Electron Carriers: Proteins and Cofactors in Oxidative Phosphorylation

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Biological electron transfer is mediated by a heterogeneous group of redox cofactors. Most of these compounds are firmly bound by proteins, others migrate freely within membranes, and some are soluble. The orderly flow of electrons through chains of such cofactors is fundamental to the generation of metabolic energy.

Introduction

Most of the metabolic energy generated in living cells results from processes that abstract electrons from a donor molecule, channel them through an electron transport chain, and finally deliver them to an acceptor molecule. When the donor is NADH and the acceptor is oxygen, the overall reaction is strongly exergonic and ATP can be generated (oxidative phosphorylation). In the light reaction of photosynthesis, electrons are taken from water and eventually transferred to $NADP^+$ to yield NADPH. This is an endergonic process, and therefore has to be driven by the absorption of light energy. The resulting overall reaction becomes sufficiently exergonic to yield significant amounts of ATP in addition to reduced NADP (photophosphorylation).

Both electron-transfer chains are membrane-associated and encompass a large number of redox cofactors of different chemical make-up. In general, spontaneous electron transfer requires that the reduction potential of the electron acceptor be higher (i.e. more positive) than that of the donor. Thus the electron carriers of a given chain are usually arranged in order of increasing reduction potential. In biological electron transfer, electrons not only jump from one cofactor to the next, but they also travel significant distances through the structural elements of the proteins that harbour these cofactors, or they are transported by mobile carriers that travel from one redox protein to the next.

Overview of Oxidative Phosphorylation and Photophosphorylation

The components of the electron transport chains associated with oxidative phosphorylation and photosynthesis are organized in a number of supramolecular complexes, each consisting of many protein subunits and multiple redox cofactors of different types. **Figure 1** shows, in a schematic way, the general topology of both chains and the approximate size of the individual complexes as determined by X-ray crystallography or electron microscopy. The types and numbers of the cofactor molecules participating in electron transport are also indicated. Their chemical properties are discussed in more detail below.

The respiratory chain

Primary electron donors of respiratory electron transport in the mitochondria are either $NADH + H^+$ or metabolites that can be oxidized by FAD-dependent dehydrogenases (**Figure 1a**). NADH delivers reducing equivalents to NADH:ubiquinone oxidoreductase (complex I), a large complex of at least 40 subunits with a mass of almost 1×10^6 Da. It transfers the electrons to ubiquinone (coenzyme Q, UQ), and at the same time translocates protons from the matrix (N phase) to the intermembrane space (P phase). Complex I contains flavin mononucleotide (FMN), more than six iron–sulfur clusters and bound ubiquinones.

A second reaction feeding the UQ pool is catalysed by succinate dehydrogenase. This enzyme, actually a component of the Krebs cycle, is an integral component of the inner mitochondrial membrane, and therefore commonly classified as complex II of the chain. As in complex I, electron transfer involves a flavin (flavin–adenine dinucleotide, FAD, in this case), several iron–sulfur clusters, and bound quinones. In addition, the enzyme contains a *b*-type cytochrome of unknown function. Further electron donors for the reduction of UQ are metabolites such as acyl–CoA, α-glycerophosphate or choline. In these cases, the electron transfer flavoprotein (ETF) serves as an intermediate electron carrier between the respective dehydrogenase and ubiquinone.

