Insights into the evolution of the antenna domains of Type-I and Type-II photosynthetic reaction centres through homology modelling

Paul K. Fyfe[a], Michael R. Jones[a], Peter Heathcote[b,*]

[a] *Department of Biochemistry, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK*
[b] *School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK*

Received 26 July 2002; revised 9 September 2002; accepted 9 September 2002

First published online 25 September 2002

Edited by Gunnar von Heijne

Abstract **The (bacterio)chlorophylls of photosynthetic antenna and reaction centre complexes are bound to the protein via a fifth, axial ligand to the central magnesium atom. A number of the amino acids identified as providing such ligands are conserved between the large antenna of the cyanobacterial Type-I reaction centre and smaller antennas of the Type-I reaction centres of green sulphur bacteria and heliobacteria, and these numbers match closely the estimated number of antenna bacteriochlorophylls in the latter. The possible organisation of the antenna in the latter reaction centres is discussed, as is the mechanism by which the more pigment-rich antenna of the cyanobacterial reaction centre evolved. The homology modelling approach is also extended to the six-helix antenna proteins CP47 and CP43 associated with the Photosystem II reaction centre.**

Key words: Reaction centre; Photosynthesis; Light harvesting; Membrane protein; Chlorophyll; Bacteriochlorophyll

1. Introduction

Reaction centres are integral membrane protein complexes that convert light energy into a biologically useful form. They consist of a protein scaffold that encases a number of cofactors involved in the harvesting of light energy and electron transfer. Reaction centres show considerable variety in terms of their protein and cofactor composition (see [1] for a recent review). However, at the most basic level they have a common design and operate according to a common mechanism, in that light energy is used to drive a series of electron transfer reactions that are linked to vectorial proton translocation across the membrane, generating a transmembrane protonmotive force [1].

The energy-transducing domain of all known reaction centres consists of a protein structure formed by two sets of five membrane-spanning α-helices. This protein scaffold encases six (bacterio)chlorin and two quinone cofactors that are arranged in two pseudo-symmetrical membrane-spanning branches. These cofactors catalyse the photochemical transmembrane electron transfer reaction that is the key to the photosynthetic process. Added to this basic structural blueprint are a variety of protein–cofactor structures, such as light-harvesting (antenna) complexes, the oxygen-evolving complex or proteins containing iron–sulphur centres, that represent further adaptations [1].

Reaction centres have been classified into two basic types [2]. Type-II reaction centres are found in purple bacteria, green filamentous bacteria and in the Photosystem-II complex of oxygenic photosynthetic organisms – plants, algae and cyanobacteria. The terminal electron acceptor in Type-II reaction centres is a quinone species that is able to dissociate from the complex in the form of the doubly reduced and doubly protonated quinol. The Type-II reaction centres engage in non-covalent protein–protein interactions with separate membrane protein complexes that contain light-harvesting pigments such as (bacterio)chlorophylls ((B)Chls) and carotenoids. In the particular case of the Photosystem-II complex, the reaction centre is closely associated with two antenna pigment–protein complexes, termed CP43 and CP47. Each of these antenna proteins has six membrane-spanning α-helices that are connected by loops, and this protein scaffold encases the light-harvesting chlorophylls (Chls) and carotenoids. The structure of a preparation of the Photosystem-II complex from spinach, containing CP47 but not CP43, has been reported at resolution of 8 Å [3]. More recently, the structure of a preparation of Photosystem-II containing both CP47 and CP43, from the thermophilic cyanobacterium *Synechococcus elongatus*, has been reported at a resolution of 3.8 Å [4].

Type-I reaction centres are found in the strictly anaerobic green sulphur bacteria and heliobacteria, and in the Photosystem-I complex of plants, algae and cyanobacteria. The two main structural polypeptides that make up Type-I reaction centres are larger than their counterparts in the Type-II complexes, and each can be divided into two regions [5–8]. The C-terminal regions consist of five membrane-spanning α-helices that associate together to form a pseudo-symmetrical dimeric structure that encases the two branches of electron transfer cofactors. This core domain has strong structural homologies to the Type-II reaction centres described above [5–8]. The N-terminal region of each of the two main structural polypeptides has six membrane-spanning α-helices that form a domain that binds antenna (B)Chls and carotenoids. These six-helix antenna domains are located on either side of the core domain, and have structural homologies to the antenna CP47 and CP43 pigment proteins of Photosystem-II [3,7], but do not have counterparts in the purple bacteria or green sulphur bacteria. The electron transfer chain of Type-I

*Corresponding author. Fax: (44)-20-8983 0973.
E-mail address: p.heathcote@qmul.ac.uk (P. Heathcote).

Abbreviations: BChl, bacteriochlorophyll; (B)Chls, bacteriochlorophylls and/or chlorophylls; Chl, chlorophyll

0014-5793/02/$22.00
PII: S0014-5793(02)03436-1

reaction centres terminates in low potential iron–sulphur centres.

