Discussion Letter

MECHANISM OF REACTIONS INVOLVING SINGLET OXYGEN AND THE SUPEROXIDE ANION

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1. Introduction

Recently it was shown [1] on thermodynamic grounds that singlet oxygen could be a product of the following redox reactions:

$$O_2^- + O_2^- + 2H^+ \longrightarrow {}^1\Delta_gO_2 + H_2O_2$$

$$O_2^- + H_2O_2 + H^+ \longrightarrow {}^1\Delta_gO_2 + H_2O + OH^\cdot$$

$$O_2^- + OH^\cdot + H^+ \longrightarrow {}^1\Sigma_g^+O_2 + H_2O$$

$$H_2O_2 + H_2O_2 \longrightarrow {}^1\Sigma_g^+O_2 + 2H_2O$$

Since superoxide anions and hydrogen peroxide are formed in living systems and since singlet oxygen as well as the hydroxyl radical are considered to be harmful species, it is important to know in which reactions the formation or involvement of these species is kinetically feasible. For instance, does the dismutation of O_2^- yield ${}^1\Sigma_g^+$, ${}^1\Delta_g$ or ${}^3\Sigma_g^-O_2$? In two recent papers by Khan [2,3] it was calculated that ${}^1\Sigma_g^+O_2$ would be formed if O_2^- were surrounded by six or more water molecules. It is obvious that the calculations of Khan require the strict control of many parameters; therefore we maintain that the formation of ${}^1\Sigma_g^+O_2$ as a product of the dismutation reaction is most improbable [1]. To decide whether oxygen will be formed in its ${}^1\Delta_g$ or ${}^3\Sigma_g^-$ state we must know the relative rates of the respective reactions.

2. Discussion

According to the well-known Arrhenius equation

$$k = Ae^{-Ea/RT}$$

the rate constant, k, of a reaction is determined by (i) A, a frequency factor, which is influenced by effects such as diffusion and electrostatic interaction, as well as the entropy of the activated complex with respect to the reactants, and (ii) E_a, the activation energy, which can be related to the height of the energy barrier. For an electron transfer reaction it is necessary that during the transition state overlap should occur between the electron donating and the electron accepting orbital [4]. This is possible if they have the correct topology and symmetry. These effects are not considered in the Marcus theory [5]. If such an overlap does occur, making mixing in of higher states unnecessary, we expect the reaction to have a small activation energy and a high rate constant.

O_2^- has the electron configuration:

$$(\sigma_g1s)^2(\sigma_u^*1s)^2(\sigma_g2s)^2(\sigma_u^*2s)^2(\sigma_g2p)^2$$

$$(\pi_u2p)^4(\pi_g^*2p)^3(\sigma_u^*2p)^0$$

In O_2^- one of the two π_g^* orbitals is filled and the other one contains one electron. It is the latter orbital which can easily overlap with a half-filled π_g^* orbital of HO_2 (figs. 1a–c). After electron transfer, an oxygen molecule will be formed which has an empty and a filled π_g^* orbital: ${}^1\Delta_gO_2$ (figs. 2a–c). A mechanism leading to ${}^3\Sigma_g^-$ or – if it were thermodynamically

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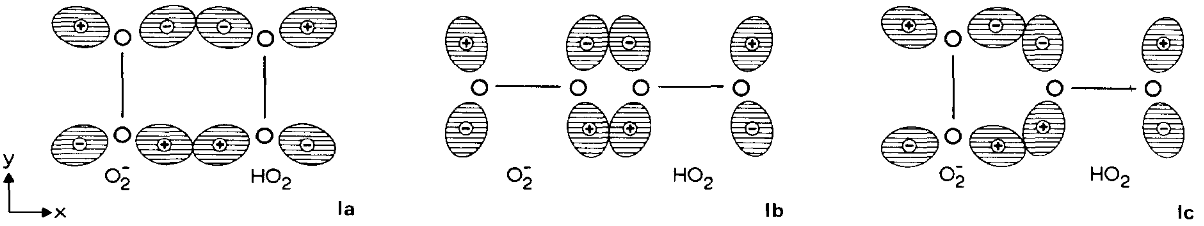


Fig.1a–c. Schematic representation of orbitals involved in overlap. Empty orbitals are not shaded, orbitals containing one electron are half-shaded and filled orbitals are shaded. The orbitals in the *zx*- and *zy*-plane are not shown, nor are the empty σ_u^* orbitals in the *xy*-plane.

