5.2: Chemistry of Dioxygen

Thermodynamics

The reduction potential for the four-electron reduction of dioxygen (Reaction 5.1) is a measure of the great oxidizing power of the dioxygen molecule.[8] However, the reaction involves the transfer of four electrons, a process that rarely, if ever, occurs in one concerted step, as shown in Reaction (5.2).

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-,2H^+} H_2O_2 \xrightarrow{e^-,H^+} H_2O + OH \xrightarrow{e^-,H^+} 2H_2O \tag{5.2}$$

$$dioxygen \xrightarrow{e^-} superoxide \xrightarrow{e^-,2H^+} hydrogen\ peroxide \xrightarrow{e^-,H^+} water + hyrdoxyl\ radical \xrightarrow{e^-,H^+} water \tag{5.2.1}$$

Since most reducing agents can transfer at most one or two electrons at a time to an oxidizing agent, the thermodynamics of the one- and two-electron reductions of dioxygen must be considered in order to understand the overall mechanism.

In aqueous solution, the most common pathway for dioxygen reduction in the absence of any catalyst is one-electron reduction to give superoxide. But this is the least favorable of the reaction steps that make up the full four-electron reduction (see Table 5.1) and requires a moderately strong reducing agent. Thus if only one-electron pathways are available for dioxygen reduction, the low reduction potential for one-electron reduction of O_2 to O_2^- presents a barrier that protects vulnerable species from the full oxidizing power of dioxygen that comes from the subsequent steps. If superoxide is formed (Reaction 5.3), however, it disproportionates quite rapidly in aqueous solution (except at very high pH) to give hydrogen peroxide and dioxygen (Reaction 5.4). The stoichiometry of the overall reaction is therefore that of a net two-electron reduction (Reaction 5.5). It is thus impossible under normal conditions to distinguish one-electron and two-electron reaction pathways for the reduction of dioxygen in aqueous solution on the basis of stoichiometry alone.

$$2O_2 + 2e^- \rightarrow 2O_2^- \tag{5.3}$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \tag{5.4}$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \tag{5.5}$$

Table 5.1: Standard reduction potentials for dioxygen species in water. a) The standard state used here is unit pressure. If unit activity is used for the standard state of O_2, the redox potentials for reactions of that species must be adjusted by +0.17 V.[8,9]

Reaction		E°, V vs. NHE, pH 7, 25 °C
$O_2 + e^- \rightarrow O_2^-$	(5.2.2)	-0.33[a]
$O_2^- + e^- + 2H^+ \rightarrow H_2O_2$	(5.2.3)	+0.89
$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH$	(5.2.4)	+0.38
$OH + e^- + H^+ \rightarrow H_2O$	(5.2.5)	+2.31
$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$	(5.2.6)	+0.281[a]
$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$	(5.2.7)	+1.349
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	(5.2.8)	+0.815[a]

The thermodynamics of dioxygen reactions with organic substrates is also of importance in understanding dioxygen reactivity. The types of reactions that are of particular interest to us here are hydroxylation of aliphatic and aromatic C—H bonds and epoxidation of olefins, since these typical reactions of oxygenase enzymes are ones that investigators are trying to mimic using synthetic

reagents. Some of the simpler examples of such reactions (plus the reaction of H_2 for comparison) are given in the reactions in Table 5.2. It is apparent that all these reactions of dioxygen with various organic substrates in Table 5.2 are thermodynamically favorable. However, *direct* reactions of dioxygen with organic substrates in the absence of a catalyst are generally very slow, unless the substrate is a particularly good reducing agent. To understand the sluggishness of dioxygen reactions with organic substrates, we must consider the kinetic barriers to these reactions.

Table 5.2: Examples of hydroxylation and epoxidation reactions.

