Review

Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: Redox tuning to survive life in O2

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Abstract

The energy-converting redox enzymes perform productive reactions efficiently despite the involvement of high energy intermediates in their catalytic cycles. This is achieved by kinetic control: with forward reactions being faster than competing, energy-wasteful reactions. This requires appropriate cofactor spacing, driving forces and reorganizational energies. These features evolved in ancestral enzymes in a low O2 environment. When O2 appeared, energy-converting enzymes had to deal with its troublesome chemistry. Various protective mechanisms duly evolved that are not directly related to the enzymes’ principal redox roles. These protective mechanisms involve fine-tuning of reduction potentials, switching of pathways and the use of short circuits, back-reactions and side-paths, all of which compromise efficiency. This energetic loss is worth it since it minimises damage from reactive derivatives of O2 and thus gives the organism a better chance of survival. We examine photosynthetic reaction centres, bc1 and b6f complexes from this view point. In particular, the evolution of the heterodimeric PSI from its homodimeric ancestors is explained as providing a protective back-reaction pathway. This “sacrifice-of-efficiency-for-protection” concept should be generally applicable to bioenergetic enzymes in aerobic environments.

1. Introduction

Biological energy conversion mainly operates through membrane-spanning enzymes that build up a transmembrane electrochemical potential using the driving force provided by exergonic reactions [1]. As with all enzymes, those involved in energy conversion act as catalysts. Thus, even though the reaction pathways may involve a series of intermediates, completion of the enzyme cycle brings the enzyme back to its ground state. As energy-converting devices, their reaction pathways involve high-energy intermediates, which react exergonically providing the work required to drive the cycle. As energy-converting de

The transiently-stored energy would thus be released without any work being extracted from the overall process.

Owing to the thermodynamic reversibility of the forward, productive reactions, intermediates may simply decay by back-reacting to the ground state of the enzyme via the thermally-activated repopulation of higher energy states (Fig. 1). The transiently-stored energy would thus be released without any work being extracted from the overall process.

Productive energy conversion can be lost by processes other than simple back-reactions (Fig. 1). Such processes could involve charge recombination from a high energy state directly to the ground state without retracing the steps of forward electron transfer. Equally they could involve the formation of a lower energy form of the same component, such as a change in the protonation state or a secondary change in the redox state, reactions common in quinone chemistry. These may be termed “short-circuits” or “by-passes” depending on rates, routes, distances and semantics. In other cases, the lower energy intermediates may involve components different from those involved in the productive pathway. Such routes can be called “side pathways”, in some case these could fall into the category of “futile cycles”. The range of routes that lead to energy loss is broad and this is reflected by the loosely fitting terminology (Fig. 1).

An additional route of energy loss is worth mentioning specifically: the leak. In this category the main focus of attention is the 1-electron reduction of O2 by reducing components of the electron...
transfer pathways to produce superoxide radical, $O_2^-$ (Fig. 1). Superoxide is one of the so-called “reactive oxygen species”, it is easily reduced to form even more reactive species (peroxide and thence OH•) [2] that damage biological material and it is implicated in ageing and disease (see e.g. [3]). Superoxide is also thought to play an important role in signalling pathways within the cell [4].

The structures of all the major actors in the photosynthetic and respiratory electron transfer chains are now known at levels of resolution sufficient to identify the redox cofactors involved in the intracomplex electron transfer and to estimate the distances between them. This provides a robust framework for the theoretical determination of the electron transfer rates between a given donor and acceptor pair [5,6]. In addition, the functional characterization of some of these enzymes under various (usually inhibited) conditions has allowed the study of the non-productive pathways (short-circuits/by-passes/side-pathways etc). Thus a rather detailed picture has emerged, within which can be discerned the basic principles that determine the competition between productive and non-productive reactions and thus their respective yields. Taking Photosystem I, Photosystem II and the cytochrome $b_6f$ complexes as examples, in this review we shall highlight the mechanisms that tune the yields of the competing pathways and discuss the possible role of short-circuits etc as protective routes under adverse conditions.

2. Photosynthetic reaction centres: an overview

All known photosynthetic reaction centres share the common structural feature of pseudo-C2 symmetry both at the level of the protein backbone and of the arrangement of the redox cofactors (Fig. 2) (see e.g. [7–11]). This reflects their evolution from a common ancestral reaction centre which was made up of a homodimer of core protein subunits [12]. Despite this basic structural similarity, two classes of reaction centres are defined based on their terminal electron acceptors. Type II reaction centres use light to drive the reduction of quinone, while Type I reaction centres use light to drive the reduction of ferredoxin (or flavodoxin).

In Type II reaction centres, there are two bound quinones, $Q_A$ and $Q_B$, which act in series as electron acceptors. $Q_A$ acts as a 1-electron carrier, while $Q_B$ undergoes two sequential reductions and associated protonations to form the quinol, $Q_BH_2$ [13]. The quinol then exchanges with an oxidized quinone from the membrane. This function requires that the semiquinone, $Q_B^•$, formed on the first photochemical turnover, remains stable until a second electron arrives upon the subsequent photochemical turnover. In Type I reaction centres, the terminal electron acceptors are iron-sulfur clusters but two bound quinones are also present. In this case, the quinones are both 1-electron carriers, relaying electrons from photoreduced chlorophyll to iron-sulfur clusters (see [14] for a review).
Thus, whereas Type I reaction centres work as purely monoelectronic devices that are not coupled to protons, Type II reaction centres couple the monoelectronic photochemical charge separation to the two-electron (and two-proton) reduction of a quinone to a quinol. As will be discussed below, this essential functional difference results in a strong mechanistic constraint that has shaped the evolution from Type I to Type II reaction centres.

The energy wasteful reactions are a particular problem for photosynthetic reaction centres because the photochemistry gives such high energy intermediates. This problem is mainly dealt with through kinetic control; i.e. the forward reactions are faster than the back reactions. This is achieved by having cofactors appropriately spaced within the protein to allow rapid vectorial electron transfer across the membrane, separating the positive and negative charges from each other. Small energy losses occur on the forward reactions thus making back-reactions thermodynamically unfavourable. In contrast, the direct recombination reactions of the radical pairs are strongly exergonic. In this case however the standard free energy gap is so big that the reactions fall in the “Marcus inverted region” and are thus relatively slow [15]. In addition, as the distance increases between the two charges of the radical pair, direct charge recombination electron transfer routes become slower: short-circuits decrease [16].

When highly reducing intermediates are formed they will have a tendency to react with O$_2$ if it is present. This is a particular problem for PSI where even the terminal acceptors are more reducing (−520 mV) than the O$_2$/O$_2^−$ couple (−330 mV under 10$^5$ Pa of O$_2$ and −160 mV for O$_2$ in aqueous solution, with [O$_2$] = 1 M [21]). When very oxidizing species are formed, and this is a particular problem of Photosystem II, the adventitious oxidation of cofactors (and perhaps proteins and lipid) can occur and may propagate out from the reaction centre. Both of these can be considered as “leaks”.

