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Superoxide Does React with Peroxides: Direct Observation of the Haber-Weiss Reaction in the Gas Phase

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Abstract
Reactions of superoxide with hydrogen peroxide and organic hydroperoxides are observed as efficient processes in the gas phase and yield products – including the ozonide anion – that are consistent with the one-electron reduction of the peroxide.

Keywords
Haber-Weiss reaction, peroxides, radical ions, reaction mechanisms, reactive species

Disciplines
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Superoxide Does React with Peroxides: Direct Observation of the Haber-Weiss Reaction in the Gas Phase

Stephen J. Blanksby, Veronica M. Bierbaum, G. Barney Ellison, Shuji Kato**

((Dedication----optional))

The fact that nature provides specific enzymes to selectively remove superoxide ($O_2^{\bullet-}$) in aerobic organisms, namely the superoxide dismustases,[1] has lead to the suggestion that this radical may cause the oxidative damage associated with degradative disease and aging.[2] Intriguingly, however, superoxide itself is relatively unreactive toward most cellular components, which suggests that dismutase enzymes may ultimately protect the cell against more pernicious oxidants formed from superoxide. As such, there is increasing interest in the endogenous chemistry of superoxide and pathways *via* which it might beget more reactive oxygen species. Protonation of superoxide to form the hydroperoxyl radical ($HOO^{\bullet}$) and dismutation to hydrogen peroxide (HOOH) with subsequent metal-catalyzed reduction to the hydroxyl radical ($HO^{\bullet}$) are well characterized processes, with both $HOO^{\bullet}$ and $HO^{\bullet}$ radicals significantly more reactive than their common progenitor.[2] Recent examples, however, have also linked superoxide to the putative production of singlet oxygen[3] and ozone,[4,5] although the definitive characterization of these chemistries in the cellular milieu has proved challenging.[6,7]

The one-electron reduction of hydrogen peroxide by superoxide has also been invoked as a potential source of reactive hydroxyl radicals. First proposed more than 75 years ago,[8] Scheme 1 has become known as the Haber-Weiss reaction. This scheme has been exhaustively investigated and it is now generally accepted that the Haber-Weiss reaction does *not* occur in the absence of metal catalysis.[9,10] Measurements for the archetypical reaction (Scheme 1) and related reactions of alkyl and lipid hydroperoxides[12] find it prohibitively slow in aqueous solution (*e.g.*, Scheme 1, $k = 0.13 \pm 0.07\ M^{-1}s^{-1}$).[11] Here we report the direct observation of a facile reaction between superoxide and hydrogen peroxide as well as several organic hydroperoxides in the gas phase. These observations demonstrate, for the first time, the efficacy of the *intrinsic* Haber-Weiss reaction, where the influence of enzymes, transition metals and solvent can be rigorously excluded and thus suggests a reassessment of the potential role of this reaction *in vivo,* particularly in membranes and hydrophobic protein domains.

$$O_2^{\bullet-} + H_2O_2 \longrightarrow O_2 + HO^{\bullet} + HO^{-}$$

Scheme 1.

Figure 1(a) shows a spectrum of mass selected superoxide anion (*m/z* 32) following reaction with hydrogen peroxide in the gas phase. The two major primary product ions are observed at *m/z* 34 and *m/z* 48 corresponding to the oxide-water cluster $[O^{\bullet-}\cdots H_2O]$ and ozonide anions $[O_3^{\bullet-}]$, respectively. From the measured rate constant of 6 x 10^{-10} $cm^3\ s^{-1}$ a reaction efficiency of 29% of the theoretical collision rate was calculated.[13] The proposed reaction mechanism is outlined in Scheme 2 and can be considered directly analogous to the original Haber-Weiss reaction scheme. In the gas phase, the electron transfer from superoxide to hydrogen peroxide as outlined in Scheme 1 is endothermic[14] by 18.6 kcal mol^{-1} in contrast to the exoergic aqueous reaction (11.1 kcal mol^{-1}) predicted from redox potentials.[9]

