a distribution of relaxation processes, corresponding to the reactions represented in eq 4 and 5.

Acknowledgments. We are indebted to Professor R. Fernández-Prini for valuable discussions. The support of the Center of Materials Research of the University of Maryland and the Office of Saline Water, U. S. Department of Interior, is acknowledged. Computing time was supported by the University of Maryland Computer Science Center under National Aeronautics and Space Administration Grant NsG-398.

Direct Spectroscopic Evidence for a Deuterium Solvent Effect on the Lifetime of Singlet Oxygen in Water

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Abstract: The $^1\Delta_g$ oxygen dimol emission produced from the decomposition of hydrogen peroxide has been studied in H_2O and in D_2O. These studies provide direct spectroscopic proof that there is a solvent deuterium effect on the lifetime of singlet oxygen and thus confirm our earlier results which were based on indirect laser photolysis measurements. The pH dependence of the H_2O_2 decomposition and the chemiluminescence have also been studied to provide more information about the nature of the reaction.

In 1971 Merkel and Kearns developed a laser flash photolysis method for determining the lifetime of singlet oxygen in solution in which the decay of singlet oxygen was followed indirectly by its reaction with a colored acceptor (DBBF).[1,2] One of the interesting results of their measurements is that there is a considerable isotope effect on the lifetime of $^1\Delta_g$ (2 μsec in H_2O and 20 μsec in D_2O). The isotope effect as well as the variation of the lifetime in different solvents was well accounted for in terms of an energy transfer mechanism in which $^1\Delta_g$ transfers its electronic excitation energy to the vibrational energy levels of the solvent molecules.[3] These initial results have now been confirmed independently,[4,5] and while there is no reason to doubt they are correct, it was realized that lifetime measurements were indirect. Because of the important role which solvent isotope effects are beginning to play in elucidating the role of singlet oxygen in other photochemical and photobiological processes,[6–10] we believed it was desirable to have an alternative, and, if possible, direct, method for checking the solvent isotope effect on the lifetime of singlet oxygen. In the present study we have accomplished this by measuring the chemiluminescence arising directly from singlet oxygen generated by decomposition of hydrogen peroxide and sodium hypochlorite.

It is well established that singlet oxygen molecules are produced by mixing H_2O_2 aqueous solution and NaOCl aqueous solution, and $^1\Delta_g$ oxygen molecules show a characteristic dimol chemiluminescence at 6334 and 7032 Å and emission from $^1\Sigma$ at 7620 Å.[11–13] From an analysis of rotational structure Khan and Kasha were able to establish that $^1\Sigma$ emission only occurs from the bubbles,[11] and no emission has ever been observed from oxygen molecules dissolved in solution.[11] Since the concentration of excited oxygen in the bubbles depends strongly on the rate at which they decay in the solvent before reaching a bubble, we had hoped that it might be possible to obtain a quantitative estimate of the lifetime of singlet oxygen in H_2O and D_2O. While this turned out not to be possible, we were able to obtain direct spectroscopic evidence that the lifetime of singlet oxygen is longer in D_2O than in H_2O. We have also investigated the pH dependence of the chemiluminescence and the rate of evolution of molecular oxygen and in this way obtained additional information regarding the mechanism of the reaction.

Experimental Section

Materials and Methods. Commercial bleaching reagent "Chlorox" was used as sodium hypochlorite solution and titration with a 0.1 *N* standard As_2O_3 solution[14] showed that it contained 1.55 *N* (or 0.78 *M*) NaOCl. Hydrogen peroxide (30% Mallinckrodt Analytical Reagent) and deuterium oxide (Diaper Inc. 99.7% D_2O) were used without further purification. A D_2O solution of sodium hypochlorite was made as follows. About 30 g of calcium hypochlorite, $Ca(OCl)_2$ (B & A Technical Grade), was dissolved into 300 ml of D_2O. After about 1 hr the undissolved material was removed and the solution was combined with a D_2O solution of Na_2CO_3 (20 g in 89 ml). After precipitation of the white $CaCO_3$ more of the Na_2CO_3 solution was added until no further precipitation of $CaCO_3$ was observed. After about 1 hr the precipitated

