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Generation of Superoxide Radicals by Photolysis of Oxygenated Ethanol Solutions

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Abstract: Vacuum-UV photolysis of oxygenated aqueous ethanol solutions with a Xe-plasma lamp yields relatively high concentrations of superoxide radical in short time periods. The superoxide radical in this system is identified by its absorption spectrum (λ_{max} = 240 nm, ϵ = 2350 M^{-1} cm^{-1}), ESR spectrum in a glass at low temperatures with the characteristic low-field $g_{\parallel}$ = 2.072 and high-field $g_{\perp}$ = 2.006, second-order decay kinetics (k_{obsd} = 22 M^{-1} s^{-1} in 12 M ethanol containing 0.01 M KOH; k_{obsd} = 8.7 × 10^5 M^{-1} s^{-1} in 12 M ethanol containing 0.085 M H_2SO_4), and reactivity with cytochrome *c*. Two mechanisms for O_2^- formation, consistent with experimental results, are proposed for the photolysis of dilute and concentrated ethanol solutions.

The generation of superoxide radicals by vacuum-UV photolysis of oxygenated aqueous formate solutions was reported earlier.[1] In an attemtp to extend this technique to the study of compounds insoluble in water, we have applied it to solutions containing up to 95% ethanol. Ethanol is a good solvent which is miscible with water in all proportions. Its optical transparency down to 200 nm is useful for spectroscopic studies of many compounds. It is relatively inert and can be obtained commercially in pure form. Since it melts at −117 °C it can also be used for low-temperature experiments where reaction rates are greatly reduced.

The present study demonstrates that vacuum-UV photolysis with a Xe-plasma lamp of oxygenated ethanolic solutions yields relatively high concentrations of superoxide radicals in very short time periods.

The identity of the superoxide radical in this new system is characterized by its absorption spectrum, ESR spectrum in a glass at low temperatures, decay kinetics, and rate of reaction with cytochrome *c*.

Experimental Section

The solvents used in this study were water, purified in a Milli-Q ultrafiltration system (Millipore Corp.), and 95% Punctilious ethanol, USP 190 Proof (US Industrial Chemical Corp.), distilled from Mg turnings over a 50-plate column. The KOH used in preparation of alkaline solutions was low chloride Baker analyzed product (Baker Chemical Co.). The EDTA and sodium formate were purified by repeated crystallization as in previous studies.[2] The sulfuric acid used in this study was a distilled product "Aristar" from BDH Chemicals Ltd. Oxygen-saturated solutions were prepared by bubbling with UHP oxygen 99.99% (Matheson Products).

Cytochrome *c* (Sigma Type III) was analyzed at 550 nm, using $\Delta\epsilon_{\mathrm{F2}^{2+}-\mathrm{Fe}^{3+}}$ = 2.11 × 10^4 M^{-1} cm^{-1} cm^{-1}.[3] Acetaldehyde was analyzed by the method of Johnson and Scholes,[4] using a corrected molar extinction coefficient of ϵ = 1.91 × 10^4 M^{-1} cm^{-1}[5] at 430 nm for the diphenylhydrazone derivative.

The Xe-plasma attached to a modified Durrum Model D-110 stopped-flow spectrophotometer was described earlier.[1] A recent inovation provides for water cooling, so that the emerging photolyzed solution is at ambient temperature and does not form schlieren. Very slow rate measurements were taken on a Cary 210 spectrophotometer. The ESR spectrum of O_2^- in frozen glasses was recorded on a Varian E 12 spectrometer.

Results

The identity of a transient (O_2^-) formed during the photolysis of aqueous ethanol solutions was investigated by measuring its absorbance as a function of wavelength in a stopped-flow spectrophotometer. The photolyzed solution was always presaturated with oxygen (1.3 mM) and contained 0.01 M KOH, 20 μM EDTA, and varying amounts of ethanol. After photolysis in the Xe-plasma lamp the solution was thermostated for 30 s at 24 °C before mixing with an equal volume of nonphotolyzed solution and recording of its absorbance. The results for 16.2 M and 10.2 M ethanolic solutions photolyzed for 4 s at a microwave power of 70 W are shown in Figure 1. The solid line is the spectrum of O_2^- in water. Since the absorbance monitored at 270 nm was identical for a 4-s photolysis of solutions containing either 5 mM sodium formate or ethanol in the range from 5 mM to 16.2 M, which implied that the O_2^- yield is independent of ethanol concentration in this concentration range, the results shown in Figure 1 were normalized in terms of O_2^- absorbance in water reported earlier.[6] When an extinction of ϵ = 1490 M^{-1} cm^{-1}[6] for O_2^- at 270 nm was used, the average yield observed for these solutions was 211 ± 26 μM. Measurements were taken preferentially at 270 nm since acetaldehyde formed during photolysis contributes slightly (a few percent) to the absorbance below 260 nm.

