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.

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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.

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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.

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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.

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