When high energy radical pairs involving chlorophyll recombine, they can form chlorophyll triplet states that can react with O$_2$ to form singlet oxygen, a reactive oxygen species that is much more damaging than superoxide. This problem is common to all reaction centres in aerobic environments. This can be considered a short-circuit (chlorophyll triplet formation), resulting in a leak (triplet-sensitized $^{1}$O$_2$ formation, a leak of energy not of an electron), in some cases preceded by a back-reaction (e.g. P$^+$Q$^−_A$ to P$^+$Phe$^−$). In this case the energy loss to the leak does not drive the short-circuit, as the triplet state would decay, albeit more slowly, without reacting with oxygen. The consequences of $^{1}$O$_2$ formation are presumably so negative that a range of strategies are employed to prevent this route from occurring.

3. Type I reaction centres

Photosystem I is a plastocyanin/ferredoxin photooxidoreductase (in some species and conditions the donor may be cytochrome c$_6$ and the acceptor flavodoxin) and is present in plants, algae and cyanobacteria. It is thought to have evolved from an ancestral homodimeric reaction centre that resembled those in the present day Helio bacteria and Chlorobiaceae, both of which grow in anaerobic conditions [17]. These homodimeric reaction centres are composed of two identical subunits, each bearing a redox chain capable of light-driven charge separation [18,19].

Even in the absence of a crystallographic structure, the main structural features of the Helio bacteria and Chlorobiaceae reaction centres can be deduced given the similarities to PSI. The two electron transfer branches diverge from a (bacterio)chlorophyll pair,
which is close to one side of the membrane, and converge at the
level of the \( F_x \) iron sulfur cluster on the other side of the membrane
\[12,20–22\]. In a homodimer, with a truly symmetric structure,
both pathways are expected to function symmetrically.

3.1. Heterodimeric PSI: adaptive redox tuning to deal with life in \( O_2 \)

Existing PSI has evolved to have greater asymmetry, with dupli-
cation of the core reaction centre gene and separate evolution of
the two resulting genes giving rise to a heterodimeric reaction cen-
tre \[17,23\]. The two near-symmetrical electron transfer branches
of PSI, which have around 60% identity between the A and B sub-
units, thus show differences at the amino-acid side chain level
resulting in several functional differences (see Figs. 2 and 3).

Under normal circumstances, when PSI undergoes photochem-
istry, reduced \( F_A/F_B \) is rapidly oxidized by ferredoxin or flavodoxin.
Prior to the activation of the \( CO_2 \)-fixation enzymes however, the
amount of oxidized ferredoxin is limited and it builds up in its re-
duced form. The back-reaction \( F_A/F_B \) with \( P_{700} \) is 40 ms, but under
normal circumstance reduced donors are available and electron
donation prevents the back-reaction. \( F_A/F_B \) will thus accumulate,
when fully reduced, further light excitations will result in forma-
tion of \( F_x \) and the two \( A^- \) acceptors, all three of which are rather
close in energy and these will back-react with \( P_{700} \) when present

![Diagram](image)

**Fig. 3.** Scheme illustrating the dominance of the A-branch as a back-reaction pathway in Photosystem I. Both panels show the standard free energy levels of the radical pairs
formed by charge separation in Photosystem I (estimate from \[14,24\]) and a structural scheme of the same reactions \[8\]. The order of the forward reactions is indicated by
numbered red arrows. Broken arrows show back-reactions with grey designating the disfavoured reactions. Panel A shows charge separation initiated on the A branch and the
dominant back-reactions are shown, illustrating the idea that charge recombination occurs mainly between \( A^- \) and \( P^+ \) thereby minimizing the formation of \( 3P \) triplet state
and thence \( ^3O_2 \). Panel B shows charge separation on the B branch and here too the dominant back-reactions pathway is suggested to be the non-triplet generating A branch as
explained in the text. Note that charge separation is considered to occur between the Chl and \( A_0 \) pigments followed by rapid donation from the \( P \) chlorophylls in accordance
with \[45,138\].
These back-reactions take place with rates ranging from milliseconds to hundreds of microseconds. Again, unless the plastocyanin pool is fully oxidized, electron donation to $P_{700}$ should be fast enough to trap the acceptors in the reduced form. Thus when the soluble electron acceptors are limited, electrons linger on the terminal electron acceptors and upon further turnovers the preceding acceptors can become reduced. Leaks and back reactions are then predicted to occur.

When the PSI electron acceptors are reduced, their very low potentials mean that $O_2$ can easily be reduced to $O_2^-$. This leak occurs not only at the stromal surface but also perhaps within the membrane, presumably from the $A_1$ semiquinones when the iron sulfur centres are reduced (see [26]). At the onset of illumination, before the enzymes for $CO_2$ fixation are activated, nearly all the electrons coming through the electron transfer chain end up forming $O_2^-$. In recent years $O_2$ has been recognised as a signalling molecule (in addition to its established reputation as a potentially damaging reactive oxygen species) and so this leak is not necessarily a bad thing. But it is something that needs to be regulated and exactly how that is done is still not clear in detail. It does seem likely that the regulation of electron donation into and out of the reaction centre in both linear and cyclic electron transfer, are all important and that redox tuning may also play a role.

One feature of PSI that may reflect redox tuning to limit $O_2$ reduction is the fact that the more stable, highest potential acceptor is not the $F_X$, the exposed terminal acceptor that interacts with ferredoxin, but rather the $F_A$ centre which is buried inside the protein (see [27]). The more sequestered location of the reduced $F_X$ could slow its reaction with $O_2$. Interestingly in the green sulphur bacterial reaction centre this $F_A$-type acceptor appears to be the more stable of the two iron-sulfur centres [21]. This would make sense since it lives in an anaerobic environment and thus does not need to protect itself against $O_2$.

The back-reactions occurring when the electron acceptor side of PSI is blocked can result in chlorophyll triplet formation and hence singlet $O_2$ formation. This might be one of reasons underlying the photosensitivity of PSI in mutants lacking PGR5 [28], a protein shown to promote cyclic electron flow and proposed to be involved in the formation of supercomplexes comprising all the players required for an efficient cyclic electron flow around PSI [29,30]. Indeed, such redox cycling supercomplexes, which function without the diffusion of soluble electron carriers such as ferredoxin or plastocyanin, are expected to be less susceptible to the electron acceptor-side limitations described above. Below we propose that back-reaction pathways leading to the chlorophyll triplet formation are specifically minimized by redox tuning.

Asymmetry in PSI also exists at the level of the phylloquinones (compare Fig. 3A and B). The forward electron transfer rates for the two phyllosemiquinones, $A_{1A}$ and $A_{1B}$, to $F_X$ differ by an order of magnitude (200 and 20 ns respectively) [31-34] due to differences in the reduction potential of the two phyllosemiquinones (estimated to be $-671$ and $-844$ mV respectively [35], although functional studies point to the difference being smaller [36,37]). The origins of these very low potentials and the difference in the two potentials are discussed in detail elsewhere [25], briefly it results from a combination of electrostatic effects notably those from the $F_X$ and $F_A$ and from the protein environment with the asymmetry arising from differential effects of the protein backbone and asymmetry in specific ionisable amino acids [35].

