Oxidation–Reduction Reactions

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Oxidation–reduction reactions in nature are usually catalysed by enzymes classified broadly as oxidoreductases. These enhance the rate of electron transfer from an oxidizable substrate, typically a hydrogen donor, to a reducible acceptor.

Secondary article

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Introduction

Oxidation is the loss of electrons from an atom or molecule, whereas reduction is the process of gaining electrons. Since the loss of electrons by one substance must be coupled to the gain by another, oxidation and reduction occur together. As with most processes involving chemical reactions in living organisms, enzymes serve as catalysts to enhance the rates of reactions. Those enzymes that catalyse oxidation–reduction (redox) reactions are classified as oxidoreductases (as defined in Enzyme Nomenclature, 1992, compiled by the International Union of Biochemistry and Molecular Biology (IUBMB)). These enzymes utilize the intermediacy of cofactors that are organic redox coenzymes, inorganic transition metal ions, or both to enhance the rate of electron transfer between oxidizable donor molecules and reducible acceptor molecules. In the systematic (IUBMB) nomenclature for such enzymes as periodically updated by the Enzyme Commission (EC), the first number (1) designates that they are oxidoreductases; the second number indicates the group in the hydrogen donor that becomes oxidized (1 denotes an alcohol, 2 an aldehyde or ketone, etc.); the third number usually specifies the type of acceptor (1 for $NAD(P)^+$, 2 for a cytochrome, 3 for molecular oxygen, etc.); the fourth number specifies the particular enzyme. For example, 1.2.3.1. is the EC number for aldehyde oxidase, an oxidoreductase that oxidizes an aldehyde and utilizes O_2. Among the hundreds of oxidoreductases that have been identified from diverse organisms, most are driven directly or indirectly by the energetically favourable reduction of oxygen to water in aerobic organisms, or the reduction of some other acceptor species, e.g. nitrogen or sulfur to ammonia or hydrogen sulfide respectively, in certain anaerobic bacteria.

After discussion of redox states and potentials, major types of oxidoreductases, the mechanisms by which they operate, and representative examples will be given.

Redox States and Potentials

Most potential substrates encountered in biological systems exist as relatively stable molecules with singlet ground states. Hence their oxidation often proceeds through two-electron steps, e.g. oxidation of a primary alcohol to an aldehyde, which have lower energy barriers than the one-electron steps that produce less stable radical species. These latter are less common but none the less important as, for example, in ribonucleotide reductases. Those systems that use transition metal ions, e.g. Fe^{2+} or Cu^+, to achieve single-electron transfers are, of course, energetically favoured by the structural features of the surrounding ligand. There are also some 4-electron and even 6-electron redox reactions, as with the net reduction of molecular oxygen to water and of sulfite to sulfide, respectively.

The tendency for a redox reaction to proceed is expressed in terms of the reduction potential, which is the electrical difference measured when an electrochemical half-cell containing oxidized and reduced forms of a redox compound is connected to some reference half-cell, typically the standard hydrogen electrode. Some representative reduction potentials are given in **Table 1**.

The coupling of any two of the half-reactions will run in the direction expected on the basis of the reduction potentials, namely the half-reaction with the more positive (less negative) E_0^1 has the greater tendency to proceed towards reduction, whereas the half-reaction with the less positive (more negative) E_0^1 will tend towards oxidation. The combination of the acetaldehyde to ethanol half-reaction ($E_0^1 = -0.16$) with that for NAD^+ to NADH ($E_0^1 = -0.32$) will result in reduction of acetaldehyde by the NADH, which becomes oxidized as indicated in eqn [1].

$$CH_3CHO + NADH + H^+ \rightleftharpoons CH_3CH_2OH + NAD^+ \quad [1]$$

This reaction is catalysed by NAD-dependent alcohol dehydrogenase (EC 1.1.1.1), which is also named aldehyde reductase since the reaction is actually favoured in the direction of acetaldehyde reduction.