Recently a 2.5 Å resolution X-ray crystal structure has been determined for the Photosystem-I complex of *S. elongatus* [9,10]. This new and detailed structure of a Type-I reaction centre from an oxygenic organism has provoked new interest in the mechanism of energy and electron transfer in the Type-I reaction centre complexes. In addition to variations in structure and organisation, differences in the mechanism of transmembrane electron transfer between Photosystem-I and the Type-II reaction centres are already becoming apparent, in particular the likelihood that electron transfer in Photosystem-I can occur along either of the two pseudo-symmetrical branches of electron transfer cofactors [11–15]. Furthermore, the new structural information has provided insights into the control of function in reaction centres that operate at very low redox potentials [9,10], and has prompted new queries about the evolution of photosynthesis and photosynthetic reaction centres (see [16] for a recent review).

In this report the possible structures of the antenna domains of the Type-I reaction centres from green sulphur bacteria (*Chlorobium limicola*) and heliobacteria (*Heliobacillus mobilis*) have been investigated (see [17,18] for recent reviews of these Type-I reaction centres). The modelling takes into account evolutionary conservation of residues that provide the fifth (axial) ligand to the (B)Chl molecules of the antenna. We show that it is possible to account for the reported bacteriochlorophyll (BChl) composition of the antenna domains of these bacterial Type-I reaction centres through this simple approach. Implications for chlorophyll (Chl) binding by the CP47 and CP43 antenna complexes of Photosystem-II are also discussed.

2. Results and discussion

2.1. Donors of the axial ligands to the antenna Chls in the cyanobacterial reaction centre

Comparison of the antenna domains of the Type-I reaction centres was based on a protein sequence alignment constructed by Baymann and co-workers [16]. This alignment was derived from computer-based secondary structure predictions and alignment of structural elements, with final assignments being made manually, taking into account knowledge relating to important residues within the complex. The alignment compares the sequences for the PsaA and PsaB polypeptides from *Synechocystis* PCC6803, the PscA polypeptide from *C. limicola*, the PshA polypeptide from *H. mobilis* and the PsbB (CP47) and PsbC (CP43) polypeptides from *Synechocystis* PCC6803 [16].

To model the antenna domains of the *C. limicola* and *H. mobilis* reaction centres, and the CP47 and CP43 complexes of Photosystem-II, the sequences of the *Synechocystis* PCC6803 PsaA, PsaB, PsbB and PsbC polypeptides were replaced by the highly homologous ($\geq 80\%$) sequences for the same polypeptides from *S. elongatus* [19–21]. Residues of the PsaA and PsaB polypeptides that provide axial ligands to the Chls of the antenna domains were then identified from the X-ray crystal structure of the *S. elongatus* Photosystem-I complex (Protein Structure Database, deposition code 1JBO [9,10]). These PsaA and PsaB residues, and the equivalent residues in the aligned sequences, are shown in Fig. 1.

The X-ray crystal structure of the *S. elongatus* Photosystem-I complex includes six Chls in the two transmembrane electron transfer chains of the reaction centre core, and 90 additional antenna Chls. Of the latter Chls, 40 are bound by the PsaA polypeptide, 39 by the PsaB polypeptide and a further 11 by minor polypeptides [9]. The latter do not have equivalents in the Type-I reaction centres from green sulphur bacteria and heliobacteria, and were not included in the analysis.

As can be seen from Fig. 1, a total of 32 of the Chls bound by PsaA have a histidine residue as the liganding amino acid, with two ligands provided by glutamine and one provided by the backbone carbonyl of a threonine (shown in bold, shaded in Fig. 1) [9]. The remaining five Chls have an axial ligand provided by a water molecule, and were not considered in the analysis. Eleven of the liganding residues are located in the five-helix C-terminal (reaction centre) domain of PsaA, and the remainder in the six-helix N-terminal (antenna) domain of PsaA. In the case of PsaB, 29 axial ligands are provided by histidines, one by aspartic acid, one by glutamine and one by tyrosine (shown in bold in Fig. 1) [9]. The remaining seven Chls have an axial ligand provided by a water molecule, and again were not considered in the analysis. Once again, 11 of the liganding residues are located in the reaction centre domain of PsaB, and the remainder in the antenna domain of PsaB.

A striking feature of the data in Fig. 1 is the correspondence between the histidine ligands of PsaA and PsaB. Of the