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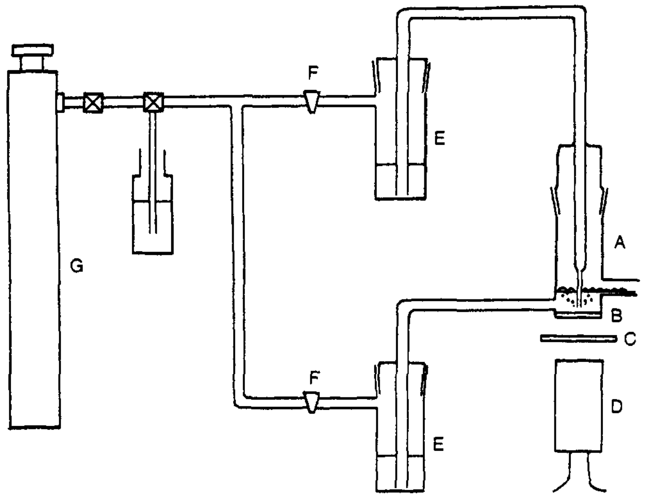

Figure 1. Flow system for chemiluminescence observation: (A) reaction cell, (B) Pyrex window, (C) interference filter, (D) photomultiplier, (E) solution container with siphon, (F) needle valve, (G) N_2 gas.

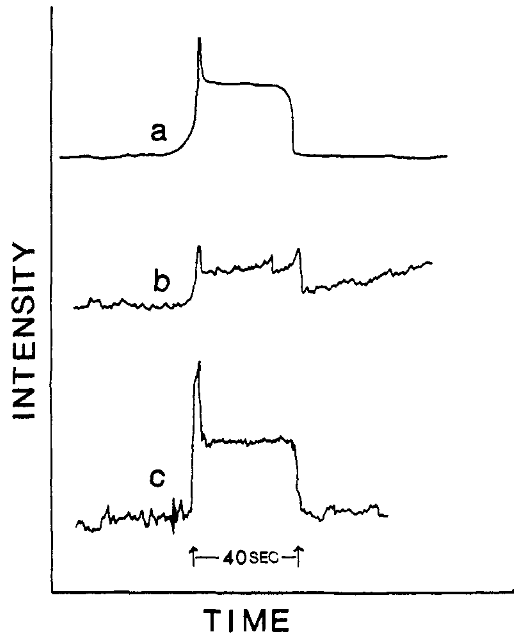

Figure 2. Some of the recorded chemiluminescence intensity: (a) H_2O_2 0.02%, NaOCl 0.38 N in D_2O; (b) H_2O_2 0.02%, NaOCl 0.38 N in H_2O; (c) H_2O_2 0.06%, NaOCl 0.38 N in H_2O. Intensity scales for three figures are different, so they cannot be directly compared. However, a and b were measured with the same conditions and the ratio of the intensity scales is 10. b is the weakest one measured in the present work. The figures also show the initial spike due to the transient initial conditions of flow rate and concentrations of reacting mixtures.

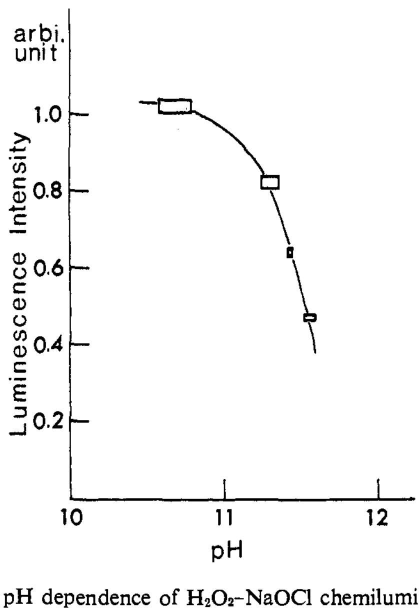

Figure 3. pH dependence of H_2O_2–NaOCl chemiluminescence intensity: H_2O_2 = 0.075%; NaOCl = 0.78 N.

