Gas-Phase Reactions

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

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The fact that nature provides specific enzymes to selectively remove superoxide ($O_2^{\bullet-}$) from aerobic organisms, namely, the superoxide dismutase enzymes,[1] has led to the suggestion that this radical ion 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 the pathways by which it might beget more reactive oxygen species. Protonation of superoxide to form the hydroperoxyl radical ($HOO^{\bullet}$) and dismutation of the same species to hydrogen peroxide (HOOH), with subsequent metal-catalyzed reduction to the hydroxyl radical ($HO^{\bullet}$), are well-characterized processes in which both the $HOO^{\bullet}$ and $HO^{\bullet}$ radicals are 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 radical ions. Scheme 1 was first proposed more than 75 years ago[8] and 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 catalysts.[9,10] Measurements for the archetypical reaction (Scheme 1) and for related reactions of alkyl and lipid hydroperoxides[12] show that these processes are prohibitively slow in aqueous solution (for example, in the case of Scheme 1, $k = 0.13 \pm 0.07\,\text{M}^{-1}\,\text{s}^{-1}$).[11] Here, we report the direct observation of a facile reaction between superoxide and hydrogen peroxide—as well as several other 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 suggest 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. Haber–Weiss reaction.

Figure 1a shows a spectrum of mass-selected superoxide anion (*m/z* 32) after reaction with hydrogen peroxide in the gas phase. The two major primary product ions, namely, the oxide–water cluster [$O^{\bullet-}\cdots H_2O$] and the ozonide anion [$O_3^{\bullet-}$], are observed at *m/z* 34 and *m/z* 48, respectively. From the measured rate constant of $6\times10^{-10}\,\text{cm}^3\,\text{s}^{-1}$, 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

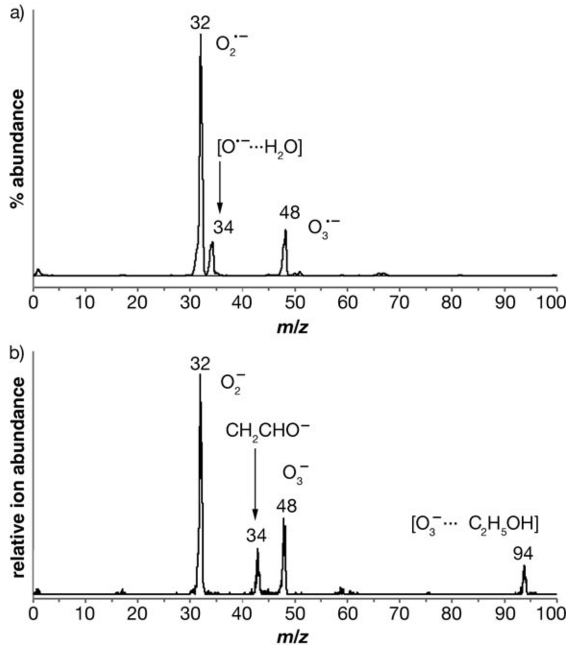

Figure 1. Flowing afterglow-selected ion flow tube spectra showing the products of the gas-phase reactions: a) $O_2^{\bullet-} + H_2O_2$ and b) $O_2^{\bullet-} + CH_3CH_2OOH$.

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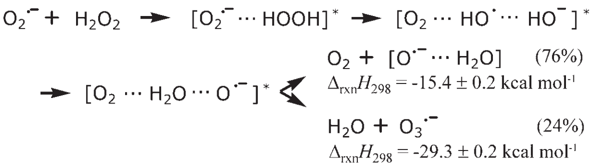
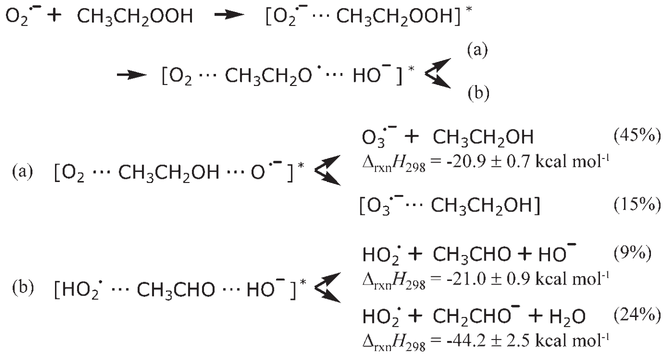


Scheme 2. Proposed mechanism for the reaction of superoxide with hydrogen peroxide in the gas phase (from Figure 1 a).