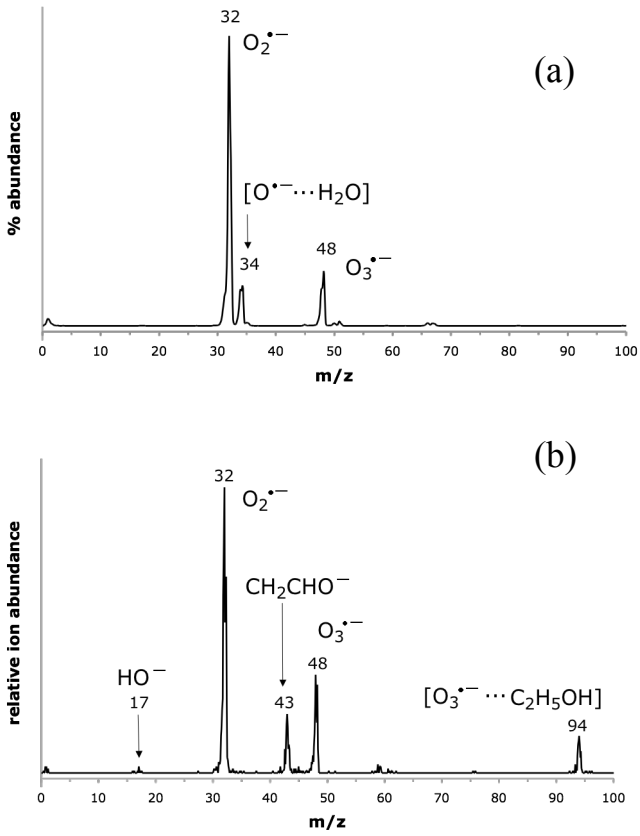
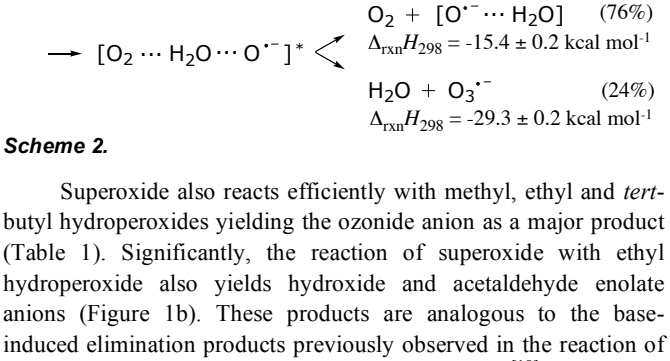


Figure 1. Flowing afterglow-selected ion flow tube spectra showing the products of the gas phase reactions; (a) $O_2^{\bullet-}$ + H_2O_2 and (b) $O_2^{\bullet-}$ + CH_3CH_2OOH.

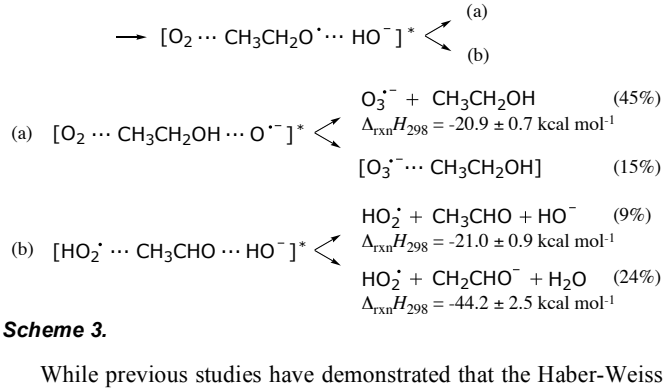
In spite of the gas phase endothermicity, the electron transfer is facilitated *via* the formation of the ion-dipole complex $[O_2^{\bullet-}\cdots HOOH]^*$ (see Scheme 2). That is, the electron transfer to form the Haber-Weiss intermediate complex $[O_2\cdots HO^{\bullet}\cdots HO^-]^*$ is fuelled by energy from the initial solvation of superoxide by hydrogen peroxide (*i.e.*, solvation energy is ≥ 18.6 kcal mol^{-1}). The solvation enthalpy of the analogous superoxide-water cluster has been measured[15] to be 18.4 kcal mol^{-1} strongly suggesting that solvation of superoxide by hydrogen peroxide should provide sufficient energy to drive rearrangement within the complex and access exothermic product channels. The two most energetically accessible channels are (i) hydrogen atom transfer between the hydroxide anion and the hydroxyl radical (or the converse proton transfer) to form the solvated oxide anion $[O^{\bullet-}\cdots H_2O]$ with loss of dioxygen and

(ii) addition of the oxide anion to dioxygen within the complex to form the ozonide anion and water.[14,16] The association reaction of the atomic oxygen anion and neutral dioxygen has been observed at low temperature and is facilitated by termolecular interactions and solvation of the reactive anion, which is analogous to the intra-complex process proposed here.[17]



Scheme 2.

Superoxide also reacts efficiently with methyl, ethyl and *tert*-butyl hydroperoxides yielding the ozonide anion as a major product (Table 1). Significantly, the reaction of superoxide with ethyl hydroperoxide also yields hydroxide and acetaldehyde enolate anions (Figure 1b). These products are analogous to the base-induced elimination products previously observed in the reaction of even electron anions with alkyl hydroperoxides,[18] although a Haber-Weiss-type mechanism involving electron transfer (Scheme 3) is much more likely in this instance due to the poor gas phase basicity of $O_2^{\bullet-}$. The observation of this reaction suggests that superoxide can decompose alkyl hydroperoxides to aldehydes or ketones depending on the initial substitution of the peroxide.