The thermodynamic tendency for any redox reaction to proceed (conventionally with reactants and products written from left to right) is calculated from the difference in the potentials for the two half-reactions, viz. $\Delta E_0^1 = E_{0(\text{red})}^1 - E_{0(\text{ox})}^1$. In the example with the reduction of

Table 1 Reduction potentials of some redox half-reactions[a]

Reduction half-reaction	Standard potential E_0^1(V)[b]
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.82
cyt a_3 $(Fe^{3+}) + 1e^- \rightarrow$ cyt a_3 (Fe^{2+})	0.55
$\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow H_2O_2$	0.30
cyt c $(Fe^{3+}) + 1e^- \rightarrow$ cyt c (Fe^{2+})	0.25
$Cu^{2+} + 1e^- \rightarrow Cu^+$	0.15
Ubiquinone + 2-$H^+ + 2e^- \rightarrow$ ubiquinone-H_2	0.10
Acetaldehyde + $2H^+ + 2e^- \rightarrow$ ethanol	−0.16
FMN or FAD + $2H^+ + 2e^- \rightarrow FMNH_2$ or $FADH_2$	−0.18
GSSG + $2H^+ + 2e^- \rightarrow$ 2GSH	−0.23
NAD^+ or NAD-$P^+ + 2H^+ + 2e^- \rightarrow$ NADH or NADPH + H^+	−0.32
$H^+ + 1e^- \rightarrow \frac{1}{2}H_2$	−0.42
Ferredoxin $(Fe^{3+}) + 1e^- \rightarrow$ ferredoxin (Fe^{2+})	−0.43
Acetate + $2H^+ + 2e^- \rightarrow$ acetaldehyde	−0.60

[a]Examples selected from Segel (1975).
[b]Based on unit activity of component at pH 7 and 1 atm (101 kPa) pressure.

acetaldehyde by NADH, $\Delta E_0^1 = (-0.16) - (-0.32) = +0.16$. The positive net value indicates the reaction does proceed from left to right. The ΔE_0^1 value relates to the free energy as $\Delta G' = -nF\Delta E_0^1$ where n = number of electrons transferred per mole and F = the Faraday constant (96.496 kJ V^{-1} equivalent^{-1}); since $\Delta G' = -RT \ln K_{eq}$, the free energy change under standard conditions is $\Delta G'_0 = -(2.3\,RT/nF) \log K_{eq}$ at 25°C, and $\Delta E_0^1 = (0.059/n) \log K_{eq}$. Hence, in the example of acetaldehyde reduction, $0.16 = (0.059/2)\,K_{eq}$, $\log K_{eq} = 5.4$, and $K_{eq} = 2.6 \times 10^5$.

A perusal of the ranking of reduction potentials in **Table 1** leads to the expectation that the electron-to-oxygen transfer that occurs in membrane respiratory chains would generally follow a cascade from pyridine nucleotide (NAD^+ and $NADP^+$) systems, which have relatively more negative potentials and are thus prone to serve as reductants, to flavin-dependent systems, to ubiquinone, to cytochromes, to cytochrome oxidase, and then to molecular oxygen with the most positive potential.

For some mechanistic understanding of how principal types of oxidoreductases function, they are considered in the following sections.

NAD/NADP-dependent Dehydrogenases

Oxidoreductases that use the nicotinamide-containing coenzymes nicotinamide–adenine dinucleotide (NAD^+) and its 2′-phosphate ($NADP^+$) are among the most diverse in terms of substrates used as hydrogen donors (Dolphin *et al.*, 1987). The groups that undergo oxidation include alkyl functions, alcohols, oxo functions, amines, thiols, diphenols, and hydrogen. In most cases the coenzyme is not bound so avidly by the enzyme as to alter markedly the redox potential; hence, they usually serve as cosubstrates. However, dehydrogenases that bind nicotinamide-containing coenzymes have a conserved double β-α-β-α-β motif (Lesk, 1995). Most commonly the mechanism involved is a single 2-electron process involving stereospecific transfer of a hydride ion (H^-) to and from one (A or *re*) or the other (B or *si*) side at the *p*-(4-) position of the nicotinamide moiety (McCormick, 1996). This is illustrated in **Figure 1** with a generalized substrate and prochiral *R* (A) and *S* (B) hydrogens shown on reduced coenzyme.

