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Biochemistry of Dioxygen

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Preface

This book is written for the research biochemist who needs to know more about the particular field of dioxygen metabolism, whether this be for designing lectures for a graduate level course or for his or her own research needs. We hope researchers in a given area of dioxygen metabolism will gain knowledge of related fields of dioxygen metabolism.

We have decided to use the term *dioxygen* to distinguish molecular oxygen from divalent oxygen in water and organic compounds, dioxygen being a simpler term than molecular oxygen. We do not intend to review the metabolism of all compounds that contain oxygen, since this would include all of biochemistry.

An understanding of dioxygen chemistry is essential to the discussion of the biochemistry of dioxygen. While this statement could be made about any biochemical constituent, the chemistry of dioxygen is so unusual that interpretations without detailed chemical background are futile. Prediction of dioxygen reaction products by analogy with other oxidants is impossible.

The partial reduction products of dioxygen, superoxide ion and peroxides, develop naturally in the chemistry of dioxygen. It would be difficult to discuss dioxygen biochemistry without first discussing these partial reduction products.

The first chapters stress the chemistry of dioxygen. These are followed by chapters that delve into the intricacies of dioxygen metabolism. This procedure has tended to divide the book into two sections. The sections are purposely not well defined because certain biological reactions of dioxygen and the reduction products of dioxygen are better discussed in the early chemical chapter.

After an introductory chapter, the discussion is focused on the chemistry and physical properties of ground-state triplet dioxygen. An important aspect of dioxygen chemistry is the kinetic barrier to oxidations by dioxygen. Because of this kinetic barrier, there is a need for a mechanism to activate dioxygen before it can oxidize most substrates.

The activation of dioxygen in biological systems is usually accomplished by a metalloenzyme. These form metal–dioxygen complexes that are commonly diamagnetic. These (singlet-state) metal–dioxygen complexes perform the great majority of the biological oxidations. Singlet metal dioxygen complexes react

much more like singlet dioxygen than like ground-state dioxygen, so that knowledge of singlet-dioxygen chemistry facilitates an understanding and appreciation of dioxygen biochemistry. The reactions of singlet dioxygen that may have relevance to biological dioxygen metabolism are discussed thoroughly in Chapter 3. There are also many reactions of singlet dioxygen that have no relevance to biological reactions at this time, but the potential importance of these reactions to dioxygen metabolism dictates that they be included. These reactions are included as an addendum designated Chapter 21.

The discussion of triplet and singlet dioxygen is followed by a discussion of the chemistry of the reduction products of dioxygen, superoxide ion and peroxides. Superoxide dismutase and prostaglandins very naturally fit into these chapters. The assumption is made that the reader is aware of the physiological function of prostaglandins but wishes to know more about the chemistry and biosynthesis. Discussions of catalases and peroxidases logically follow the chemistry and biochemistry of peroxides. A familiarity with the enzyme intermediates in catalase and peroxidase is helpful in the understanding of enzyme intermediates in dioxygen reactions.

The discussion returns to dioxygen again to focus on the important problem of activation. Activation by metals makes an easy transition to metal carriers for dioxygen, both models and actual biological carriers.

Once dioxygen is activated, it can react in three different ways: (1) as a one-, two-, or four-electron acceptor from the substrate; (2) as a two-electron acceptor plus an oxygen atom donor; or (3) as a donor of two oxygen atoms. These are discussed in order beginning with the four-electron oxidases. The two-electron-plus-oxygen-atom donors are called monooxygenases. These are classified for discussion with respect to the cofactor involved: flavins, pterins, copper, and iron. The monooxygenases are followed by the enzymes that donate two oxygen atoms, the dioxygenases. The next two chapters discuss the special topics of bioluminescence and toxicity, followed by the remainder of the singlet-dioxygen chemistry in Chapter 21.

It is hoped that the reader will have time to read the whole book in order to appreciate the complete story of dioxygen metabolism. However, this book should be useful as a reference book when the reader desires information on a given aspect of dioxygen chemistry or metabolism.

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Introduction

1.1 Chemical Reactions of Dioxygen

Dioxygen is a fascinating molecule for biologists, chemists, and physicists. Its utilization is necessary for all higher animals, and it is either produced or utilized, or both, by much of the rest of the living world. To the layman, oxygen utilization is almost synonymous with life. Dioxygen has several characteristics that make it ideal for a terminal oxidant in a biological system: its high oxidizing potential, its barrier to oxidation, and the fact that it forms innocuous products.

