Biocatalysis: Electrochemical Mechanisms of Respiration and Photosynthesis

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I. INTRODUCTION: KINETIC ASPECTS OF SYNCHRONOUS MULTIELECTRON REACTIONS

Vectorial charge transfer and a molecular recognition at the interface between two dielectric media are important stages in many bioelectrochemical processes such as those mediated by energy-transducing membranes [1–4]. Many biochemical redox reactions take place at aqueous medium/membrane interfaces and some of them are multielectron processes. About 90% of the oxygen consumed on Earth is reduced in a four-electron reaction catalyzed by cytochrome c oxidase. Multielectron reactions take place in photosynthesis, which is the most important process on earth [5–10]. Life on Earth began as photosynthesis.

Synchronous multielectron reactions may proceed without formation of intermediate radicals, which are highly reactive and can readily enter a side reaction of hydroxylation and destruction of the catalytic complex. Since multielectron reactions do not poison the environment with toxic intermediates and they are ecologically safe, they are used by Nature for biochemical energy conversion in respiration and photosynthesis [11,12]. In the multielectron reaction that takes place in a series of consecutive one-electron stages, the Gibbs energy necessary per single electron transfer obviously cannot be completely uniformly distributed over the stages [7]. The energy needs for various stages will be different and the excess energy in the easier stages will be converted into heat. In a synchronous multielectron reaction the energy will be used very economically [11].

An important parameter in the quantum theory of charge transfer in polar media is the medium reorganization energy $E_s$ that determines activation energy [1,12–17]. The energy of medium reorganization in systems with complicated charge distribution was calculated by Kharkats [18]. Reagents and products can be represented by a set of $N$ spherical centers arbitrarily distributed in a polar medium. The charges of each of the reaction centers in the initial and final state are $z^1$ and $z^f$, respectively. Taking $R_k$ to represent co-ordinates of the centers and $\varepsilon_k$ for dielectric constants of the reagents it follows that

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where \( \delta z_k = z^0_k - z^f_k \), \( R_{pk} = R_p - R_k \), \( z^0_k, z^f_k \) are charge numbers of particle \( k \) in the initial and final states, respectively, \( a^p \) is the radius of particle \( p \), \( R_k \) is the co-ordinate of the \( k \) - particle center, and \( \varepsilon_i \) is the dielectric constant of the reactant. Reactions with synchronous transfer of several charges present a particular case of Eq. (1).

It follows from Eq. (1) that \( E_s \) is proportional to the square of the number of charges transferred. This factor makes multielectron processes impossible in the majority of homogeneous redox reactions due to the high activation energy resulting from a sharp rise in the energy of solvent reorganization. For multielectron reactions the exchange currents of \( n \)-electron processes are small compared to those of one-electron multiple step processes, which makes the stage-by-stage reaction mechanism more advantageous. Therefore, multielectron processes can proceed only if the formation of an intermediate is energetically disadvantageous. However, conditions can be chosen which reduce \( E_s \) during transfer of several charges to the level of the reorganization energy of ordinary one-electron reactions. These conditions require systems with a low dielectric constant and large reagent radii. Furthermore, the substrate must be included in the co-ordination sphere of the charge acceptor, with several charge donors or acceptors bound into a multicenter complex. Recent papers [14–17] have presented theoretical studies on the kinetics of heterogeneous multielectron reactions at water/oil interfaces, which proved to be capable of catalyzing multielectron reactions and sharply reducing the activation energy.

The most effective coupling of ATP synthesis to electron transport can be obtained if the activation energy of the coupled process is lower than that of the charge transfer in the electron-transport chain. It is obvious from Eq. (1) that this requires a simultaneous transfer of opposite charges, so that the second and the third terms of the equation are negative. An optimal geometry between the centers of charges of donors and acceptors must also be chosen [19].

