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Spotlight

Redox Tuning in Photosystem II

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In photosynthesis, oxygen is liberated from water, not from CO_2; however, this model has been silent on why photosynthesis requires bicarbonate. Rutherford and colleagues solve this problem elegantly: bicarbonate tunes water-oxidising photosystem II to make onward electron transfer efficient; an absence of bicarbonate retunes, redirects, and safely shuts down energy flow.

Electron transfer is the universal currency of biological energy transduction. Loss of an electron is oxidation; gain of an electron is reduction. Any reduction–oxidation ('redox') reaction has a direction determined by the electrochemical potentials of its participating atoms or molecules. Each has a 'midpoint redox potential' (E_m) – the electrical potential, measured in volts (V), when the electrons leave as fast as they arrive. At equal concentrations of their oxidised and reduced states, the donor of an electron has a lower E_m than its acceptor. The central event in photosynthesis breaks this rule because a photon absorbed by chlorophyll forces an electron from a reluctant donor, with a high (positive) E_m, to a reluctant acceptor at lower (more negative) potential. Physical separation of electric charge captures the energy of the photon – and light energy is converted into electrochemical potential.

Reaction Centres

At this photochemical 'reaction centre' there is a tendency for the electron to return whence it came – to the newly photo-oxidised chlorophyll molecule. The chemistry employed by photosynthesis is to pass the electron, instead, along a chain of secondary acceptors, much like those in a respiratory chain. Each link in the chain accepts and donates at a slightly higher E_m value than the last, while each forward electron transfer must be fast enough to out-compete the return of the electron to willing acceptors in wasteful and potentially destructive 'back reactions'.

Photosystem II

In most photosynthetic organisms a chlorophyll *a* molecule is the primary electron donor in both photosystems I and II. In photosystem II [1] the E_m of its redox couple is +1.2 V – sufficient to remove four electrons eventually from water, liberating oxygen, at an E_m of +0.82 V. The primary electron acceptor of photosystem II, pheophytin, at an E_m of −0.5 V [2], passes one electron to a quinone, Q_A, giving $Q_A^{\bullet-}$. From there the electron passes to a second quinone, Q_B. A second electron, from $Q_A^{\bullet-}$ to $Q_B^{\bullet-}$, allows the formation of a stable, protonated molecule of plastoquinol, QH_2 [3]. This two-electron gate thus accepts two protons from the bacterial cytoplasm or chloroplast stroma in a second type of energy coupling – from electron transport to transmembrane proton motive force.

To understand how energy is conserved or dissipated it is essential to know the E_m of the couple $Q_A/Q_A^{\bullet-}$. Frustratingly, the literature records widely-differing measured values, all from carefully executed experiments on well-defined cyanobacterial or chloroplast membranes or membrane fractions.

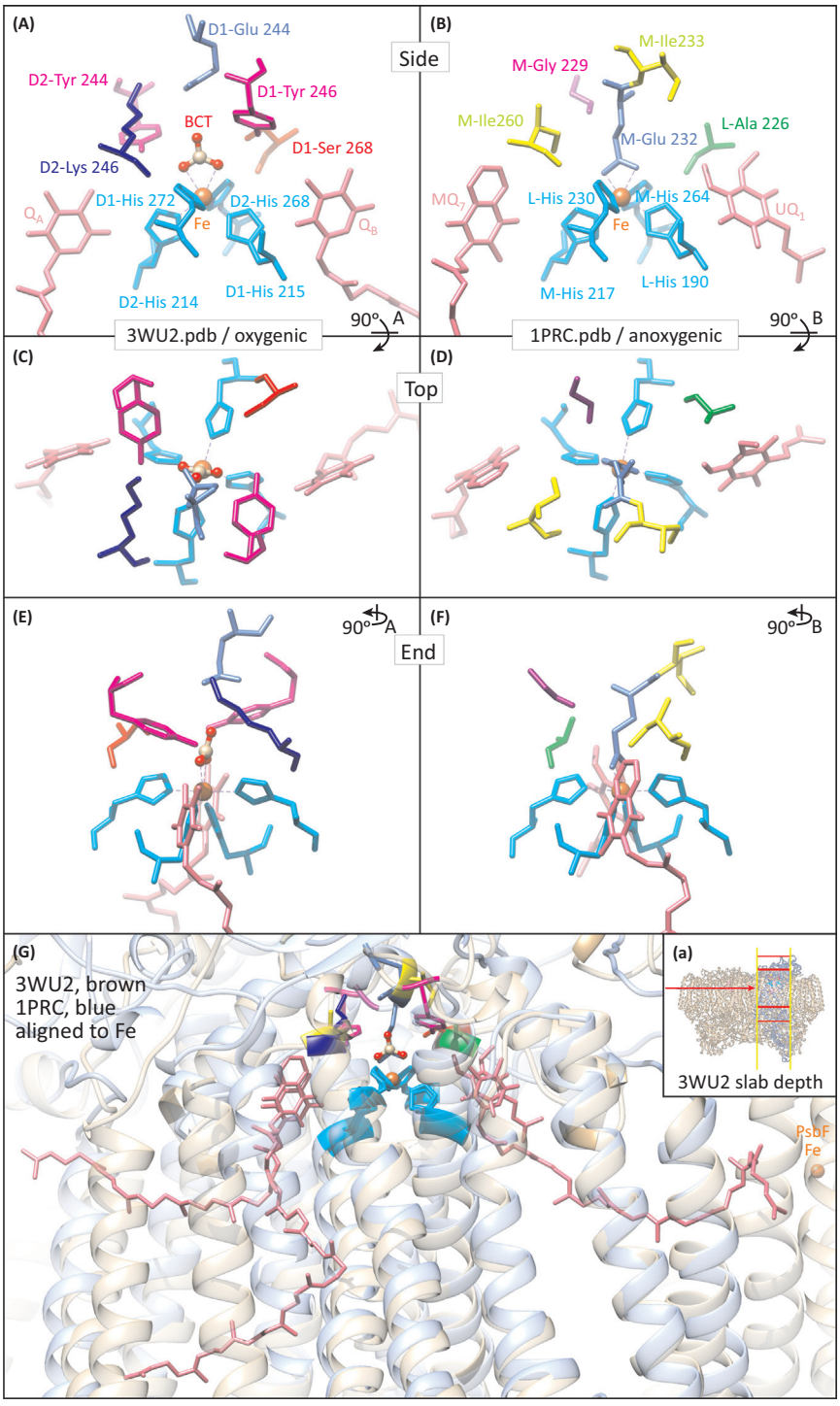
Brinkert *et al.* [4] provide an explanation of these various E_m values, and connect loose ends regarding the protection of photosynthesis from high light intensity, the emergence of oxygenic from anoxygenic photosynthesis, and the strange dependency of oxygen evolution on the presence of CO_2.