Reduced ubiquinone (ubiquinol, UQH_2) delivers two hydrogen atoms to ubiquinol:cytochrome *c* reductase (complex III) where the electrons are channelled to the next mobile carrier, cytochrome *c*, while the protons are
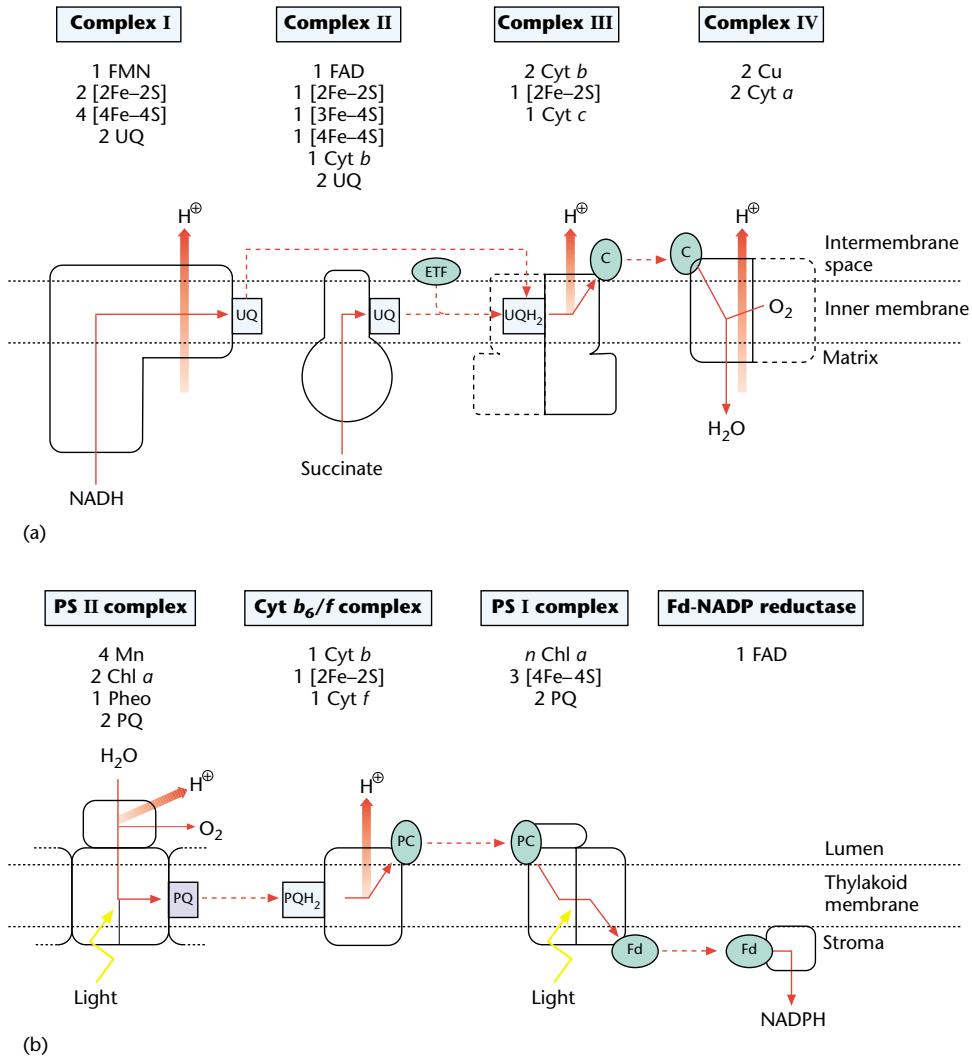
Figure 1 Overview of oxidative phosphorylation and photophosphorylation. Electron transfer steps are indicated by thin red arrows, and proton transport by thick red arrows. Dashed red arrows indicate transfer by mobile carriers. (a) Scheme of the respiratory chain; UQ/UQH$_2$, ubiquinone/-ol; ETF, electron transfer flavoprotein; c, cytochrome c. (b) Scheme of photosynthetic electron transport. PQ/PQH$_2$, plastoquinone/-ol; PC, plastocyanin; Fd, ferredoxin.

translocated into the intermembrane space. This pathway, which is known as the 'Q-cycle', involves two separate binding sites for UQ and/or UQH$_2$ (Q$_o$ and Q$_i$), both connected to the UQ pool. Two b-type cytochromes (b_H and b_L), the 'Rieske' [2Fe–2S] iron–sulfur protein and a c-type cytochrome mediate the transfer of electrons from Q$_o$ to Q$_i$ or to cytochrome c, respectively.

The respiratory chain is completed by cytochrome c oxidase (complex IV), which transfers electrons to the final acceptor O$_2$, again pumping protons from the matrix side into the intermembrane space. The electrons delivered by cytochrome c first pass a copper centre (Cu$_A$) and haem a before they reach a binuclear Cu$_B$–haem a_3 site, where

molecular oxygen is ultimately reduced to water in a stepwise fashion.

Photosynthetic electron transport

The photosynthetic thylakoid membranes of higher plants, algae and cyanobacteria harbour an electron-transport chain that resembles the respiratory chain in many respects. However, as mentioned above, photosynthetic electron flow is 'uphill' and needs an input of energy in the form of visible light. Therefore, photosynthetic electron transfer, in addition to the electron carriers found in respiration, includes pigments that allow the trapping of

light energy and its conversion to chemical energy, i.e. energized electrons at low reduction potential. This task is performed by chlorophyll molecules in the 'reaction centres' of two separate photosystems. The mobile carriers involved in photosynthesis are similar to those of the respiratory chain: there are quinones (called 'plastoquinones') within the membrane and small redox-active carrier proteins (plastocyanin and ferredoxin) on its surface.