The mechanistic significance of the kinetic and redox asymmetry associated with the quinones is unexplained. A rate of 200 ns compared to 20 ns is not expected to have any functional significance since these rates are both much faster than the competing back-reaction $P^+ A_{1A}$ recombination occurs in 200 µs. However, one can predict that the back-reaction pathway from $F_X$ will be dominated by the A-side route since $A_{1A}$ is slightly downhill from $F_X$, while the $F_X$ to $A_{1A}$ step is uphill. It seems possible that this characteristic of PSI could be beneficial under high-light conditions.

It is known that chlorophyll triplets can form in the PSI reaction centre when illuminated under reducing conditions [38] or when the secondary acceptors are removed [39]. Under physiological conditions it seems possible that PSI would encounter conditions in which electron donors and acceptors would be insufficient to prevent charge recombination within PSI, under supersaturating light intensities for example, and this may be expected to result in triplet formation. Fig. 3 illustrates how the A-side, with its high potential phylloquinone, would be favoured for the back-reaction from $F_X$, compared to the uphill transfer from $F_X$ to $A_{1B}$.

On the B-side, the energy gap between $P^+ A_{1A}$ and $P^+ A_{1B}$ is smaller than the equivalent reaction on the A-side. This means the population of $P^+ A_{1A}$ from $P^+ A_{1B}$ will be rapid and hence triplet formation will be favoured. On the A-side however, the equivalent back-reaction will be slower and the triplet formation will be disfavoured provided that the $P^+ A_{1B}$ recombination reaction occurs directly to ground state with a rate that out-competes the back-reaction to $P^+ A_{1A}$. Therefore, if the back-reaction occurs mainly down the A branch, as suggested above, then triplet generation overall will be minimized (see Fig. 3). The lowered triplet yield could constitute a significant advantage. A corollary of this idea is that native PSI should show less triplet formation per back reaction than seen in the anaerobic, homodimeric reaction centres of Helobacteria and Chlorobiaceae. In these bacteria, however, triplet formation would be less of a problem as these they grow in strictly anaerobic conditions and thus singlet oxygen will not be formed.

When Ishikita et al. [35] calculated the potentials for the quinones, they found that a key amino acid responsible for the asymmetric potentials was Asp575-PSaB. This group is closer to $A_{1A}$ than $A_{1B}$ and it was suggested that it undergoes a deprotonation in response to the formation of the semiquinone, thereby stabilizing the semiquinone. Given the very rapid forward electron transfer in PSI however, the protonation may not have time to influence the forward reactions, and indeed site-directed mutations of this particular residue had little effect on the reoxidation rates of the phyllosemiquinones [40]. However under conditions where charges accumulate and back-reactions occur, it seems possible that this protonation does occur. This could switch $A_{1A}$ to an even higher potential and thus the protective mechanism suggested above would be even more effective. Intriguingly Asp575-PSaB is changed to an Asn in the unusual gene variant present in nitrogen fixing cyanobacteria and expressed in heterocysts [41]. It seems clear the high potential $A_{1A}$ and the tuning switch that we suggested above would not be needed in PSI functioning in the anaerobic conditions encountered during nitrogen fixation. If there is a change to two-sided low-potential PSI in anaerobic conditions, we must suppose that there is an advantage in doing this. A comparison of this anaerobic PSI with normal PSI might show differences in efficiency as well as susceptibility to $O_2$ related damage.

Current views of the evolution of Type II reaction centres propose that the Type II reaction centres diverged from the Type I reaction centres while both were homodimeric. This is particularly compelling since the majority of features (the cofactors and their environments) that differentiate Type I RCs from Type I RCs are symmetrical over both sides of the reaction centre. These differences were therefore almost certainly present in an ancestral homodimeric Type II reaction centre [42]. The heterodimericity of PSI, should the word exist, most likely evolved relatively recently compared to the separation of Type I and Type II reaction centres. Indeed the two sides of PSI, PsaA and PsaB, have not diverged greatly from each other (60% identity). In the preceding text, the asymmetry existing in PSI was rationalised in terms of protection and regulation in the context of reactions with oxygen.
We therefore suggest that heterodimerisation in PSI occurred after O₂ appeared in the environment, i.e. after the evolution of water splitting PSI and probably in the same membrane as the nascent water oxidizing reactions. Based on other arguments, a similar conclusion has been arrived at independently (John Golbeck personal communication). The tuning of electron transfer needed to deal with leaks and damaging back-reactions seems to be a requirement associated with life in the presence of oxygen.

4. Type II reaction centres

4.1. No charge separation in the B-branch of Type II reaction centres: a requirement for an efficient two-electron gate

In Type II reaction centres the quinone, QA, is the last electron acceptor in the chain and Q₈ must be stable until another photochemical turnover provides the second electron required to complete its 2-electron reduction. At this stage there is no forward reaction which can compete with the back-reaction and so kinetic control is not an option to prevent the back-reaction. The simplest way to prevent the energy loss is to slow-down the backward rate. This is done by making the back-reaction, or at least one step in the back-reaction pathway, strongly uphill in energy.

The shortest route for electrons to get to P⁰⁺ from QA⁻⁺ is via the Pheo₂, the pheophytin on the “non-functional” B-side of the reaction centre. The distance between QA and Pheo₂ is thought to be very large in all Type II centres (the potential of Pheo₂ has not been determined but is considered to be more negative than that of Pheo₃ and the potential of QA is around 100 mV higher than QA see [43,44]). Thus no P³⁺ QA back-reaction takes place by this route. This is a major factor contributing to the long lifetime of QA. In the evolution of the Type II reaction centres, the switching-off of the B branch presumably occurred by a mutation or mutations that lowered the potential of Pheo₂, simultaneously switching-off the charge separation on the B-side and blocking QA⁻⁺ from back-reacting through Pheo₂. Interestingly, it has been shown in Photosystem I that raising the potential of QA, which is analogous to Pheo₂ in Photosystem II, lowers the yield of the B-branch without affecting the overall quantum yield of charge separation, suggesting that the proposed evolutionary tinkering does not impact the overall charge separation efficiency [45].

The evolution of a large energy gap between QA and PB contrib- buted to a more efficient reaction centre by elimination of this direct back-reaction route. Several other features of the current Type II reaction centres can be seen as greatly increasing the efficiency of reaction centre as a quinol-producing device, compared to the ancestral homodimeric quinol-producing reaction centre. These have been dealt with in detail elsewhere [42,46]. Basically a homodimeric quinone-reducing reaction centre would suffer inefficiencies associated with Pheo⁻ encountering semiquinone (which would be awaiting the second electron) or an empty site (due to quinol/quinone exchange and incomplete occupancy). The heterodimer evolved (i) a specialised QA, which is always bound and only does rapid, 1-electron chemistry and (ii) a special- ised QA site that stabilises a semiquinone adjacent to the non-functional PheoB.