To illustrate this point, we can consider two instances of multielectron reactions: simultaneous transfer of \( n \) charges from one donor to an acceptor and simultaneous transfer of several charges (one from each of the centers) to \( m \) acceptors (\( m \leq n \)). In the former case \( E_s \) is proportional to \( n^2 \), while in the latter it may be significantly lower (depending on the sign of the charge being transferred and the reciprocal positions of reagents). A multicenter process with \( E_s \approx n \) is also possible. The concerted multicenter mechanism of multielectron reactions markedly reduces \( E_s \), and hence the activation energy, compared to a two-center multielectron process. Electrostatic interactions between reactants also reduces the activation energy of multielectron processes at the interface. Such electrostatic energies in a heterogeneous process can never be equal to zero due to interactions with image charges. However, by appropriate arrangement of the reactants the electron-transfer activation energy in a heterogeneous multielectron reaction can be much lower than the energy of medium reorganization.
II. CYTOCHROME OXIDASE: ELECTROCHEMISTRY OF RESPIRATION

The function of the enzymes of the mitochondrial respiratory chain is to transform the energy of redox reactions into an electrochemical proton gradient across the hydrophobic barrier of a coupling membrane. Isolated oligoenzyme complexes of the respiratory chain of mitochondria, cytochrome c oxidase, succinate–cytochrome c reductase, and NADH–CoQ reductase, are able to catalyze charge transfer in model systems, e.g., at a water/octane interface, which can be followed by a change in the interfacial potential at this interface [20–22]. A necessary condition for this measurement is the presence of the enzymes and substrates in the aqueous phase and a charge acceptor in the octane phase [22].

Cytochrome oxidase (EC 1.9.3.1) is the terminal electron acceptor of the mitochondrial respiratory chain. Its main function is to catalyze the reaction of dioxygen reduction to water using electrons from ferrocytochrome c:

\[
4\text{H}^+ + \text{O}_2 + 4\text{e}^- \xrightarrow{\text{respiration}} 2\text{H}_2\text{O}
\]

Reaction (2) is exothermic, and the energy can be used to transport protons across the mitochondrial membrane (Fig. 1). Mitochondrial cytochrome c oxidase is a dimer, each monomer being composed of 13 subunits. The enzyme contains cytochromes a and a3, one binuclear copper complex Cuₐ, one mononuclear copper site Cuₕ and one bound Mg²⁺ per monomer [23]. It has a molecular weight of about 180,000 – 200,000 kDa for the most active form. Cytochrome oxidases can transport up to eight protons across the membrane per four electrons. Four of the protons bind to the reaction complex during dioxygen reduction to water and up to four other protons are transported across the membrane. The resulting chemiosmotic proton gradient is used in ATP synthesis.

Respiration is the reduction of O₂ to H₂O during the oxidation of carbohydrate to CO₂. There are two types of respiration in photosynthetic organisms: a dark respiration and a photorespiration [3]. Dark respiration includes O₂ reduction and the oxidation of NADH and FADH₂ in mitochondrial membranes, glycolysis, the Krebs cycle, and the oxidative pentose phosphate pathway. Respiration is commonly subdivided into two functional components: growth respiration, supplying energy for new biomass production, and

![Diagram of Cytochrome c and c Oxidase Monomer](image)

**FIG. 1** Scheme of the structural organization of cytochrome c and cytochrome c oxidase monomer in the inner mitochondrial membrane.
maintenance respiration providing energy to maintain the integrity of already existing structures and their turnover. In the present chapter we will consider the thermodynamics of oxygen reduction in plant mitochondria.

Plant mitochondria have two membranes: a smooth outer membrane that surrounds an invaginated inner membrane. The respiratory chain of mitochondria is an integral part of the inner mitochondrial membrane. It is composed of four electron-transporting protein complexes (NADH dehydrogenase complex I, succinate dehydrogenase complex II, cytochrome reductase complex III, and cytochrome c oxidase complex IV), ATP synthase (complex V), and the mobile electron carriers ubiquinone and cytochrome c. Plant mitochondria have additional enzymes not found in the mitochondria of animals: the cyanide-insensitive alternative oxidase, an internal rotenone-insensitive NADPH dehydrogenase, and an externally located NADPH dehydrogenase, which do not conserve energy. The alternative oxidase catalyzes the oxidation of ubiquinol to ubiquinone and the reduction of oxygen to water and is inhibited by salicylhydroxamic acid. In some photosynthetic cells the carbohydrates formed during photosynthesis can serve as the Gibbs free energy source for respiration, which leads to ATP synthesis and water and CO\textsubscript{2} production. Oxygen reduction, catalyzed by cytochrome c oxidase accounts for a significant portion of the water eliminated from the mitochondria.