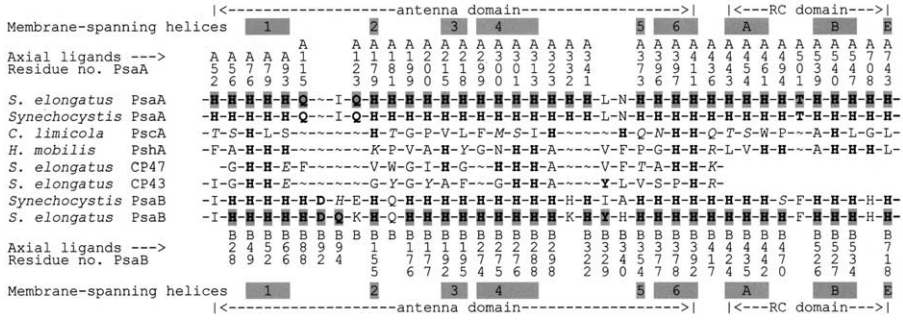

Fig. 1. Conservation of amino acids donating axial ligands to the antenna (B)Chls in Type-I reaction centres and in the Photosystem-II CP43 and CP47 antenna proteins. Numbering is as for the Chl axial ligands of the PsaA and PsaB proteins from *S. elongatus*. Partition of the residues between the antenna and reaction centre domains is shown, as is the location of membrane-spanning helices (numbering as in [16]). Key: Bold, shaded – axial ligands in the *S. elongatus* PsaA or PsaB proteins; bold – conserved residues in other protein sequences; italics – non-conserved residues capable of providing an axial ligand; a gap in the alignment is indicated by the symbol ~.

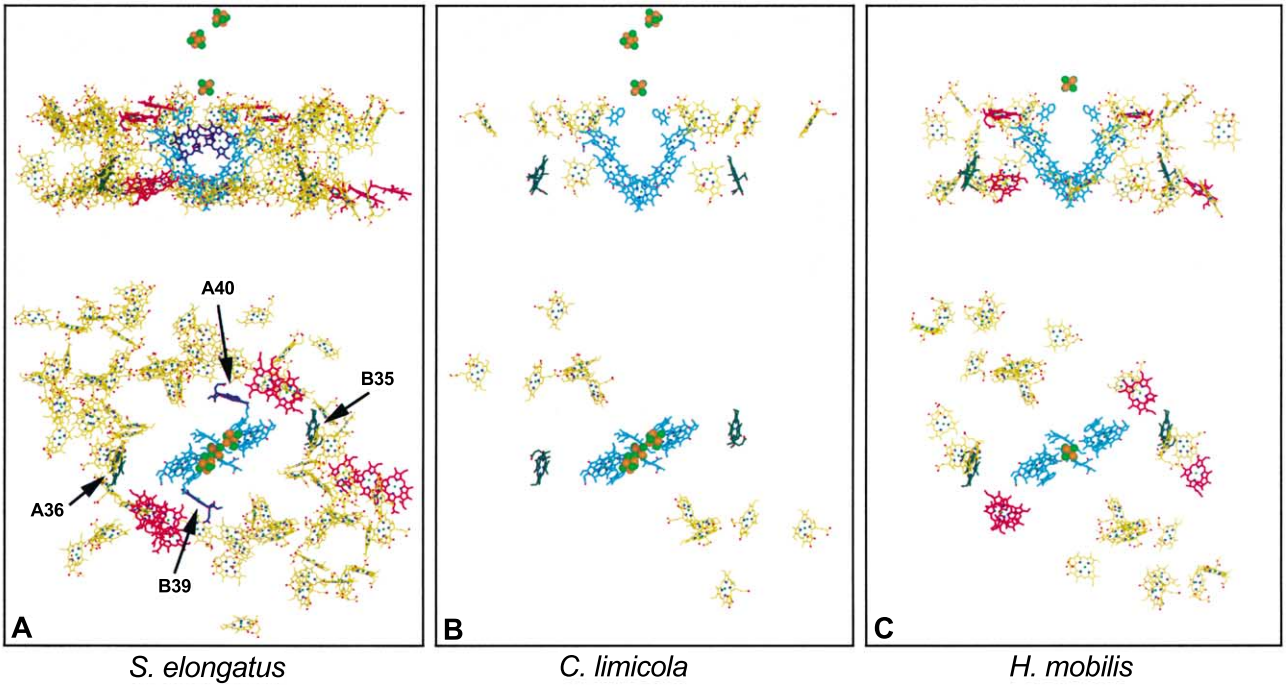

Fig. 2. Organisation of the cofactors of the *S. elongatus* Photosystem-I complex, and the proposed organisation of their counterparts in the *C. limicola* and *H. mobilis* complexes. a: In *S. elongatus* the electron-transferring Chls (cyan), quinones (cyan) and iron–sulphur centres (green and orange spheres) are surrounded by antenna Chls (cpk colours) and carotenoids (not shown). Linker Chls A40 and B39 are highlighted in dark blue, Chls A36 and B35 are highlighted in dark green, and candidates for red-shifted Chls are highlighted in red. b: The same view of the modelled organisation of the antenna BChls in the *C. limicola* complex. c: The modelled organisation of the antenna BChls in the *H. mobilis* complex. The figure was constructed using Molscript [36] and Raster3D [37].

32 and 29 histidine ligands in PsaA and PsaB, respectively, 28 occupy equivalent positions in the aligned sequences of the PsaA and PsaB polypeptides. This points to a high degree of symmetry in the arrangement of the Chls in the two antenna domains which, as revealed by the X-ray crystal structure, are related by the two-fold symmetry axis that runs through the middle of the reaction centre core domain [9]. This symmetry is evident from the views of the complex in Fig. 2a. The data in Fig. 1 also highlights the strong conservation of these residues between *S. elongatus* and *Synechocystis*, with only four differences in PsaB and none in PsaA. In the case of PsaB, the residues Gln B94, Tyr B329, His B340 and His B470 in *S. elongatus* are replaced by His, Ile, Ala and Ser, respectively.