Reaction	ΔH in kcal/mol	Reference
$CH_{4(g)} + \frac{1}{2}O_{2(g)} \rightarrow CH_3OH_{(g)}$ (5.2.9)	-30	10
$C_6H_{6(g)} + \frac{1}{2}O_{2(g)} \rightarrow C_6H_5OH_{(g)}$ (5.2.10)	-43	11,12
$C_6H_5OH_{(g)} + \frac{1}{2}O_{2(g)} \rightarrow C_6H_5(OH)_{2(g)}$ (5.2.11)	-42	12,13
$C_2H_{4(g)} + \frac{1}{2}O_{2(g)} \rightarrow C_2H_4O_{(g)}$ (5.2.12)	-25	10
$C_5H_5N_{(g)} + \frac{1}{2}O_{2(g)} \rightarrow C_5H_5NO_{(g)}$ (5.2.13)	-13	14
$H_{2(g)} + \frac{1}{2}O_{2(g)} \rightarrow H_2O_{(g)}$ (5.2.14)	-58	10

Kinetics

The principal kinetic barrier to direct reaction of dioxygen with an organic substrate arises from the fact that the ground state of the dioxygen molecule is triplet, i.e., contains two unpaired electrons.[15,16] Typical organic molecules that are representative of biological substrates have singlet ground states, i.e., contain no unpaired electrons, and the products resulting from their oxygenation also have singlet ground states. Reactions between molecules occur in shorter times than the time required for conversions from triplet to singlet spin. Therefore the number of unpaired electrons must remain the same before and after each elementary step of a chemical reaction. For these reasons, we know that it is impossible for Reaction (5.6) to go in one fast, concerted step.

$$\frac{1}{2}\,{}^3O_2 + {}^1X \rightarrow {}^1XO \tag{5.6}$$

$$\uparrow\uparrow \quad \downarrow\uparrow \quad \downarrow\uparrow \tag{5.2.15}$$

The arrows represent electron spins: ↓↑ represents a singlet molecule with all electron spins paired; ↑↑ represents a triplet molecule with two unpaired electrons; and ↑ (which we will see in Reaction 5.13) represents a doublet molecule, also referred to as a free radical, with one unpaired electron. The pathways that do not violate the spin restriction are all costly in energy, resulting in high activation barriers. For example, the reaction of ground-state triplet dioxygen, i.e.,$^3O_2$, with a singlet substrate to give the excited triplet state of the oxygenated product (Reaction 5.7) is spin-allowed, and one could imagine a mechanism in which this process is followed by a slow spin conversion to a singlet product (Reaction 5.8).

$$\frac{1}{2}\,{}^3O_2 + {}^1X \rightarrow {}^3XO \tag{5.7}$$

$$\uparrow\uparrow \quad \downarrow\uparrow \quad \uparrow\uparrow \tag{5.2.16}$$

$${}^3XO \xrightarrow{slow} {}^1XO \tag{5.38}$$

$$\uparrow\uparrow \quad \downarrow\uparrow \tag{5.2.17}$$

But such a reaction pathway would give a high activation barrier, because the excited triplet states of even unsaturated molecules are typically 40-70 kcal/mol less stable than the ground state, and those of saturated hydrocarbons are much higher.[17] Likewise, a pathway in which O_2 is excited to a singlet state that then reacts with the substrate would be spin-allowed (Reactions 5.9 and 5.10). The high reactivity of singlet dioxygen, generated by photochemical or chemical means, is well-documented.[18,19] However, such a pathway for a reaction of dioxygen, which is initially in its ground triplet state, would also require a high activation energy, since the lowest-energy singlet excited state of dioxygen is 22.5 kcal/mol higher in energy than ground-state triplet dioxygen.[15,16]

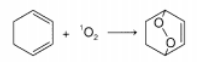
$$^3O_2 + 22.5\ kcal/mol \rightarrow\ ^1O_2 \tag{5.9}$$

$$\uparrow\uparrow \qquad\qquad\qquad\qquad \downarrow\uparrow \tag{5.2.18}$$

$$\frac{1}{2}\ ^1O_2 + \ ^1X \rightarrow\ ^1XO \tag{5.10}$$

$$\downarrow\uparrow \qquad \downarrow\uparrow \qquad\qquad \downarrow\uparrow \tag{5.2.19}$$