Kharkats and Volkov first presented proofs that cytochrome c oxidase reduces molecular oxygen by synchronous multielectron mechanism without \textit{O\textsubscript{2}} intermediate formation [15,17–19,24]. Our calculations predicted that the first step in dioxygen reduction by cytochrome c oxidase should be a concerted multielectron process although oxygen intermediates at room temperature were not detected before our estimations. As the field progresses after these pioneering observations, it became clear that the first step of dioxygen reduction is a two-electron concerted process. In the present chapter a possible molecular concerted 2:1:1-electron and 2:2 proton pump mechanisms for cytochrome c oxidase functioning are discussed.

The 1:1:1:1-electron mechanisms of oxygen reduction by cytochrome oxidase are the most frequently discussed in biochemistry. The reaction implies that the Gibbs free energy of the first electron transfer from cytochrome oxidase to \textit{O\textsubscript{2}} is positive (Fig. 2). As a result,

![FIG. 2](image)

**FIG. 2** Energy diagram for possible routes of the reaction \( \text{O}_2 + 4\text{H}^+ \leftrightarrow 2\text{H}_2\text{O} \); \( \Delta G_m \) is the reaction Gibbs free energy at pH 7.
this route should either not be followed or the reaction rate should be extremely low. Since the Gibbs free energy of O₂ binding in the catalytic site of cytochrome oxidase is −21 kJ mol⁻¹ [25], the cytochrome c redox potential is 0.25 V, and the Gibbs free energy of the first electron donation to oxygen at pH 7 is +33 kJ mol⁻¹, the Gibbs free energy of the reaction:

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- \]  

in a cytochrome oxidase catalytic site is equal to +78 kJ mol⁻¹. The activation energy for O₂ reduction by fully reduced cytochrome oxidase amounts to 16 kJ mol⁻¹ [26]. Since the Gibbs free energy of the endothermic reaction (3) is five times the measured activation energy for O₂ reduction by cytochrome oxidase, the 1-electron mechanisms 1:1:1:1, 1:2:1, 1:1:2, and 1:3 at room temperature are unlikely. They could only be feasible if the energy of the 1-electron intermediate binding were negative, but greater than 52 kJ mol⁻¹ in magnitude. Such significant energy of covalent binding allows this intermediate to be experimentally detected. However, it has not been detected thus far.

The fact that the first electron addition to O₂ is endothermic accounts for the relative chemical inertness of oxygen in Nature and permits the existence of life on Earth. The high-energy binding of oxy-intermediate provides a strong restriction on the energetics of the next step of second electron transfer and formation of a peroxide intermediate. The probability of such a pathway is too small due to kinetic and thermodynamic limits. Therefore, evolution could reserve either ecologically clean sequential 2:1:1 mechanism, with intermediates tightly bound at the catalytic site. A possible mechanism of dioxygen reduction by cytochrome c oxidase is outlined in Fig. 3 and will be considered in detail after discussing the thermodynamic and kinetic aspects of the problem.

**FIG. 3** Scheme of 2:1:1 electron oxygen reduction mechanism at the cytochrome c oxidase active site. (From Refs. 15,17–19, and 24.)
A. Reaction Center Architectonics

Equation (1) lays down conditions for the structure of cytochrome c oxidase catalytic sites necessary for dioxygen reduction to occur by the concerted n-electron mechanism. To lower the medium reorganization energy and thus activation energy, it is necessary that:

- The dielectric constant of the medium where oxygen reduction takes place should be low, i.e. the catalytic site should be immersed in the hydrophobic phase of the membrane (protein).
- There should be n spatially separated electron donors. For the mechanism proposed, these are hemes and protein–copper complexes.
- It is desirable that electron transfer via cytochrome c oxidase be accompanied by the transport of cations (e.g., protons). It follows from Eq. (1) that, when charges of opposite signs are transferred simultaneously in close directions, the medium reorganization energy could be lowered due to the third and fourth terms in Eq. (1). Equation (1) implies that the coupling of the electron and proton pumps in cytochrome oxidase can be attained if medium reorganization is neutralized by the simultaneous transfer of unlike charges in close directions. If electron transfer via cytochrome oxidase is coupled with proton transport across the mitochondrial membrane, the energy liberated in reaction (2) will be consumed for useful work instead of being converted into heat.
- The radii of electron donors should be sufficiently large. This is achieved not by using free metal ions, but their organic complexes (hemes and cysteines) with systems of conjugated bonds and ligands capable of participating in redox reactions.