The duration of photolysis affects the yield of O_2^- in alcohol solutions (Figure 2). Two concentrations of ethanol were tested, 10 mM and 16.2 M, both containing the standard amounts of KOH, EDTA, and oxygen. The major difference observed was the fall of the yield in the more concentrated alcohol with longer exposures to photolyzing light. Although it was observed that upon prolonged photolysis the solutions heat up and expand, chemical effects like depletion of oxygen in the photolyzed zone and backreactions cannot be excluded. Because of these observations all critical experiments were carried out with short photolysis times, e.g., optimum 5 s.

Figure 2 also contains results of the measurements of yields of HO_2 generated in similar experiments. Aqueous oxygenated ethanol (12 M) containing 0.01 M KOH was photolyzed and mixed rapidly with a similar alcohol solution containing 0.09 M sulfuric acid. After the solution was mixed all the O_2^- was converted to HO_2, while the alcohol concentration remained at 12 M. The amounts of HO_2 shown in Figure 2 are those observed in a 0.5-cm optical cell of the stopped-flow spectrophotometer. These are lower than the amounts produced in the mixer because of the dead time between mixer and optical port. Since the concentrations of the precursor O_2^- can be determined accurately because of its slow decay, analysis of the rates of decay of the HO_2 absorbance showed that the dead time between the mixer and optical cell was 5 ± 1.5 ms for this particular set of experiments (65 pounds of pressure per inch applied to the syringe drive). These experiments were monitored at 250 nm and the molar extinction of HO_2 was taken as 900 M^{-1} cm^{-1}.[6]

The above experiments were also used to evaluate the rates of decay of O_2^- and HO_2 in alcohol solutions. For both species the

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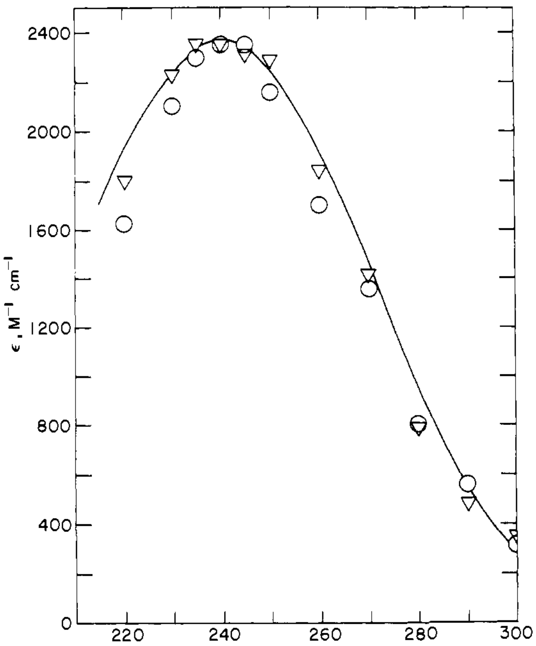

Figure 1. Absorption spectra of O_2^- in aqueous 0.01 M KOH (solid line) and alkaline (.01 M KOH) ethanol solutions (○, 95% EtOH; ▽, 60% EtOH).

