

The potential diagram for oxygen at pH 7

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Successive one-electron reductions of molecular oxygen yield the superoxide radical ($O_2^{\cdot-}$), H_2O_2 , the hydroxyl radical (OH^{\cdot}) and water. Redox potentials at pH 7 for one-, two- and four-electron couples involving these states are presented as a potential diagram. The significance of each of these potentials is explained. The complete potential diagram enables complex systems to be rationalized, such as production of OH^{\cdot} by H_2O_2 plus Fe^{3+} .

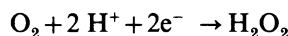
In inorganic chemistry, diagrams showing potentials between different oxidation states of an element are widely used as summaries and teaching aids. These potentials are variously known as redox potentials, oxidation–reduction potentials and standard electrode potentials.

Fig. 1 presents a potential diagram for oxygen at the biochemical standard state of pH 7. Successive one-electron reductions of molecular oxygen give the superoxide radical anion ($O_2^{\cdot-}$), H_2O_2 , the hydroxyl radical (OH^{\cdot}) and water. The standard sign conventions lead to redox potentials being cited for reductive reactions:



However, the potential diagram is equally useful for discussion of oxidations.

The voltages shown apply to standard states; as explained by myself (Wood, 1987), these approximate to 10^5 Pa (0.987 atm) of O_2 , $1 \text{ mol} \cdot \text{l}^{-1}$ concentrations of $O_2^{\cdot-}$, H_2O_2 and OH^{\cdot} , and pure liquid water. The values given are derived from diagrams for pH 0 and pH 14 in a recent compilation (Hoare, 1985). Data for OH^{\cdot} are based on the mean of two recent determinations: E_0 (OH^{\cdot}/H_2O) = +2.72 V (Schwarz & Dodson, 1984) and +2.74 V (Klänning *et al.*, 1985). As an example of the conversion to pH 7, consider E_0 (O_2/H_2O_2) = +0.695 V. The equation is written as a reduction:



For this reaction, $\Delta G'_0 = \Delta G_0 - RT \ln [H^+]^2$. Since

$$\Delta G = -nEF$$

where F is Faraday's constant ($96485 \text{ J} \cdot \text{V}^{-1} \cdot \text{mol}^{-1}$), $E'_0 = E_0 + (RT/2F) \ln 10^{-14}$. For 25 °C, $E'_0 = E_0 - 0.414 \text{ V}$. In converting to pH 7, care must be taken to choose appropriate protonation states, or include pK values (Hoare, 1985). Thus $O_2^{\cdot-}$ becomes protonated at acid pH ($O_2^{\cdot-} + H^+ \rightarrow HO_2^{\cdot}$; $pK = 4.8$), whereas OH^{\cdot} and H_2O_2 both lose a proton at high pH ($HO^{\cdot} \rightarrow O^{\cdot-} + H^+$, $pK = 11.8$; $H_2O_2 \rightarrow HO_2^{\cdot} + H^+$, $pK = 11.7$). The potentials shown in the diagram all fall with increasing pH, apart from E_0 ($O_2/O_2^{\cdot-}$), which is pH-independent.

A useful property of potential diagrams is that alternative connections between different states have the

same value for $\sum nE$, where n is the number of electrons transferred in a reductive step with redox potential E . For example, $-0.33 + 0.89 = 2 \times (+0.28)$. As has been mentioned, for each step, $\Delta G = -nEF$. Alternative pathways must have the same overall ΔG change, implying the same value for $\sum nE$.

The following paragraphs will discuss the steps constituting the diagram in turn. Put simply, a reductive step on the diagram implies oxidation of a donor with a similar or lower redox potential, whereas an oxidative step on the diagram implies reduction of an acceptor with a similar or higher redox potential.

The complete four-electron reduction, $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$, E'_0 (O_2/H_2O) = +0.815 V, constitutes the terminal reaction of the aerobic respiratory chain. The converse reaction provides the electron source for plant photosynthesis.

The reduction of molecular oxygen to H_2O_2 is less energetically favourable than the complete reduction to water, but requires only two electrons in place of four. For this reason H_2O_2 is the product of oxygen reduction by many oxidases.