2.2. Conservation of axial ligands to antenna Chls in the reaction centre of green sulphur bacteria

The Type-I reaction centre of green sulphur bacteria is composed of a homodimer of a polypeptide termed PscA, rather than the PsaA/PsaB heterodimer as found in Photosystem-I [22] (for a recent review of this complex see [18]). Although the PscA polypeptide is comparable in size to PsaA and PsaB it binds far fewer pigments, with only eight BChls *a* and two Chls *a* (esterified to 2,6-phytanediol) bound per PscA [23]. Allowing for the binding of one BChl and two Chls per PscA in the electron transfer chains of the reaction centre core [18], this indicates the presence of 14 antenna BChls in the complex, the symmetry implying that seven BChls are bound to each copy of the PscA polypeptide. This is in marked contrast to the total of 90 antenna Chls bound by the PsaA and PsaB polypeptides in Photosystem-I. Similarly there are also only two carotenoids per reaction centre in green sulphur bacteria, 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 residues in the PscA polypeptide from the green sulphur bacterium *C. limicola* that align with the ligand donors in the cyanobacterial PsaA and PsaB polypeptides are shown in Fig. 1. Of the 35 PsaA residues listed in Fig. 1, six residues – all histidines – were conserved in the aligned sequence of the PscA polypeptide (in bold in Fig. 1). All of these histidines are also conserved between PsaA and PsaB. A seventh histidine, equivalent to PsaB residue His B340, is also highlighted in Fig. 1. Residue His B340 is located near to the central magnesium of antenna Chl 1221 and adjacent to residue Tyr B329, which appears to be the ligand donor in the cyanobacterial complex. However in the absence of Tyr B329 the adjacent His B340 would be a good candidate to provide an axial ligand, being close to the central magnesium, and for this reason the latter is included in the alignment. Six of the conserved His residues are located in the putative N-terminal (antenna) domain of PscA (including the His equivalent to His B430), and the seventh in the C-terminal (reaction centre) domain of PscA. The alignment also highlights a number of non-histidine residues that could act as ligand donors in PscA (shown in italics). However, it is possible to account for the predicted 14 BChls of the *C. limicola* antenna by considering only the conserved histidines.

2.3. Model of the antenna BChls in the C. limicola reaction centre

Using the sequence alignment data outlined above, a model of the antenna BChls in the *C. limicola* reaction centre was

constructed, based on the X-ray crystal structure of the *S. elongatus* reaction centre. Fig. 2a shows the cofactors of the electron transfer chain of the *S. elongatus* reaction centre, and the arrangement of the antenna Chls. Energy transfer from the antenna to the electron transfer chain is rapid and extremely efficient [24], facilitated by the proximity of the antenna Chls to each other in what is a densely packed pigment array. The Chls liganded to residues A743 and B718 (labelled A40 and B39 in Fig. 2a) have been identified as a possible conduit for excitation energy transfer between the bulk of the antenna Chls and the Chls of the electron transfer chain (the so-called 'linker' Chls) [9,10]. In addition a sub-set of the antenna Chls in Fig. 2a absorb at lower energies than the remainder of the antenna Chls (the so-called long-wavelength or red Chls). These could be responsible for capturing light energy at the red end of the spectrum when *S. elongatus* is shaded by other photosynthetic organisms, and/or directing and retaining excitation energy close to the electron transfer chain (reviewed in [25]). The X-ray crystal structure of the cyanobacterial Photosystem-I complex [9,10] revealed a number of tightly interacting antenna Chls that form dimers and trimers (highlighted in red in Fig. 2a). These could be excitonically coupled, and are possible candidates for some of the long-wavelength Chls [9,10]. The relevance of the Chls labelled A36 and B35 is explained below.

Fig. 2b shows the same view of the modelled organisation of the antenna BChls in *C. limicola*. The model was constructed by taking the X-ray crystal structure of the *S. elongatus* reaction centre, and deleting those antenna Chls whose axial ligand donor is not conserved in *C. limicola*. The resulting model contained 14 antenna BChls that are liganded by conserved His residues. The proposed linker Chls of Photosystem-I (A40 and B39 in Fig. 2a) are not conserved in the model, nor are equivalents of the tightly interacting Chls proposed to be the long-wavelength Chls (except for one single BChl shown at the top, right in Fig. 2b).

Only two of the seven BChls modelled into each *C. limicola* PscA protein are located on the same side of the membrane as the pair of BChls that form the primary donor in the electron transfer chain (P840) (Fig. 2b). One of these is the single BChl located in the C-terminal antenna domain, and the second is bound to the outer surface of the reaction centre core domain (labelled A36 and B35 in Fig. 2b). The remaining five BChls are located on the opposite side of the membrane, with a group of three separated from two single BChls by approximately 10 and 15 Å.