The first step in photosynthetic electron transport (**Figure 1b**) is the excitation by light of the reaction centre of photosystem II (PS II). Excited electrons are generated and immediately passed on to phaeophytines and then to bound plastoquinones (PQ) before they reach the external PQ pool. The electron 'holes' resulting from this transfer are filled by electrons derived from water. The oxygen-evolving complex (OEC) responsible for the reaction involves a cluster of four manganese ions as functional components.

Reduced plastoquinone (PQH_2) is reoxidized by the Cyt b_6/f complex, which shows striking similarity to complex III of the respiratory chain. It contains a haem of type b (in cytochrome b_6) and a c-type haem (in cytochrome f), as well as a Rieske iron–sulfur protein, and catalyses proton transfer according to a 'Q-cycle' mechanism. The mobile carrier reduced by the Cyt b_6/f complex is plastocyanin (PC), a small copper-containing protein present on the lumenal side of the membrane.

Photosystem I (PS I) catalyses the reduction of ferredoxin (Fd) by electrons derived from PC. Ferredoxin is a [2Fe–2S] iron–sulfur protein located on the stromal side of the thylakoids. Since its reduction potential is considerably lower than that of PC, a second excitation by light is required for the reaction. PS I contains at least six

chlorophyll molecules, several iron–sulfur clusters and two bound plastoquinones.

The final reaction of photosynthetic electron transfer, catalysed by the flavoenzyme Fd:$NADP^+$ reductase, yields $NADPH + H^+$, which can be used for the light-independent synthesis of glucose from carbon dioxide, the so-called 'dark reactions'.

Electron Carriers: NADH, Flavoproteins, Cytochromes, Iron–Sulfur Proteins, Quinones and Others

The electron-transferring cofactors involved in energy metabolism belong to a number of chemically diverse groups of compounds. Most of them act as prosthetic groups, i.e. they remain tightly bound to the proteins that use them. Exceptions to this rule are the quinones (see below) and the pyridine nucleotides NAD^+ and $NADP^+$. The latter are water-soluble cofactors that shuttle between the enzymes that reduce or reoxidize them. Reduced $NADH + H^+$ carries reducing equivalents from catabolic pathways to the respiratory chain, while $NADP + H^+$ is used as the reductant in biosynthetic processes. The quinone cofactors are lipid-soluble and move electrons within membranes.

Pyridine nucleotides

Nicotinamide adenine dinucleotide (NAD^+) and the closely related nicotinamide adenine dinucleotide phosphate ($NADP^+$, **Figure 2**) consist of two nucleoside 5'-monophosphates linked by a phosphoric acid anhydride

NAD(P)$^\oplus$ NAD(P)H + H$^\oplus$

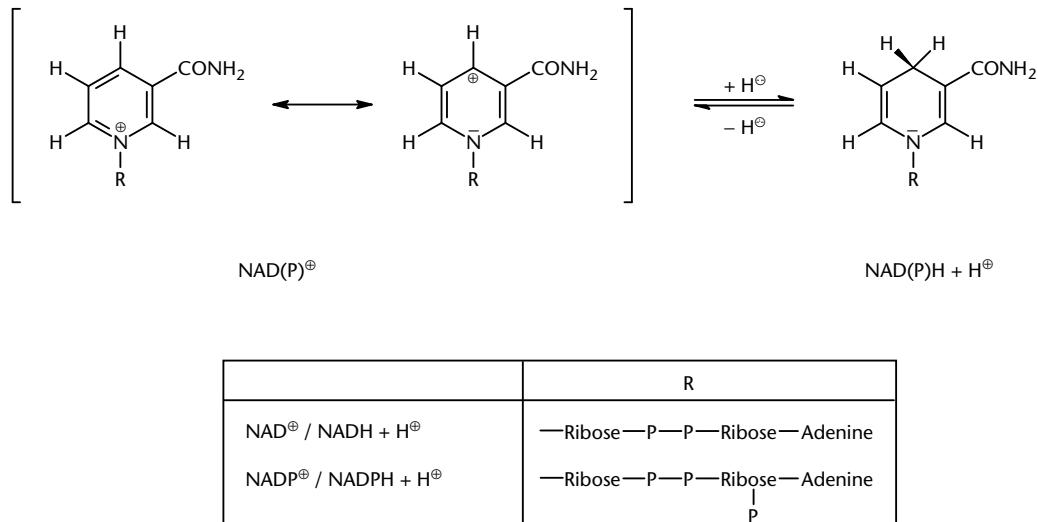|	R
$NAD^\oplus$ / $NADH + H^\oplus$	—Ribose—P—P—Ribose—Adenine

$NADP^\oplus$ / $NADPH + H^\oplus$	—Ribose—P—P—Ribose—Adenine
	                    │
	                    P

Figure 2 Pyridine nucleotides.

bond. NAD^+ is made up of 5′-AMP and a nucleotide with nicotinamide as the base. In $NADP^+$, the 3′-OH group of the ribose residue linked to adenine is additionally esterified with phosphoric acid.