B. Bridge Electron-Transfer Mechanism

Electrons generated in the oligoenzyme complexes of the mitochondrial respiratory chain are transferred to the cytochrome c oxidase active site by the 1-electron bridge mechanism. The reduction of the oxygen molecule to water requires the stepwise transfer of four electrons from cytochrome c to cytochrome a and a3 as well as to two Cu-containing proteins, Cuα and Cuβ. A quantum mechanical calculation has been made of the probability of electrons transfer via an intermediate virtual state as a possible model of an electron mechanism with an activated outer sphere and a bridge ion.

The stepwise transfer of electrons from cytochrome c to cytochrome a3 via cytochrome a is kinetically favorable due to a substantial decrease in the medium reorganization energy for direct electron transfer from cytochrome c to cytochrome a3. The redox potential of Feα may not be smaller, but even greater than the redox potential of Feα. It is essential that only the minimum of the intermediate term on the reaction energy diagram be below the cross-point of the initial and final terms.

C. Activation Energy and Mechanism of Dioxygen Reduction

Studies on the temperatures dependence of the oxygen reduction rate have revealed that cytochrome oxidase exists in two conformations—“hot” (h) and “cold” (c). The respective activation energies $E_{ah}$ and $E_{ac}$ are equal to 16 kJ mol$^{-1}$ (at 23–35°C) and 60 kJ/mol (below 20°C) [27]. A phase transition accompanied by changes in conformation and the absorption spectrum takes place between 18°C and 23°C. The temperature $T^*$ depends on the
composition of the surrounding lipid. The low $E^h$ value means that the 1-electron mechanisms 1:1:1:1, 1:2:1, 1:3, and 1:1:2 are unlikely at temperatures higher than $T_c$ since the enthalpy for the transfer of the first electron from reduced cytochrome oxidase to dioxygen is five times the measured activation energy. If the energy of binding of one electron intermediate $a_3-O_2$ is less than $-52$ kJ mol$^{-1}$ it gives thermodynamic and kinetic possibilities for a multistep one-electron mechanism of oxygen reduction. However, the energy of reorganization of the medium for one-electron transfer in cytochrome oxidase in “hot” and “cold” conformations cannot be different in four times. From Eq. (1) it follows that if we change the distance $h_{12}$ of electron transfer from $h_{12} = a_1 + a_2$ to $h_{12} > a_1 + a_2$ the energy of reorganization changes by less then 50%. $E_a$ can be equal to 16 and 60 kJ/mol for the one-electron mechanism of oxygen reduction according to Eq. (1) only if $E_a > 770$ kJ/mol when $E_{\text{comp}} < -52$ kJ/mol$^{-1}$. Such a high value of $E_a$ also shows that 1:1:1:1-electron mechanism of oxygen reduction in vivo is unlikely. For the multielectron reaction 2:1:1, according to Eq. (1), $E_a$ for 2-electron reactions between $O_2$, $a_3$, and $Cu_b$ strongly depends on the geometry and distances in a catalytic site. Only 2:1:1 mechanism of oxygen reduction by cytochrome oxidase can be realized in vivo in both “hot” and “cold” conformations. Consider the molecular mechanism of dioxygen reduction outlined in Fig. 3 in more detail.