The antenna BChls bound to the outer surface of reaction centre core are rather isolated, approximately 25 Å from the nearest other antenna Chl. These Chls, labelled A36 and B35 in Fig. 2b, make the closest approach between the seven antenna BChls and the six chlorins of the reaction centre core, being approximately 15 Å from the reaction centre Chl located approximately half-way across the membrane, in the position assigned to the A_0 electron acceptor (and equivalent to the (bacterio)pheophytin cofactors in Type-II reaction centres)). An interesting point is that the histidine that binds this Chl is conserved in the D1 (His 118) and D2 (His 117) polypeptides of the Photosystem-II reaction centre (see [4,16]), and as a result the Photosystem-II reaction centre can be prepared with eight bound chlorins (six in the electron transfer chain and the two others bound to the outside of the core). These Chls have been proposed as a possible conduit for energy transfer from the antenna Chls of CP43 and CP47 to the Chls of the reaction centre core in Photosystem-II [4], and a redox role for one or both of these Chls has also been suggested [26,27].

2.4. Conservation of axial ligands to antenna Chls in the reaction centre of heliobacteria

The Type-I reaction centre of heliobacteria is also homodimeric, consisting of two copies of the PshA polypeptide [28]. Once again, although the PshA polypeptide is of comparable size to PsaA and PsaB it binds fewer pigments, although more than the PscA polypeptide of the green sulphur bacteria. The heliobacterial reaction centre is usually described as containing about 35 BChl *g* molecules (reviewed in [17]), although there is some uncertainty over the correct number and it may be rather larger than this (R. Blankenship, personal communication). Presumably it must contain an even number of such pigments since it is composed of a homodimeric pair of core PshA proteins. Van de Meent and co-workers [29] identified an 8′-hydroxychlorophyll *a* as a functional reaction centre pigment in heliobacteria, and determined an 8′-hydroxychlorophyll *a*:BChl *g* molar ratio of 1:17. On the basis that there are two 8′-hydroxychlorophyll *a* and four BChl *g* per electron transfer chain (two of which form the electron donor P798 and are presumably 13^2 epimers of BChl *g*), it therefore seems reasonable to assume that there are 30 antenna BChl *g* molecules per reaction centre. As can be seen from Fig. 1, the PshA polypeptide of *H. mobilis* contains 13 conserved His residues, which would account for 26 antenna BChls. The alignment also contains three additional residues, Lys, Tyr and Arg, that could increase the number of potential ligand donors to 32. Five of the seven His ligand donors conserved in the *C. limicola* PscA protein are also conserved in the *H. mobilis* PshA protein.

Of the 11 potential additional ligand donors observed when comparing PshA with PscA (see Figs. 1 and 2c), six are located in the N-terminal antenna domain, and five are located in the C-terminal reaction centre domain. In the antenna domain, all six of the additional ligand donors are located in the membrane-spanning α-helices (two in helix I, two in helix III, two in helix IV). In the reaction centre domain, three of the additional ligand donors are located in the membrane-spanning α-helices, one in an amphipathic connecting helix and one in a loop region.

Turning to the cyanobacterial complex (see Figs. 1 and 2a), of the additional Chl ligands present in the PsaA and PsaB polypeptides (compared to PshA and PscA), almost all of these are located in loop regions of either the antenna or reaction centre domains, rather than in the membrane-spanning α-helices.

2.5. Development of the light-harvesting capacity of the Type-I reaction centres

The protein sequence analysis suggests a evolutionary scenario in which the basic light-harvesting capacity represented by the 14 antenna BChls seen in the reaction centres of the extant green sulphur bacteria was enhanced in the organisms that led to the extant heliobacteria through the binding of an additional set of BChls, largely to the membrane-spanning α-helices of both the C-terminal and N-terminal domains of the complex. This enhancement presumably occurred in re-