possible — $^1\Sigma_g^+O_2$ would involve overlap between the filled π_g^* orbital of O_2^- and the half-filled π_g^* orbital of HO_2 and is therefore unlikely. Experimental evidence for the formation of $^1\Delta_gO_2$ has been presented by Mayeda and Bard [6]. HO_2, which has a p*K* of 4.9 [7], reacts relatively fast with O_2^- : $k_1 = 8.5 \cdot 10^7\ M^{-1}\ s^{-1}$. The reaction of O_2^- with itself is much slower: $k < 0.3\ M^{-1}\ s^{-1}$ [8]. This low-rate constant cannot be caused by electrostatic interaction alone. One can calculate, using Debye's correction factor [10], that it should not be less than 0.25 k_1. We believe that the slow rate is caused by another effect. In the case of a reaction of O_2^- with O_2^-, an O_2^{2-} will be formed after electron transfer, and in the case of a reaction of O_2^- with HO_2, a HO_2^- ion. Clearly the HO_2^- ion is a more feasible intermediate than the double-charged O_2^{2-} ion. In fact H_2O_2 has a p*K* of 11.8, such that around and above pH 11 HO_2^- exists as a relatively stable species. The reaction of HO_2 with itself ($k = 7.6 \cdot 10^5\ M^{-1}\ s^{-1}$) is slower than the reaction of HO_2 with O_2^- [7]. We assume that this is caused by the stabilizing effect of the proton on the electron donating orbital and the effect of this proton on hindering the overlap.

The concept of overlap between electron donating and accepting orbital helps us to understand the course of other reactions, see table 1. Some of the reactions need comment.

Reactions (5) and (6) form the Haber–Weiss cycle [20] of which reaction [5] has been proposed as a source of OH$^\cdot$ radicals in biochemical systems [21]. Recently McClune and Fee [9], Halliwell [14] and Rigo et al. [22] have investigated this reaction. Of these authors McClune and Fee [9] and Rigo et al. [22] did not appreciate the fact that reaction (5) forms part of a cycle, but this has had no effect on their results. These results as well as those of Halliwell [14] show that reaction (5) has a very low rate constant. If reaction (5) is to occur, the rate determining step will probably be the transfer of an electron from the π_g^* orbital of O_2^- to the σ_u^* orbital of H_2O_2. It can be seen in fig.4 that the filled π_g^* orbitals of H_2O_2 hinder the overlap. If reaction (5) occurs, it will yield $^1\Delta_g$ or $^3\Sigma_gO_2^-$, depending on whether the electron donating orbital is the filled or half-filled one.

The disproportionation reaction of hydrogen peroxide, reaction (4), is very similar to reaction (5). Smith and Kulig [13] have found evidence for $^1\Delta_gO_2$ production through the base catalysed disproportionation of H_2O_2 under conditions which show that this reaction has an appreciable amount of activation energy.

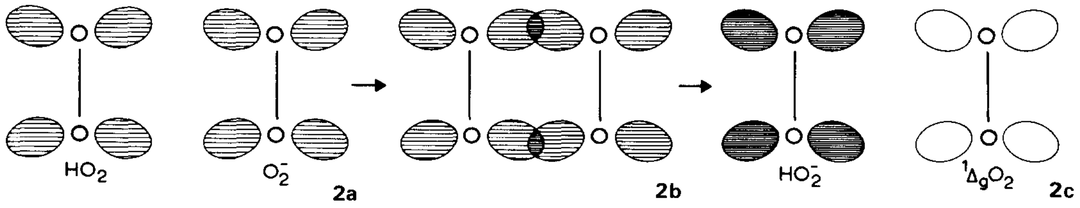


Fig.2a–c. Encounter, overlap and products of reaction (1).