The high redox potential for H_2O_2 reduction to water ($E'_0 = +1.349 \text{ V}$) implies a latent oxidizing power, put to use by peroxidases. For many peroxidases, H_2O_2 reacts with the resting ferric enzyme to yield a 'Compound I', in which the iron has undergone a one-electron oxidation to a ferryl ($Fe^{IV}=\text{O}$)²⁺ state, whereas the second electron is abstracted from another group close by (Dunford & Stillman, 1976; Penner-Hahn *et al.*, 1983):



The two one-electron redox couples, ($P^{\cdot+}/P$) and (Fe^{IV}/Fe^{III}), may both have potentials approaching +1.0 V (Hayashi & Yamazaki, 1979). This is sufficient for one-electron oxidation of many aromatic compounds to yield a radical cation, for instance during lignin biosynthesis or lignin breakdown by wood-rotting fungi (Prince & Stiefel, 1987).

The preceding two paragraphs have considered the two-electron couples O_2/H_2O_2 and H_2O_2/H_2O . The large differences in redox potential between these processes provides a driving force for H_2O_2 dismutation, catalysed by catalase.

Abbreviations and symbols used: $O_2^{\cdot-}$, superoxide radical; OH^{\cdot} , hydroxyl radical; HO_2^{\cdot} , hydroperoxy radical; $O^{\cdot-}$, oxygen anion radical; HO_2^- , peroxide anion; OH^- , hydroxyl anion; E_0 , standard electrode potential; ΔG , molar Gibbs energy change; E'_0 , electrode potential standard at pH 7.0; $\Delta G'_0$, standard Gibbs energy at pH 7.0; F , Faraday constant; E , redox potential; n , number of electrons transferred; $E_{m,7}$, midpoint potential in a redox titration at pH 7.0.

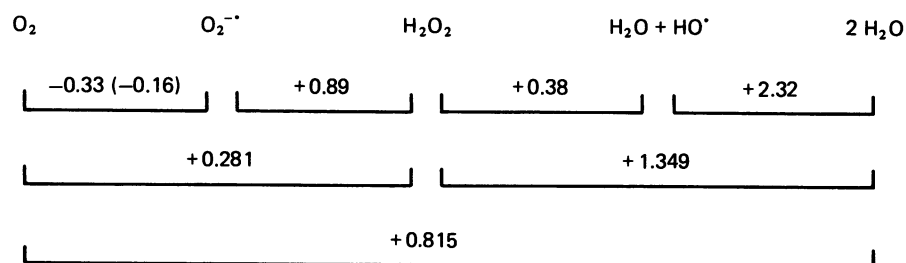
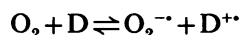


Fig. 1. Potential diagram for oxygen at pH 7

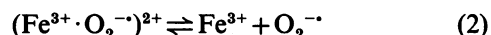
The units are volts (V). The value in parentheses is for 1 mol of $O_2 \cdot l^{-1}$ as standard state, instead of 10^5 Pa of O_2 . For sources, see the text.

Turning to one-electron couples, the reduction of molecular oxygen to $O_2^{\cdot -}$ has a lower redox potential than for H_2O_2 formation. However, only a single electron need be transferred. For this reason, $O_2^{\cdot -}$ formation is a characteristic of autoxidation. By contrast, $O_2^{\cdot -}$ production by neutrophils is a deliberate act of biological warfare (Rossi, 1986). The diagram shows two redox potentials for the molecular oxygen/superoxide couple ($O_2/O_2^{\cdot -}$). I (Wood, 1987) have discussed the relationship between these values and why both are useful. The redox potential relative to 10^5 Pa of O_2 is the standard potential, $E_0(O_2/O_2^{\cdot -}) = -0.33$ V. However, the redox potential relative to 1 mol of $O_2 \cdot l^{-1}$, $E_0[O_{2(aq.)}/O_2^{\cdot -}] = -0.16$ V, is relevant for reversible one-electron exchange:



Superoxide dismutases contain metal ions with one-electron redox potentials between those for $O_2^{\cdot -}$ oxidation and reduction, enabling dismutation to take place. For example, the copper atoms of CuZn-superoxide dismutase have an $E_{m,7}$ of +0.42 V (Fee & DiCorleto, 1973).

