

Singlet molecular oxygen evolution upon simple acidification of aqueous hypochlorite: Application to studies on the deleterious health effects of chlorinated drinking water

(singlet oxygen/hypochlorite decomposition/chlorinated water supply/DNA mutation)

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ABSTRACT A study of the pH profile of the decomposition of aqueous hypochlorite has revealed the evolution (onset at pH 8) of single ($^1\Delta_g$) molecular oxygen (singlet spin state dioxygen) detected spectroscopically (1268 nm), prior to the appearance of chlorine (onset at pH 5.5). The possible mechanism of the singlet state dioxygen evolution is presented, and the origin of its chloride ion dependence is discussed, especially in reference to chloride ion dependence of singlet molecular oxygen evolution in biological systems. Recent epidemiological analyses of the correlation of human cancer with chlorinated water supplies focus attention on the singlet oxygen mechanisms of DNA lesion formation.

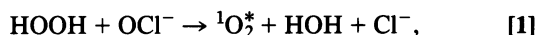
Singlet Molecular Oxygen Generation

In a general study of singlet molecular oxygen generation, we discovered that simple acidification of hypochlorite solutions yielded typical IR emission characteristic of singlet molecular (excited) oxygen. Currently, an epidemiological study (1) has correlated the statistics of human cancer with use of chlorinated water supply. The demonstration of singlet oxygen attack on DNA components by several laboratories (2–4) suggests a possible mechanistic role for the singlet oxygen generated in acidic environments in the correlation between chlorinated water and cancer.

A study of the pH profile of the decomposition of aqueous hypochlorite has revealed the evolution (onset at pH 8) of singlet ($^1\Delta_g$) molecular oxygen (singlet spin state dioxygen) detected spectroscopically (1268 nm), prior to the appearance of chlorine (onset at pH 5.5). This result is surprising because it has been overlooked in the long history of research on the chlorine–hypochlorite equilibria, kinetics, and mechanisms. These observations suggest another focus on mechanisms of redox reactions involving H_2O_2 and singlet oxygen species, in both chemical and biological systems.

Experimental Results on Acidification of Hypochlorite

The generation of singlet molecular oxygen by the chemiluminescent reaction of hydrogen peroxide with aqueous hypochlorite is well known and is now a standard method (5–9),



the singlet oxygen being detected spectroscopically. Initially, this was observed (5, 6) as the 634-nm simultaneous transition ($^1\Delta_g$) ($^1\Delta_g$) \rightarrow ($^3\Sigma_g^-$) ($^3\Sigma_g^-$) for the electron exchange collision of excited molecular pairs. The preferred and more efficient

method is the direct detection of the $^1\Delta_g \rightarrow {}^3\Sigma_g^-$ emission at 1268 nm in the near infrared, and this method (10, 11) has been used in the present research. We used a Ge-photodiode detector with a dedicated near-IR spectrometer in the 1- to 1.7- μ m region, with electronic and data-processing peripherals (12). Fig. 1 shows the typical 1268-nm chemiluminescence spectra recorded upon the acidification of aqueous hypochlorite with HCl at room temperature.

The pH profiles of 1O_2 and Cl_2 evolution upon acidification of aqueous hypochlorite solution at room temperature are shown in Fig. 2 (reagent-grade sodium hypochlorite solution, acidified by reagent grade hydrochloric acid). The pH of the solution was measured with a Fisher Accumet pH meter before the acidification and after the completion of the singlet oxygen emission experiment. In each case, the initial hypochlorite solution was at pH 11, an aliquot of acid was added, the integrated (900 sec) luminescence intensity at 1268 nm was recorded, and the final pH was measured and recorded.

The highest emission intensity recorded at 1268 nm was observed by adding 200 μ l of concentrated HCl (12.6 M, Mallinckrodt analytical reagent) to a 5-ml aqueous solution of 0.6 M sodium hypochlorite [analytical reagent from Alpha 1 Biomedicals (Culver City, CA)] in an optical cuvette, which was stirred continuously with a Teflon-covered magnetic stirrer; final pH was approximately 5. The limit of detectability of the 1268-nm emission in the current procedure was observed for 0.06 M sodium hypochlorite (5 ml) acidified with 150 μ l of 1 M HCl.