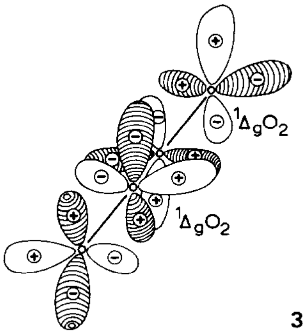
Table 1

Effect of orbital symmetry and topology on the rate of reaction between oxygen containing radicals and/or molecules

Reaction	Donor orbital	Acceptor orbital	Overlap possible	Figure	State of oxygen if a product	Experimental evidence for this state	Rate constant ($M^{-1}\ s^{-1}$)
(1) $O_2^- + HO_2 + H^+ \rightarrow H_2O_2 + O_2$	$(\pi_g^*)^1$	$(\pi_g^*)^1$	yes	1(a–c)	$^1\Delta_g$	yes, [6]	$8.5 \cdot 10^7$ [7]
(2) $^1\Delta_gO_2 + {}^1\Delta_gO_2 \rightarrow 2O_2 + h\nu$	$(\pi_g^*)^2$	$(\pi_g^*)^0$	yes, 2 ×	3	$^3\Sigma_g^-$	yes	unknown, high [2]
(3) $O_2^- + {}^1\Delta_gO_2 \rightarrow O_2 + O_2^-$	$(\pi_g^*)^2$	$(\pi_g^*)^0$	yes		$^3\Sigma_g^-$	yes	$(3.6 \pm 0.1) \cdot 10^7$, $1.6 \cdot 10^9$ [11,18][a]
(4) $H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$	$(\pi_g^*)^2$	$(\sigma_u^*)^0$	difficult		$^1\Delta_g$	yes, [13]	unknown, very low [12]
(5) $O_2^- + H_2O_2 + H^+ \rightarrow O_2 + OH^{\cdot} + H_2O$	$(\pi_g^*)^2$	$(\sigma_u^*)^0$	difficult	4	$^3\Sigma_g^-$	no	$< 10^{-4}\ M^{-1}\ s^{-1}$ [22]
	$(\pi_g^*)^1$	$(\sigma_u^*)^0$	difficult		$^1\Delta_g$	no	
(6) $H_2O_2 + OH^{\cdot} \rightarrow H_2O + O_2^- + H^+$	$(\pi_g^*)^2$	$(p_u)^1$	no				$2.3 \cdot 10^7$ [16]
(7) $O_2^- + OH^{\cdot} + H^+ \rightarrow H_2O + O_2$	$(\pi_g^*)^1$	$(p_u)^1$	yes		$^1\Delta_g$	yes[b], [15]	$1.0 \cdot 10^{10}$ [17]
(8) $OH^{\cdot} + OH^{\cdot} \rightarrow H_2O_2$	$(p_u)^1$	$(p_u)^1$	yes				$5.5 \cdot 10^9$ [19]

[a] In aprotic media

[b] The $^1\Delta_gO_2$ in these particular experiments may have been produced from the reaction of O_2^- with HO_2



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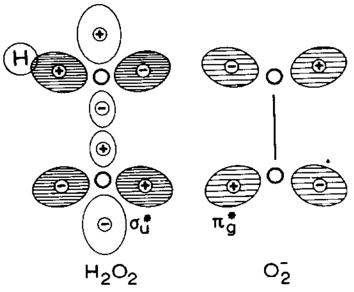

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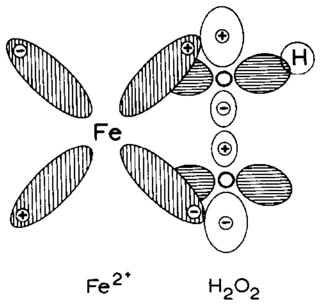

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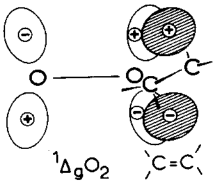

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Fig.3. Double overlap between π_g^* orbitals of two $^1\Delta_gO_2$ molecules, reaction (2).

Fig.4. Electrons in π_g^* orbitals of H_2O_2 (left) hinder overlap between π_g^* orbital of O_2^- (right) and σ_u^* orbital of H_2O_2, reaction (5).

Fig.5. Overlap of a d orbital of Fe^{2+} with the σ_u^* orbital of H_2O_2, reaction (12).

Fig.6. Overlap between the empty π_g^* orbital of $^1\Delta_gO_2$ and the filled π_u orbital of an alkene, reaction (10).

The oxidation of H_2O_2 by $OH^\cdot$ is very slow for an $OH^\cdot$-reaction [23]. No direct overlap is possible and therefore it is likely that the reaction proceeds by a mechanism that involves a high-activation energy. The effect of direct overlap is nicely illustrated by the reaction of O_2^- with $OH^\cdot$ [7]: this reaction is 450 times faster than reaction (6). Note that we expect $^1\Delta_gO_2$, not $^1\Sigma_g^+O_2$ to be formed, even though $^1\Sigma_g^+O_2$ is thermodynamically possible.

