1 Introduction

Photosynthesis is one of the most fundamental processes occurring in the biosphere. It is the main “planetary” machinery responsible for the conversion of energy, which is emitted by the Sun, and the simultaneous natural oxygen production, allowing the existence of all oxygen-consuming life on the Earth [1].

1.1 Evolution of Photosynthesis

At early stages of Earth’s history, the atmosphere was reducing, thereby allowing only those forms of life, which could deal with electron donors such as H₂, H₂S and some small organic compounds. In fact, about three billion years ago [2, 3] (Fig.1), primitive forms of life faced the problem of exhausting these kinds of resources, forcing them to seek alternative sources of energy. The most obvious and ubiquitous source was (and still is) the “Universe lamp” – the Sun, therefore not surprisingly different mechanisms to fix the sunlight evolved soon thereafter [4-6]. Anoxygenic photosynthesis became the only choice for autotrophic organisms to survive and was widely adapted by early forms of life already 3.5 billion years ago [5-7].

![Figure 1. Important events in the evolution of photosynthesis, adapted from [8].](image-url)
The actual revolution in photosynthesis happened around 3-2.5 billion years ago (Fig. 1), when Nature decided to utilize water as an abundant source of electrons with concomitant oxygen evolution, giving a rise to all subsequent evolution on the Earth. Despite the fact that oxygen is in principle a toxic compound, protective mechanisms against reactive oxygen species (ROS) were born, stimulating the evolution of aerobic respiration.

To maintain charge separation a special pair of chlorophylls is needed, presumably linked to protein and/or other cofactor modifying the properties of chlorophylls. The evolution of reaction centres (RCs) started from the primitive RC with only one “special” chlorophyll (Chl), acting as a light-driven oxidoreductant, leading to highly-specialized descendants [8] (see Fig. 2). The ancestral RC probably was a five transmembrane-helices (TMH) protein, which might have been fused with the light-harvesting protein [9] (binding chlorophylls and carotenoids), that evolved to provide the ability to capture sunlight, which became the major source of energy. Due to gene duplication, dimeric RC evolved, carrying the two special Chl molecules that compose the special pair still found in all RC up to date. The dimeric RC originally was a homodimer (now only chlorobium and heliobacteria show such arrangement), that further developed into a heterodimer (Fig. 2), indicating the possible advantages of such construction [8].

![Diagram of RC evolution](image)

**Figure 2.** Possible evolution of reaction centers (RC) in anoxygenic photosynthetic bacteria, cyanobacteria, algae and plants, adapted from [8]. Red rhombus indicates chlorophyll molecule of special pair, green ovals – protein subunits. RCI and RCIi indicate two different types of RC (see text), which developed into two different photosystems PSI and PSII. CP43 and CP47 are chlorophyll-binding (antenna) subunits (see text).
There are two types of RC known – type I RC harness iron-sulphur clusters as terminal electron acceptors (found in green sulfur bacteria, helicobacteria and in photosystem I (PSI) of cyanobacteria, algae and higher plants), whereas type II RC (found in purple and green filamentous nonsulfur bacteria and in photosystem II (PSII) of cyanobacteria, algae and higher plants) employ quinone molecules as terminal electron acceptors.

It is not possible to determine which type of RC preceded, and taking into account the fact that there is a high structural similarity between them it might be that they coevolved having the same proto-RC. There are two hypotheses [8] trying to explain the evolution of RC that are currently accepted. The fusion hypothesis suggests that two types of bacteria with different RC types evolved separately from the same ancestor, without explanation of the reasons forcing such separation. The next assumption should be made to extend this hypothesis – one type of RC might have been transferred into an organism with the other type of RC [10]. The fission hypothesis supposes that two types of RC was a result of gene duplication in one organism with the advantage to use both photosystems in series, which allows generating sufficient amount of energy for water splitting. Later one type of RC was probably laterally transferred to other bacteria.