The oxidized catalytic site of cytochrome oxidase composed of cytochrome $a_3$ and $Cu_b$ is reduced via the bridge mechanism by two electrons supplied from the electron reservoir of the respiratory chain to form a reduced complex, which then binds an oxygen molecule. The reaction center is oxidized to the initial state in a 2-electron reaction with the formation of a peroxide bridge between $a_3$ and $Cu_b$. The partially reduced (to peroxide) oxygen molecule must be bound in the reaction center since cytochrome oxidase is known to reduce dioxygen to water without the release of any intermediates from the membrane. After that, the catalytic complex accepts two electrons in turn from the electron reservoir $Fe(c) \rightarrow a_3$. At the next step, the peroxide bridge undergoes 1:1-electron reduction and protonation to water. The concerted electron-transfer step ($10^{-15} - 10^{-13}$ s) is followed by enzyme relaxation with a complex set of characteristic times and constants. Rate constants for such processes can range from very low (1 s$^{-1}$) to very high ($10^9$ s$^{-1}$). Studying the relaxation of fully reduced cytochrome oxidase on its interaction with dioxygen allowed the following characteristic constants to be resolved: $7 \times 10^7$ M$^{-1}$s$^{-1}$, $6.8 \times 10^4$ M$^{-1}$ s$^{-1}$, $1.7 \times 10^4$ M$^{-1}$ s$^{-1}$, and $1.1 \times 10^3$ M$^{-1}$ s$^{-1}$. Such a complex relaxation pattern has led some authors to suggest that electron transfer to $O_2$ is stepwise and proceeds via the 1:1:1:1 mechanism. However, since no intermediate that should be formed on the first electron donation to $O_2$ has been detected in the native enzyme at a temperature higher than 21°C, this spectrum of characteristic times can be attributed to the relaxation of the metalloenzyme during 2:1:1-electron oxygen reduction to water. The redox potentials of cytochromes $a$ and $a_3$ as well as of $Cu_a$ and $Cu_b$ are about the same, which means that the energy states of all the four metal centers in the reaction complex of the native enzyme are similar. This also favors concerted reactions.

Sucheta et al. [28] published the experimental proofs of the theoretical mechanism of cytochrome $c$ oxidase functioning proposed originally be Kharkats and Volkov [15,17–19,24]. Using time-resolved optical absorption difference spectra and singular value decomposition analysis, Sucheta et al. [28] found the presence of peroxo and ferryl intermediates at room temperature during reduction of oxygen by cytochrome $c$ oxidase and measured the rate constants.
D. Proton Pump

Water molecules released in the course of oxygen reduction are removed from the hydrophobic catalytic site to the aqueous phase. The product being continuously removed from the reaction center will shift the equilibrium of reaction (2) to the right. Energy liberated in the exothermic reaction (2) is sufficient for transporting eight $\text{H}^+$ ions across the membrane. Four of them are used to form with $\text{O}_2$ two $\text{H}_2\text{O}$ molecules. The remaining $\text{H}^+$ ions can be transported across the hydrophobic zone of the membrane and used for ATP synthesis via the ATP-synthetase complex, with the cytochrome oxidase $\text{H}^+$ pump serving only to transform the energy of ferrocytochrome c oxidation. Proton translocation can be direct if the ligands to redox centers provide the protons, or indirect if the redox reactions cause conformational changes transmitted to proton-donating groups remote from the redox centers. As follows from thermodynamics (Fig. 2), energy for the $\text{H}^+$-pump functioning is liberated only at the last steps of water formation on the addition of third and fourth electrons independently of the reaction route. The functioning of the protons' pump after formation of ferryl intermediate is possible only if the difference between the Gibbs energy of ferryl and peroxy intermediates' binding is more negative than $-35 \text{ kJ/mol}^{-1}$.

The energy of binding of ferryl intermediate is negative and sufficiently high, which gives a possibility to the functioning of a proton pump not only at last stage of addition of fourth electrons, but also after formation of a 3-electron oxygen intermediate. The stoichiometry of proton pumping by cytochrome oxidase can be 0:2:2 if the ferryl intermediate has $-35 \text{ kJ}$ more negative energy of binding than the peroxy intermediate.

As follows from Eq. (1), media reorganization energy corresponding to simultaneous transfer of electrons and protons will be minimal in the case when the directions of their transfers are close. In the case of charge transfer in cytochrome oxidase the donor of electrons is situated on side $C$ and the protons come from side $M$. In this case the minimal activation energy will be achieved at the maximally possible given geometry of the systems angle between the directions of electrons and proton transfers.