It is only the nicotinamide ring of the pyridine nucleotide coenzymes that is involved in redox reactions. Nicotinamide is the acid amide of pyridine-3-carboxylic acid (nicotinic acid). In the oxidized form, the ring is aromatic and carries a positive charge. Solely to emphasize this fact, the oxidized coenzyme is designated NAD^+, although its net charge is negative. During the dehydrogenase-catalysed oxidation of substrates, two hydrogen atoms (i.e. two electrons and two protons) are removed from the substrate. However, it is only a hydride ion (H^- one proton and two electrons) that is transferred to NAD^+. The target of this transfer is the carbon in *para* position to the ring nitrogen. Upon reduction, an alicyclic CH_2 group is formed at this position, the double bonds are rearranged, and the positive charge disappears. The proton released during hydride transfer to NAD^+ must not be ignored, and therefore the correct designation for the reduced coenzyme is $NADH + H^+$ (not $NADH_2$). The fact that the reduced nicotinamide ring strongly absorbs UV light of wavelengths around 340 nm can be utilized to follow NAD(P)-dependent reactions.

Flavin nucleotides

Like the pyridine cofactors, the flavin coenzymes FMN and FAD are nucleotides. Their redox-active group is the yellow heterocycle flavin (isoalloxazine). Flavin mononucleotide (FMN) carries the phosphorylated sugar alcohol ribitol at the flavin ring, while flavin–adenine dinucleotide (FAD) consists of FMN linked to 5′-AMP. In animals, the *de novo* synthesis of flavin coenzymes depends on the uptake of vitamin B_2 (riboflavin).

The flavin moiety can be reduced by accepting one or two electrons and the same number of protons (**Figure 3**). In contrast to NAD(P)H, reduced flavins are easily reoxidized by dioxygen. Flavin coenzymes are, therefore, versatile cofactors involved in respiratory and photosynthetic electron transport, as well as in reactions catalysed by oxidases, monooxygenases and dehydrogenases. Oxidized flavins (FMN or FAD) are more intensely yellow than the fully reduced forms ($FMNH_2$ and $FADH_2$), while the semiquinone radical intermediates ($FMNH\cdot$ and $FADH\cdot$) are reddish or purple in colour. These spectral differences facilitate the study of flavin-dependent redox reactions.

All flavoproteins bind their cofactors rather tightly, usually in a noncovalent manner (an exception is succinate dehydrogenase, where FAD is covalently linked to a histidine residue of the enzyme). This keeps free flavins in the cell at very low levels and reduces the formation of superoxide (O_2^-), which can arise from the interaction of flavin semiquinones with O_2.

Cytochromes

Cytochromes are electron-transferring haem proteins. Their reduced forms show characteristic absorption bands at wavelengths between 510 and 610 nm, which once led to the classification of cytochromes as types *a*, *b*, *c* or *d*. Some cytochromes still contain the wavelength of such a band in their name, e.g. Cyt b_{562} or Cyt c_{550}. Another way to classify the cytochromes is according to the type of coordination of the haem iron.