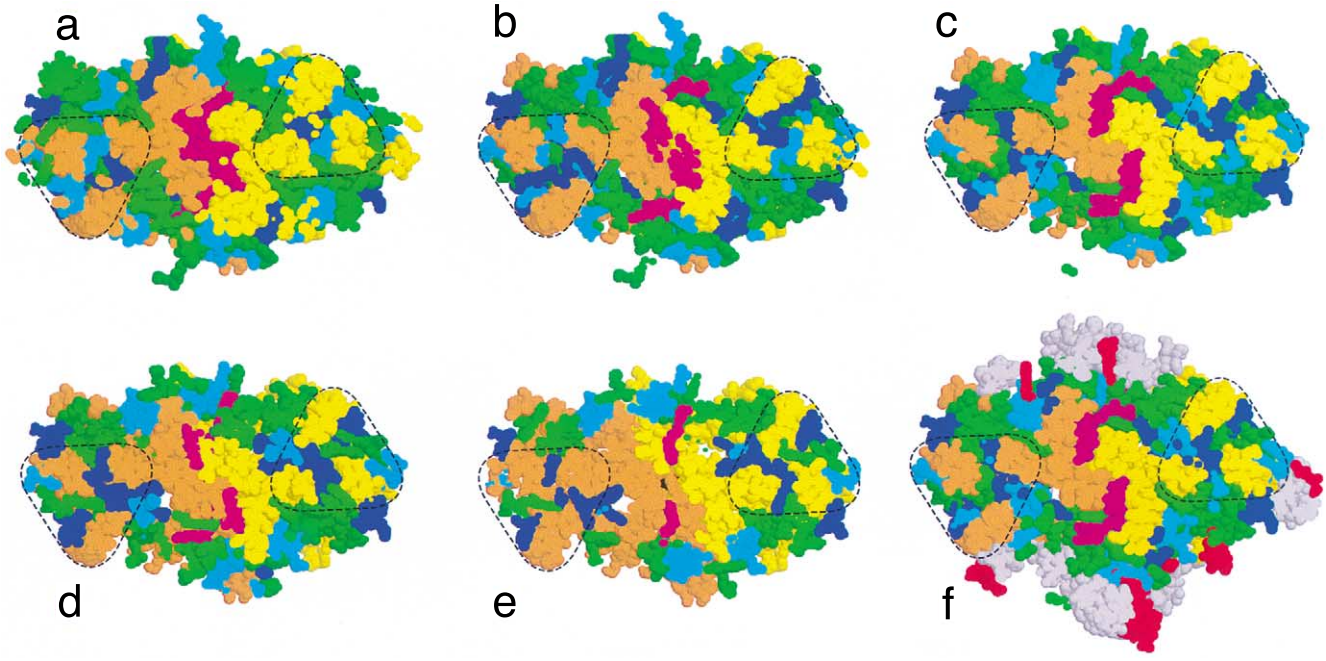

Fig. 3. Spatial location of different groups of Chl molecules in the Photosystem-I complex from *S. elongatus*. All views are along the axis of two-fold symmetry, and show cross-sections of the complex at different depths in the membrane. a–e: Space-fill representations of the PsaA (yellow) and PsaB (orange) proteins, and associated cofactors. The (B)Chls and quinones of the electron transfer chains are shown in magenta. The antenna BChls proposed to be present in the *C. limicola* complex are in dark blue, the additional antenna BChls proposed to be present in the *H. mobilis* complex are in cyan, and the Chls proposed to be exclusive to the cyanobacterial complex are in green. Cross-sections are taken at the level of (a) the P700 primary electron donor Chls located close to the near side of the membrane, (c) the A_0 Chl electron acceptor located approximately half-way across the membrane, (e) the A_1 quinone electron acceptor located close to the far side of the membrane and (b, d) two intermediate positions. Panel (f) shows the same cross-section as in (c), but includes the additional polypeptides of the Photosystem-I complex (grey) and the associated antenna Chls (red).

sponse to physiological pressure for increased light-harvesting capacity in these bacteria, which do not contain any light-harvesting pigment proteins other than PshA. In the line that led to the extant green sulphur bacteria, the acquisition of accessory light-harvesting structures such as the chlorosome presumably made a similar enhancement unnecessary, and the BChl content of the reaction centre remained at a more basic level. In the ancestors of the modern-day cyanobacteria, the light-harvesting capacity of the reaction centre was further enhanced through the binding of Chls to (mainly) inter-helix loop regions of the protein.

Fig. 3a–e shows the positions of the different groups of Chls in the cyanobacterial Photosystem-I complex, coloured according to their proposed presence in the *C. limicola* and/or *H. mobilis* complex. The view is down the two-fold symmetry axis of that part of the complex formed by the PsaA and PsaB proteins, with cross-sections taken at different depths in the membrane. The protein is colour coded in yellow (PsaA) and orange (PsaB), with the 10-helix heterodimeric reaction centre domain in the middle. The two flanking six-helix antenna domains are indicated by the dotted lines. In the membrane, these antenna domains are built from three pairs of helices, dubbed a 'trimer of dimers' [7], that have a triangular arrangement in cross-section.

The spatial arrangement of the different groups of Chls shown in Fig. 3 supports the proposal, strongly implied by the protein sequence alignments, that the basic protein fold of the antenna and reaction centre domains is conserved across the different Type-I reaction centres. The Chls equivalent to the 14 BChls proposed to be present in the *C. limicola* complex are highlighted in dark blue, and are mainly attached to (in some cases encased by) the helices of the antenna domains. The additional BChls proposed to be present in the *H. mobilis* complex are highlighted in cyan. Some of these are again attached to (or partially encased by) the helices of the antenna domains, whilst the remainder are attached to the outer faces of the reaction centre domain. Finally, the Chls proposed to be exclusive to the cyanobacterial complex are highlighted in green. With a couple of exceptions, these are located in more peripheral regions on the outer faces of the complex formed between PsaA and PsaB, and in particular fill up much of the space between the antenna domains and the central reaction centre domain. This arrangement is consistent with the idea that the (B)Chl complement of the antenna could be built up through the addition of extra (B)Chls to increasingly peripheral regions of the complex, rather than through more drastic changes in the backbone fold of the antenna domains of the protein.