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

Peroxide	Rate constant $[cm^3 s^{-1}]$[a] (efficiency [%])	Primary products (branching ratio [%])
HOOH	6×10^{-10} (29)	$[O^{\bullet-}\cdots H_2O]$ (76), $O_3^{\bullet-}$ (24)
CH_3OOH	3×10^{-10} (14)	HO^- (42), HCO_2^- (10), $O_3^{\bullet-}$ (44), $[O_3^{\bullet-}\cdots HOCH_3]$ (4)
CH_3CH_2OOH[b]	4×10^{-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)
$(CH_3)_3COOH$	1×10^{-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.



Scheme 3. Proposed mechanism for the reaction of superoxide with ethyl hydroperoxide in the gas phase (from Figure 1 b).

original Haber–Weiss reaction scheme. In the gas phase, the electron transfer from the superoxide to the hydrogen peroxide—as outlined in Scheme 1—is endothermic[14] (by 18.6 kcal mol^{-1}), in contrast to the exoergic aqueous reaction (namely, 11.1 kcal mol^{-1}) predicted from the redox potentials.[9]

In spite of the gas-phase endothermicity, the electron transfer is facilitated by the formation of the ion–dipole complex $[O_2^{\bullet-}\cdots HOOH]^*$ (see Scheme 2). The electron transfer to form the Haber–Weiss intermediate complex $[O_2\cdots HO^\bullet\cdots HO^-]^*$ is fueled by energy from the initial solvation of superoxide by hydrogen peroxide (that is, the 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}, which strongly suggests that solvation of superoxide by hydrogen peroxide should provide sufficient energy to drive rearrangements within the complex and access exothermic product channels. The two most energetically accessible channels are: 1) hydrogen-atom transfer between the hydroxide anion and the hydroxyl radical ion (or the converse proton transfer) to form the solvated oxide anion $[O^{\bullet-}\cdots H_2O]$, with loss of dioxygen, and 2) addition of the oxide anion to dioxygen within the complex to form the ozonide anion and water.[14,16] The association reaction between the atomic oxygen anion and neutral dioxygen has been observed at low temperatures and is facilitated by termolecular interactions and solvation of the reactive anion, which is analogous to the intracomplex process proposed here.[17]

Superoxide also reacts efficiently with methyl, ethyl, and *tert*-butyl hydroperoxides, thereby 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 1 b). 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 because of the poor gas-phase basicity of the $O_2^{\bullet-}$ species. The observation of this reaction suggests that superoxide can decompose alkyl hydroperoxides into aldehydes or ketones depending on the initial substitution of the peroxide.

While previous studies have demonstrated that the Haber–Weiss reaction is negligible in aqueous solution,[11,12] the data presented here indicate that the reaction is intrinsically facile in the gas phase (with efficiencies of up to 47%). These results suggest that aqueous solvation of the 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 the reaction of superoxide with peroxides in selected organic solvents, which 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 with a peroxide, an efficient reduction of the latter would occur. What would the biochemical consequences of such chemistry be? The reduction of lipid peroxides by superoxide would produce genotoxic and mutagenic aldehydes and ketones.[21] Migration of the $O_3^{\bullet-}$ species to an aqueous environment of pH < 8 would yield the hydroxyl radical ion and dioxygen[22] through the intermediacy of the $HO_3^\bullet$ radical[23] (that is, $O_3^{\bullet-} + H^+ \rightarrow [HO_3^\bullet] \rightarrow HO^\bullet + O_2$). This would represent a novel pathway for hydroxyl radical production and perhaps an additional pathway for superoxide toxicity. It is also interesting to note that neutral ozone itself has been recently suggested as an endogenous oxidant.[5,7]

Experimental Section

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

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