There are numerous examples of dehydrogenases that use one or the other side in the transfer of the prochiral hydrogen to a reducible substrate (Creighton and Murthy, 1990). Alcohol dehydrogenase removes and adds hydrogen from the A side of NAD^+, whereas D-glyceraldehyde-3-phosphate dehydrogenase operates from the B side. With $NADP^+$, dihydrofolate reductase utilizes the A side, but D-glucose-6-phosphate dehydrogenase operates from the B side. There is also a conformational preference for the coenzyme that relates to the side from which hydrogen abstraction occurs. An *anti*- orientation of the nicotinamide ring (with 3-carboxamido function away from the ribofuranosyl moiety) is found with A-side dehydrogenase/reductases, and a *syn*-conformation with B-side enzymes. This preferential transfer of a *pro-S* or *pro-R* hydrogen, relating to *syn*- and *anti*- configuration, leads to

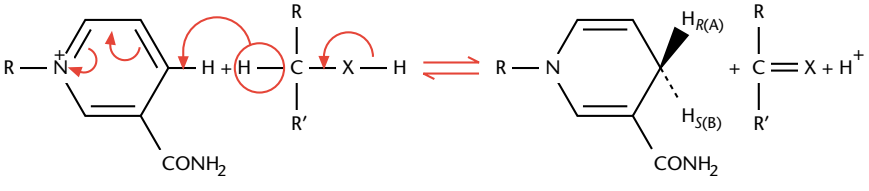


Figure 1 Stereospecific hydride ion transfer to/from the nicotinamide moiety in NAD(P)-dependent oxidoreductases.

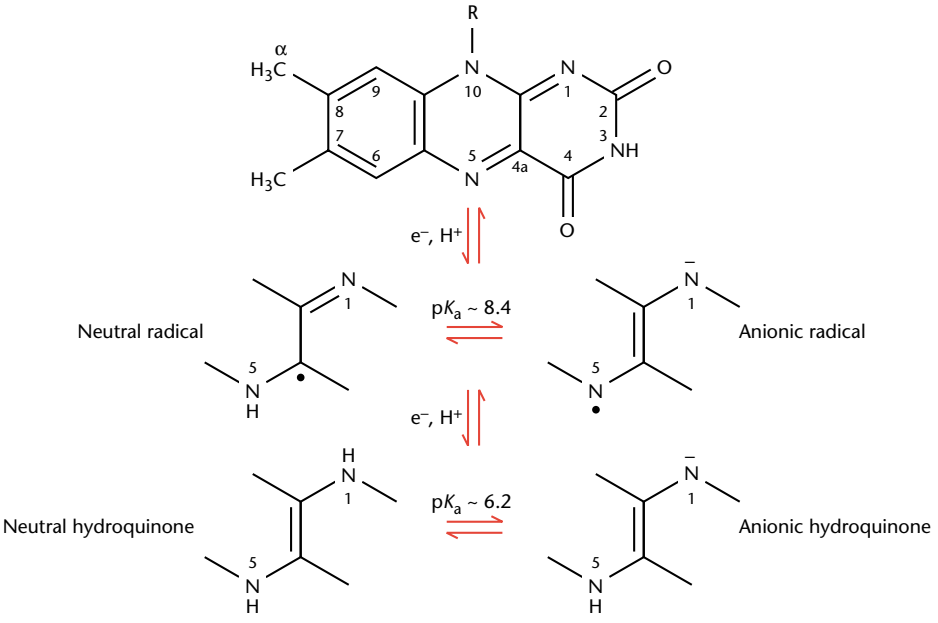


Figure 2 Biological redox states of flavocoenzymes with pK_a values for interconversions of free species.

the expectation that the dihydronicotinamide ring is stabilized in a boat conformation in which there is orbital overlap between the lone pair of electrons on the ring nitrogen and the anti-bonding σ*(C–O) orbital of the ribosyl moiety. Because of orbital overlap in the transition state, the pseudoaxial hydrogens (*pro-R* for *anti* and *pro-S* for *syn*) are most easily transferred as hydride ions.

Flavoprotein Dehydrogenases

There are a large number of flavocoenzyme-dependent oxidoreductases, the properties of which are detailed in the twelve volumes resulting from symposia spanning thirty years of work. The latest proceeding from the most recent symposium are in Stevenson *et al.* (1996). Most flavoproteins have α-β-barrel motifs (Scrutton, 1994). The flavocoenzyme most commonly encountered in flavoproteins is flavin–adenine dinucleotide (FAD), which is usually bound noncovalently to the apoenzymatic protein to constitute a functional holoenzyme. There are, however, some instances where linkage from an electronegative atom (N,S,O) of a side-chain residue of the protein (histidyl, cysteinyl, tyrosyl) is to the 8α-position of FAD (Decker, 1991). The other, less frequently encountered, flavocoenzyme is flavin mononucleotide (FMN). The versatility of flavoproteins in covering a wide range of redox reactions is that both 1-electron and 2-electron transfers can be accomplished by the flavin, which additionally varies considerably in the reduction potential, which is markedly affected by binding within the catalytic site of the protein (Stankovich, 1991). Among nine chemically discerned acid–base and redox forms of the isoalloxazine ring system in flavins, those five that have been found to operate in flavoproteins are illustrated in **Figure 2**.