With the overcoming the difficult chemistry of water splitting and oxygen accumulation in the atmosphere, Nature started to conquer the land. Non-surprisingly, the higher forms of life also used advantages of autotrophic grow allowed with photosynthesis. It is supposed by the endosymbiont theory that chloroplasts of algae and higher plants evolved by the engulfment of precursor of cyanobacteria into non-photosynthetic eukaryotic hosts [11-13].

1.2 Principles of oxygenic photosynthesis

The first step of any type of photosynthesis is the absorption of light energy. A great variety of cofactors has been chosen by Nature for this purpose. There are three general types of light-absorbing cofactors: chlorophylls, which are cyclic tetrapyrrole-derivatives; phycobilins, which are acyclic forms of tetrapyrroles; and carotenoids, which are linear polyenes with or without terminal rings (see Fig. 3 for examples).
Figure 3. Examples of light-absorbing cofactors: A) β-carotene; B) phycocyanobilin; C) Chlorophyll α.

Chlorophyll a (Chl a) and Pheophytin a (demetallized form of Chl a) (Pheo a) are the only cyclic tetrapyrroles found in cyanobacteria. Chl a shows absorption maxima at 650-680 nm (Q_y region) and additionally covers the Soret region at 430-480 nm. Phycocyanobilin, allophycocyanobilin, phycoerythrocyanobilin and phycoerythrobilin represent phycobilins in cyanobacteria. Phycobilins are carried by special phycobiliproteins, which are aggregated in phycobilisomes [14-16] serving as extrinsic antenna, attached to PSII at the cytoplasmic side.

In cyanobacteria phycobilisomes usually have a hemi-discoidal shape and consist of two parts, the allophycocyanin core and rod elements. The core maintains direct excitation energy transfer (EET) connection from phycobiliproteins to the antenna Chl a in the thylakoid membrane complexes. This class covers the 500-650 nm zone of absorption spectra. The third class – carotenoids (Car) [17, 18] (mainly β-carotene) is employed to fill the blue gap in the
absorption spectra in the area of 400-500 nm. Moreover they serve as efficient protective system against ROS by suppressing the formation of reactive singlet oxygen species with triplet carotenoid formation ($^3\text{Car}$) or by triplet Chl $a$ ($^3\text{Chl}~a$) quenching. In both cases fast radiationless decay of populated $^3\text{Car}$ states to ground state occurs [19, 20]. All types of cofactors also play stabilizing role for the protein matrix, e.g. chlorophyll-containing proteins are significantly destabilized upon Chl removal [21].

After light absorption has occurred, the energy must be transferred to the RC. As outlined by Renger [22], there are two possible ways of interactions between Chl molecules in excited and ground state: a) radiationless EET and b) electron transfer. In (a) the electronically excited state is transferred from one molecule to another in ordered arrays (proper to cofactors forming light-harvesting antennae), while in (b) an ion radical pair $P1^+P2^-$ is formed as a result of electron transfer from the pigment in the excited state ($^1\text{P1}^*$) to the electron-accepting pigment in ground state (P2) (proper to cofactors forming RC). This type of transfer is the key step of transformation of solar energy into electrochemical equivalents in photosynthesis.

The next stage is an actual charge separation, which occurs in two steps: formation of the radical pair by electron transfer from the primary electron donor to the primary electron acceptor; and stabilization of the charge separation by rapid electron transfer.

The overall process might be described as

$$P_{\text{RC}}\text{Acc}_1\text{Acc}_2 \xrightarrow{hv} P_{\text{RC}}^*\text{Acc}_1\text{Acc}_2 \xrightarrow{k_{pc}} P_{\text{RC}}^{**}\text{Acc}_1^*\text{Acc}_2 \xrightarrow{k_{stab}} P_{\text{RC}}^{**}\text{Acc}_1\text{Acc}_2^*$$

Where $P_{\text{RC}}$ is the photoactive pigment in the RC, Acc$_1$ and Acc$_2$ are the primary and secondary electron acceptors, respectively; $k_{pc}$ is the rate constant of the primary charge separation and $k_{stab}$ is the rate constant for stabilization of the charge separation.