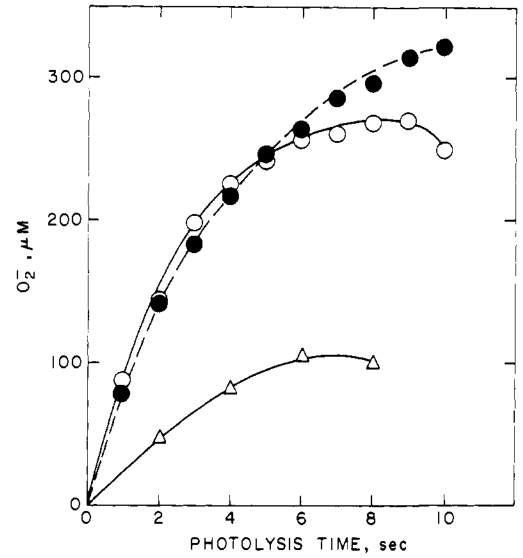

Figure 2. Yields of O_2^- as a function of photolysis time at 70 W microwave power in alkaline (0.01 M KOH) ethanol solutions (●, 10 mM EtOH; ○, 16.2 M EtOH). The yield of perhydroxyl radical (△) represents the amount of HO_2 that survived the dead space between mixer and optical port after mixing of 12 M ethanol (0.01 M KOH) containing O_2^- with a similar alcohol solution containing 0.09 M sulfuric acid.

rates were bimolecular. The O_2^- decayed slowly in 12 M ethanol which had a measured pH of 11.6. The rate of decay was monitored in a 1-cm cell at 270 nm in a Cary 210. The rate constant was calculated to be 22 $M^{-1}\ s^{-1}$, which is close to the value of 12.5 in water at the same pH.[6] The acid form of the radical decayed with a rate of $(8.7 \pm 1.4) \times 10^5\ M^{-1}\ s^{-1}$ in ethanol close to the $9 \times 10^5\ M^{-1}\ s^{-1}$ measured in acidified water.[6]

The dependence of the amount of O_2^- generated on microwave power is illustrated in Figure 3. The curve shows saturation about 50 W, but the actual yield depends strongly upon the design of the lamp as well as the tuning of the apparatus. In these experiments a 5-s photolysis period was used.

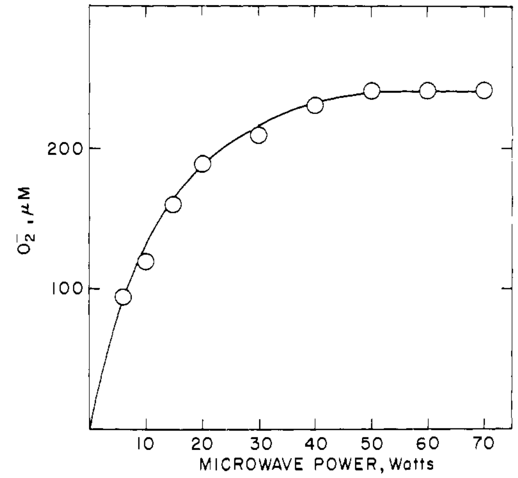

Figure 3. Yields of O_2^- as a function of microwave power in a 12 M ethanol solution (O_2 presaturated; 0.01 M KOH; photolysis time 5 s).

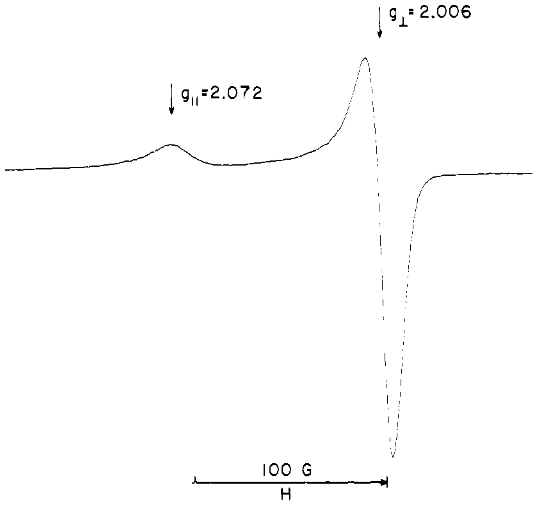

Figure 4. Electron spin resonance spectrum of O_2^- at 95 K in a 16.2 M ethanol glass.

The other main product of the photolysis is acetaldehyde, and the relative amounts of O_2^- and acetaldehyde produced were measured under different conditions. Two series of solutions were photolyzed, one 10 mM and the other 16.2 M in ethanol. A flow-through Xe-plasma lamp was used to photolyze a continuous stream of solution, which was collected and analyzed. Concentrations of O_2^- were determined by extrapolation of the slowly decaying absorbance at 270 nm to the time of photolysis. In 10 mM ethanol, the ratio of concentrations $[O_2^-]/[CH_3CHO]$ was 2.05. In 16.2 M ethanol this ratio was 0.91.