All haem groups are coordination complexes of the tetrapyrrole porphyrin with iron. Subtypes *a–d* only differ in the substituents at the porphyrin ring (**Figure 4**). The most common haem, type *b*, is derived from protoporphyrin IX, the product of porphyrin *de novo* synthesis. Haem *b* is found in *b*-type cytochromes, but also in haemoglobin, myoglobin, and in enzymes such as catalase. As in all haem groups, its porphyrin contains two adjacent propionyl groups. The other substituents are methyl and vinyl groups. Haems of type *a* are typical constituents of cytochrome oxidase. They contain a formyl residue in position R_1 and an isoprenoid farnesyl side-chain as R_2.
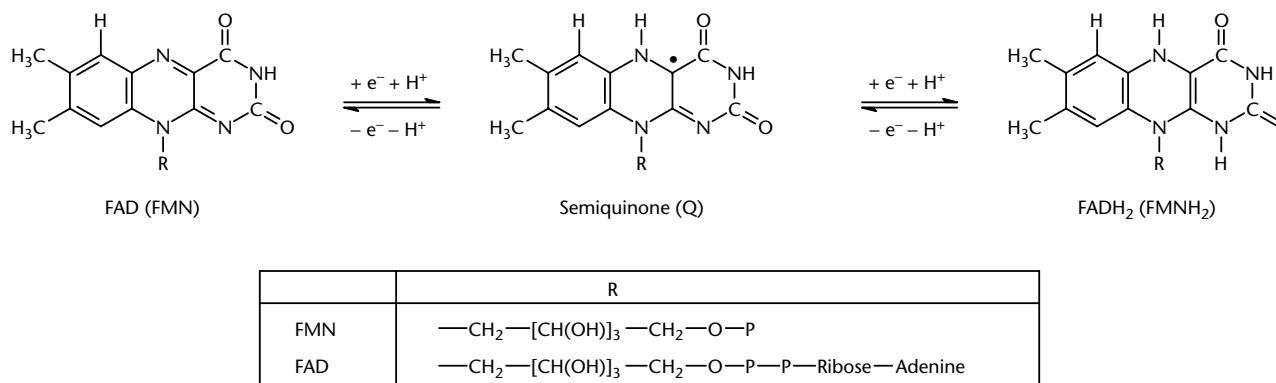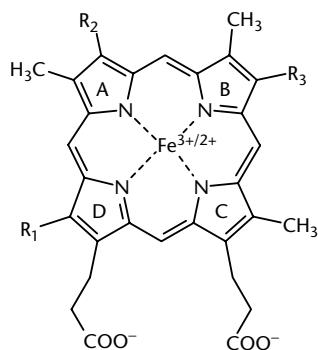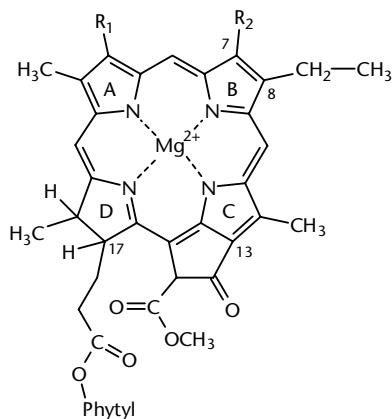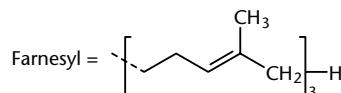

	R
FMN	—CH_2—[$CH(OH)$]$_3$ —CH_2—O—P
FAD	—CH_2—[$CH(OH)$]$_3$ —CH_2—O—P—P—Ribose—Adenine

Figure 3 Flavin cofactors.

Type	Substituents
Haem a	$R_1 = $ —CHO $R_2 = $ —CH(OH)—CH$_2$—Farnesyl $R_3 = $ —CH=CH$_2$
Haem b	$R_1 = $ —CH$_3$ $R_2 = $ —CH=CH$_2$ $R_3 = $ —CH=CH$_2$
Haem c	$R_1 = $ —CH$_3$ $R_2 = $ —CH(CH$_3$)—S—R′ $R_3 = $ —CH(CH$_3$)—S—R′

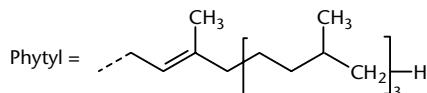| Type	Substituents
Chl a	$R_1 = $ —CH=CH$_2$ $R_2 = $ —CH$_3$
Chl b	$R_1 = $ —CH=CH$_2$ $R_2 = $ —CHO
Bchl a	$R_1 = $ —CO—CH$_3$ $R_2 = $ —CH$_3$ 7,8 - dihydro

Figure 4 Tetrapyrrole compounds.

Haem c, which is found in c-type cytochromes and a few enzymes, is covalently linked to cysteine residues of the host protein by thioether bonds. Notwithstanding its name, cytochrome f, a component of the Cyt b_6/f complex, contains a c-type haem.

The properties of the redox centres in cytochromes are strongly influenced by the ligands coordinated to the central iron. Nearly all complexes are hexacoordinate; four positions are invariably occupied by the pyrrole nitrogen atoms. In cytochromes, the remaining two ligands are usually amino acids (His, Met or Tyr). A notable exception is provided by haem a_3 in cytochrome oxidase, where the sixth coordination site is occupied by molecular oxygen or one of its reduction products.