4.2. Back-reactions in Type II reaction centres: the purple bacterial reaction centres

For QA⁻⁺ the first step in the back-reaction is electron transfer back to QA. In this case however, these two components are not far apart in energy, the two semiquinones equilibrate (K = 20). It is on the next back-reaction step that a big energy gap exists: the QA⁻⁺ to Pheo step requires several hundred meV [47,48]. Across the Type II reaction centres this energy gap varies, having marked effects on back-reaction rates and this has clear mechanistic significance.

There are several examples that illustrate the extent to which the lifetime of the radical pair involving QA⁻⁺ depends on the free energy difference associated with the electron transfer from QA⁻⁺ to the nearby Pheo₈. In Rhodobacter sphaeroides, the lifetime of the radical pair changes as a function of the energy gap between QA and Pheo and this has been studied by substituting different quinones for QA and by imposing an external field [47,49,50]. When the energy gap is smaller than around 350 meV then repop- ulation of the P⁻Pheo⁻ state dominates; when the energy gap is larger than that, the direct tunnelling recombination reaction dom- inates [47,49,50].

Variations in this energy gap and hence the back-reaction kinetics are seen in different species of purple bacteria. In R. sphaeroides or Rhodobacter capsulatus, where QA is a ubiquinone (UQ), the lifetime of QA is significantly longer than in Rhodobacter viridis, a bacteriochlorophyll b-containing species in which QA is a menaquinone (MQ) [49,51,52]. This is explained by the smaller energy barrier for the repopulation of Pheo⁺⁻ from QA⁻⁺ in R. viridis due to the higher potential of the Pheo (and perhaps a contribution from the slightly lower potential of MQ as QA). The smaller energy gap here is mainly a consequence of R. viridis’s use of longer wave-length light for photosynthesis. The energy available from 960 nm light (1.292 eV) is significantly less than 870 nm light (1.425 eV) used by R. sphaeroides and yet the reaction centre bacteriochloro- phyll b gives rise to a P⁻⁺ cation that has approximately the same oxidizing power as that from R. sphaeroides (+450 mV). This means that it has around 130 mV less reducing power on the acceptor side and this is mainly seen as a diminution in the Pheo to QA energy gap. The rapid recombination via repopulation of the P⁻Pheo⁻ is expected to give rise to a high yield of the triplet P (see Fig. 4).

R. sphaeroides does not suffer from this energetic squeeze and so is able to have an energy gap between QA and Pheo₈ that is more than enough to prevent the back-reaction by that route. Instead the P³⁺ QA⁻⁻ recombination reaction takes place via a slow reaction that involves a tunnelling process. We shall see in the following section that PSI is like R. viridis insofar as it has insufficient energy in the absorbed photon to allow it to maintain an energy gap between Pheo and QA that is big enough to render the Pheo to QA step irreversible. In PSI the energy squeeze is not caused by a lower energy photon (indeed it uses the highest energy photon of all the photosynthetic reaction centres) but by the high energy requirement at the oxidizing side of the reaction centre. PSI needs all the energy it can get to take electrons out of water with a reasonable over-potential.

4.3. Two back reaction pathways in PSI: redox switching

PSI contains two charge recombination pathways for P³⁺ QA⁻⁻, one of which is comparable to that in R. viridis and the other more similar to that in R. sphaeroides [48,53]. As described in the purple bacterial reaction centre, the size of the energy gap between QA and the Pheo determines the back-reaction rate and thus the recombination route. Here however this has more important mechanistic implications and remarkably PSI is able to modulate the size of the energy gap and hence the yields of these pathways in order to mitigate damage and optimize function.

As described above for R. viridis, the indirect pathway in PSI takes place with the formation of the P⁺Pheo⁻ radical pair, which then decays to the P₃ triplet state ([54,55] and see [56]), for a review of the bacterial case see [57]). Unlike R. viridis, however, PSI is far from anaerobic, indeed it makes the O₂ and its P₃ triplet state...
The free energy level of $P^+\text{Pheo}^-$ or by decreasing the energy level of $P^+\text{Q}^-$.

Each of these strategies may compromise the overall efficiency of the energy converter. Indeed, since Photosystem II is a “shallow photochemical trap” [61,62] (another consequence of being energy squeezed), raising the free energy level of the primary radical pair impacts the quantum yield [63–65]. Similarly, decreasing the free energy level of $Q_A^-$ (relative to $Q_b$ and or the quinone pool) will increase its steady state concentration, thereby impacting the overall energetic efficiency of the system [53,66]. There is thus a trade-off between the need to limit potentially harmful but, under standard conditions, rare (chlorophyll triplet generating) back-reactions and the optimization of the energy conversion yield. The latter seems to have been favoured since the relative yield of the indirect, back-reaction pathway in Photosystem II, under functional conditions is rather high and accounts for ~70% of the charge recombination process [67]. Under normal functional conditions electrons are plentiful from water splitting and these potentially damaging reactions occur infrequently. When however conditions are encountered in which back-reactions are more frequent, then this damaging route can be essentially switched off [53].

While this switching process involves kinetic control, the switch itself is through a conformational change and this can be viewed as kinetic gating. Photosystem II is fully assembled in the membrane as a photosynthetic reaction centre without its active site, the Mn$_4$O$_5$Ca cluster. The cluster is assembled by a process known as photoactivation. Prior to and during photoactivation, electrons are much less readily available to stabilize the oxidizing equivalent resulting from photochemistry, thus charge recombination would be expected. However when the cluster is absent, the reduction potential of $Q_A$ is higher by 150 mV than in the functional enzyme [69]. Consequently, the free energy gap between $P^+\text{Q}_A^-$ and $P^+\text{Pheo}^-$ is large and the direct charge recombination pathway is favoured, while the indirect pathway, and its troublesome $3^P$ intermediate, is avoided [53] (see Fig. 4).

It is known that the simple absence of Ca$^{2+}$, rather than the whole cluster, is responsible for the switching effect [68,69]. It is possible that this occurs under physiological conditions, for example in the presence of high local proton concentrations [68,69] and in certain $S$ states [70], and this would result in the same kind of redox switching. This would protect PSII should this occur but it also could be a regulatory mechanism in high light conditions.

It is assumed that the structural modifications resulting from the absence of the Mn$_4$Ca cluster, or indeed just the Ca$^{2+}$, propagate over to the other side of the protein (almost 40 Å away) and induces a down-shift of the midpoint of the $\text{Q}_A/\text{Q}_b$ couple [53,69,71]. The nature of this change is not clear however it has been suggested to be related to the presence of an H-bond from threonine (217 of D$_2$) to the carbonyl on $Q_b$ that is proximal to the non-heme iron [72]. The presence of this H-bond has been calculated to produce just such an up-shift in potential. The simple rotation of the OH group of the threonine could make or break this bond. It is not clear how the binding status of the Ca$^{2+}$ 40 Å away would influence this rotation. Alternatively, the ionization of amino acids in the region of $Q_A$, perhaps the bicarbonate/carbonate that ligands the non-heme Fe, could also be responsible for this redox shift [46]. A well resolved crystal structure of the Mn$_4$Ca-depleted PSII may help understand this effect and how it is propagated across the protein.