The ESR spectrum of O_2^- at liquid-nitrogen temperature in a 16.2 M ethanol glass is given in Figure 4. The signal shown was obtained when the ethanol solution was photolyzes for 3 s in the stopped-flow spectrophotometer and immediately transferred to a 3 mm (i.d.) suprasil quartz tube and frozen in liquid nitrogen. Concurrant optical measurements indicated that the frozen samples were approximately 130 μM in O_2^-. As is apparent the signal to noise ratio of the ESR spectrum is exceptionally high and the line shape is similar to that in ice,[7,8] that is the low-field parallel component is $g_\parallel = 2.072$ and the high-field absorption $g_\perp = 2.006$. ESR signals for similarly prepared samples of frozen alkaline water containing 0.01 M sodium formate or 10.2 M ethanol were identical except for slightly lower intensities in the case of water.

A further check of the identity of the transient produced in ethanol was provided by a study of its reaction with cytochrome *c*. Five milliliters of an alcoholic solution photolyzed while flowing through a Xe-plasma lamp at a rate of 1.7 mL/min were rapidly

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mixed into 15 mL of a 0.1 mM aqueous cytochrome *c* solution in a beaker. The amount of cytochrome *c* reduced at pH 9.8 by the O_2^- generated photolytically in 10.2 M ethanol was measured at 550 nm. When this yield was checked against the quantity calculated from direct measurements of the undiluted photolyzed solution at 270 nm, by extrapolating to the time of photolysis and using the extinction coefficient of O_2^- in water, the two quantities were identical. Similar results were obtained with 16.2 M ethanol solutions, showing that the molar absorbance of O_2^- remained constant up to that concentration of alcohol. The experiment with cytochrome *c* was repeated in the stopped-flow apparatus, where a 10.2 M photolyzed solution of ethanol was mixed with buffered protein solutions. The rate of reduction of cytochrome *c* by O_2^- was measured at 550 nm under pseudo-first-order conditions. The corresponding second-order rate constant for this reaction was found to be $k \simeq 6.0 \times 10^4\ \mathrm{M^{-1}\ s^{-1}}$. The agreement with the value reported by Simic et al.[9] ($k = 6 \times 10^4\ \mathrm{M^{-1}\ s^{-1}}$) is rather coincidental since the rate constant decreases rapidly with pH in water and the value of pH in 10.2 M ethanol cannot be assigned with certainty.

Discussion

It had been shown in an earlier study[1] that in aqueous solutions, vacuum-UV photolysis has an effect similar to high-energy ionizing radiation; that is, water is dissociated into H and OH radicals:

$$\mathrm{H_2O} + h\nu \rightarrow \mathrm{OH + H} \qquad (1)$$

In the presence of oxygen and formate the primary radicals are converted to superoxide radicals, the quantum yield of which is twice the quantum yield of reaction 1:

$$\mathrm{H + O_2 \rightarrow HO_2} \qquad (2)$$

$$\mathrm{HO_2 \rightleftharpoons H^+ + O_2^-} \qquad (3)$$

$$\mathrm{H + HCOO^- \rightarrow H_2 + CO_2^-} \qquad (4)$$

$$\mathrm{OH + HCOO^- \rightarrow H_2O + CO_2^-} \qquad (5)$$

$$\mathrm{O_2 + CO_2^- \rightarrow O_2^- + CO_2} \qquad (6)$$

^{60}Co γ-ray studies by Jayson et al.[10] and Hummel and Allen[11] have shown that substitution of ethanol for formate also yields superoxide radicals. The mechanism they had proposed for HO_2 formation was later confirmed by pulse radiolysis studies.[12,13] Rabani et al.,[13] who studied oxygenated aqueous methanol solutions in great detail, proposed an overall mechanism in which the primary radicals (H, OH) react with alcohol to yield the transient $\cdot CH_2OH$ radical which reacts rapidly with molecular oxygen to produce the peroxy radical $\cdot O_2CH_2OH$. The latter species undergoes an OH^- catalyzed ionization ($O_2CH_2OH \xrightarrow{OH^-} \cdot O_2CH_2O^-$) followed by a rapid decomposition to CH_2O and O_2^-.