The $O_2^{\cdot -}/H_2O_2$ couple has a high redox potential. For this reason, the one-electron oxidation of H_2O_2 to $O_2^{\cdot -}$ can only be brought about by strong oxidizing agents. As an example, consider the effect of high concentrations of H_2O_2 on horseradish peroxidase. The enzyme is converted into a state known as 'Compound III' (Dunford & Stillman, 1976; Nakajima & Yamazaki, 1987). This is an oxy complex, but lacks the stability of oxymyoglobin. Its formation takes place from Compound II, which contains Fe^{IV} :



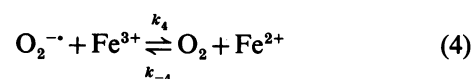
These two reactions represent a one-electron oxidation of H_2O_2 , made possible by the high potential of the (Compound II/ferriperoxidase) redox couple, namely +0.96 V (Hayashi & Yamazaki, 1979).

In 1894, Fenton described the strong oxidizing properties of a mixture of H_2O_2 and Fe^{2+} (Fenton's reagent). The active species was shown by Haber & Weiss (1934) to be OH^{\cdot} , formed by one-electron transfer from Fe^{II} :



A wide range of states of ferrous iron are active (Cohen, 1985). At pH 7, many iron complexes have a redox potential near 0 V [e.g. EDTA, +0.114; oxalate, +0.002; protoporphyrin-IX (free haem), about -0.12 V; Clark (1960)], making electron transfer to H_2O_2 a 'downhill'

reaction. The traces of unprotected iron present in many biological systems can be sufficient, provided there is a means of regenerating the ferrous state (Cohen, 1985). This can be accomplished by one-electron reduction of Fe^{3+} by $O_2^{\cdot -}$ (McCord & Day, 1978):



Provided the Fe^{III}/Fe^{II} redox potential is above -0.16 V [$E_0(O_{2(aq.)}/O_2^{\cdot -})$], k_4 is greater than k_{-4} (Wood, 1987). The combination of eqn. (4) plus eqn. (3) is the Haber-Weiss reaction (Haber & Weiss, 1934; Cohen, 1985). OH^{\cdot} is liable to be formed whenever $O_2^{\cdot -}$ is produced in the absence of adequate levels of catalase and superoxide dismutase (Wolff *et al.*, 1986; Halliwell & Gutteridge, 1986).

A final example shows how the whole potential diagram can be used to rationalize a complex system. If the Fe^{2+} of Fenton's reagent is replaced by Fe^{3+} , similar oxidations can still take place, although less rapidly (Gutteridge & Wilkins, 1983; Gutteridge & Bannister, 1986). In the literature the activating reaction is often said to be a one-electron oxidation of H_2O_2 , coupled to reduction of ferric iron (Walling, 1982; Halliwell & Gutteridge, 1984):



However, as has been stated, the Fe^{III}/Fe^{II} redox potential at pH 7 is generally near 0 V, compared with +0.89 V for the $O_2^{\cdot -}/H_2O_2$ couple. As I explained previously (Wood, 1987), a one-electron transfer can have $\Delta G'_0$ positive, but not too positive. For $\Delta E'_0 = -0.9$ V, $\Delta G'_0$ will be about +86000 J·mol⁻¹. Such a process can scarcely be significant. A more plausible activating reaction would be a two-electron reduction of H_2O_2 : $E'_0(H_2O_2/H_2O) = +1.349$ V. The iron will either be oxidized to Fe^{IV} , with the second electron taken from a complexing ligand (as in a peroxidase), or Fe^V may be formed transiently (Dunford & Stillman, 1976; Rush & Bielski, 1986). Once a high oxidation state of iron has been produced, there is no difficulty in oxidation of H_2O_2 to superoxide [reactions (1) and (2) above]. Reactions (4) and (3) complete a pathway for hydroxyl-radical formation, in which all steps are energetically downhill.

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