We can now discuss two other reactions which are of biological importance: the oxidation of a thiol and the 'ene' reaction of $^1\Delta_gO_2$ with an alkene.

The oxidation of a thiol by $^3\Sigma_g^-$ oxygen is brought about by the availability of the $3d_g$ state of sulphur. A half-filled π_g^* orbital of $^3\Sigma_g^-O_2$ can overlap with a $3p_u \rightarrow 3d_g$ excited sulphur atom:

$$^3\Sigma_g^-O_2 + R\text{–}S\text{–}H\,(3p_u \rightarrow 3d_g) \longrightarrow O_2^- + RS^\cdot + H^+ \qquad (9)$$

The superoxide anion produced [25] may react with another thiol group in exactly the same manner. The products of this reaction will be another sulphur radical and hydrogen peroxide. The reaction of singlet oxygen with a thiol is of particular interest in that the sulphur atom need not be activated. A direct overlap between an empty π_g^* orbital of $^1\Delta_gO_2$ and the filled $3p_u$ orbital of the sulphur will directly lead to a reaction giving the same products as reaction (9).

$^3\Sigma_g^-O_2$ and O_2^-, having no empty π_g^* orbitals, cannot easily react with a double bond. As shown in fig.6 $^1\Delta_gO_2$ has one empty π_g^* orbital which can overlap with the π_u orbital of the double bond‡. Addition

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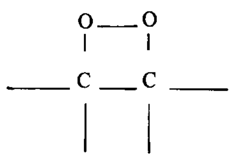
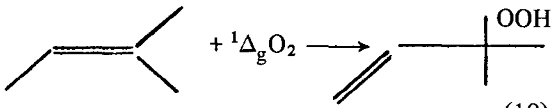
‡Overlap between the filled π_g^* orbital of $^1\Delta_gO_2$ and the empty π_g^* of the alkene does not seem likely in view of the difference in electronegativity between oxygen and carbon; however if it occurred, the compound formed would be a dioxetane:



will preferably occur at that carbon atom which has the higher electron density. The reaction product is a hydroperoxide:



(10)

Experimental evidence shows that when $^1\Delta_gO_2$ reacts with alkenes in organic solvents the expected product is found but not exclusively. The rate constants range from $4.4 \cdot 10^3$ to $2.4 \cdot 10^7$ M^{-1} s^{-1} and the activation energies are low: 5.4–0.5 kcal/mol [26]. Since the reaction of O_2^- with HO_2 will yield $^1\Delta_gO_2$, reaction (10) may be of particular importance with respect to lipid peroxide formation, provided $^1\Delta_gO_2$ is produced near a membrane; its lifetime in water is very short: $\tau = 2$ μs [27]. In the lipid bilayer this may be increased, permitting selective reactions. It will be noted that we do not regard O_2^- in itself as a harmful radical. From reaction (9) and (10) it is clear that we expect O_2^- to behave like triplet oxygen.

As shown above, it is unlikely that OH˙ radicals can be formed from the direct reaction of O_2^- with H_2O_2. However, iron compounds may be able to catalyze this reaction:

$$\text{Fe(III)complex} + O_2^- \longrightarrow \text{Fe(II) complex} + O_2 \qquad (11)$$

Evidence for reduction of Fe(III)EDTA by O_2^- has been presented by Halliwell [28]. Overlap between a d-orbital of iron and the σ_u^*-orbital of H_2O_2 is just possible as shown in fig.5, making reaction (12) possible:

$$\text{Fe(II)complex} + H_2O_2 \longrightarrow \text{Fe(III)complex} + OH^{\bullet} + OH^- \quad (12)$$

This leads to a modified Haber–Weiss cycle (see also [29], [30] and [31]) which would explain the results of Haber and Weiss and the fact that iron compounds appear to be necessary for lipid peroxide formation [31–33].

The production of hydroxyl radicals via the modified Haber–Weiss reactions (11) and (12), and of singlet oxygen from the dismutation reaction (1) and the disproportionation reaction (4) can occur simultaneously. Therefore it is not surprising that both superoxide dismutase and catalase are needed to protect living systems [34,35].

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