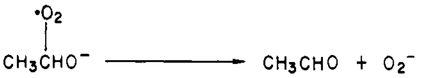
The present photolysis results show that the yield of O_2^- is independent of the ethanol concentration in the range from 5 mM to 16.2 M. If one assumes that total light absorption takes place in the medium, then the depth of penetration is unimportant for short photolysis times (before back-reactions set in) since all photons are absorbed and converted to O_2^-. To account for the similar yields of O_2^- in dilute and concentrated ethanol solutions, we find it reasonable to conclude that the photolytic decomposition of ethanol also yields two molecules of superoxide radical and that the quantum efficiencies in water and alcohol are similar.

In dilute solutions it can be shown that oxygen effectively competes for the H atom with ethanol ($k_H + k_{O_2} = 2 \times 10^{10}\ \mathrm{M^{-1}\ s^{-1}}$, $k_H + k_{EtOH} = 4 \times 10^7\ \mathrm{M^{-1}\ s^{-1}}$).[14] Hence, following the formation of a superoxide radical in reactions 1 and 2, an additional O_2^- is formed by a squence of reactions involving ethanol:

$$\mathrm{CH_3CH_2OH + OH \longrightarrow CH_3\dot{C}HOH + H_2O} \qquad (7)$$

$$\mathrm{CH_3\dot{C}HOH + O_2 \longrightarrow CH_3\overset{\overset{\cdot O_2}{|}}{C}HOH} \qquad (8)$$

$$\mathrm{CH_3\overset{\overset{\cdot O_2}{|}}{C}HOH + OH^- \longrightarrow CH_3\overset{\overset{\cdot O_2}{|}}{C}HO^- + H_2O} \qquad (9)$$



$$\mathrm{CH_3\overset{\overset{\cdot O_2}{|}}{C}HO^- \longrightarrow CH_3CHO + O_2^-} \qquad (10)$$

Although we were unable to check for short-lived transients ($CH_3CH_2O\cdot$, $CH_3\dot{C}HOH$, etc.) with our present experimental setup, numerous studies support $CH_3\dot{C}HOH$ radical formation in reaction 7.[10-18] The overall mechanism (reactions 1, 2, and 7–10) is supported by the observed ratio of $[O_2^-]/[CH_3CHO] = 2.0$.

Direct photolysis of ethanol in concentrated solutions containing oxygen can be described by eq 11 and 12 followed by eq 8–10:

$$\mathrm{CH_3CH_2OH} + h\nu \rightarrow \mathrm{CH_3\dot{C}HOH + H} \qquad (11)$$

$$\mathrm{CH_3CH_2OH + H \rightarrow CH_3\dot{C}HOH + H_2} \qquad (12)$$

Since in the presence of high alcohol concentrations the H atom reacts preferentially with an α hydrogen of ethanol,[15] this leads to the formation of one additional molecule of acetaldehyde and the same amount of O_2^- as in dilute solutions. Such a mechanism is consistent with an observed ratio for $[O_2^-]/[CH_3CHO]$ of close to unity.

If photolysis of ethanol by vacuum-UV (160–195 nm used in this study) should by any chance also yield ethoxy radicals ($CH_3CH_2O\cdot$), they would be rapidly converted to α-ethanol radicals by the excess of ethanol in the medium:

$$\mathrm{CH_3CH_2O\cdot + CH_3CH_2OH \rightarrow CH_3CH_2OH + CH_3\dot{C}HOH} \qquad (13)$$

The occurrence of reaction 13 has been known for some time.[10-21] Its effect upon product distribution in the photoxidation of alcohols by ferric iron was discussed recently by Walling and Humphreys.[22]

This study shows that the photolysis of oxygenated ethanol is a fast and efficient method for the production of relatively concentrated superoxide solutions (25–30% of all available oxygen in the system is converted into O_2^-) and also an efficient way of converting ethanol into acetaldehyde.

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Registry No. O_2^-, 11062-77-4; ethanol, 64-17-5.

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