At the higher concentrations used for the data in Figs. 1 and 2, an estimate of the quantum yield of singlet oxygen generated was made by comparing the integrated 1268-nm emission against that observed for the OCl^-/H_2O_2 chemiluminescence reaction (Eq. 1). Because of the very large difference in the rate of the two reactions, OCl^-/H^+ and OCl^-/H_2O_2 , photo yield comparisons were made for the same OCl^- concentration consumed, but for differing final volumes. In a typical experiment, 200 μ l of 0.6 M sodium hypochlorite solution was allowed to diffuse slowly out of a disposable glass micropipette (Accu-Fill 90, Micropet) into 5 ml of 20 mM H_2O_2 solution for approximately 20 sec, and the emission intensity was integrated. The integrated emission intensity at 1268 nm over 1200 sec from the stirred solution of 5 ml of 0.6 M NaOCl solution containing 200 μ l of 12.6 M HCl was adjusted for equal amounts of OCl^- . The average results indicated a value of 0.30 ± 0.05 for the ratio of singlet oxygen yield for the OCl^-/H^+ vs. OCl^-/H_2O_2 reactions.

The pH profile for Cl_2 evolution was obtained by acidifying hypochlorite solution with concentrated HCl. To 20 ml of 0.6 M sodium hypochlorite/20 ml of CCl_4 in a separatory funnel was added an aliquot of 12.6 M HCl, the funnel was quickly capped and vigorously shaken for 15 min, and the CCl_4 was then allowed to separate for 30 min. The absorption spectrum

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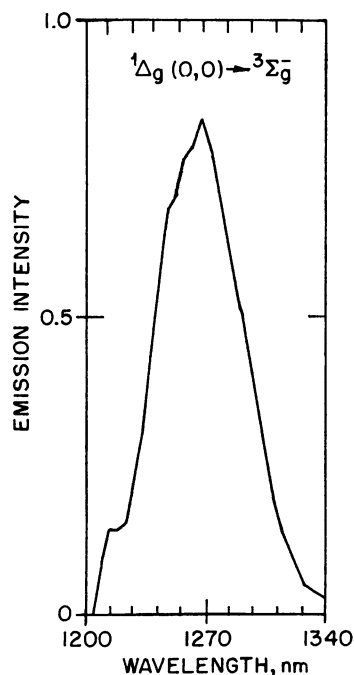


FIG. 1. Near-IR chemiluminescence spectrum of singlet dioxygen observed upon acidification of 0.6 M aqueous sodium hypochlorite solution with 12.6 M HCl at room temperature.

of the bottom CCl_4 layer was measured with a UVIKON 820 double-beam spectrophotometer. The absorption at 3300 Å was taken as a measure (13) of dissolved Cl_2 in solution. The final pH of the upper aqueous phase was measured with a pH meter. Each of the Cl_2 evolution points shown in Fig. 2 was obtained by plotting the final pH of the aqueous phase against the absorption of the CCl_4 layer at 3300 Å.

Mechanism of Singlet Oxygen Evolution

In the exhaustive study of the mechanism for reaction (1) by kinetic methods by Connick (14) and by isotopic labeling by Cahill and Taube (15), it had not been noticed that the

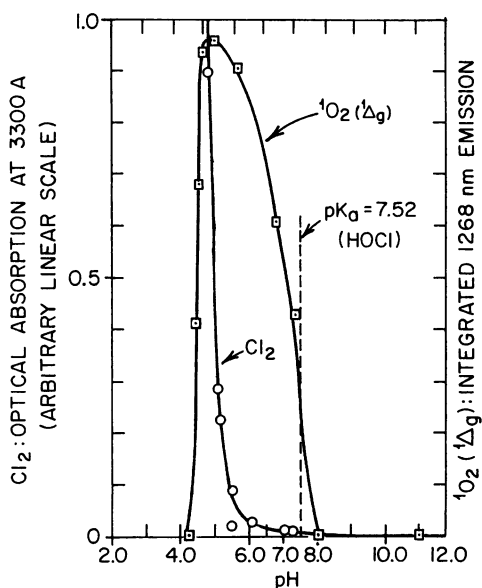
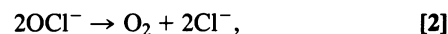
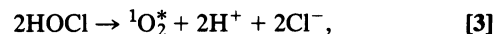


FIG. 2. pH profiles of singlet state dioxygen (□) and chlorine evolution (○) upon acidification of 0.6 M sodium hypochlorite aqueous solution with HCl at room temperature. A, Å.