Scheme 3.

While previous studies have demonstrated that the Haber-Weiss reaction is negligible in aqueous solution,[11,12] the gas phase data presented here indicate that the reaction is intrinsically facile with efficiencies of up to 47%. This suggests that aqueous solvation of superoxide anion actually hampers the Haber-Weiss reaction. Such a conclusion is supported by the observation of related one-electron reduction reactions in polar aprotic solvents[19] but contrasts with previous studies of superoxide with peroxides in selected organic solvents that failed to provide convincing evidence for the electron transfer reaction.[20] Nevertheless, the observation of efficient Haber-Weiss chemistry in the perfect hydrophobic environment of the gas phase suggests that this reaction may need to be reconsidered as a viable pathway for the reduction of peroxides in membranes and hydrophobic protein domains. For example, if an enzyme were to provide the environment to facilitate the interaction of "naked" superoxide and a peroxide, an efficient reduction of the latter would occur. What would the biochemical consequences for such chemistry be? The reduction of lipid peroxides by superoxide would produce genotoxic and mutagenic aldehydes and ketones.[21] Migration of $O_3^{\bullet-}$ to an aqueous environment of pH < 8 would yield hydroxyl radical and dioxygen[22] *via* the intermediacy of the $HO_3^{\bullet}$ radical[23] (*i.e.*, $O_3^{\bullet-} + H^+ \rightarrow [HO_3^{\bullet}] \rightarrow HO^{\bullet} + O_2$). This would represent a novel pathway for hydroxyl radical production and perhaps an additional pathway for superoxide toxicity. It is also intriguing to note that neutral ozone itself has been recently suggested as an endogenous oxidant.[5,7]

Table 1. The rate constants and reaction efficiencies, given as a percentage of theoretical collision rate[13] in the gas phase reaction $O_2^{\bullet-}$ + ROOH.

Peroxide	Rate constant ($cm^3\ s^{-1}$)[a] and efficiency (%)	Primary products (Branching ratio%)
HOOH	6 x 10^{-10} (29%)	$[O^{\bullet-} \cdots H_2O]$ (76%), $O_3^{\bullet-}$ (24%)
CH_3OOH	3 x 10^{-10} (14%)	HO^- (42%), HCO_2^- (10%), $O_3^{\bullet-}$ (44%), $[O_3^{\bullet-} \cdots HOCH_3]$ (4%)
CH_3CH_2OOH[b]	4 x 10^{-10} (17%)	HO^- (9%), CH_2CHO^- (24%), $O_3^{\bullet-}$ (45%), $CH_3CO_2^-$ (2%), $CH_3CH(O^-)OH$ (1%), $[O_3^{\bullet-} \cdots CH_3CH_2OH]$ (15%)
$(CH_3)_3COOH$	1 x 10^{-9} (47%)	$O_3^{\bullet-}$ (63%), $CH_3COCH_2^-$ (2%), $(CH_3)_2C(O^-)OH$ (5%), $[O_3^{\bullet-} \cdots HOC(CH_3)_3]$ (29%)

[a] The rate constants have systematic uncertainties of ± 20 %. Relative rates between different reactions, however, remain unchanged. [b] A minor unidentified ion (~ 4%) was observed at *m/z* 45.

Experimental Section

The experiments were performed using a flowing afterglow-selected ion flow tube.[24] Superoxide anions were prepared by electron transfer from the propenide anion[25] then mass-selected using a quadrupole mass filter and injected into a flow reactor buffered by 0.5 Torr of helium at 300 K. Neutral peroxides, obtained commercially or synthesized by standard methods,[26] were introduced *via* inlets in the flow tube and the abundance of the charged reaction products were recorded using a quadrupole mass spectrometer as a function of reaction time or reagent flow-rate. Reaction rate constants and branching ratios for the primary products were determined from analysis of the kinetics data.

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Superoxide Reactivity

Stephen J. Blanksby, Veronica M. Bierbaum, G. Barney Ellison, Shuji Kato**

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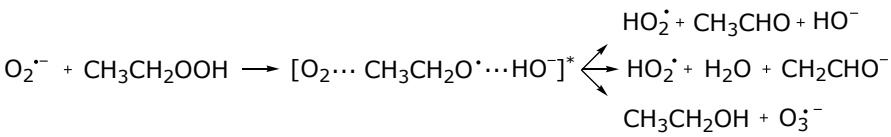

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