In PSII, $P_{\text{RC}}$ is called P680 (due to a characteristic edge in the difference absorption spectrum at 680 nm) and most probably occurs as a cluster of pigments with formula (Chl $a$)$_x$Pheo$_x$ [23, 24], where $x$ might be 0, 1, 2. Acc$_1$ was assigned to a Pheo $a$ molecule [25] and Acc$_2$ to the plastoquinone Q$_A$ molecule, whereas it was shown that the main electron donor most probably is Chl$_{D1}$ [26-31] (Fig. 4).

In PSI, $P_{\text{RC}}$ is denoted as P700 and composed by a special pair of chlorophylls Chl $a$ and Chl $a'$ (13$^2$-epimer of Chl $a$) with the primary electron acceptor $A_0$ (Chl $a$ molecule) and with the secondary electron acceptor $A_1$ (phylloquinone molecule).
Figure 4. Schematic view of the electron transfer chain in PSII. The electron flow from a water molecule to the mobile electron carrier \( Q_B \) is shown with the red arrows. Distances are given in Å. Cofactors are colour-coded: chlorophylls in green, pheophytines in yellow, carotenoids in orange, plastoquinones in magenta, haems and non-haem iron in turquoise. Subscript D1 or D2 indicates that cofactor is ligated by D1 or D2 protein subunit respectively.

The water is the main electron supplier in oxygenic photosynthesis. The driving force for water splitting into molecular oxygen, four protons and four electrons is generated in PSII by formation of \( P680^+ \), which has the strongest positive midpoint potential of +1.25 V [32] known for biological systems. The site of water splitting is the unique \( Mn_4CaO_x \) cluster, which undergoes consecutive light-dependent transitions, termed Kok cycle [33] (see Fig. 5).

Figure 5. S-state cycle of water oxidation, adapted from [34].
P680\(^{+}\) and Mn\(_4\)Ca cluster are linked via redox active Tyr\(_Z\) (Tyr161 of D1 (PsbA) subunit) [35, 36]. Electrons originated from water cleavage are transferred to the terminal electron acceptor Q\(_B\) and by the latter to cytochrome b\(_{6f}\) (cyt b\(_{6f}\)), from which another electron carriers plastocyanin / cytochrome c\(_6\) transfer electrons further to PSI. The charge separation in PSI leads to reduction of bound ferredoxin, acting as electron carrier to ferredoxin-NADP\(^{+}\)-reductase, which finally reduces NADP\(^{+}\) to NADPH.

The overall reactions of linear electron transport from water molecule to NADP\(^{+}\) might be summarized as:

\[
\begin{align*}
4H^+_{stroma} + 2H_2O + 2PQ & \xrightarrow{hv} 2PQH_2 + O_2 + 4H^+_{lumen} \\
4H^+_{stroma} + 2PQH_2 + 4PC_{ox} & \xrightarrow{\text{dark}} 2PQ + 4PC_{red} + 8H^+_{lumen} \\
2H^+_{stroma} + 4PC_{red} + 2NADP^{+} & \xrightarrow{hv} 4PC_{ox} + 2NADPH
\end{align*}
\]

or in the form of Z-scheme (Fig. 6).

---

**Figure 6.** Energy diagram of light-driven reactions in cyanobacteria (Z-scheme). Abbreviations as follows: S0 to S4 – different states of the Mn\(_4\)Ca cluster (see Fig. 5 for details); Tyr\(_Z\) – redox active tyrosin residue of D1 subunit; P680 (P700) and P680\(^{+}\) (P700\(^{+}\)) – ground and excited states of P\(_{RC}\) in PSII (PSI) (see text); Chla, Pheoa, Q\(_A\) and Q\(_B\) – different electron acceptors in PSII: chlorophyll, pheophytine and two plastoquinone molecules respectively; PQ\(_{pool}\) – pool of free plastoquinone molecules; PC and FD – mobile electron carriers plastocyanin and ferredoxin respectively; A\(_b\), A\(_1\), F\(_X\), F\(_A\), F\(_B\) – different electron acceptors in PSI: chlorophyll, phylloquinone and three iron-sulfur clusters respectively.