In addition to the single-electron interconversions shown, a single-step, 2-electron transfer could arise from hydride ion transfer from a reduced pyridine nucleotide or a carbanion generated by base abstraction of a substrate proton. In these cases, attack of the nucleophile is expected at the oxidized flavin N5. Some nucleophiles such as hydrogen peroxide anion add at the adjacent C4a position with its frontier orbital. The stereochemistry for transfer of hydrogen onto and off N5 can take place to and from one or the other face of the isoalloxazine ring system. When visualized with the benzenoid ring to the left and side chain at top (as at the top of **Figure 2**), orientation is to the *si* face with *re* on the opposite side. A fair number of flavoproteins have been categorized on this basis (Creighton and Murthy, 1990). Among reductase/dehydrogenase types, general acyl-CoA dehydrogenase operates from the *re* side, whereas D-lactate dehydrogenase from *Megasphaera elsdenii* uses *si* orientation.

Those flavoprotein enzymes that do not use oxygen as an electron acceptor are generally regarded as dehydrogenases. In simplest form, the stoichiometry of reactions with a reduced substrate (XH_2) and an oxidase flavoprotein (F·E) yields the product and a reduced flavoprotein (eqn [2]).

$$XH_2 + F{\cdot}E \rightleftharpoons X + FH_2{\cdot}E \qquad [2]$$

These are usually involved in a series of reversible electron transfer reactions. Often 1-electron acceptors, e.g. quinones, cytochromes, or nonhaem iron–sulfur clusters, are used. Among examples of flavoprotein dehydrogenases are acyl-CoA dehydrogenases such as are involved in β-oxidation of fatty acids, the so-called electron transferring flavoprotein (ETF) and other simple electron-transferring enzymes such as flavodoxins and the microsomal NADH–cytochrome b_5 reductase, and a rather diverse list of complex dehydrogenases that include flavocytochrome b_2, succinate dehydrogenase, the adrenodoxin reductase complex, cytochrome P450 reductase, complex dehydrogenases containing disulfide and sulfhydryl groups, and the multiple cofactor-containing xanthine dehydrogenase/oxidase system.

Disulfide Reductases

There is a relatively small but important group of nicotinamide coenzyme-dependent oxidoreductases that use a disulfide compound as acceptor. These enzymes, catalogued as EC 1.6.4, are mainly FAD-dependent flavoproteins containing a redox-active disulfide (Ghisla and Massey, 1989). Examples include NADPH-utilizing reductases for glutathione, thioredoxin, trypanothione and mercury(III) ion, and the lipoyl dehydrogenase of the multienzymatic α-ketoacid dehydrogenase complexes. There is much similarity in the mechanisms used by those enzymes operating on glutathione, thioredoxin, and the lipoyl moiety. The basic steps are illustrated in **Figure 3**.

Catalysis begins with hydride ion transfer from NADPH to the FAD N5 to generate the dihydroflavin anion, which adds to the active-site disulfide of the enzyme via a labile C(4a)–cysteinyl linkage to generate a thiolate anion that can then undergo thiol–disulfide interchange with the substrate disulfide. In the case of mercury(III) reductase, the metal ion is ligated to four cysteinyl sulfides and the reduced flavin can then operate to form the labile sulfur-bridged complex with generation of Hg^{o}.

In passing it can be noted that another group of oxidoreductases (EC 1.8.4) also utilizes disulfides as acceptors, but these enzymes are conventionally considered as transhydrogenases, as for glutathione–homocystine and a protein–disulfide reductase that has also been called glutathione–insulin transhydrogenase. Both the iron–sulfur-containing ribonucleotide diphosphate reductase from most prokaryotes and the CoB_{12}-dependent ribonucleotide triphosphate reductase from eukaryotes use disulfide receptor centres.