The high oxidizing potential is valuable for the simple reason that oxidations by dioxygen make large amounts of energy available to the organism. A poor oxidizing agent would make less efficient use of foods.

The kinetic barrier to reaction is also an important factor because without it, foodstuffs and the organism itself would be oxidized indiscriminately by the terminal oxidant. It is difficult to picture life in an atmosphere of chlorine.

The production of innocuous products is an important but seldom appreciated factor. The products water and carbon dioxide are neutral and unreactive. A terminal oxidase system that produced hydrochloric or hydrobromic acid would pose some difficult problems for any conceivable organism.

However, there are also problems connected with the use of dioxygen as a terminal oxidant in biological systems. The desirable high oxidizing potential of dioxygen also means that dioxygen and some of its partial reduction products are potentially hazardous to the cell. Certain compounds that react with dioxygen without requiring prior enzymatic activation of the dioxygen can be toxic to the cell. The one- and two-electron reduction products of dioxygen pose a particular problem because they are often reactive. Thus, the cell must have enzymes that reduce the level of these partially reduced products of dioxygen that may be

toxic to the cell. The two notable enzymes in this category are catalase and superoxide dismutase.

The kinetic barrier that is a benefit to the organism when storing dioxygen presents a problem when the organism needs to use the dioxygen to produce energy. The forms of catalysis that reduce this barrier for living systems illustrate nature's novelty at its highest level.

Nature faces another problem with dioxygen: It has a relatively low solubility. Water at 20°C will dissolve only 3.1% of its volume of dioxygen (STP). Dioxygen is more soluble in organic solvents. For example, ethanol will dissolve 41.5% of its volume in dioxygen. Nature not only needs enzymes to activate dioxygen, but also must have carriers to get the dioxygen to the point of reaction. The problems in designing a dioxygen carrier are an important facet of the metabolism of dioxygen.

The kinetic barrier to oxidations by dioxygen is the result of two properties of dioxygen. One is that ground-state dioxygen is a triplet state. The other is that the lowest orbital available to accept an electron is an antibonding orbital.

Ground-state dioxygen does not react as a normal double bond, but as a diradical because of the triplet ground state. To maintain spin conservation during the reaction, dioxygen must either react with another molecule with unpaired electrons or produce a triplet-state product. Stable triplet states are unusual, so this places a kinetic restriction on reactions of dioxygen. Electron transfer requires that the first electron be placed in a partially filled antibonding π-orbital. Again, this places a barrier to electron-transfer reactions.

1.2 Biological Reactions of Dioxygen

In the previous section, we saw the need for activation of ground-state dioxygen for reaction. This activation is commonly accomplished in biological systems by forming a complex with a transition metal. The complex eliminates both of the aforementioned barriers. The complex is a singlet state instead of a triplet state, and back-bonding from the metal to the dioxygen adds electrons that make the antibonding π-orbitals more like electron pairs to allow for electron acceptance without a barrier.

The reactions of the metal–dioxygen complexes are much different from those of ground-state triplet dioxygen. Ground-state dioxygen commonly reacts in chain reactions. Chain reactions are difficult to control and consequently would tend to burn up more of the cell than simply the intended energy source. Singlet-dioxygen and biological dioxygen reactions tend to be reactions in which only one substrate is oxidized and the reaction stops. Metal–dioxygen complexes are not singlet dioxygen, but the reactions are similar.

1.3 Reduction Products of Dioxygen

An integral part of dioxygen chemistry is the one- and two-electron reduction products. The one-electron product, superoxide ion, and its conjugate acid, HO_2, have fascinating properties. Hydrogen superoxide is thermodynamically unstable with respect to dihydrogen and dioxygen. The reduction of dioxygen to superoxide ion has a negative potential, but the one-electron potential at superoxide ion is highly positive. Dioxygen becomes a stronger one-electron oxidant by adding an electron. Despite the positive potential, superoxide ion tends not to react as an oxidant. This is because the addition of one more electron is repelled by the existing negative charge on the superoxide ion. Superoxide can act as a base because the favorable disproportion reaction consumes hydrogen ions:

$$2H^+ + 2O_2^- \longrightarrow H_2O_2 + O_2$$

The real danger of superoxide ion appears to be in the metal-ion-catalyzed Haber–Weiss reaction, which produces the very reactive, and consequently toxic, hydroxyl radical:

$$O_2^- + H_2O_2 \xrightarrow{\text{Metal ion}} OH^- + \cdot OH + O_2$$

Very little of the dioxygen consumed in a cell produces superoxide, but there are extraneous reactions that are not part of a normal dioxygen metabolism that do produce superoxide in the cell. Superoxide is kept at a low concentration in the cell by the disproportionation reaction catalyzed by the enzyme superoxide dismutase.

