Experimental systems based on bacteria of the genus *Rhodobacter* (*R*), particularly *R. sphaeroides* and *R. capsulatus*, have produced many insights into the mechanism of light energy transduction in photosynthesis, including the structural basis of light harvesting and photochemical charge separation. Structural and spectroscopic studies have been greatly assisted by the availability of bacterial strains with altered photosystems, such as mutants lacking one or both types of light harvesting complex for example, or with an altered complement of carotenoids (so-called green or blue-green mutants), together with the ability of the bacterium to assemble the photosynthetic apparatus when growing in the dark under conditions of low aeration. The present work involves a systematic study of the structural and functional consequences of (1) expression of structural genes *in trans* in deletion mutants, (2) variation in growth conditions, (3) deletion of one or more light harvesting complexes, (4) changes in the carotenoid composition of the photosystem and (5) removal of the PufX protein. Particular attention is given to the effects of such changes on the composition of the so-called "core complex" formed between the reaction centre and the LH1 antenna protein, and the ability of the bacterium to grow under photosynthetic conditions.

doi:10.1016/j.bbabio.2008.05.090

S2/4 The 2-methoxy group of ubiquinone is essential for function of the acceptor quinones in reaction centers from *R. sphaeroides*

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The orientation of a methoxy substituent is known to substantially influence the electron affinity and vibrational spectroscopy of benzoquinones, and has been suggested to be important in determining the function of ubiquinone as a redox cofactor in bioenergetics. Ubiquinone functions as both the primary (Q_A) and secondary (Q_B) quinone in the reaction centers of many purple photosynthetic bacteria, and is almost unique in its ability to establish the necessary redox free energy gap for 1-electon transfer between them. The role of the methoxy substitution in this requirement was examined using monomethoxy analogues of ubiquinone-4, which were reconstituted into quinone-depleted reaction centers from the purple photosynthetic bacterium, *Rhodobacter sphaeroides*. The analogues used were 2-methoxy-3,5-dimethyl-6-isoprenyl-1,4-benzoquinone (2-MeO-Q) and 3-methoxy-2,5-dimethyl-6-isoprenyl-1,4-benzoquinone (3-MeO-Q) and only 2-MeO-Q was able to simultaneously act as Q_A and Q_B. The necessary redox potential tuning was shown to occur in the Q_B site. In the absence of active Q_B, the IR spectrum of the monomethoxy quinones was examined *in vitro* and in the Q_A site, and a novel distinction between the two methoxy groups was tentatively identified, consistent with the unique role of the 2-methoxy group in distinguishing Q_A and Q_B functionality.

doi:10.1016/j.bbabio.2008.05.091

S2/5 The photosystem I reaction centre of oxygenic photosynthesis

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Photosystem I of oxygenic photosynthesis is a large multi-protein complex binding 100 chlorophylls. At its core are two related polypeptides which each bind symetrically-related electron transfer chains. We present evidence from studies utilising spectroscopic approaches in combination with site-directed mutagenesis that demonstrate that light-initiated electron transfer occurs on both branches of electron transfer.

doi:10.1016/j.bbabio.2008.05.092

S2/6 The unusual configuration of the quinone reduction site of the cytochrome b_6f complex

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Complex cytochrome b_6f couples electron transfer with proton translocation through the thylakoid membrane in oxygenic photosynthesis. Unlike its counterpart in mitochondria and proteobacteria, it exhibits three additional cofactors whose functions are not understood: a chlorophyll a, whose phytyl chain lies at the entrance of site Q_o, a β-carotene and an additional haem, called c_i. X-ray crystal structures in the presence of inhibitors suggest that the putative substrate binding pocket of the Q_i site is near the iron atom of haem c_i, providing an unusual site for a quinone involved in an oxidoreduction reaction, where interaction near the edge of the protoporphyrin ring is sufficient for electron transfer. 2-*n*-nonyl-4-hydroxyquinoline N-oxide (NQNO) binds into the Q_i pocket and is able to coordinate the iron of haem c_i. Functional implications need to take into account the configuration of the Q_i site: haem c_i has no axial ligand contributed by the protein, one side is occupied by a water molecule or hydroxide in interaction with haem b_H, while the other face of haem c_i is protected by a phenylalanine, which can be displaced to allow ligand coordination. This raises questions on a mechanism that is thought to be very similar to the Q-cycle of the bc_1 complex.

doi:10.1016/j.bbabio.2008.05.093

(S2) Photosystems symposium abstracts (poster and raised abstracts)

S2.7 The bacteriochlorophyll B_A is missing in H(L153)Y *Rhodobacter sphaeroides* RC

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The reaction center of *R. sphaeroides* is a membrane-bound pigment–protein complex where the photosynthetic charge separation occurs. Direction and effectiveness of photochemical reactions within RC depends on coordination of the cofactors with each other and with the protein environment. His L153 serves as the axial ligand for the monomer bacteriochlorophyll B_A. We describe four site-directed RC mutants of *R. sphaeroides* with His L153 replaced by Cys, Met, Leu, and Tyr. The most prominent effect of the mutation on the RC properties was observed in H(L153)Y mutant. Because of the instability of these mutant RCs their properties were studied without isolation from the photosynthetic membranes using *R. sphaeroides* RCO strains. The absorption spectra of membrane-bound and isolated mutant RCs H(L153)M and H (L153)C were essentially the same as those of the WT RCs with the absorption bands of the monomer BChls being clearly resolved. In contrast, in the spectra of membrane-bound RCs H(L153)Y the 802 nm absorption band was absent. The results of the pigment analysis confirm that the B_A molecule is missing in the H(L153)Y RC. Nevertheless, being associated with photosynthetic membranes, these RCs were able to accomplish the photochemical charge separation showing quantum yield approximately 7% comparing to that of the WT RCs.