The relationship between the redox potential of $Q_A$ (and hence the $P^+\text{Q}_A^-\text{P}^+\text{Ph}^-\text{energy gap}$) and the generation of $O_2$ has been established experimentally using spin trap EPR methods. This was done in a site-directed mutant that lowered the potential of $Q_b$ and it duly gave rise to more $O_2$ [73]. Another demonstration was done using herbicides. The binding of herbicides in the $Q_b$ site results in changes in the potential of $Q_b$ and this again affects the
Qₐ to Pheo energy gap [74]. The yield of singlet oxygen increased when the binding of phenolic herbicides decreased the size of the energy gap due to the increased yield of the indirect pathway via the Pheo⁻ giving rise to ³P formation [59]. These herbicides not only block electron transfer but they also redox tune Qₐ, favoring the back-reaction up to a high energy intermediate (P⁺Pheo⁻), this reacts by a short-circuit (charge recombination) to form a reactive state (³P) that reacts with O₂ (a leak) and this kills the plant [75].

In the Qₐ site, phenolic herbicides seem to H-bond strongly to the imidazole that ligands the non-heme iron, this effect may be relayed to the H-bonded Qₐ on the other side of the imidazole–Fe-imidazole motif leading to a weaker H-bond to the Qₐ and thus generating the lower potential [76]. In line with this, the affinity of the Qₐ pocket for phenolic herbicides depends on the redox state of Qₐ [77]. This herbicide-induced modulation of the Qₐ potential is additive to the Ca-induced effect, so clearly the chemical origin of the redox effects are different [74]. These observations also could indicate that the native occupant of the Qₐ site, PQ (its presence and absence and each of its different redox states), could influence the reduction potential of Qₐ and thus tune the forward and back reactions. This has yet to be studied.

Based on the effect of Ca²⁺ on the potential of Qₐ [78] and the fact that Ca²⁺ binding changes during the S-state cycle [79], it has been suggested that the potential of Qₐ could be tuned to suit specific properties of the S states [46]. For example a short-lived S₃TyrZ state may have less chance of back-reacting if the Qₐ potential were increased in S₃.

4.4. Back reactions in PSII: modulating the potential of pheophytin

The standard free energy level of the Pheo⁻ state can be modified depending on the strength of the H-bond to the C₅₋₁₋₋ₐ⁻⁻O of Pheo₉ from the amino acid side chain at D₁-Gln130. In nature this residue can be Glu or Gln but a range of site-directed mutants have been made and studied. The lifetime of Qₐ decreased as the H-bond was strengthened (as the Ph potential became more positive) and the corresponding increase in the lifetime of Qₐ occurred when the H-bond was weakened (as the potential of Ph became more negative) [48,65]. As expected these studies showed the correlation between the size of the standard free energy gap between Pheo⁻ and Qₐ and the lifetime of the semiquinone.

The impact of modulating the free energy level of the Pheo⁻ is not limited to the back electron transfer rate from Qₐ⁺ but indeed the forward reactions are also affected. Site-directed mutants at the position D₁-130 position showed the rate and quantum yield of the primary charge separation were dependent on the H-bond strength, with increased rates and yields when the H-bond strength was decreased [63,64,79]. Lowering the potential of Pheo leads to lower yields of charge separation as well as slower back reactions.

In nature only the high potential form of Pheo⁻ but also the electron transfer rate between P⁺Pheo⁻ and P⁻Pheo⁻. They proposed that increasing the midpotential of Pheo would indeed favour the thermally activated repopulation of P⁺Pheo⁻ from P⁻Qₐ⁻ but in addition it would favour radical pair recombination from the singlet over the triplet route. This can be rationalized in terms of Marcus theory: the very large driving force for singlet recombination to the ground state (above 1.6 eV) would put the reactions into the inverted region (i.e. a decrease in driving forces accelerates the reaction), while recombination to the triplet, with a driving force around 0.2–0.3 eV, behaves conventionally (i.e. a decrease in driving force slows the reaction). Thus a positive shift in the potential of Ph (i.e. with the stronger H-bond from D₁-Glu130) would decrease the driving force for P⁺Pheo⁻ recombination, accelerating singlet recombination to the ground state but slowing triplet formation. This then is positive protective effect. While this is plausible, it is not wholly satisfying since the rate effects are not expected to be large and the increased decay of P⁺Qₐ⁻ by the dangerous, indirect back-reaction route has to be compensated for before any protective benefit can be gained by this mechanism. Below we suggest some alternatives.

The modulation of the energy gap as a method of minimizing the formation of the PSII triplet, as originally formulated by John-son et al [53], was seen as a protection strategy for preventing P⁺Pheo⁻ formation when populated from P⁺Qₐ⁻, i.e. to prevent electrons from returning to Pheo from Qₐ⁻ (or from Qₐ⁻ via Qₐ⁻). This is a particular risk when the enzyme is unable to provide electrons: i.e. when the water splitting function is absent (before photoassembly of the MnCa cluster) or disabled (after photodamage or when Ca²⁺ is lost) or when the light intensities is so low that S₃TyrZ or S₃Qₐ⁻ charge recombination can take place [53,55]. Under high light intensities, however, the situation is quite different. Under high light water splitting works and the acceptor side rapidly becomes unable to keep up with the electron input. Under these conditions, the Qₐ⁻ will accumulate, and despite the “shallow trap” lowering the quantum yield of charge separation, the
P^+Pheo^−Q_X state will still be formed [61,62]. Recombination of this state will produce triplet [61]. Now, returning to the change in the potential of Ph associated with the H-bonding Glu/Gln at D1-130, we suggest that a smaller driving force from P^+Pheo^− state (with stronger Glu 130 H-bond) may simply diminish the triplet yield and favour singlet recombination. For this to occur then, like Vass and Cser [86], we resort to the Marcus theory. In this case however, recombination is from P^+Pheo−Q_X^− (rather than P^+Pheo−Q_X) because of the electrostatic effect of the charge on Q_X^− on Ph+, this radical pair would be at an even higher energy, even further into the Marcus inverted region for singlet recombination to the ground state and thus its rate would be more susceptible to a small changes in the driving force. The Glu 113 H-bonded Ph would thus be an advantage in the high light strains. The difference between this model compared to that to Vass and Cser [86] is that here the energy gap between P^+Q_X^− and P^+Pheo^− is irrelevant to the proteic influence mechanism in high light, the dominant problem is not repopulation of P^+Pheo^− from P^+Q_X^−, but simply the decay route of P^+Pheo^− (formed by the forward reaction from P^+).