Fig. 3f shows the same cross-section as Fig. 3c, but with the additional polypeptides of the cyanobacterial Photosystem-I complex shown in grey, and the Chls bound by these polypeptides highlighted in red. These additional polypeptides provide a protein environment for the antenna Chls that are attached to the periphery of the main PsaA/PsaB complex [9,10]. The Type-I complexes from green sulphur bacteria and heliobacteria do not have this additional complement of antenna Chls, and lack these additional polypeptides and associated Chls.

2.6. Homologies with the CP43 and CP47 antenna proteins of Photosystem-II and related six-helix antenna proteins

The alignment constructed by Baymann and co-workers also includes the PsbC (CP43) and PsbB (CP47) polypeptides of the *Synechocystis* Photosystem-II complex. A low resolution (3.8 Å) X-ray crystal structure for this complex from *S. elongatus* has been described [4], that included 12 antenna Chls bound to CP43 and 14 antenna Chls bound to CP47. Both CP43 and CP47 show the six-helix 'trimer of dimers' arrangement that is seen for the antenna domains of the Type-I complexes [3,4], which taken together with their position relative to the reaction centre core suggest a common evolutionary origin for the CP43/CP47 and the antenna domains of Type-I complexes [7,30,31].

In the present work, the sequences of the CP43 and CP47 proteins from *S. elongatus* were added to the alignment of Baymann and co-workers, and the resulting conservation of Chl ligands is shown in Fig. 1. According to the alignment, four His residues are absolutely conserved between the three Type-I reaction centres, CP43 and CP47 (equivalent to PsaA residues A76, A301, A313 and A411), with a fifth found in all sequences except PscA (A79). The CP47 sequence contains three additional His residues of note; the first is found in all sequences except CP43 (A397), the second is found in all sequences apart from CP43 and PshA (A215), and the third is found in PsaA, PsaB and CP47 only. In addition to these, the alignment indicates three alternative residues in CP47 and five in CP43. This produces a total of 11 potential ligand donors for the CP47 complex and 10 for the CP43 complex, compared with the Chl contents of 14 and 12, respectively, as determined from the X-ray crystal structure. Thus this approach also makes a reasonable prediction of the Chl content of these Photosystem-II antenna complexes, despite the rather weak sequence homologies between the CP43/CP47 complexes and the antenna domains of the Type-I complexes.

Six-helix antenna proteins that are related to CP43 have been identified in the phycobilisome-containing cyanobacteria (the CP43′ protein, encoded by the iron-stress-induced *isiA* gene), and in non-phycobilisome containing oxyphotobacteria such as *Prochlorococcus* (the Pcb protein, encoded by the *pcb* gene). Recent reports that these proteins can form rings of antenna pigment–protein around Photosystem-I [32–35] have prompted considerable interest in these other representatives of the family of six-transmembrane α-helical antenna proteins. The sequences of the CP43′ and CP43 proteins of *Synechocystis* 6803 have been compared, and shown to have nine His residues in common in the (predicted) membrane-spanning helices [33]. Comparison with the alignment described above shows that three of these His residues are also conserved in PsaA and PsaB of the cyanobacterial Photosystem-I, PscA of *C. limicola* and PshA of *H. mobilis*, whilst two others are present in all but the *C. limicola* PscA protein.

2.7. Conclusions

In summary, the X-ray crystal structure of the cyanobacterial Photosystem-I complex has revealed the amino acid residues responsible for binding the central magnesium atom of the antenna Chls. The sequence alignment outlined above shows that a sub-set of these residues are either identical, or are conserved as residues with the potential to provide a ligand, in the Type-I reaction centres from green sulphur bacteria and heliobacteria. For both of these reaction centres, the number of predicted liganding residues matches closely the estimated number of antenna BChls, and the data has been used to model the possible organisation of the antenna Chls in these reaction centres. This approach can also account for most of the antenna Chls in the CP43 and CP47 antenna proteins of Photosystem-II. Analysis of the location of the (B)Chls predicted to be present in green sulphur bacteria, heliobacteria and cyanobacteria suggests mechanisms by which the antenna of this type of reaction centre was developed from a relatively simple precursor into the complex arrangement seen in modern-day cyanobacteria.

Acknowledgements: This work was supported by the Biotechnology and Biological Sciences Research Council of the United Kingdom and a European Union TMR Programme (Contract No. FMRX-CT98-0214).