Moreover, the products of typical reactions of singlet-state dioxygen with organic substrates (Reactions 5.11 and 5.12, for example) are quite different in character from the reactions of dioxygen with organic substrates catalyzed by oxygenase enzymes (see Section V):



(5.11)

(5.12)

One pathway for a direct reaction of triplet ground-state dioxygen with a singlet ground-state organic substrate that can occur readily without a catalyst begins with the one-electron oxidation of the substrate by dioxygen. The products of such a reaction would be two doublets, i.e., superoxide and the oneelectron oxidized substrate, each having one unpaired electron (Reaction 5.13). These free radicals can diffuse apart and then recombine with their spins paired (Reaction 5. 14).

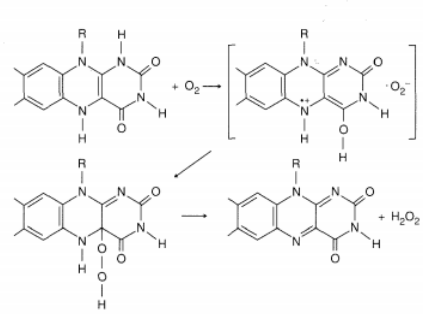
$$^3O_2 + \ ^1X \rightarrow\ ^2O_2^- + \ ^2X^+ \tag{5.13}$$

$$\uparrow\uparrow \qquad \downarrow\uparrow \qquad \uparrow \qquad \uparrow \tag{5.2.20}$$

$$^2O_2^- + \ ^2X^+ \rightarrow\ ^2O_2^- + 2X^+ \rightarrow\ ^1XO_2 \tag{5.14}$$

$$\uparrow \qquad \uparrow \qquad\qquad \uparrow \qquad \downarrow \qquad\qquad \downarrow\uparrow \tag{5.2.21}$$

Such a mechanism has been shown to occur for the reaction of dioxygen with reduced flavins shown in Reaction (5.15).[20]



(5.15)

However, this pathway requires that the substrate be able to reduce dioxygen to superoxide, a reaction that requires an unusually strong reducing agent (such as a reduced flavin), since dioxygen is not a particularly strong one-electron oxidizing agent (see Table 5.1 and discussion above). Typical organic substrates in enzymatic and nonenzymatic oxygenation reactions usually are not sufficiently strong reducing agents to reduce dioxygen to superoxide; so this pathway is not commonly observed.

The result of these kinetic barriers to dioxygen reactions with most organic molecules is that uncatalyzed reactions of this type are usually quite slow. An exception to this rule is an oxidation pathway known as free-radical autoxidation.

Free-Radical Autoxidation

The term free-radical autoxidation describes a reaction pathway in which dioxygen reacts with an organic substrate to give an oxygenated product in a free-radical chain process that requires an initiator in order to get the chain reaction started.[21] (A free-radical initiator is a compound that yields free radicals readily upon thermal or photochemical decomposition.) The mechanism of free radical autoxidation is as shown in Reactions (5.16) to (5.21).

Initiation: $$X_{2} \rightarrow 2X \cdotp \tag{5.16}\]

$$X\cdot + RH \rightarrow XH + R\cdot \tag{5.17}$$

Propagation: $$R \cdotp + O_{2} \rightarrow ROO \cdotp \tag{5.18}\]

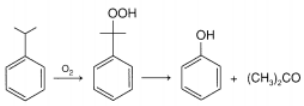
$$\downarrow \quad \uparrow\uparrow \quad \uparrow \tag{5.2.22}$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot \tag{5.19}$$