The two-electron reduction product of dioxygen, hydrogen peroxide, is formed by both enzymatic and nonenzymatic reactions. Hydrogen peroxide is a reactive compound, so it must be kept at a low concentration in the cell. This is accomplished by the enzyme catalase, which decomposes hydrogen peroxide to water and dioxygen. It is interesting to note that the structure of the intermediate in hydrogen peroxide decomposition is probably the same as that of the intermediate in certain hydroxylation reactions.

1.4 Dioxygen Enzymes

The main reactions of dioxygen metabolism can be classified into three broad groups. The first group includes those reactions in which oxygen acts by merely accepting electrons from the substrate. The second group is comprised

of reactions in which an oxygen atom is donated to a substrate and two electrons are accepted from one of the substrates to form water. These reactions appear to be quite diverse in mechanism and utilize several types of cofactors including copper, iron, flavins, and pterins. The third group of reactions includes those in which two oxygen atoms are added to the substrate in one step. These reactions are reminiscent of those of singlet dioxygen.

Finally, the high potential of dioxygen as an oxidizing agent automatically causes it to be a dangerous chemical despite the kinetic barriers. The toxicity of dioxygen is an important subject.

Dioxygen is the oxidant in bioluminescence reactions. The dioxygen actually acts as in a dioxygenase reaction, but the uniqueness of the reactions gives reason for grouping them in a separate category.

1.5 Oxygen-17 Nuclear Magnetic Resonance Spectroscopy of Oxygen

To study dioxygen metabolism, it is necessary to be able to trace the pathway of the oxygen atom. In early work, this was usually performed by mass spectroscopy of ^{18}O. Many newer studies utilize the NMR detection of ^{17}O. Some of the applications of ^{17}O NMR spectroscopy to oxygen metabolism are reviewed here.

Oxygen exists as three natural isotopes, ^{16}O, ^{17}O, and ^{18}O, as 99.759, 0.037, and 1.204% of natural oxygen. The ^{17}O isotope is rather expensive because of its low natural abundance, but the cost is compensated for by a nuclear spin of $^5/_2$ that allows it to be detected by NMR. The ^{18}O isotope is more readily available than ^{17}O, but can be detected only by mass spectrometry. There is a radioactive isotope of oxygen, ^{15}O, but its half-life of 124 sec precludes it from having much use in tracer chemistry.

Oxygen-17 NMR spectroscopy is a promising analytical technique for the study of oxygen environments and reaction mechanisms. The technique has not been used extensively in the past due to the low natural abundance of ^{17}O and to the fact that the nucleus is a quadrapole. In a large number of cases, these disadvantages have been offset by the advent of availability of Fourier-transform NMR apparatus and ^{17}O enriched H_2O and O_2. Signal-to-noise ratios are raised experimentally by maximizing the rate of molecular tumbling, i.e., by raising the temperature or lowering the solution viscosity.

Oxygen-17 NMR has proved very informative in those cases in which the difficulties have been overcome. The resonances cover a very wide range of chemical shift even within, for instance, the category of oxygen atoms bonded to carbon (Sugiwara *et al.*, 1979). These values range from about -30 to $+600$ ppm from H_2O. In this series, and also in those compounds with nitrogen–oxygen bonds, there is a good correlation between bond order and chemical shift, so

that alcohols appear at the −30 ppm end of the scale and aldehydes in the neighborhood of +600 ppm. Ethers, esters, carbonates, anhydrides, acids, acyl halides, amides, and other compounds fall in various regions between these extremes, depending on resonance and hydrogen bonding. Nitrogen-bonded oxygens absorb as far as 915 ppm downfield. The chemical shift for alcohols can be correlated with the O–H stretching frequency (Takasuka, 1981).

In other types of compounds, the position of absorption is more difficult to predict. Ozone, for example, has two resonances—one at 1598 ppm (terminal oxygen) and the other at 1032 ppm. Oxygen atoms bound to NMR-active nuclei show split patterns so long as solvent exchange is slow compared with the observation time scale.