References

[1] Heathcote, P., Fyfe, P.K. and Jones, M.R. (2002) Trends Biochem. Sci. 27, 79–87.
[2] Blankenship, R.E. (1992) Photosynth. Res. 33, 91–111.
[3] Rhee, K.-H., Morris, E.P., Barber, J. and Kühlbrandt, W. (1998) Nature 396, 283–286.
[4] Zouni, A., Witt, H.T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P. (2001) Nature 409, 739–743.
[5] Fromme, P., Witt, H.T., Schubert, W.D., Klukas, O., Saenger, W. and Krauss, N. (1996) Biochim. Biophys. Acta 1275, 76–83.
[6] Krauss, N., Schubert, W.D., Klukas, O., Fromme, P., Witt, H.T. and Saenger, W. (1996) Nat. Struct. Biol. 3, 965–973.
[7] Schubert, W.-D., Klukas, O., Saenger, W., Witt, H.T., Fromme, P. and Krauss, N. (1998) J. Mol. Biol. 280, 297–314.
[8] Klukas, O., Schubert, W.D., Jordan, P., Krauss, N., Fromme, P., Witt, H.T. and Saenger, W. (1999) J. Biol. Chem. 274, 7351–7360.
[9] Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. and Krauss, N. (2001) Nature 411, 909–917.
[10] Fromme, P., Jordan, P. and Krauss, N. (2001) Biochim. Biophys. Acta Bioenerg. 1507, 5–31.
[11] Joliot, P. and Joliot, A. (1999) Biochemistry 38, 11130–11136.
[12] Guergova-Kuras, M., Boudreaux, B., Joliot, A., Joliot, P. and Redding, K. (2001) Proc. Natl. Acad. Sci. USA 98, 4437–4442.
[13] Purton, S., Stevens, D.R., Muhiuddin, I.P., Evans, M.C.W., Carter, S., Rigby, S.E.J. and Heathcote, P. (2001) Biochemistry 40, 2167–2175.
[14] Muhiuddin, I.P., Heathcote, P., Carter, S., Purton, S., Rigby, S.E.J. and Evans, M.C.W. (2001) FEBS Lett. 503, 56–60.
[15] Rigby, S.E.J., Muhiuddin, I.P., Evans, M.C.W., Purton, S. and Heathcote, P. (2002) Biochim. Biophys. Acta Bioenerg. 1556, 13–20.
[16] Baymann, F., Brugna, M., Muhlenhoff, U. and Nitschke, W. (2001) Biochim. Biophys. Acta Bioenerg. 1507, 291–310.
[17] Neerken, S. and Amesz, J. (2001) Biochim. Biophys. Acta Bioenerg. 1507, 278–289.
[18] Hauska, G., Schoedl, T., Remigy, H. and Tsiotis, G. (2001) Biochim. Biophys. Acta Bioenerg. 1507, 260–277.
[19] Golden, S.S. and Stearns, G.W. (1988) Gene 67, 85–96.
[20] Kulkarni, R.D., Mueller, U.W. and Golden, S.S. (1993) Biochim. Biophys. Acta 1173, 329–332.
[21] Muhlenhoff, U., Haehnel, W., Witt, H. and Herrmann, R.G. (1993) Gene 127, 71–78.
[22] Büttner, M., Xie, D.L., Nelson, H., Pinther, W., Hauska, G. and Nelson, N. (1992) Biochim. Biophys. Acta Bioenerg. 1101, 154–156.
[23] Permentier, H.P., Schmidt, K.A., Kobayashi, M., Akiyama, M., Hager-Braun, C., Neerken, S., Mille, M. and Amesz, J. (2000) Photosynth. Res. 64, 27–39.
[24] Gobets, B. and van Grondelle, V. (2001) Biochim. Biophys. Acta Bioenerg. 1507, 80–99.
[25] Fleming, G.R. and van Grondelle, R. (1997) Curr. Opin. Struct. Biol. 7, 738–748.

[26] Stewart, D.H., Cua, A., Chisholm, D.A., Diner, B.A., Bocian, D.F. and Brudvig, G.W. (1998) Biochemistry 37, 10040–10046.
[27] Wang, J., Gosztola, D., Ruffle, S.V., Hemann, C., Seibert, M., Wasielewski, M.R., Hille, R., Gustafson, T.L. and Sayre, R.T. (2002) Proc. Natl. Acad. Sci. USA 99, 4091–4096.
[28] Liebl, U., Mockensturm-Wilson, M., Trost, J.T., Brune, D.C., Blankenship, R.E. and Vermaas, W. (1993) Proc. Natl. Acad. Sci. USA 90, 7124–7128.
[29] Van de Meent, E.J., Kobayashi, M., Erkelens, C., van Veelen, P.A., Amesz, J. and Watanabe, T. (1991) Biochim. Biophys. Acta 1058, 356–362.
[30] Vermaas, W.F.J. (1994) Photosynth. Res. 41, 285–294.
[31] Rutherford, A.W. and Nitschke, W. (1996) in: Origin and Evolution of Biological Energy Conversion (Baltscheffsky, H., Ed.), pp. 143–176, VCH Publishers, Berlin.
[32] Bibby, T.S., Nield, J. and Barber, J. (2001) Nature 412, 743–745.
[33] Bibby, T.S., Nield, J. and Barber, J. (2001) J. Biol. Chem. 276, 43246–43252.
[34] Bibby, T.S., Nield, J., Partensky, F. and Barber, J. (2001) Nature 413, 590.
[35] Boekema, E.J., Hifney, A., Yakushevska, A.E., Piotrowski, M., Keegstra, W., Berry, S., Michel, K.P., Pistorius, E.K. and Kruip, J. (2001) Nature 412, 745–748.
[36] Kraulis, P.J. (1991) J. Appl. Cryst. 24, 946–950.
[37] Merritt, E.A. and Bacon, D.J. (1997) Methods Enzymol. 277, 505–524.