There is another possible explanation which is not necessarily exclusive. It is known that strong light under reducing conditions results in the second reduction of Q_X forming the Q_H2 state [87,88]. This results in an increased yield of charge separation, because the electrostatic influence of Q_X on the energy level of P^+Pheo^− is removed, the energy gap between this state and P^+ increases and so does the quantum yield of charge separation: in short the shallow trap effect is reversed [61]. As a result of the increased quantum yield of P^+Pheo^− formation, the yield of the P^+ is greatly increased. Furthermore, the proposed Q_X electron transfer quenching of P^+ will also be lost, allowing the triplet lifetime to increase by more than 2 orders of magnitude [61,89]. This would give rise to severe oxidative damage if O2 were present. This has been suggested to occur under some photoinhibitory conditions [87,90]. The increase in the potential of the Pheo due to the presence of H-bonded D1Glu-130 would decrease the driving force for this second reducing step, Ph−Q_X^−+2H^+→PhQH2, and might be expected to slow it down. This would constitute a significant advantage for selecting of D1Glu-130 in high light conditions.

4.5. Side path, futile cycle and oxidative leaks in PSII

The very high potentials involved in water photolysis can result in the adventitious oxidation of nearby organic species in and around the PSII reaction centre. The longest-lived of the chlorophyll cations formed during primary charge separation, because the electrostatic influence of Q_X on the energy level of P^+Pheo^− is removed, the energy gap between this state and P^+ increases and so does the quantum yield of charge separation: in short the shallow trap effect is reversed [61]. As a result of the increased quantum yield of P^+Pheo^− formation, the yield of the P^+ is greatly increased. Furthermore, the proposed Q_X electron transfer quenching of P^+ will also be lost, allowing the triplet lifetime to increase by more than 2 orders of magnitude [61,89]. This would give rise to severe oxidative damage if O2 were present. This has been suggested to occur under some photoinhibitory conditions [87,90]. The increase in the potential of the Pheo due to the presence of H-bonded D1Glu-130 would decrease the driving force for this second reducing step, Ph−Q_X^−+2H^+→PhQH2, and might be expected to slow it down. This would constitute a significant advantage for selecting of D1Glu-130 in high light conditions.

Electron donation from this β-carotene to P^+ (probably via ChlD2) occurs in a few ms [96]. This side-pathway donation is slow compared to the main forward reactions for P^+ reduction (e.g. electron donation from TyrZ can occur in tens of ns). Nevertheless it is predicted to occur with a very low quantum yield under normal conditions and with an increased quantum yield whenever P^+ life-times are longer (i.e. when electron donation from water is inhibited or absent). The carotenoid is a 20 Å-long cofactor and it gets within a short distance of Cyt b559 and makes van der Waals contact with ChlZD2. Given its low potential relative to the ChlZD2, Cyt b559 if reduced, donates electrons rapidly to the β-carotene cation. The oxidized Cyt b559 is relatively stable but can be reduced slowly by plastoquinol [97]. This completes a futile cycle. Not much is known about the reduction of the Cyt b559 except that it is slow and is blocked by the same herbicides that bind to the Qb site. Given the long distance, electron transfer is expected to take around a second to occur from the Qb site to the heme of Cyt b559. It seems possible that faster electron donation may occur under other circumstances through a less well-defined quinone site that is closer to the Cyt b559 (a “Qc site”) and that is also herbicide sensitive [98,99]. A third functional quinone in isolated reaction centres [100] was detected by crystallography bound in a channel close to the heme of Cyt b559 [92], although electron transfer from here to the heme could be rapid, it seems somewhat unlikely that this corresponds to the earlier defined Qc site [46].

This inefficient Cyt b559-mediated cycle has been proposed to protect against oxidative damage caused by P680 [101] or by Car− (see citations in [34]). In marine plankton a much more efficient futile cycle exists under high light [102]. This may represent a souped-up version of the Cyt b559 cycle described here. In order to work so much more efficiently, some kind of redox switch seems to be required. It was suggested recently [46] that this could involve a perturbation of the relative redox potentials of the core chlorophylls so that the chlorophyll cation may be distributed onto ChlD2, i.e. closer to the carotenoid. This could occur by for example the electrostatic influence of accumulated oxidising species, say TyrZ or at least its proton [46].

When the cytochrome b559 is already oxidized, the carotenoid cation can be reduced by chlorophyll, most likely ChlZD2, which is calculated to have a uniquely low potential [103]. This branch of the futile cycle may serve as a trap for the cation and as a fluorescence quencher that will protect against over excitation of PSII.

It is possible that other pigments further from the reaction centre may undergo oxidation due to further oxidation of side-path components. Reports exist in the literature of multiple chlorophylls and carotenoids undergoing slow bleedings with prolonged illumination [104]. Such oxidations, should they occur under physiologically relevant conditions, may be considered as oxidative leaks. The oxidation of carotenoids in the antenna will remove their protective (triplet quenching) influence from the nearby chlorophylls and this will start a chain reaction of light-driven, triplet-mediated 1/2O2 damage [75]. These oxidative leaks (and several other oxidative leaks occurring under other circumstances) are clearly to be avoided and the futile cycle and the up-shift in the Qa potential when water splitting is non-functional, as described above, are both useful for that.

5. The cytochrome bc1/buf

In the Q cycle of cytochromes bc1/buf, a reversible oxidation of quinol in the catalytic Qa site delivers one electron into the high potential c-chain and the other into the low potential b-chain, giving rise to a charge-separated state in the enzyme [105]. This reaction relies on i) the energetic coupling of the two reduction/oxidation reactions, one involving the FeS center of the c-chain, the other heme b6 of the b-chain, and ii) on the split between the midpoint potentials of the quinol/semiquinone and semiquinone/quinone redox couples illustrated in Fig. 5, estimated to be at least ~800 mV [106,107]. The electrons are then transferred from the FeS center to the heme c1/f in the c-chain and from the heme b6, via the heme b4 to the second catalytic quinone site, the Qb site in the b-chain. While in mitochondria and purple bacteria the
enzyme works as a quinol:cytochrome oxidoreductase, there are examples of bacteria which rely for their growth on reverse electron flow from cytochrome c to quinone [108].

Despite a great deal of research, the mechanism of the Q site catalysis and the way the two chains are connected are still not fully understood and remain the subject of intense debate (for recent references see for example [110–112]). The connection between the two chains has an additional level of complexity arising from the fact that the enzyme has a homodimeric structure with each monomer containing one c- and one b-chain that together form an H-shaped electron transfer system.

From a kinetic point of view, the direction of electron flow through the two coupled chains would be expected to depend exclusively on the rates of all partial reactions, including the QH2 exchange rate to and from the catalytic sites, provided that all the reactions within the chains are fully reversible. The equilibrium of one reaction in the coupled chains influences the equilibrium of all other reactions. A kinetic model based on this is sufficient to explain the re-equilibration reactions occurring following a flash-induced change in redox level of quinone pool and the cytochrome c pool [113].

However, rapid reversibility (within the catalytic timescale) of all partial reactions, including the initial charge separation at the Q site [114], raises a mechanistic problem in understanding how the productive (reversible) two-electron oxidation of quinol is kinetically separated from wasteful short-circuits (when single electrons directly flow from the low to the high potential chain), or leaks (when electrons are transferred to oxygen) (discussed in [114–117]), as shown in Fig. 5.