$CaCO_3$ was filtered off using a medium glass filter Titration with 0.1 N standard AS_2O_3 solution showed that this solution contained 0.38 N NaOCl. Because of the $Ca(OH)_2$ and other impurities present in crude $Ca(OCl)_2$ reagent, the final D_2O solution also contained 10% protons as indicated by nmr. A deuterium oxide solution of hydrogen peroxide was prepared by dissolving 30% aqueous H_2O_2 in D_2O. Since dilute solutions (mostly less than 0.3%) were used in most experiments, the H concentration from this solution was much less than that of D_2O solutions of sodium hypochlorite. The simple flow system shown in Figure 1 was employed to measure the chemiluminescence. Typically 100 ml of hydrogen peroxide solution and 100 ml of sodium hypochlorite solution were driven by nitrogen gas from two identical vessels (E) into the reaction cell, the bottom of which was a flat Pyrex window. The flow rate was controlled by needle valves to about 100 ml/40 sec. An EMI 9558A photomultiplier was used to detect the red dimol emission of $(^1O_2{}^*)_2$, through an interference filter (No. 3139-633 of Optics Technology, Inc.) which had a transmission maximum at ~637 nm and a transmission efficiency at both 610 and 700 nm of only 2% of the maximum value at 637 nm. The photomultiplier output was amplified by a DC amplifier and recorded on a L & N Speedomax Type H recorder. No further effort was made to increase the sensitivity of the detecting system. Some of the recorded data are shown in Figure 2. Except for the initial and final part of the experiment, in which the flow of solutions is not constant, the chemiluminescence intensity remained fairly constant. Sometimes there was considerable fluctuation in the intensity due to instability of flow and rather vigorous bubbling of evolving oxygen, but those data were discarded, and the intensity values obtained from data with moderate fluctuations (less than ±15%) were used.

In the course of our experiments, we observed that the chemiluminescence intensity depends on the pH of the reaction mixture. This pH effect could be due in part to OH^- quenching of $^1O_2$ since other nucleophilic reagents such as N_3^- are known to be efficient singlet oxygen quenchers.[14] Secondly, it was possible that the pH effect on the chemiluminescence was due to a pH effect on the rate of decomposition. In order to distinguish between these and other mechanisms and also in order to determine the relative reaction rates in H_2O and D_2O, we measured the reaction rates using KI–starch titration.[15,16] The pH of the reaction mixture was adjusted by adding sodium hydroxide to sodium hypochlorite solution before mixing with the H_2O_2. Sodium hydroxide was not added to hydrogen peroxide solution because the hydrogen peroxide was unstable in highly alkaline solution. Fifty milliliters of H_2O_2 solution of about 10^{-4} M and 50 ml of NaOCl solution of the same concentration were mixed at room temperature (23–25°). Aliquots (10 ml) of this reaction mixture were taken out at four or five different times after mixing, and 1 ml of 1:17 H_2SO_4 containing 0.2% ammonium molybdate and 1 ml of 17% KI solution were immediately added to each aliquot. After the yellow color of I_2 developed in the solution 1 ml of starch solution was added and the O.D. of the resulting blue solution was measured at 570 nm by a Cary recording spectrophotometer (Model 14). Both 1 mol of H_2O_2 and 1 mol of NaOCl produce 1 mol of I_2 and no serious interference effect was observed in the case of reaction in H_2O.

Results and Discussion

The pH dependence of chemiluminescence intensity is shown in Figure 3. The pH of hypochlorite solution with added NaOH was measured before reaction and

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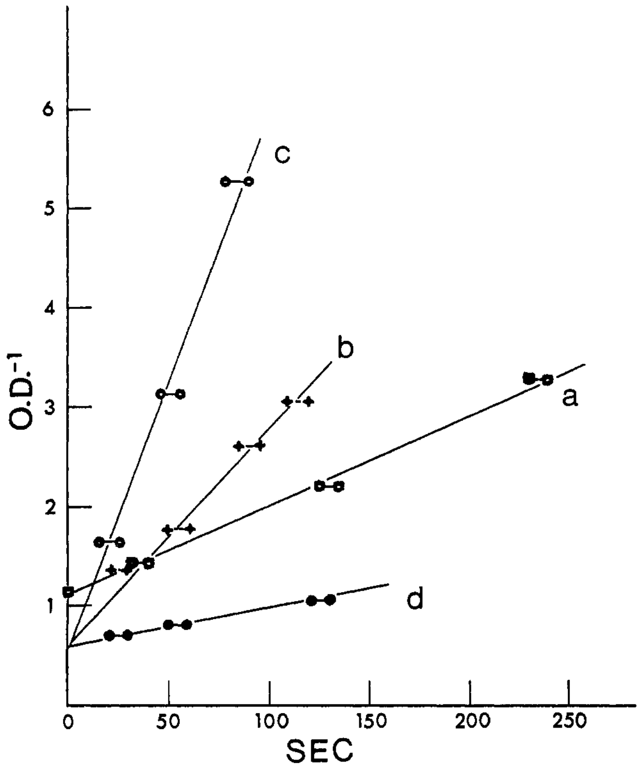