Oxidases and Oxygenases

In both oxidases and oxygenases, O_2 is the electron acceptor; however, with oxygenases, one atom of the O_2 molecule is incorporated into the substrate of a monooxygenase, or both into the substrate of a dioxygenase (Walsh, 1979).

Among oxidases are those that use flavocoenzymes, e.g. FAD with oxidases for glucose or D-amino acid and FMN with those for glycolate or pyridoxine (pyridoxamine) 5′-phosphate. Such reactions proceed by the abstraction of hydrogen and electrons by the enzyme-bound flavin (F) acting on initial substrate followed by the reaction of O_2 at the C(4a)-position of reduced flavin to form a labile 4a-

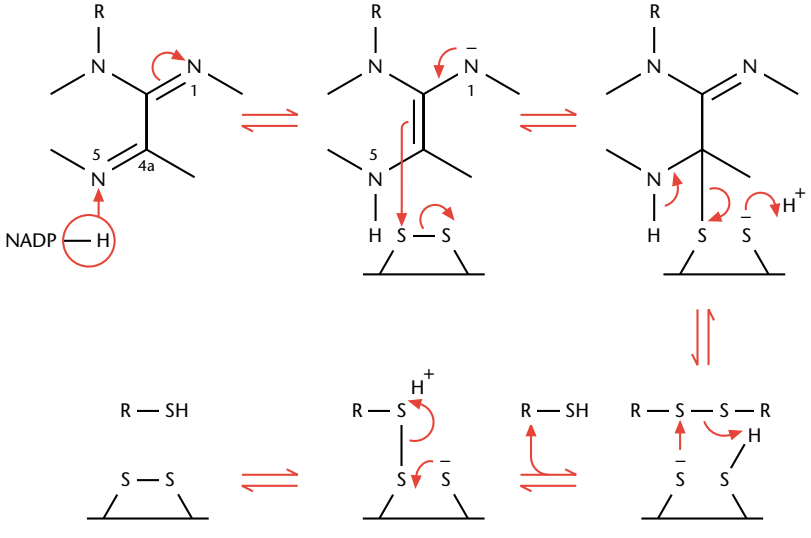


Figure 3 Catalytic mechanism of a typical NADPH-dependent flavoprotein disulfide reductase with the intermediacy of a flavin C(4a)-thio adduct.

hydroperoxide. This precedes release of H_2O_2 as a product (Massey, 1994). In this type of reaction, an electron is transferred from singlet reduced flavin to triplet O_2 to form a caged radical pair which undergoes spin inversion to collapse into the flavin hydroperoxide (Kemal *et al*., 1977). Equation [3] summarizes the events.

$$XH_2 + F{\cdot}E \rightleftharpoons X + FH_2{\cdot}E \rightleftharpoons F{\cdot}E + H_2O_2 \qquad [3]$$

The overall 2-electron reaction is essentially irreversible because of the high redox potential of the O_2/H_2O_2 couple, near $+0.3$ V, versus that of F/FH_2, near -0.2 V.

Another large group of oxidases is represented by metalloenzymes that use ions of transition metals such as copper and iron, which have unpaired electrons and generate molecular orbitals via overlap of the metal ion d orbital and the oxygen p orbital, rendering O_2 more singlet-like in reactivity. The organic cofactors and ligands associated with these metalloprotein oxidases range from the rather simple tocaquinone (6-hydroxydopa residue) found in the copper-containing plasma amine oxidase (Janes *et al*., 1990) to the complex protoporphyrin IX of other Cu^{2+}-oxidases. Some operate by 2-electron oxidations of amines and galactose to reduce O_2 to H_2O_2 while undergoing redox shuttling of the copper; others operate by 4-electron oxidations to form H_2O, e.g. L-ascorbate oxidase and cytochrome oxidase, the latter of which contains both copper and iron.