Termination: $$R \cdotp + ROO \cdotp \rightarrow ROOR \tag{5.20}\]

$$2ROO\cdot \rightarrow ROOOOR \rightarrow O_2 + ROOR \tag{5.21}$$

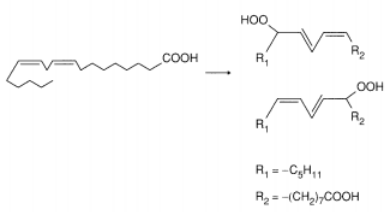
(plus other oxidized products, such as ROOH, ROH, RC(O)R, RC(O)H). This reaction pathway results in oxygenation of a variety of organic substrates, and is not impeded by the spin restriction, because triplet ground-state dioxygen can react with the free radical R• to give a free-radical product ROO•, in a spin-allowed process (Reaction 5.18). It is a chain reaction, since R• is regenerated in Reaction (5.19), and it frequently occurs with long chain lengths prior to the termination steps, resulting in a very efficient pathway for oxygenation of some organic substrates, such as, for example, the oxidation of cumene to give phenol and acetone (Reaction 5.22).[22]



(5.22)

When free-radical autoxidation is used for synthetic purposes, initiators are intentionally added. Common initiators are peroxides and other compounds capable of fragmenting readily into free radicals. Free-radical autoxidation reactions are also frequently observed when no initiator has been intentionally added, because organic substrates frequently contain peroxidic impurities that may act as initiators. Investigators have sometimes been deceived into assuming that a metal-complex catalyzed reaction of dioxygen with an organic substrate occurred by a nonradical mechanism. In such instances, the reactions later proved, upon further study, to be free-radical autoxidations, the role of the metal complex having been to generate the initiating free radicals.

Although often useful for synthesis of oxygenated derivatives of relatively simple hydrocarbons, free-radical autoxidation lacks selectivity and therefore, with more complex substrates, tends to give multiple products. In considering possible mechanisms for biological oxidation reactions used *in vivo* for biosynthesis or energy production, free-radical autoxidation is not an attractive possibility, because such a mechanism requires diffusion of highly reactive free radicals. Such radicals, produced in the cell, will react indiscriminately with vulnerable sites on enzymes, substrates, and other cell components, causing serious damage.[6] In fact, free-radical autoxidation is believed to cause certain deleterious reactions of dioxygen in biological systems, for example the oxidation of lipids in membranes. It is also the process that causes fats and oils to become rancid (Reaction 5.23).[23,24]



(5.23)

How do Enzymes Overcome These Kinetic Barriers?

We see then the reasons that uncatalyzed reactions of dioxygen are usually either slow or unselective. The functions of the metalloenzymes for which dioxygen is a substrate are, therefore, to overcome the kinetic barriers imposed by spin restrictions or unfavorable one-electron reduction pathways, and, for the oxygenase enzymes, to direct the reactions and make them highly specific. It is instructive to consider (1) how these metalloenzymes function to lower the kinetic barriers to dioxygen reactivity, and (2) how the oxygenase enzymes redirect the reactions along different pathways so that very different products are obtained. The first example given below is cytochrome c oxidase. This enzyme catalyzes the four-electron reduction of dioxygen. It overcomes the kinetic barriers to dioxygen reduction by binding dioxygen to two paramagnetic metal ions at the dioxygen binding site, thus overcoming the spin restriction, and by reducing dioxygen in a two-electron step to peroxide, thus bypassing the unfavorable one-electron reduction to form free superoxide. The reaction occurs in a very controlled fashion, so that the energy released by dioxygen reduction can be used to produce ATP. A second example is provided by the catechol dioxygenases, which appear to represent substrate rather than dioxygen activation, and in which dioxygen seems to react with the substrate while it is complexed to the paramagnetic iron center. Another example given below is the monooxygenase enzyme cytochrome PASO, which catalyzes the reaction of dioxygen with organic substrates. It binds dioxygen at the paramagnetic metal ion at its active site, thus overcoming the spin restriction, and then carries out what can be formally described as a multielectron reduction of dioxygen to give a highly reactive high-valent metal-oxo species that has reactivity like that of the hydroxyl radical. Unlike a free hydroxyl radical, however, which would be highly reactive but nonselective, the reaction that occurs at the active site of cytochrome P-450 can be highly selective and stereospecific, because the highly reactive metal-oxo moiety is generated close to a substrate that is bound to the enzyme in such a way that it directs the reactive oxygen atom to the correct position. Thus, metalloenzymes have evolved to bind dioxygen and to increase while controlling its reactivity.
