with $\Delta^2,6$ -phytyadienol. *Photosynth. Res.* **63**, 269–280.
- Kraulis, P. J. 1991 MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr.* **24**, 946–950.
- Krauss, N., Schubert, W. D., Klukas, O., Fromme, P., Witt, H. T. & Saenger, W. 1996 Photosystem-I at 4 Å resolution represents the first structural model of a photosynthetic reaction centre and core antenna system. *Nature Struct. Biol.* **3**, 965–973.
- Luneberg, J., Fromme, P., Jekow, P. & Schlodder, E. 1994 Spectroscopic characterization of PS-I core complexes from thermophilic *Synechococcus* sp: identical reoxidation kinetics of A_1^- before and after removal of the iron-sulfur-clusters F_A and F_B . *FEBS Lett.* **203**, 225–229.
- McRee, D. E. 1992 XTALVIEW: a visual protein crystallographic software system for XII/Xview. *J. Mol. Graph.* **10**, 44–46.

- Merritt, E. A. & Bacon, D. J. 1997 RASTER3D: photorealistic molecular graphics. *Meth. Enzymol.* **277**, 505–524.
- Moenne-Loccoz, P., Heathcote, P., MacLachlan, D. J., Berry, M. C., Davis, I. H. & Evans, M. C. W. 1994 Photoaccumulation in photosystem I does produce a phylloquinone (A_1^-) radical. *Biochemistry* **33**, 10 037–10 042.
- Moser, C. C. & Dutton, P. L. 1996 Outline of theory of protein electron transfer. In *Protein electron transfer* (ed. D. S. Bendall), pp. 1–21. Oxford: BIOS Scientific Publishers.
- Muhiuddin, I. P., Rigby, S. E. J., Evans, M. C. W., Ames, J. & Heathcote, P. 1999 ENDOR and Special TRIPLE resonance spectroscopy of photoaccumulated semiquinone electron acceptors in the reaction centres of green sulphur bacteria and heliobacteria. *Biochemistry* **38**, 7159–7167.
- Muhiuddin, I. P., Heathcote, P., Carter, S., Purton, S., Rigby, S. E. J. & Evans, M. C. W. 2001 Evidence from time resolved studies of the $P700^+/A_1^-$ radical pair for photosynthetic electron transfer on both the PsaA and PsaB branches of the photosystem I reaction centre. *FEBS Lett.* **503**, 56–60.
- Neerken, S. & Ames, J. 2001 The antenna reaction center complex of heliobacteria: composition, energy conversion and electron transfer. *Biochim. Biophys. Acta Bioenergetics* **1507**, 278–289.
- Nuijs, A. M., Vasmel, H., Joppe, H. L. P., Duysens, L. N. M. & Ames, J. 1985 Excited states and primary charge separation in the pigment system of the green photosynthetic bacterium *Prosthecochloris aestuarii* as studied by picosecond absorbance difference spectroscopy. *Biochim. Biophys. Acta* **807**, 24–34.
- Permentier, H. P., Schmidt, K. A., Kobayashi, M., Akiyama, M., Hager-Braun, C., Neerken, S., Mille, M. & Ames, J. 2000 Composition and optical properties of reaction centre core complexes from the green sulfur bacteria *Prosthecochloris aestuarii* and *Chlorobium tepidum*. *Photosynth. Res.* **64**, 27–39.
- Powls, R. & Redfearn, E. R. 1969 Quinones of the chlorobacteriaceae properties and possible function. *Biochim. Biophys. Acta* **172**, 429–437.
- Purton, S., Stevens, D. R., Muhiuddin, I. P., Evans, M. C. W., Carter, S., Rigby, S. E. J. & Heathcote, P. 2001 Site-directed mutagenesis of PsaA residue W693 affects phylloquinone binding and function in the photosystem I reaction center of *Chlamydomonas reinhardtii*. *Biochemistry* **40**, 2167–2175.
- Rigby, S. E. J., Evans, M. C. W. & Heathcote, P. 1996 ENDOR and special triple resonance spectroscopy of A_1^- of photosystem I. *Biochemistry* **35**, 6651–6656.
- Rigby, S. E. J., Muhiuddin, I. P., Evans, M. C. W., Purton, S. & Heathcote, P. 2002 Photoaccumulation of the PsaB phyllosemiquinone in photosystem I of *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta Bioenergetics* **1556**, 13–20.
- Scheller, H. V., Jensen, P. E., Haldrup, A., Lunde, C. & Knetzel, J. 2001 Role of subunits in eukaryotic photosystem I. *Biochim. Biophys. Acta Bioenergetics* **1507**, 41–60.
- Schmid, R., Goebel, F., Warnecke, A. & Labahn, A. 1999 Synthesis and redox potentials of methylated vitamin K derivatives. *J. Chem. Soc. Perkin Trans. 2*, 1199–1202.
- Schubert, W.-D., Klukas, O., Saenger, W., Witt, H. T., Fromme, P. & Krauss, N. 1998 A common ancestor for oxygenic and anoxygenic photosynthetic systems: a comparison based on the structural model of photosystem I. *J. Mol. Biol.* **280**, 297–314.
- Shen, J. R. & Kamiya, N. 2000 Crystallization and the crystal properties of the oxygen-evolving photosystem II from *Synechococcus vulcanus*. *Biochemistry* **39**, 14 739–14 744.
- Swallow, A. J. 1982 Physical chemistry of semiquinones. In *Function of quinones in energy conserving systems* (ed. B. L. Trumpower), pp. 59–72. New York: Academic.
- Van de Meent, E. J., Koayashi, M., Erkelens, C., Van Veelen, P. A., Otte, S. C. M., Inoue, K., Watanabe, T. & Ames, J. 1992 The nature of the primary electron acceptor in green sulfur bacteria. *Biochim. Biophys. Acta* **1102**, 371–378.
- Van der Est, A., Bock, C., Golbeck, J., Brettel, K., Setif, P. & Stehlik, D. 1994 Electron transfer from the acceptor A_1 to the iron-sulfur centers in photosystem I as studied by transient EPR spectroscopy. *Biochemistry* **33**, 11 789–11 797.
- Vassiliev, I. R., Antokine, M. L. & Golbeck, J. H. 2001 Iron-sulfur clusters in type-I reaction centres. *Biochim. Biophys. Acta Bioenergetics* **1507**, 139–160.
- Xu, W., Tang, H., Wang, Y. & Chitnis, P. R. 2001 Proteins of the cyanobacterial photosystem I. *Biochim. Biophys. Acta Bioenergetics* **1507**, 32–40.
- Zouni, A., Witt, H. T., Kern, J., Fromme, P., Krauss, N., Saenger, W. & Orth, P. 2001 Crystal structure of photosystem-II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* **409**, 739–743.

GLOSSARY

- ENDOR: electron nuclear double resonance
 EPR: electron paramagnetic resonance
 ESP: electron spin polarized