It is to be noted that cytochrome oxidase can reduce $\text{O}_2$ without concomitant proton transfer. In such a case the enzyme would work as a machine converting the energy of electron transfer to heat. It may be that evolution has reserved only the $\text{e}^-\text{H}^+$-form of cytochrome oxidase with minimum energy dissipation.

III. PHOTOSYNTHETIC SYSTEMS

The annual consumption of energy by mankind is currently about $4 \times 10^{17}$ kJ, rising rapidly and doubling every 20 years. The known reserves of fossil fuels are limited to an estimated energy equivalent of $5 \times 10^{19}$ kJ, so new sources of energy are of fundamental importance. One obvious possibility is solar energy. The amount of solar energy incident on the Earth is about $5 \times 10^{21}$ kJ per year, of which $3 \times 10^{18}$ kJ is converted into chemical energy by photosynthesis in plants and micro-organisms. In water-oxidizing photosynthesis two large membrane-integrated protein complexes photosystem II (PS II) and photosystem I (PS I) are operating in series [3]. The electron transfer starts in both photosystems vectorially across the membrane. Light energy is harvested by photosynthetic pigment systems in which the electronic structure of excited-state chlorophyll donates an electron to a primary acceptor pheophytin, the first component of an electron-transport chain. The electron carries with it the energy of the original photon of light that was absorbed, and in
the process of electron transport the energy is captured in two ways. The first involves coupling a proton pump mechanism with the sequential redox reactions in one part of the electron-transport chain, so that a proton gradient is established across the thylakoid membrane. The electrochemical energy of the proton gradient is then used to drive ATP synthesis by the ATP synthase enzymes embedded in the membrane [1]. The second energy capture occurs when an acceptor molecule such as NADP is reduced to NADPH, which in turn is used to reduce carbon dioxide in the Calvin cycle. Systems modeling photosynthesis should have the capability of carrying out relatively simple versions of these fundamental reactions.

The last part of this chapter focuses on electrochemical mechanisms of water oxidation in the PS II of green plants.

IV. STRUCTURE AND COMPOSITION OF THE OXYGEN-EVOLVING COMPLEX IN VIVO

The redox map of photosynthesis in green plants can be described in terms of the well-known Z-scheme proposed by Hill and Bendal [29]. The main advantage of the currently accepted Z-scheme depicted in Fig. 4 lies in the specific mechanism of charge transfer at the stage of water oxidation, which is a multielectron reaction mechanism involving no unknown intermediates [6,12].

The molecular organization of a thylakoid membrane is shown in Fig. 5. The spectral characteristics of PS II indicate that the primary electron donor is the dimer of chlorophyll P680 with absorption maxima near 680 and 430 nm. Water can be oxidized by an oxygen-evolving complex (OEC) composed of several chlorophyll molecules, two molecules of pheophytin, plastoquinol, several plastoquinone molecules, and a manganese–protein complex containing four manganese ions. The OEC is a highly ordered structure in which a number of polypeptides interact to provide the appropriate environment for cofactors such as manganese, chloride, and calcium, as well as for electron transfer within the complex. Figure 6 shows the electronic equivalent circuit of PS I and PS II.

Manganese-binding centers were first revealed in thylakoid membranes by EPR methods, and it is now understood that four manganese ions are necessary for oxygen evolution during water photo-oxidation. Plastoquinone (PQ) acts as a transmembrane carrier of electrons and protons between reaction centers of two photosystems in the case of noncyclic electron transfer and may also serve as a molecular “tumbler” that switches between one-electron reactions and two-electron reactions. Pheophytin is an intermediate acceptor in PS II. Direct formation of P680 pheophytin ion radical pairs was revealed by experiments on magnetic interactions between pheophytin and PQ reflected in the EPR spectra.

V. THERMODYNAMICS OF WATER OXIDATION

The photocatalytic oxidation of two molecules of water to dioxygen cannot be a single-quantum process since the total energy expenditure of a catalytic cycle cannot be less than 476 kJ mol⁻¹. However, there is no fundamental reason why one quantum should not induce the transfer of several electrons. For instance, a two-quantum process would require light with a wavelength of less then 504 nm while a four-quantum process
would involve a sequential mechanism in which each light quantum is used to transfer one electron from the photocatalyst to an electron acceptor. The threshold wavelength for the oxidation of water in this case is 1008 nm. The eight-quantum scheme which is actually used in photosynthesis can be explained by the need to compensate for energy losses in a long electron-transfer chain of redox reactions.