If one considers all possible types of short-circuits reactions that could potentially occur in this two-chain system (two- or three-step one-electron reaction sequences as discussed in detail in Refs. [114,115,118]), there are just two general solutions that ensure that all short-circuits occur with kinetics slower than the catalytic timescale. The first possibility is that the Q site allows the sequential oxidation of quinol when the two electron acceptors (i.e. the FeS and heme b) are both oxidized. This requires double gating of reactions at the Q site so that formation of semiquinone at the Q site, SQ, takes place only in a strictly defined set of redox conditions [114,115]. Since the reactions are reversible the same must apply to quinone reduction at the Q site, which would require both FeS and heme b to be present in the reduced form at the same time.

The second possibility is that the site favours a concerted, two-electron oxidation of the quinol that does not involve a semiquinone intermediate [114,115,119], or at least keeps its concentration extremely low. Again this would apply to the reverse reaction, quinone reduction.

Deciding between these two possibilities, as well as developing a precise understanding of the molecular mechanisms occurring awaits further studies. One interesting line of investigation has opened up recently with the reports of methods for the trapping of the semiquinone formed at the Q site [106,107].

The short-circuit reactions are traditionally referred to as “unwanted” because they dissipate energy and thus lower the energetic efficiency of the system. Recent studies however, indicate that their occurrence on a much longer time-scale (seconds) may have physiological relevance [120–122]. This relates to the redox conditions in which the cofactors remain in the reduced state for long periods, for example, when the Q site is unable to accept electrons from the reduced b hemes. As discussed below, under these circumstances short-circuits might compete effectively with electrons leaking onto O2 and thus would diminish O2 formation [120,121]. Furthermore, they might even allow quinol oxidation at the Q site to remain functional at a residual, yet physiologically competent, level [122].

5.1. Competition between short-circuits and leaks of electrons

Superoxide, O2-, is formed by the cytochrome bc1 complex under certain circumstances. If, as seems likely, the reaction of SQ with oxygen is directly responsible for the generation of O2 [106,107,123], then the probability of this reaction will increase when the reduced forms of the heme b or FeS remain present long enough to prevent complete oxidation of the quinol. A similar argument applies for the reverse reaction, where complete reduction of the quinol will not be achieved if either heme b or FeS is not reduced upon arrival of the quinol in the Q site. But SQ or may also engage in competitive reactions that retain electrons within enzyme rather than reducing oxygen. As described in detail in [120,121], several of those reactions result in short-circuits (see Fig. 5).

Because semiquinone in the Q site can be formed in two ways, either by the withdrawal of electron from QH2 by FeS (the semiforward reaction) or the reduction of Q by heme b (the semireverse reaction), both of these reaction can potentially generate O2-. Experimental analysis of O2 production in various mutants of Rb. capsulatus cytochrome bc1, combined with modelling, suggested that the dominant reaction responsible for O2- formation is electron transfer from heme b to quinone [121]. This leads to a build-up of steady state levels of SQ, which can react with O2 [121]. According to this model, when SQ is formed at a time when the head domain of the mobile FeS is away from the Q site, the FeS cluster cannot immediately react to neutralize SQ. Thus the lifetime of SQ is relatively long and the probability that SQ will reduce oxygen is therefore greater. If however the FeS cluster is close to the Q site when in its oxidized form it simply picks up the electron from the SQ (resulting in a short-circuit), while if it is in its reduced form it will donate an electron to SQ (completing full quinone reduction, i.e. the reverse reaction). In either case SQ is rapidly removed by internal reactions occurring within the Q site before it can react with O2.

This type of kinetic competition between short-circuits and leaks also probably occurs in the mitochondrial system, where the electron transfer from heme b to Q has also been proposed to be responsible for generation of reactive oxygen species by

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**Fig. 5.** Scheme illustrating the various electron transfer reactions occurring in the bc1/bcf complexes discussed in the text. Consistent with Fig. 1 the forward reactions are shown in red, solid arrows. The backward reactions are shown in dotted blue arrows, the short-circuits in dashed green arrows and the leak toward oxygen as the dashed violet arrow. The reactions discussed in the text are specifically indicated. As a convention the arrows start from the redox couple that provides the electron donor and points toward the redox couple that provides the electron acceptor.

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cytochrome $b_{1}$ [124,125]. Such competition may conceivably work as a protective mechanism with which the living cells would minimize the deleterious formation of O$_2$ through the use of competing energy-wasting but leak-proof and safer short-circuits [120]. But on the other hand, under some conditions the O$_2$ production could occur as part of a signalling pathway, reflecting the redox state of the electron transfer chain so the leak itself, when it occurs, could be physiologically relevant [126].

5.2. Short circuits in the broken Q-cycle

We have discussed above the possibility of the back-reaction between reduced $b_1$ and quinone and how this can lead either to the reduction of the oxidized FeS by the resulting SQo, or to leaks to oxygen. As shown in Fig. 5, another possible short-circuit involving the reduced $b_1$ is the electron transfer from $b_1$ to SQo. Such a situation might occur when the oxidized FeS oxidizes the quinol yielding SQo, while $b_1$ is reduced.

Owing to the large separation between the potentials of the quinol/semiquinone and semiquinone/quinone couples (at least ~800 mV [106,107]), the unstable SQo is both an efficient electron donor and acceptor and it is thus capable of accepting an electron from the reduced $b_1$ yielding a quinol.

The occurrence of this reaction was demonstrated recently in the $b_{1}f$ complex when the Q site was inhibited [118] or disabled, and it was found that the reduction of SQo, at the expense of $b_1$ was very slow (~250 ms [122]). Despite its sluggishness, the very existence of this short-circuit is important because it represents an “emergency exit” pathway which bypasses the Q-cycle. This reaction thus allows the quinol-plastooyxanin oxidoreductase activity to occur and thus the entire photosynthetic chain to function, even with a non-functional Q cycle.

This short circuit mechanism may be relevant to wild-type cytochrome $bc$ and $b_{1}f$ complexes under steady-state conditions. As the proton motive force builds up in the light, the driving force for the electrogenic electron transfer from $b_1$ to SQo decreases, thereby increasing the steady state level of reduced $b_1$ and thus the relative yield of the short-circuit pathway.

The slow rate of electron transfer from reduced $b_1$ to SQo under these conditions is not understood. Notably it is much slower than electron transfer from SQo to oxidized $b_1$ (~250 ms for the former vs 3 ms for the latter [122]). The slower electron transfer rate might be simply due to the standard free energy change for the electron transfer from $b_1$ to SQo being much larger than that of SQo to $b_1$, putting the latter reaction into the “inverted region” of the Marcus curve [15]. According to the current available estimates for the $\Delta G^*$’s of these reactions, however, their reorganization energies would have to be unusually low for this to occur. Alternatively, and more likely, the rate-limiting step may not be electron transfer, but limited by a change in the binding of the quinol or of SQ in the site, indeed the appropriate configuration of the site for the formation of SQo may depend on the redox state of $b_1$ (see [116,117] for a discussion of such possibilities).