Figure 4. KI-starch analysis of H_2O_2-NaOCl reaction in H_2O: (a) pH ~6.9, (b) pH ~8.2, (c) pH ~10.1, (d) pH ~12.6.

the pH of reaction mixture was also measured after chemiluminescence observation by a Corning pH meter (Model 7). We also observed the chemiluminescence of pH values lower than 10 by adding HCl to Chlorox solution to adjust the pH. Buffers were not used because they might cause some undesirable interference.[16] Owing to a low dissociation constant of HOCl, a rather large amount of HCl was needed to lower the pH value of Chlorox solution below 10, and consequently, during the reaction (and consumption of HOCl and H_2O_2), the pH value of the reaction mixture decreased markedly. For example, in the case of reactions of 100 ml of 1% H_2O_2 in H_2O solution and 100 ml of 0.78 *N* Chlorox solution, with initial pH adjusted to 8.4 or 5.8, the pH values after the chemiluminescence measurement were 2.3 and 1.9, respectively. Because of this large pH change, the chemiluminescence intensity data in the pH region lower than 10 were not included in Figure 3. However, relative to the pH 10.8 solution the intensity was six times weaker than a solution whose initial pH was 5.8. The low intensity at pH 5.8 is not due to the decomposition of HOCl despite the fact that HOCl is somewhat unstable in acidic solution. We could regain the full chemiluminescence intensity by neutralizing the acidic Chlorox solution up to pH 10.5 by NaOH. Thus our data show that the chemiluminescence is indeed weaker in acidic solution in contradiction to an earlier report by Seliger.[17] We cannot make any definite comments on this discrepancy because Seliger gave neither basic data nor experimental conditions which led to his conclusion that the efficiency of the reaction is increased in acid solution. More recent later work by Khan and Kasha[13] seems to show that the chemiluminescence intensity is stronger in alkaline solution. Stauff and his colleague[18] also reported pH effects on oxygen chemiluminescence and pointed out that chemiluminescence is stronger in acidic solution. However, since they worked with different reactions (Ce^{4+} or Ti^{3+} with H_2O_2) their results cannot be directly compared with our present work.

Owing to incomplete ionization of NaOH (degree of ionization 0.73 at 18°) and also to low dissociation constants of H_2O_2 and HOCl (2.4×10^{-2} at 25°[19] and 3.4×10^{-8} at 25°,[16] respectively), the pH change during the reaction was not so very large. In each case, however, the uncertainty of pH is also indicated in Figure 3.

The pH dependence of reaction rate in H_2O is shown in Figure 4. The following procedure was used to obtain these reaction rates. We assumed that the reaction is of second order

$$\frac{d[H_2O_2]}{dt} = \frac{d[NaOCl]}{dt} = -k[H_2O_2][NaOCl]$$

and adjusted the initial concentration of H_2O_2 and NaOCl to a same value C_0.

In this case

$$\frac{1}{[H_2O_2]} = \frac{1}{[NaOCl]} = -kt + \frac{1}{C_0}$$

Because the measured optical density is proportional to $[H_2O_2] + [NaOCl]$

$$(\text{O.D.}) = -\frac{k}{2\alpha}t + \frac{1}{(\text{O.D.})_0} \qquad (1)$$

where $(\text{O.D.})_0$ is the optical density of the reaction mixture immediately after mixing. From this linear relation (eq 1) between $1/(\text{O.D})_0$ and t we can obtain the relative reaction rate $k/2\alpha$. 2α was determined to be about 4×10^4 O.D./(mol/l.) by measuring the optical density of sodium hypochlorite solution which was prepared by dilution of a Chlorox solution whose concentration was known by standard 0.1 *N* As_2O_3 titration. Some of the measured curves are shown in Figure 5. Because of the time necessary for sampling and some instability in intensity of the blue color of I_2–starch solution (due to some oxidation of KI by oxygen in air or by some other impurities), the observed points show some deviation from linearity in the low concentration region. However, they fit sufficiently well to eq 1 to give unambiguous reaction rates.