Authors acknowledge the support by the Russian Basic Research Foundation (06-04-48686 and 06-04-48668), Russian Academy of Science.

doi:10.1016/j.bbabio.2008.05.094

S2.8 Substitution of ILE L177 by His in *Rhodobacter sphaeroides* reaction center affects interaction of BCHL molecule with the surrounding protein environment

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In photosynthetic organisms the conversion of light energy takes place in the membrane-bound pigment–protein complex termed reaction center (RC). It is known that all RC cofactors interact with surrounding protein by relatively weak contacts and so can be easily extracted by organic solvents. In *R. sphaeroides* RC the isoleucine L177 was substituted by histidine. Our results show that placement of His in the vicinity of P_A and B_B bacteriochlorophyll (BChl) molecules strongly affects the spectral properties of the RC. The RC I (L177)H was found to be active in charge separation with the formation of the $P^+Q_A^-$ state with the quantum efficiency of this process close to 57%. Pigment analysis revealed that one BChl molecule was missing in the acetone–methanol extract of the I (L177)H RCs. SDS-PAGE demonstrated that a BChl molecule could not be extracted by organic solvents apparently because of its stable covalent attachment to the mutant RC L-subunit. Our data indicate that the attached bacteriochlorophyll is one of the special pair BChls, P_A. The chemical nature of this covalent interaction remains to be identified.

This work was supported by MCB grant from Russian Academy of Sciences and by grant 06-04-48686 from the Russian Foundation for Basic Research.

doi:10.1016/j.bbabio.2008.05.095

S2.9 Hydrogencarbonate is not a structural part of the Mn_4O_xCa cluster in photosystem II

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Since the end of the 1950s hydrogencarbonate (‘bicarbonate’) is discussed as a possible cofactor of photosynthetic water-splitting, and in a recent x-ray crystallography model of photosystem II (PSII) it was displayed as a ligand of the Mn_4O_xCa cluster. In this study, we provide strong evidence that hydrogencarbonate (‘bicarbonate’) is not a tightly bound ligand to the water oxidizing complex (WOC) of PSII. This is demonstrated by performing formate and NH_2OH additions into PSII samples and simultaneously monitoring the released gaseous products by membrane-inlet mass spectrometry (MIMS). The addition of formate into the PSII samples induces the release of ~0.3 HCO_3^- per reaction center of PSII. Employing Mn-depleted PSII samples we show that formate does not replace HCO_3^- from the donor side, but only from the acceptor side of PSII. In contrast, a reductive destruction of the Mn_4O_xCa cluster inside the MIMS cell by NH_2OH addition does not lead to any CO_2/HCO_3^- release. This indicates the absence of a firmly bound HCO_3^- to the WOC. We therefore conclude that HCO_3^- has only ‘indirect’ effects on water-splitting in PSII, possibly by being part of a proton relay network and/or by participating in assembly of the WOC.

doi:10.1016/j.bbabio.2008.05.096

S2.10 A glimpse into the atomic structure of plant photosystem I — 3.5 billion years of perfection

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Photosystem I (PSI) emerged as a homodimeric structure containing several chlorophyll molecules over 3.5 billion years ago, and has perfected its photoelectric properties ever since. The recently determined structure of plant PSI, which is at the top of the evolutionary tree of this kind of complexes, provided the first relatively high-resolution structural model of a super-complex containing a reaction center (RC) and a peripheral antenna (LHCI). The RC is highly homologous to that of the cyanobacterial PSI and maintains the position of most transmembrane helices and chlorophylls during the last 1.5 years of separate evolution. The LHCI is composed of four nuclear gene products (Lhca1–Lhca4) that are unique among the chlorophyll a/b binding proteins in their pronounced long-wavelength absorbance and their assembly into dimers. The current crystal structure provides a picture at near atomic detail of 16 of the 17 protein subunits with an additional subunit (PsaN) being identified for the first time on the luminal side of the supercomplex. The positions of about 3000 amino acids were assigned as were those of 168 chlorophylls (80 of them revealing the orientation of the Qx and Qy transition dipolar moments), 2 phyloquinones, 3 FeS clusters and 10 carotenoids. The structure provided a first glimpse at the architecture of the most intricate and efficient nano-photochemical machine in Nature and it tells a tale on the evolution of terrestrial life. The structure should provide a template for designing artificial systems amenable for harvesting light and utilizing its energy.

doi:10.1016/j.bbabio.2008.05.097