Water oxidation to molecular oxygen is a multielectron process that proceeds with a surprisingly high quantum efficiency. The water oxidation reaction can proceed on illumination at 680 nm, a wavelength of light that excludes one-electron mechanisms using hydroxyl and oxygen radicals (Fig. 2). For a three-electron reaction a stronger oxidant than the cation radical P680$^+$ is needed. A synchronous two-by-two 2:2-electron pathway of the reaction is thermodynamically possible if the standard free energy of binding of the two-electron intermediate is about $-40 \text{ kJ mol}^{-1}$. This value corresponds to the energy of two hydrogen bonds forming between H$_2$O$_2$ and the catalytic center. For this case a molecular mechanism can be proposed (Fig. 7) and will be discussed below. Synchronous four-electron oxidation of water to molecular oxygen (Fig. 8) is also thermodynamically possible.
One-electron mechanisms of water oxidation are likely to be operative in some model systems with a low quantum efficiency, but two- or four-electron reactions cannot occur due to kinetic limitations. The intermediates formed in these systems would be highly reactive and could enter into side reactions of hydroxylation, oxidation, and destruction of chlorophyll and other components of the reaction center.

FIG. 5  Schematic model of the electron-transport chain with most of the light-harvesting pigment–protein complexes omitted.

FIG. 6  Electronic equivalent circuit of photosystems I and II.
Membrane-bound P680 enters an excited state on illumination. In dimers and other aggregated forms of chlorophyll the quantum efficiency of triplet states is low, and it is the singlet excited states that undergo photochemical transformations. In several picoseconds, an electron is first transferred to pheophytin, then to plastoquinone Qₐ, and from plastoquinone Qₐ to another polypeptide-bound plastoquinone Qₐ in the thylakoid membrane (Fig. 4), resulting in an oxidized pigment and a reduced acceptor. The cation radical P₆₈₀⁺ successively oxidizes four manganese ions, which in turn drives the production of molecular oxygen. Formation of a cation radical of chlorophyll or oxidation of manganese ions is accompanied by dissociation of water bound to the reaction center and ejection of protons. A synchronous multielectron process that describes all four oxidizing states of the OEC was proposed earlier. The transfer of electrons in a 1:1:1:1 series from a manganese cluster to the electron-transport chain is accompanied by the ejection of 1:0:1:2 protons and the evolution of molecular oxygen.

Protons are released from reaction centers either by regulators of proton distribution or by hydrogen-bond transfer (analogous to a Grotthus mechanism) through the hydration shell of manganese ions. The hydration sphere of manganese is known to contain water molecules that rapidly exchange protons with bulk water. The presence of divalent cations at the interface between two immiscible electrolyte solutions facilitates strong

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**FIG. 7** Proposed 2:2-electron mechanism of water photo-oxidation.

**FIG. 8** Proposed four-electron mechanism of water photo-oxidation.
adsorption of water molecules belonging to the second hydration shell of ions. Thus, a portion of co-ordinatively bound water enters the compact part of the electrical double layer, which changes its differential capacity at the interface. In the case of multivalent ions with small radii, the electric field near a cation is large. This can disturb the microstructure of the adjacent intrathylakoid space and bring about dielectric saturation effects.

Manganese ions play a particularly important role in the evolution of dioxygen during photosynthesis. Although there are several manganese pools in chloroplasts, only one is involved in water oxidation. The manganese ions associated with chloroplast OEC can perform a number of functions:

- The Mn–polypeptide complex is a redox intermediate that protects the reaction center from redox and radical destruction.
- Mn clusters are redox buffers facilitating accumulation of four holes in the reaction center of PS II, which are needed to ensure water photo-oxidation.
- Hydrated multivalent Mn cations bring water to the reaction center so that rapid proton exchange and transport through the hydration shell of Mn ions in the zone of water oxidation are affected.
- Multivalent Mn ions induce dielectric saturation effects in the polar region of the reaction center of PS II, which reduces the reorganization energy of the medium during charge transfer.

REFERENCES