Returning to Figure 4, the reaction rate shows the same decreasing trend above pH 10.0 as the chemiluminescence intensity. Unfortunately, chemiluminescence intensity is determined not only by the reaction rate but also by factors such as the flow rates of the solutions, mean escaping time, and various quenching mechanisms of $^1\Delta_g$ oxygen in the bubble, and consequently we cannot prove that the decrease of chemiluminescence intensity is solely caused by decrease of reaction rate. However, the close resemblance between the two curves suggests that reaction rate is

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Table I. A Comparison of the Intensity of the Oxygen Dimol Emission in H_2O and D_2O

Peroxide concn, %	NaOCl concn, *N*	pH as measured by Corning pH meter	Chemiluminescence intensity ratio (D_2O/H_2O)
0.06	0.376	~10.2 in H_2O ~10.5 in D_2O^a	15
0.02	0.376	~9.7 in H_2O ~10.3 in D_2O^a	31

[a] Solutions contained ~5% H_2O.

the main factor responsible for the pH dependence of chemiluminescence intensity. We further conclude that the effect of OH^- on the lifetime of singlet oxygen (up to pH 11.5) is small since a much larger decrease would have been observed if OH^- quenching was becoming important. In Figure 4 we have also shown a theoretical rate curve which was calculated based on the assumption that reaction proceeds through either one of the following two rate determining steps.

$$HO_2^- + HOCl \xrightarrow{k_1} H_2O + O_2 + Cl^-$$

$$H_2O_2 + OCl^- \xrightarrow{k_2} H_2- + O_2 + Cl^-$$

The following dissociation constants were used.

$$K_1 = \frac{[H^+][HO_2^-]}{[H_2O_2]} = 2.4 \times 10^{-12} \text{ mol/l. } (25°)$$

$$K_2 = \frac{[H^+][OCl^-]}{[HOCl]} = 3.4 \times 10^{-8} \text{ mol/l. } (25°)$$

Then we obtain

$$k = k_1 \frac{K_1[H^+]}{(K_1 + [H^+])(K_2 + [H^+])} \qquad (2)$$

or

$$k = k_2 \frac{K_2[H^+]}{(K_1 + [H^+])(K_2 + [H^+])} \qquad (3)$$

where k is the apparent reaction rate determined by eq 1. Since there is no reason that k_1 or k_2 should be dependent on pH, both eq 2 and 3 give the same pH dependence for the apparent reaction rate k, and we are unable to determine which one is the true mechanism for the H_2O_2–NaOCl reaction. The calculated rate is $k_1 \approx 3 \times 10^7$ l./(mol sec) if we assume the first mechanism and $k_2 \approx 2 \times 10^3$ l./(mol sec) if we assume the second mechanism. Our results do appear to eliminate a mechanism involving a hydride ion transfer from HO_2^- to OCl^-,[20] since our results indicate that only one of the species is ionized.

$$^-O\text{-}O\text{-}H + {}^-O\text{-}Cl \longrightarrow O_2 + HO^- + Cl^-$$

The chemiluminescence in deuterium oxide was measured at two concentrations of peroxide and these results are shown in Table I. The results show first that there is a considerable enhancement of chemiluminescence in D_2O as compared with H_2O and that the enhancement is larger in the more dilute solution. If we used even more dilute solutions, we might have obtained an even greater intensity ratio. The fact that the enhancement factor is larger in the more dilute solution immediately indicates that the lifetime of $^1O_2$ is longer in D_2O than in H_2O. To say anything more quantitative it is necessary to determine the rate of decomposition of H_2O_2 in D_2O. We tried to measure the reaction rate by the same method as described before for the reaction in H_2O, except that 10 ml of peroxide and of NaOCl solution and 3 ml of reaction mixture were used for KI-starch analysis. The measured optical density was corrected to a value which would have been obtained if we used 10 ml of reaction mixture as a sample for KI-starch analysis, so that it can be directly compared with the values obtained for H_2O solution. The sodium hypochlorite solution was prepared by adding 100 μl of ~10^{-2} *M* H_2O solution of NaOCl into 10 ml of D_2O; 100 or 200 μl of 0.1–1 *N* NaOH solution was added to adjust the pH. An additional 300 or 200 μl of H_2O was added to adjust the proton concentration to 5%. The peroxide solution was prepared similarly, but sodium hydroxide was not added to peroxide solution as in the case of H_2O solution. Some of the results are shown in Figure 6. Unfortunately the experimental results do not agree with eq 1, except for the very slow reaction at pH ~7.0, where the reaction rate was about 30 times smaller than the reaction rate in H_2O solution. At other pH's there is an initial fast reaction immediately after mixing followed by a very slow reaction. We used distilled D_2O in some experiments to eliminate possible effects of impurities, but no essential change was observed in the behavior of reaction mixture. We also tried to find out whether the slow rate of reaction following the initial fast reaction was due to a large reduction in the concentration of either one of the reagents as a result of faster consumption of that reagent by some abnormal reasons.