Among monooxygenases, where one atom of O_2 ends up in H_2O, are those that are flavin- or pterin-dependent, e.g. flavoprotein hydroxylases and tetrahydrobiopterin-utilizing hydroxylase for aromatic amino acids. Typically the generalized reaction uses an obligatory reducing coenzyme (NAD(P)H) as shown in eqn [4].

$$X{-}H + O_2 + NAD(P)H \rightarrow X{-}OH + H_2O + NAD(P)^+ \qquad [4]$$

In the flavoprotein cases (Massey, 1994), a flavin C(4a)-hydroperoxide formed from reduced flavin and O_2 serves as the hydroxylating intermediate, whereby one oxygen atom is incorporated into substrate, leaving the flavin C(4a)-hydroxide, which decays to oxidized flavin and H_2O. With pterin systems, there are differences with regard to the presence of redox-active irons that cycle from Fe^{3+} to Fe^{2+} during catalysis. In phenylalanine hydroxylase there is a migration (NIH shift) of hydrogen from intermediate position 4 to 3 in the tyrosine product. This could result from an oxoiron enzyme serving as an epoxidizing agent followed by opening of the epoxide with a hydride shift from *para*- to *meta*- positions on the ring. Examples of other metalloenzymes that are monooxygenases include Cu-containing dopamine-β-hydroxylase and tyrosinases and the cytochrome P450 group that so importantly hydroxylates aliphatic and aromatic systems. A somewhat similar haem-dependent hydroxylating system is that using cytochrome b_5 with a flavoprotein reductase, NADH, and an Fe-desaturase for the oxidative desaturation of long-chain acyl-CoAs to their monounsaturated acylthioesters.

Among dioxygenases, where both atoms of O_2 are incorporated into product, again certain flavoproteins and both Cu- and Fe-dependent cases are known. FAD functions coenzymatically in the bacterial enzyme that cleaves oxidatively between ring carbons 2 and 3 in 2-methyl-3-hydroxypyridine 5-carboxylate resulting from catabolism of vitamin B_6 analogues. The 2-nitropropane dioxygenase from yeast contains both FAD and Fe^{3+}. Prolyl and lysyl hydroxylases are among Fe^{2+}-dioxygenase systems that utilize α-ketoglutarate, which becomes decarboxylated to succinate during incorporation of O_2 into both its carboxyl and the hydroxylated substrate, and L-ascorbate, which is oxidized to dehydroascorbate during maintenance of iron in the $+2$ oxidation state. This reaction type on substrate X is shown in eqn [5].

$$X + O_2 + \alpha{-}\text{ketoglutarate} \xrightarrow{\text{ascorbate}} X{-}OH + \text{oxalate} + CO_2 \qquad [5]$$

Other important Fe^{2+}-dioxygenases are prostaglandin cyclooxygenase and lipoxygenase. These catalyse oxidations of unsaturated fatty acids. Quercitinase is an example of a Cu^{2+}-dioxygenase.

Peroxidases and Catalases

Peroxidases use hydrogen peroxide and in some cases alkyl peroxides as an acceptor substrate to produce an oxidized organic product plus water (eqn [6]).

$$XH_2 + RO_2H \rightarrow X + ROH + H_2O \qquad [6]$$

Catalase is a special case where H_2O_2 is the sole substrate leading to $H_2O + O_2$. Collectively such enzymes have been termed 'hydroperoxidases'. Most are high-spin Fe^{3+}-haemoproteins which exhibit two spectrally distinguishable intermediates (compounds I and II) that are probably an Fe^{IV}-porphyrin cation and ferryl oxygen species, respectively. Among diverse biological types are peroxidases that oxidize iodide to iodine and utilize chloride to chlorinate organic compounds. The principal glutathione peroxidase has selenium at its active site and catalyses the oxidation of GSH to its disulfide GSSG concomitantly with reduction of an alkyl organic peroxide to the alcohol plus water.

Membrane Electron Transport

The bridging of metabolism to electron transport that ultimately leads to reduction of molecular oxygen in aerobic organisms, or to other ultimate acceptors in simpler chemosynthetic organisms, typically involves membrane-localized electron carriers (Lehninger *et al*., 1993).