Iron–sulfur proteins

Iron–sulfur clusters (**Figure 5**) are probably an 'early' invention of evolution. They are widely distributed in nature, where they occur in the interior of proteins (iron–sulfur proteins). Iron–sulfur clusters consist of two or
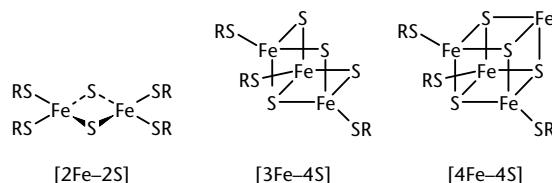

Figure 5 Iron–sulfur clusters.

more 'nonhaem' iron atoms liganded to thiol groups of cysteine residues and bridged by inorganic, acid-labile sulfide ions. The three most common types of iron–sulfur clusters are shown in **Figure 5**. The recommended designation states the iron and sulfur content in square brackets, e.g. [2Fe–2S] or [3Fe–4S]. Oxidation levels are indicated by the net charge of the complex (not including the cysteine ligands), e.g. $[4Fe–4S]^{2+}$. Not all iron–sulfur clusters are electron-transferring entities; some have catalytic functions, while others are involved in protein stabilization, or mediate transcriptional control by sensing iron or oxygen levels.

[2Fe–2S] clusters occur in most complexes of the respiratory chain, in the Cyt b_6/f complex of photosynthesis, and in the electron-transferring ferredoxins. In plants and microorganisms, [2Fe–2S] ferredoxins are involved in a number of additional pathways, such as nitrogen fixation, the reduction of nitrite and sulfite, and fatty acid desaturation. A related [2Fe–2S] protein of animal origin, adrenodoxin, acts as a cofactor of Cyt P450-catalysed steroid hydroxylation in the adrenal gland.

[4Fe–4S] clusters participate in electron transfer chains, constitute the redox-active components of ferredoxins, or assist in enzymatic catalysis. Their oxidation levels can vary between $+1$ and $+3$, which, in a formal sense, corresponds to $3Fe^{2+} + 1Fe^{3+}$, $2Fe^{2+} + 2Fe^{3+}$, and $1Fe^{2+} + 3Fe^{3+}$, respectively. [4Fe–4S] ferredoxins that undergo a redox change between $+3$ and $+2$ have particularly high redox potentials, and were formerly called high-potential iron–sulfur proteins (HiPIP).

[4Fe–4S] clusters usually adopt a more or less distorted cubane structure, i.e. the irons and the labile sulfur atoms are arranged at the corners of a cube (**Figure 5**). When one of the irons is lost from such a cube, a [3Fe–4S] cluster arises. The inactivation of the Krebs cycle enzyme aconitase is due to such a conversion. Interestingly, the long-sought iron-regulatory protein turned out to be identical with a cytoplasmic isoenzyme of aconitase. When the supply of iron is low, the enzyme loses its [4Fe–4S] cluster. The resulting apoprotein (now called IRP-1) binds to iron-responsive elements (IRE) present on the mRNAs encoding ferritin and transferrin receptor. This inhibits translation of the former and stimulates translation of the latter. Once the iron level is back to normal, IRP-1 regains the iron-sulfur cluster and, with it, aconitase activity.

Quinones

The quinones are a group of yellow-to-red pigments derived from o- or p-benzoquinone. They are easily reduced to the corresponding benzoquinols via intermediate semiquinone radicals (**Figure 6**). The quinones involved in biological electron transfer bear long isoprenoid side-chains made up from 6–10 C_5 units. As mentioned above, the hydrophobic character conferred by the side-chains renders the quinones soluble in the hydrophobic interior of membranes, where they move freely in the lateral direction. Owing to this property of the molecule, quinone 'pools' can mediate the exchange of electrons between various subcomplexes of respiratory and photosynthetic electron transfer chains. The quinones of the inner mitochondrial membrane are designated ubiquinones (UQ), due to their ubiquitous occurrence, while the term plastoquinone (PQ) is being used for quinones found in chloroplasts. UQ and PQ only differ in the number and position of substituents at the aromatic ring (cf. **Figure 6**).