Block *et al.* (1980) (see also Dyer *et al.*, 1982) observed anomalies in the ^{17}O NMR spectra of four-membered ring sulfoxides and sulfones that parallel anomalies in the ^{13}C spectra of the compounds. The values obtained for both types of signals did not follow the pattern established for decreasing ring size from six-membered to three-membered. They also observed that oxygens on asymmetrical sulfones give distinguishable signals that are very sensitive to remote substitutents. Of particular interest to biochemists are the ^{17}O NMR studies of adenine nucleotides (Gerlt *et al.*, 1982; Huang and Tsai, 1982).

Many of the experiments that have utilized ^{17}O NMR have been studies of rates of hydration and exchange in inorganic complexes. In some cases, these measurements have been made on compounds of biological interest. Mn and Mg complexes of ATP, for example, have been explored (Zetter *et al.*, 1973), as have Ni complexes of EDTA (Grant *et al.*, 1971). For cases in which solvent exchange is very rapid, the equilibrium causes broadening of the signal, which can be related to kinetic parameters. Slower reactions are observed by using isotopically enriched samples of solvent and measuring the rate of appearance or disappearance of a characteristic signal.

The cyclic phosphate esters A and B (Figure 1-1) can be distinguished (Coderre *et al.*, 1981) by their ^{17}O NMR spectra. The absolute configurations were first established by ^{17}O perturbations of the phosphorus chemical shift of derivatives of these compounds. It was then observed that the ^{17}O NMR spectra of the diastereomers differed in chemical shift, ^{31}P–^{17}O coupling constant, and line width. In phosphorus-decoupled spectra of a diastereomeric mixture, the signals were resolved. The spectra were recorded at 95°C to minimize line width.

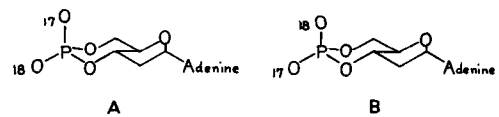

Figure 1-1. Structure of two deoxyadenosine cyclic phosphates as determined by NMR.

The limitation of ^{17}O NMR makes its feasibility for enzymatic studies remote. The tumbling rates of large molecules are quite slow, since they are related, ideally at least, to the third power of the radius. Furthermore, increasing the signal-to-noise ratio by raising the concentration also increases the viscosity, which slows the tumbling. Significant temperature elevation is also impossible.

However, model studies with this technique could be very instructive. Attempts to observe metal–dioxygen complexes have failed (Lapidot and Irving, 1972), but it has been pointed out that these procedures definitely could be improved (Klemperer, 1978). The benefits of doing so are clear. The differences between several possible metal–dioxygen complexes of biological complexes have shown (Klemperer, 1978) that the chemical shifts are related to the degree of back-bonding. Oxygen-17 NMR would clearly show whether or not the two oxygen atoms were equivalent.

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Ground-State Dioxygen

2

2.1 Properties of Dioxygen (Table 2-1)

The critical characteristic of ground state dioxygen is that it is a triplet instead of a singlet state. This fact contributes to the kinetic barrier in reactions with ground-state dioxygen and also influences the type of reactions that do occur.

The unique electronic structure of dioxygen and its resulting unique chemistry can be better understood if we consider the individual orbitals in dioxygen (see Table 2-1). The second valence shell of atomic oxygen has 6 electrons, so that dioxygen has 12 electrons in the second shell. Two of these electrons are in each 2s orbital to form lone pairs.* If we define the Z direction to be the direction of the bond between the two nuclei, we can combine the $2p_z$ orbitals to form a sigma bond with 2 electrons. The p_x and $2p_y$ are thus perpendicular to the sigma bond and form π-bonds that we will label π_x and π_y. Two electrons are placed in each of these orbitals, making a total of 10 electrons. However, we still have 2 more electrons. The $2p_x$ and $2p_y$ also form antibonding π-orbitals, π_x^* and π_y^*, which are at a lower energy than the σ^* orbital. The question is, how do we place these 2 electrons in the π_x^* and π_y^* orbitals? Placing 1 in each antibonding orbital will produce much less electron–electron repulsion than placing them both in the same antibonding orbital. The 2 electrons are in separate orbitals, as shown in Table 2-2, and as a result are unpaired, since unpairing also reduces the interaction between the electrons (Coulson, 1947). The most stable configuration of dioxygen is a triplet state, since 2 unpaired electrons form a triplet state.

Ground-state dioxygen is usually designated as $^3\Sigma_g$, indicating a triplet state with zero resultant orbital momentum. Note that in the π-orbitals of dioxygen,

* The orbitals are actually hybridized so that the lone pair is an sp^2 hybrid. Nevertheless, for simplicity, we shall add electrons to pure s and p orbitals.