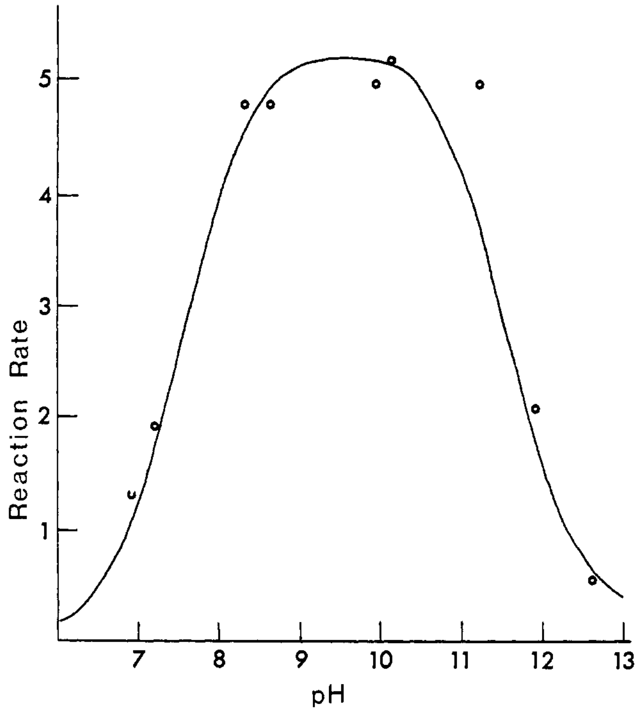

Figure 5. pH dependence of H_2O_2–NaOCl reaction rate: (○) measured values [units of (O.D. sec)$^{-1}$], (–) calculated values (see text). The unit of ordinate is for measured values. The calculated value is adjusted so that it coincides with the measured value at pH 10.

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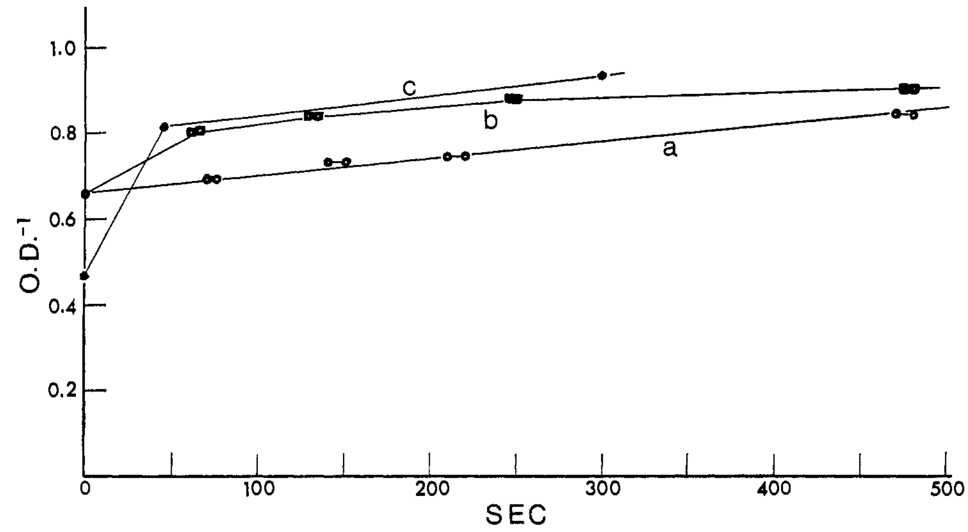

Figure 6. KI-starch analysis of D_2O_2-NaOCl reaction in D_2O: (a) pH ~7.0, (b) pH ~8.5, (c) pH ~11.6.