Others

The chlorophylls are central to all photosynthetic systems. They mediate the photochemical generation of high-potential reducing equivalents from low-potential donors. Like haem, they are derived from protoporphyrin IX. However, chlorophylls differ from haem groups in a
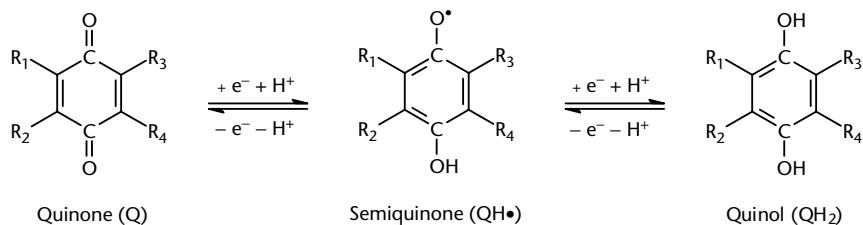
	R_1	R_2	R_3	R_4
Ubiquinones	CH_3O—	CH_3O—	—CH_3	—$[CH_2$—CH=$C(CH_3)$—$CH_2]_n$—H
Plastoquinones	CH_3—	CH_3—	—H	—$[CH_2$—CH=$C(CH_3)$—$CH_2]_n$—H

Figure 6 Quinones.

number of ways: the central metal ion is magnesium, not iron, and their side-chains are heavily modified (**Figure 4**). Most notably, there is an additional fused pentanone ring in positions 13 to 15, one of the double bonds (17–18) is reduced, and the propionate in position 17 is esterified with phytol. Chlorophyll a (Chl a) occurs in all photosynthetic eukaryotes, while Chl b is found in higher plants and green algae. In bacteriochlorophyll a (Bchl a) the porphyrin ring is further reduced (7–8), and a $-COCH_3$ group is found in position 3. When an excited chlorophyll is oxidized, the electron is not removed from the magnesium ion, but rather from the porphyrin ring, and a cationic radical is formed. The missing electron is then replaced by a low-potential electron extracted from water or another reduced donor.

Other, less common redox cofactors are functional components of specialized enzyme systems. Here, we can name just a few of them. So, for instance, coenzyme F430, a nickel-containing tetrapyrrole, is found in methyl–coenzyme M reductase, which is involved in the formation of methane by methanogenic bacteria. Nitrogenase, xanthin oxidase and other oxidoreductases employ molybdenum complexes as cofactors. The pteridine tetrahydrobiopterine (THB) acts as coenzyme of tyrosine hydroxylase and other monooxygenases, while PQQ (pyrroloquinolinoquinone) serves as the coenzyme of certain prokaryotic oxidases.

Reduction Potentials

In chemistry, a mixture of the oxidized (A_{ox}) and reduced (A_{red}) forms of a compound is called a redox system. The tendency of such a system to reduce other compounds is quantitatively expressed by its reduction potential, E (in volts, V). Reduction potentials are derived from the free energy change, ΔG, associated with electron transfer between the system in question and the redox couple 2 H^+/H_2, which has been arbitrarily defined as the reference. For such a reaction, ΔG is related to ΔE (i.e. $E_{acceptor} - E_{donor}$) by eqn [1], where n is the number of electrons transferred and F is the Faraday constant ($96\,480\,C\,mol^{-1}$).

$$\Delta G = -nF\Delta E \qquad [1]$$

As the value of ΔE depends on the concentrations of the reactants and often also on pH, reduction potentials are usually given as standard apparent potentials E'°, which refer to the standard conditions used in biochemistry ($[H^+] = 10^{-7}\,mol\,L^{-1}$, i.e. pH = 7, all other reactant concentrations at $1\,mol\,L^{-1}$). For arbitrary concentrations and pH 7 we can express the apparent reduction potential as in eqn [2], where R is the gas constant

($8.314\,J\,mol^{-1}\,K^{-1}$), and T is the absolute temperature (in K).

$$E' = E'^\circ + \frac{RT}{nF} \times \ln\frac{[A_{ox}]}{[A_{red}]} \qquad [2]$$

Table 1 summarizes standard reduction potentials for a number of redox systems of biological importance. The table allows predictions as to whether a given redox system is capable of transferring electrons to another one. As shown by eqn [1], this requires that $\Delta E > 0$. Only then can we have $\Delta G < 0$, i.e. the reaction will proceed spontaneously.

When using **Table 1**, one has to bear in mind that the reduction potentials of redox systems within proteins are tuned by the protein environment and by the binding of substrates and other ligands. Therefore observed potentials can deviate significantly from those tabulated. In the dihydrolipoyl dehydrogenase component of the pyruvate dehydrogenase complex, for instance, electron transfer proceeds in the order: lipoamide→FAD→NAD$^+$, which seems impossible from **Table 1**. In fact, the standard potential of FAD/FADH$_2$ in the enzyme is $-0.34\,V$, owing to a special environment of the coenzyme. Another interesting example is provided by nitrogenase, where binding of ATP to the iron protein lowers the reduction potential of a nearby [4S–4Fe] cluster from $-0.43\,V$ to $-0.62\,V$, thereby enabling nitrogen (N$_2$) reduction. Especially large changes in reduction potential (1–1.5 V) occur in photosynthesis when reaction centre chlorophylls are excited by light. The electrons generated in the process are at sufficiently chemical potential to reduce NADP$^+$ and to generate ATP at the same time.