5.3. Cytochrome $b_{1}$ as a functional dimer and its possible role in diminishing ROS

The recent demonstration of all electron transfer paths in the $bc$ dimer provided important functional principles. In addition to the well-known electron transfers through the $c$- and $b$-chains of each monomer, a functional electron transfer bridge connecting the two monomers formed between the two hemes $b_1$ in a core of dimers was demonstrated [127,128]. With this bridge, all cofactor paths within the dimer assemble into an H-shaped electron transfer system linking the two Q$_1$ sites on one side of the membrane with the two Q$_2$ sites on the other side of the membrane. This system distributes electrons between these four catalytic sites within a timescale of the catalytic turnover (milliseconds) and acting like a molecular-scale “bus bar” with four terminals [127]. As a result, any connection between the catalytic sites on opposite sides of the membrane allows the enzyme to be catalytically competent. It remains to be seen whether and how this “bus bar” design contributes to regulation of electron flow in respiratory and photosynthetic systems. It does seem clear that the built in redundancy will allow enzymological function even if operation or mutation disables specific component branches.

An important question concerns the relative ratio of intra- versus inter- monomer pathways in the functioning dimer. Considering electrochemical properties of the cofactors and distances between them, it is expected that under the conditions of unperturbed electron flow in all parts of the dimer (i.e. when all four terminals are “fully open”) the intra-monomer electron transfer would dominate [129]. However, as the equilibrium levels change and electron flow in parts of the dimer is suppressed (i.e. when any of the energetic and/or structural conditions lead to the bus bar terminals become “partly or entirely closed”, for example where $\Delta \Pi_\text{H}$ builds up), then the contribution of the inter-monomer electron transfer is expected to increase.

Another important question concerns a possible role of electron exchange between all four hemes b within a dimer to diminish leaks of electrons and generation of superoxide [114,127,130]. A general concept behind those possibilities assumes that the connection between hemes unites them with all four catalytic quinone oxidation/reduction sites in such a way that multiple unpaired electrons produced during the Q cycle can be collected and neutralized [114,127]. This means that the cross-dimer electron transfer may help in sweeping the b-chain of reduced heme b [114]. As discussed in paragraph 5.1, an increased level of reduced hemes b is associated with the increased probability of SQ and for O$_2$ formation. Thus, any means to diminish the level of reduced hemes b is expected to diminish the risk of O$_2$ formation.

Those two general questions set now the stage for further studies to clarify and define intra- and inter- monomer electron transfer in this system and its possible physiological role in regulating electron flow and guarding against unwanted ROS.

6. Overview and conclusions

The energy-converting enzymes evolved from ancestral enzymes that functioned in conditions of low O$_2$ concentration or anaerobicity. These ancestral proteins did not have to deal with the inevitable side-reactions (leaks) that occur when O$_2$ is present. The different redox tuning adaptations that we have discussed above can be rationalised in the context of avoiding side-reactions with O$_2$. The key physical values that seem to have had such an influence on this area of bioenergetics are ~160 mV (the Em of O$_2$/O$_2^-$ but note this will have varied depending on the concentration of O$_2$ in the atmosphere) and 1 eV, the energy difference between O$_2$ triplet and its highly reactive singlet state.

Redox reactions occurring with intermediates with potentials in the region of ~160 mV and lower face the possibility of electrons leaking out to O$_2$ and forming superoxide. This is dealt with in diverse ways, some of which, but not all, have been presented here: (i) the regulation of electron transfer to avoid the build up of reducing intermediates, e.g. cyclic electron flow around PSI, regulation of PSII; (ii) kinetic control, involving fast electron transfer steps through the most reducing states, e.g. F$_X$ and A$_1$ in PSI or $b_1$ to $b_6$ electron transfer in $bc_1$/b$_{1}f$; (iii) redox tuning by raising the potential of some intermediates, e.g. stabilising the $Q_2$ and $Q_3$, semiquinones, or switching from low potential menaquinone to
high potential ubiquinone and plastoquinone [131], (iv) changes in mechanisms/structures, e.g. the appearance of an additional heme in the quinone reduction site of the $b_{6}f$ complex with respect to its $b_{c}$ counterpart [132–135]. Another adaptation was of course to remove $O_{2}$ and peroxide with specific scavenging enzymes but this was clearly a second line of defence.

When chlorophyll-containing species found themselves in the presence of $O_{2}$ then there was a big problem: the excited singlet state and more importantly the longer-lived triplet state had more than enough energy ($1.3 \text{ eV}$) to drive the conversion of triplet $O_{2}$ to its highly reactive singlet form ($1 \text{ eV}$). Redox tuning does not help here, this is an energy question and red light is as low in energy as possible for efficient oxygenic photosynthesis. The main fix was to wheel out the carotenoids as quenchers of chlorophyll triplet states and of singlet $O_{2}$. However in the reaction centres, chlorophyll triplet could be formed by short circuits such as charge recombination. Here again carotenoid quenching has been employed where possible but this is clearly insufficient and many mechanisms exist where by these short-circuits are minimised. These include the following: (i) big energy gaps, when energetically possible, to prevent back reactions, (ii) redox switching to control the energy gap, e.g. the high and low potential of $Q_{A}$ in PSII which is related to donor side function; (iii) switching to a lower potential $P_{h}$ in PSI in high light isoforms of D1, (iv) a bigger energy gap for the specific back-reaction side of the reaction centre (the A-side) in PSI, (v) also perhaps the switching on and off of a futile cycle in PSII [see [46]].

In PSII, given the extreme oxidising chemistry associated with water oxidation and generation of $O_{2}$, there are other protective mechanisms that appear to be there to prevent over-oxidation of the cofactors and groups in their environment [see [46]]. These too may have secondary reactions associated with $O_{2}$ and its derivatives.

The basic requirements for electron transfer in biology have been established and a few basic rules have been defined [5,16]. What is required is proximity between cofactors and appropriate overall driving forces. Once these are in place, the system has little or no need for fine-tuning in order to promote productive electron transfer. Indeed, it has been shown that big variations in the energy levels of intermediates have little effect on the final (quantum) yield of the forward reactions [see e.g. [136,137]]. This provides great robustness to the system. Here however we have presented several cases (and there are others), where the fine-tuning of energy levels does occur. Among these there are examples where redox tuning is used to obtain totally different outcomes, while the same cofactor distances are maintained. These situations are specifically associated with circumstances in which the desired energy-useful outcome of catalysis becomes less important than saving the system from damaging reactions, particularly with $O_{2}$, that put the viability of the organism in danger.

Some of the key features of the bioenergetic redox enzymes can thus be understood from an evolutionary viewpoint, where enzymes that were already optimised for productive energy conversion in an anaerobic or low $O_{2}$ environment had to compromise efficiency in order to survive the arrival of $O_{2}$. Here we have dealt with only a limited number of enzymes and a limited number of their features. It seems likely that similar thinking could provide further insights not only into these complex enzymes but also into other bioenergetic redox enzymes.

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References