Titanium sulfate, which gives yellow color by reaction with hydrogen peroxide but is not colored by NaOCl,[15] was used to test this possibility, but measurements indicated that some quantity of peroxide was still left unreacted in the reaction mixture and also that peroxide concentration might be greater than sodium hypochlorite concentration after the initial fast reaction. However, the low sensitivity of this method (less than one-tenth of the KI-starch method) and some interference by NaOCl prevented us from comparing the result with that of the KI-starch method for determination of a precise concentration ratio.

Thus, while certain aspects of the reaction of deuterium peroxide and sodium hypochlorite are still a bit puzzling, the fact remains that the oxygen production rate is rather smaller in D_2O than in H_2O, because even the initial fast decay observed in D_2O is about the same as, or slower than, the decay in H_2O at corresponding measured pH. Since the oxygen chemiluminescence is *enhanced* in the D_2O solutions we may conclude that the lifetime of $^1\Delta_g$ oxygen is considerably longer in D_2O than in H_2O. Our experiment does not exclude the possibility that oxygen molecules are produced in D_2O by a totally different mechanism owing to the higher stability of the O–D bond and that two oxygen molecules are produced at the same time upon a collision of two intermediate species as suggested by Stauff.[18] This mechanism could give additional enhancement of chemiluminescence in D_2O. However, since the dimer is not strongly bound[21] the two oxygen molecules may diffuse apart immediately and the initial simultaneous production of two singlet oxygen molecules will have little effect on the total emission intensity. Furthermore, even if the two simultaneously produced oxygen molecules are bound in solution, most of them will rather quickly escape into bigger bubbles (in 10 μsec or less). These considerations and the fact that the radiative and nonradiative lifetimes of $^1\Delta_g$ oxygen in the bubble are long[22] indicate that the contribution of emission from oxygen dimers in the liquid phase must be small. From the values given Table I, t, the time that the $^1O_2$ molecule spends in solution before it escapes into a bubble, is estimated to be a few microseconds. Assuming that most of the chemiluminescence originates from bubbles, the intensity ratio may be roughly given by $(e^{-t/\tau_1}/e^{-t/\tau_2})^2$, where τ_1 is the lifetime of $^1\Delta_g$ in D_2O and τ_2 is the lifetime of $^1\Delta_g$ in H_2O. To estimate, t, τ_1 and τ_2 were set to 20 and 2 μsec, respectively.[3] The equation $(e^{-t/\tau_1}/e^{-t/\tau_2})^2 = 15$ or 31 then gives the corresponding value of t. Assuming that the diffusion constant of O_2 in water is ~1 cm^2/day, it is calculated that O_2 molecule can travel about 500 Å in 2 μsec. This seems to be reasonable considering that O_2 molecules may have to collide with each other many times before they form a bubble or escape into a bubble.

We have also observed chemiluminescence enhancement in D_2O of about the same order of magnitude as those in Table I using $Ca(OCl)_2$ solution instead of NaOCl solution, so that the results apply to other hypochlorite systems. However, this reaction produced a considerable amount of white precipitate (perhaps CaO_2) and was not suitable for chemiluminescence observation. No significant enhancement was observed when we tried Cl_2 bubbling in peroxide solution, but in this case the rate of oxygen production was too fast or oxygen molecules were produced only near the surface of a bubble and the amount of time an O_2 molecule remained in solution was too small for there to be any solvent effect on the singlet oxygen decay.

We conclude that our studies of the singlet oxygen chemiluminescence produced by decomposition of H_2O_2 provide direct spectroscopic evidence that the lifetime of singlet oxygen is longer in D_2O than in H_2O. This confirms our earlier work which was based on indirect laser photolysis experiments and further establishes the solvent deuterium effect on singlet oxygen as a powerful diagnostic test for the involvement of singlet oxygen in various photophysical and photochemical processes. We obtained some additional information about the nature of the H_2O_2 decomposition reaction, but the details of the reaction mechanism in D_2O remain to be clarified.

Acknowledgment. The support of the U. S. Public Health Service (Grant CA11459) is most gratefully acknowledged.

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