Electron Transfer Mechanisms

Two different mechanisms allow the transfer of electrons from a reduced donor to an oxidized acceptor molecule. The first one (direct transfer) works only over short distances. It requires that both molecules be sufficiently close for a filled orbital of the donor to overlap with an empty orbital of the acceptor. This is difficult when both redox systems are embedded in proteins. In these cases, the intervening parts of the respective proteins must participate in the process by bridging the distance between the donating and the receiving orbital. As the constituents of the protein matrix are generally redox-inactive, the bridge cannot be formed from a chain of overlapping redox systems. Instead, the electrons travel through the protein by a process known as electron tunnelling.

The term 'tunnelling' describes a quantum chemical effect by which an electron can cross the high energy barrier between donor and acceptor without having to 'go over the hill'. Theoretical studies showed that the activation energy, ΔG^*, of such a process depends on the thermodynamic

Table 1 Standard apparent reduction potentials at Ph 7, E'°, of redox systems involved in biological electron transfer

System	n	E° (V)
Ferredoxins		-0.27
		t-
		o -0.5
$2\,H^+/H_2$	2	-0.42
$NAD(P)^+/NAD(P)H + H^+$	2	-0.32
Lipoamide$_{ox}$/lipoamide$_{red}$	2	-0.29
FMN/FMNH$_2$ (free)	2	-0.2
FAD/FADH$_2$ (free)	2	-0.2
Cytochrome b_L (Fe^{3+}/Fe^{2+})	1	-0.10
FAD/FADH$_2$ (protein-bound)	2	0.0 to 0.1
UQ/UQH$\bullet$	1	0.03
Cytochrome b_H (Fe^{3+}/Fe^{2+})	1	0.06
UQ/UQH$_2$	2	0.06
UQ$\bullet$/UQH$_2$	1	0.19
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	1	0.22
Cytochrome c (Fe^{3+}/Fe^{2+})	1	0.25
Rieske [2Fe–2S] (Fe^{3+}/Fe^{2+})	1	0.28
Cytochrome a (Fe^{3+}/Fe^{2+})	1	0.29
Cytochrome a_3 (Fe^{3+}/Fe^{2+})	1	0.35
Cytochrome f (Fe^{3+}/Fe^{2+})	1	0.37
$\frac{1}{2}O_2 + 2\,H^+/H_2O$	2	0.82

driving force, ΔG, of the reaction and the nuclear reorganization energy, λ, of the intervening medium, as described by eqn [3].

$$\Delta G^* = (\Delta G + \lambda)^2/4\lambda \qquad [3]$$

The free energy change associated with tunnelling (ΔG) is governed by two factors: the difference in reduction potential between donor and acceptor (ΔE) and the electronic coupling strength, H_{AB}, between the donating and the receiving orbital. The latter, in turn, depends on additional factors: the coupling strength at close contact, the actual distance between the orbitals, and the rate at which coupling decreases with distance. The reorganization energy of the medium, λ, increases with increasing mobility and polarity of the intervening matrix. As shown by eqn [3], low activation energies and thus the high rates of tunnelling are reached when ΔG approximately equals λ. Under these conditions, electron tunnelling through fluctuating and polar protein segments can be quite efficient, provided that the thermodynamic driving force (ΔG) is sufficiently high.

In order to measure the rates of electron transfer through proteins, elegant semisynthetic systems have been set up. They involve metalloproteins with an internal native redox centre and a redox-active ruthenium complex covalently attached to a residue at the protein surface. These experiments showed that the secondary structure of the protein is particularly important for the rate of transfer. The data suggest that β sheets are more efficient in mediating electron transfer than α helices. In both structures hydrogen bonds play a prominent role in electronic coupling.

Further electron-transfer systems amenable to detailed experimental studies are photosynthetic reaction centres of bacterial origin. After excitation by light pulses, the various changes induced in the participating redox systems can be investigated by time-resolved spectroscopy. Moreover, the availability of crystal structures and a variety of engineered mutants facilitate interpretation of the data. The picture emerging from these studies is a complex one and still far from being complete. It shows that, in these systems, electron transfer is not only controlled by the efficiency of tunnelling. Concomitant proton transfers, ligand exchange phenomena and conformational changes of the protein seem to be of similar importance.

Further Reading

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