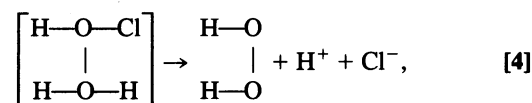
molecular oxygen was evolved in an excited singlet state (5, 6). An analogous situation exists in the exhaustive research reported on the decomposition of hypochlorite (16), in which it had not been noticed that here also a chemiluminescent reaction is involved. The initial mode of decomposition is described (17) by the following reaction



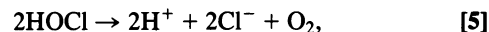
where the oxygen is presumed to be in its ground state (triplet spin state). However, hypochlorous acid is a weak acid, $\text{pK}_a = 7.52$, so that upon acidification the net reaction for the initial mode of decomposition (according to our results), as a chemiluminescent reaction, is



with the molecular oxygen in a singlet spin state. Lister (18) proposed that HOCl hydrolysis leads to an intermediate, which can then generate H_2O_2 ,



offering a path to singlet oxygen generation via reaction 1, with a pH dependence implied. Another pathway leading to oxygen evolution from hypochlorous acid has been described (19):



but the state of the liberated molecular oxygen was not identified; present knowledge would dictate that in this case the O_2 must be singlet molecular oxygen. It is, of course, not surprising that singlet oxygen was not added in the earlier research (14–17) on hypochlorite reactions and decomposition, as the observability and detection methodology were developed subsequently. The hypochlorite decomposition onset at $\text{pH} \approx 5.5$,



results in lowered pH relative to the stoichiometric value because of the escape of Cl_2 .

Biological Applications of Singlet Oxygen Research

The catalytic effects in the hypochlorite decomposition are noteworthy and suggestive of important extension. The Cl^- catalysis was noted early (16) and attributed to the kinetic salt effect, treated by the Brønsted theory. The catalysis by Cl^- was noted also by Rosen and Klebanoff (20) in their studies of evolution of singlet oxygen in the myeloperoxidase reactions. They used a chemical scavenger method for the detection of singlet oxygen (2,5-diphenylfuran conversion to *cis*-dibenzoyl ethylene) and indeed detected singlet oxygen by this method in a buffered solution of HOCl. Their onset curve for singlet oxygen evolution vs. pH parallels that shown in Fig. 2, with uncertainty of data at $\text{pH} < 6$ yielding a deviation from our results. A very strong dependence on chloride ion concentration for the efficiency of the hypochlorite decomposition was demonstrated. The direct spectroscopic detection of singlet oxygen generation in the myeloperoxidase enzyme system finally established the identity of singlet molecular oxygen in this enzyme reaction (21).

The chloride dependence of singlet oxygen production could have important extensions to a number of biological reaction mechanisms. The role of chloride in catalysis of peroxidase enzyme systems has been noted (20, 22). In the Hill reaction, the catalytic effect of Cl^- is pronounced in

various electron-transfer systems and has been discussed (23) in application to photosystem II of the chlorophyll photosynthetic apparatus. The relations of these electron transfer reactions to the present research are undefined but may lead to additional avenues of mechanistic research.

Returning to the chemical action of singlet molecular oxygen (5-7) on DNA, it has been shown that direct effects of the oxidation of guanine are mutagenesis (2, 3), loss of transforming activity, and the introduction of single-strand breaks (3, 4) in the DNA structure. Thus, the possibility of singlet molecular oxygen liberation in chlorinated water subjected to acid environment could offer a chance of generating mutagens or other reactive species, in the digestive tract, that could then diffuse further to the excretory system. This suggests that singlet molecular oxygen generated by the acidification mechanism described in this paper could be correlated with the increased risks of bladder cancer and rectal cancer deduced by the statistical epidemiological study (1).

Singlet molecular oxygen can arise from more than one source upon chlorinated-water imbibition. For example, hypochlorous acid reacts with amines, generating chloramines subject to transchlorination processes (24). Some chloramines (e.g., chloramine T) can react with hydrogen peroxide to generate singlet oxygen (25). Various other pathways to generating reactive species leading to carcinogenic oxo-halo compounds could arise from singlet oxygen mechanisms.

The present introductory research suggests that further study is needed on the chemistry of reactive oxygen species in chlorinated systems and the rapid interconversion of intermediates.

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