Two Midpoints – The Manganese Cluster

A difference of about 150 mV is seen in the E_m of $Q_A/Q_A^{\bullet-}$ centres in the presence or absence of the Mn_4CaO_5 inorganic catalyst of water oxidation at the electron-donor side of photosystem II. A physiological analogue of this shift in potential occurs during assembly of photosystem II, when the manganese cluster is added to the reaction centre protein complex in a step that requires light [5]. In photosystem II, reaction centres are heterodimers, and a bicarbonate ion is seen to sit adjacent to the iron atom that lies on the axis of symmetry between the two transmembrane polypeptide chains, D1 and D2, and therefore between Q_A and Q_B (Figure 1). The iron atom is not redox-active. Nevertheless, it plays a role in electron transfer between the two quinones. The iron atom is held in place by histidines of the bacterial L and M subunits, and of their homologues, D1 and D2, respectively, of photosystem II. In reaction centres of purple bacteria [6], which are anoxygenic, a conserved glutamate side-chain replaces the non-covalently bound bicarbonate of photosystem II (Figure 1).

Two More Midpoints – Bicarbonate

The standard method of determining E_m values is potentiometric redox titration. This technique measures the ratio of the oxidised and reduced forms of a chemical species over a range of potentials, each obtained under strictly anoxic conditions such that chemical oxidants and reductants can be added to achieve the stable, poised potential. The routine way of achieving anoxia is to bubble the reaction vessel or cuvette with inert gas (nitrogen or argon) to displace air and, if required, to expel a sample. A different technique, using a transparent, thin, electrochemical cell, confirmed the 150 mV E_m difference for $Q_A/Q_A^{\bullet-}$ between photosystem II with and without the manganese cluster [7], while the two values were both about