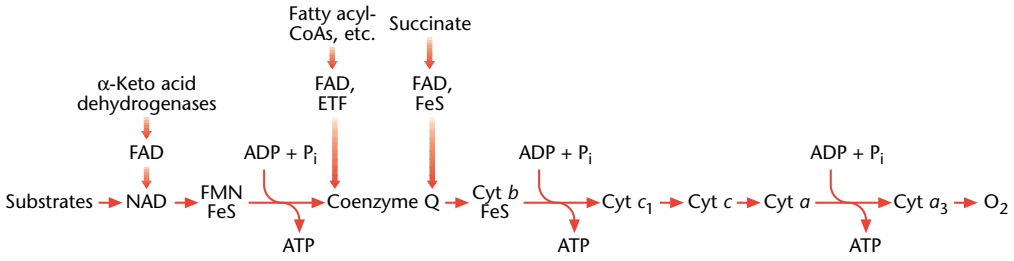
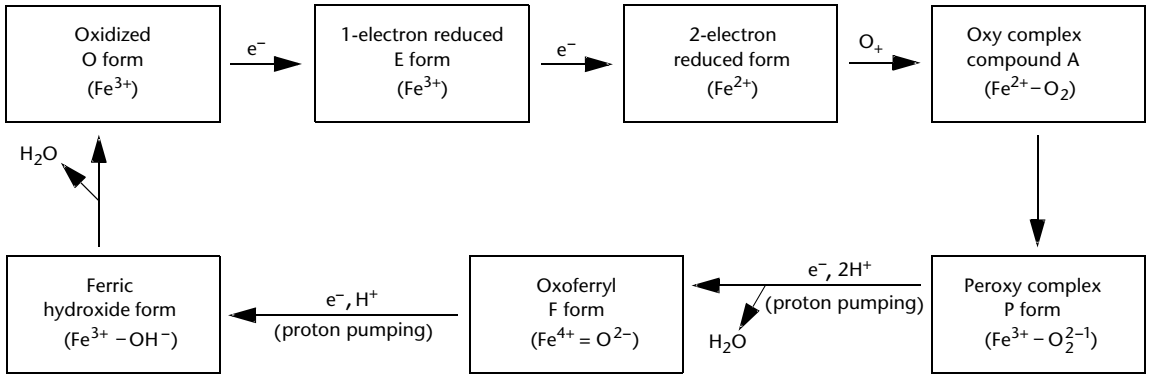


Figure 4 Principal redox participants in the flow of electrons from metabolism of substrates to mitochondrial oxidative phosphorylation.



Scheme 1

Mitochondrial electron flow, coupled to oxidative phosphorylation, is responsible for the energy-yielding synthesis of ATP in our bodies. The sequence of redox participants, following the expected potential gradients involved, is shown in **Figure 4**. The sites at which ATP is formed by synthase driven by a proton-motive force generated during electron transport are noted along the chain of components that are in the inner mitochondrial membrane. Four complexes are involved as respiratory electron carriers. Complex I catalyses electron transfer from NADH to ubiquinone; complex II from succinate to this quinone. Complex III effects electron transfer from ubiquinone to cytochrome *c*, and complex IV from cytochrome *c* to O_2.

There is now a fairly clear understanding of how respiratory cytochrome complexes link the oxidation–reduction reactions they catalyse to the electrogenic (voltage-generating) translocation of protons across the membranes in which the complexes reside (Trumpower and Gennis, 1994). The mitochondrial cytochrome *c* oxidase is a representative member of a superfamily of haem–copper, proton-pumping oxidases. The whole structure of the oxidized form of cytochrome *c* oxidase has been revealed by x-ray crystallography (Tsukihara *et al.*, 1996). There are 13 different subunits, several phospholipids, two haems A, three coppers, one magnesium, and one zinc in this complex system that exhibits several chemically and spectrally observed species as shown in **Scheme 1**.

During the operation of the oxidase, iron and copper cycle from Fe^{3+} and Cu^{2+} in the O form to Fe^{2+} and Cu^{+} in the 2-electron reduced form. O_2 then interacts with the Fe^{2+} to reoxidize both Fe^{2+} and Cu^{+} while itself becoming reduced to water. Proton pumping is coupled to the addition of the third and fourth electrons to the peroxy and oxoferryl forms.

The thylakoid membranes of chloroplasts in plant cells contain those components for the redox-coupled, light-driven synthesis of ATP in the process of photophosphorylation. Photosystem I has a reaction centre designated P700 and a high ratio of chlorophyll *a* to *b*. Photosystem II has a reaction centre P680, about equal amounts of chlorophyll *a* and *b*, and chlorophyll *c*. Following light activation of P680, electron transport proceeds from pheophytin through plastoquinones, a cytochrome *bf* complex, and plastocyanin to P700; light drives the P700 to an activated state from which electron transfer is through electron acceptor chlorophyll to phylloquinone, an iron–sulfur centre, ferredoxin, and a ferrodoxin–$NADP^{+}$ oxidoreductase, to $NADP^{+}$. Again a proton gradient couples electron flow and phosphorylation that is dependent upon an ATP synthase.

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