Trends in Plant Science

Figure 1. Comparison of Structures of Oxygenic and Anoxygenic Type II Reaction Centres at the Electron-Acceptor Side. (A) A view, within the membrane plane, of the cyanobacterial photosystem II reaction centre 3WU2.pdb [1] based upon its entry in the Orientations of Proteins in Membranes (OPM) database (http://opm.phar.umich.edu/). A 25 Å sphere of key side-chain residues is centred on the non-haem iron, Fe, of the D1 protein. (B) The corresponding view of 1PRC.pdb model of the photosynthetic reaction centre of *Rhodopseudomonas viridis* 3WU2.pdb [6] where D1 is subunit L, D2 is M, and Q_A and Q_B are menaquinone (MQ_7) and ubiquinone (UQ_1). (C) and (E) show the structure in A rotated 90°; (D) and (F) show the structure in (B) rotated 90°. (C) and (D) are 'top' views normal to the membrane plane; (E) and (F) are views parallel to the membrane plane and along the direction of electron transfer. Using the defaults of the MatchMaker structure comparison tool of University of California San Francisco (UCSF) Chimera (www.cgl.ucsf.edu/chimera/), the best-aligning pair of chains within 3WU2.pdb and 1PRC.pdb were overlaid and found to be D2 protein of 3WU2 with the M chain of 1PRC. Side-chain residues in (A) and (B) are labelled based upon chain, name, and amino acid position, for example D1-His 272. Abbreviations: BCT, bicarbonate ion; Fe, Fe(II) ion; Q_A/Q_B, plastoquinone; MQ_7, menaquinone-7; UQ_1, ubiquinone-1. Coloured labels reflect the 'residues' they represent; glutamate, cornflower blue; tyrosine, magenta; lysine, blue; BCT (the bicarbonate ion), brown with oxygen atoms in red; histidine, cyan; isoleucine, yellow; glycine, purple; serine, red; alanine, green; Fe(II) ion, brown; the quinones Q_A/Q_B/MQ7/UQ1, hot pink. For an appreciation of scale, the centre-to-centre distance of the benzene rings of the quinones Q_A and Q_B is ~22 Å; panels A–F are at the same zoom level, a sphere of ~25 Å, centred on the Fe(II) ion. (G) A zoomed out view to encompass the isoprenoid chains of the quinones, the intriguing Fe(II) ion of the PsbF subunit of cytochrome b_{559} [8], which is 18–19 Å distant from the Q_B isoprenoid tail, and the protein backbone shown as ribbons; 3WU2.pdb is coloured transparent brown, 1PRC.pdb transparent blue. The slab view depth ~75 Å and overall placement of this alignment within the 3WU2.pdb photosystem II dimer are shown as the inset (a).

80 mV lower than those previously agreed on the basis of conventional redox titration. Brinkert *et al*. [4] reasoned that the bicarbonate ion could explain the difference – bubbling with inert gas would deplete the sample of carbon dioxide and therefore of bicarbonate, HCO_3^-, from the carbonic anhydrase reaction:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

By contrast, the electrochemical cell of Shibamoto *et al*. [7] would not be expected to remove the bicarbonate that interacts with the iron atom guiding electron transfer from Q_A (Figure 1). Accordingly, Brinkert *et al*. carried out their redox titration on photosystem II reaction centres, this time in the presence and absence of 1 mM bicarbonate. Without bicarbonate the E_m value was −60 mV in the Mn-containing preparation and +64 mV with Mn depleted. With bicarbonate present, both E_m values dropped, as predicted, to −124 mV with Mn and to −22 mV without [4]. Brinkert *et al.* also demonstrate that accumulation of $Q_A^{\bullet-}$ at high light intensity decreases binding of bicarbonate to photosystem II [4].

The Safety Valve

A clear inference is that return of the electron from $Q_A^{\bullet-}$ to pheophytin can occur when bicarbonate is in place and when Q_B is present as Q_BH_2. This transfer is uphill, but the hill is less steep than the 'cliff' presented when bicarbonate is absent. Furthermore, this reduction of pheophytin produces a high proportion of its triplet state, which converts ground-state oxygen to singlet oxygen, a toxic product. Depletion of CO_2 will thus do two things. First, a slow-down of the Benson–Calvin–Bassham cycle of CO_2 assimilation will feed back, preventing oxidation of Q_BH_2 and thus allowing $Q_A^{\bullet-}$ no option for forward electron transport. Second, removal of the bicarbonate ion will increase the E_m of Q_A, favouring a safer back-reaction to chlorophyll or to the donor side of photosystem II through cytochrome b_{559} [8].

Origins of Oxygen

Photosynthesis first evolved in a world devoid of free oxygen [9], and purple bacterial photosynthesis today occurs only under anoxic conditions. There the glutamate adjacent to the iron atom (Figure 1) is fixed in place – and harmful singlet oxygen cannot be produced. When the first cyanobacterium learned to use its quinone-containing reaction centre to make oxygen [10–12], $Q_A^{\bullet-}$ had urgently to find a safe back-reaction. Replacement of the glutamate by a removable bicarbonate ion provided the solution.

In explaining the CO_2-requirement of photosystem II, Brinkert *et al.* also finally lay to rest the ghost of the long-abandoned theory that primary photochemistry splits CO_2 to give O_2 plus a C atom that becomes hydrated. The real primary event is transmembrane electron transfer, and CO_2 assimilation itself does not require light at all. Well, we knew that. Even so, the wrong model persisted in popular science writing, and it is satisfying to understand, now, that CO_2 tunes redox chemistry at the interface of our biosphere with energy from the sun.

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Forum

The First Darwinian Phylogenetic Tree of Plants

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In 1866, the German zoologist Ernst Haeckel (1834–1919) published the first Darwinian trees of life in the history of biology in his book *General Morphology of Organisms*. We take a specific look at the first phylogenetic trees for the plant kingdom that Haeckel created as part of this two-volume work.

Evolutionary Foundations and New Visualizations

Ernst Haeckel (Figure 1) was a strong proponent of integrating Darwin's ideas into existing research traditions in comparative anatomy and morphology. Haeckel was also an efficient propagandist whose ideas