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Reactions of Superoxide with Water and with Hydrogen Peroxide

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The stability of the superoxide ion, O_2^-, prepared by the electric reduction of O_2 in acetonitrile, was examined. The superoxide is decomposed, dismutated, rapidly in aqueous solutions to produce O_2 and H_2O_2, and the rate depends on the water content and/or the acidity of the medium. The addition of HCl over 10^{-3} M significantly accelerates the dismutation, while O_2^- is stabilized relatively in alkaline solutions. The addition of H_2O_2 markedly stimulated the decomposition of O_2^- to form a hydroxyl radical, ·OH, which was identified by ESR spectrometry.

Keywords——superoxide ion; hydrogen peroxide, hydroxyl radical; dismutation; Haber-Weiss reaction; spectrophotometry

Since the superoxide ion, O_2^-, is an important agent of oxygen toxicity, the implication of O_2^- in many biological processes has been stimulated much interest in its physical and chemical properties.[1,2] Generally, O_2^- is unstable in aqueous solutions. It functions as an oxidant or a reducing agent, and sometimes as a nucleophile.[3] The well-known reaction of O_2^- is self-destruction, *i.e.*, dismutation, which has been thoroughly investigated by Bielski and Allen.[4]

$$O_2^- + O_2^- \rightleftarrows O_2^{2-} + O_2 \tag{1A}$$

$$O_2^- + HO_2 \rightleftarrows HO_2^- + O_2 \tag{1B}$$

Another thermodynamically possible reaction, which is biologically relevant, is the Haber–Weiss reaction.[5] This reaction, which proceeds slowly,[6] has been proposed as a source of hydroxyl radical, ·OH, in systems producing O_2^-.[1,7,8] However, some workers

$$O_2^- + H_2O_2 \rightleftarrows O_2 + \cdot OH + OH^- \tag{2}$$

demonstrated that H_2O_2 has little effect on the rate of loss of O_2^- from aqueous solutions[9-11] and that the Haber–Weiss reaction will only proceed in the presence of traces of metal ions.[12,13] Because of the importance of ·OH generation as a mechanism for the toxicity of O_2^-, the reaction of O_2^- and H_2O_2 is of interest. In the present paper, we show that the stability of O_2^- depends on the acidity of the medium and that ·OH is undoubtedly generated by the reaction of O_2^- and H_2O_2.

Experimental

A solution of O_2^- was obtained by the electric reduction of O_2 as described previously.[14] All the chemicals used were of analytical grade, and were used without further purification. Water was once deionized and twice distilled with an all-glass apparatus.

The decay of O_2^- was followed by monitoring the optical absorption at 255 nm ($\varepsilon = 1.5 \times 10^3\ \text{M}^{-1}\ \text{cm}^{-1}$). The concentration of O_2^- was also estimated from the signal amplitude (peak height) in the $g_{//}$ region of the ESR spectrum at 77 K. H_2O_2 in the reaction mixture was determined by titration with standardized permanganate solution.

The optical and ESR spectral measurements were done on a Union SM-401 spectrophotometer and a JEOL-PE-1X spectrometer (X-band) with 100 kHz field modulation, respectively.

Results and Discussion

In order to check the stability of O_2^-, the effect of water content in the medium was examined. O_2^- was rather stable in absolute acetonitrile and maintained a constant level during the time of measurement, 30 min. When a small amount of water was added to the solution, O_2^- was lost rapidly to yield H_2O_2. The relation of the decomposition rate and the water content, which was obtained by spectrophotometry, is shown in Fig. 1. When the water content was 4% in acetonitrile, O_2^- was lost rapidly; the content of O_2^- dropped to approximately 1/4 within 1 min. These results indicate that the stability of O_2^- depends on the water content in the medium.

The acidity of the medium also affects the decomposition of O_2^-. The time courses of the decay of O_2^- in acidic (1.25×10^{-3} M HCl), neutral and alkaline (1.25×10^{-2} M KOH) media are shown in Fig. 2. In these experiments, the content of water was held constant at 1.25% (v/v) in acetonitrile. When the concentration of acid increased over 10^{-3} M, the decomposition of O_2^- was promoted significantly. The peak height of the ESR spectrum of O_2^- reduced to less than 1/3 of the initial value within 1 min. In contrast, O_2^- was stabilized in alkaline solutions. As the concentration of alkali increased over 10^{-3} M, the life time of O_2^- increased depending on the concentration of OH^-. At 1.25×10^{-2} M KOH, the ESR spectrum of O_2^- was observed explicitly for 30 hrs, while the spectrum disappeared within 15 hrs without the alkali.

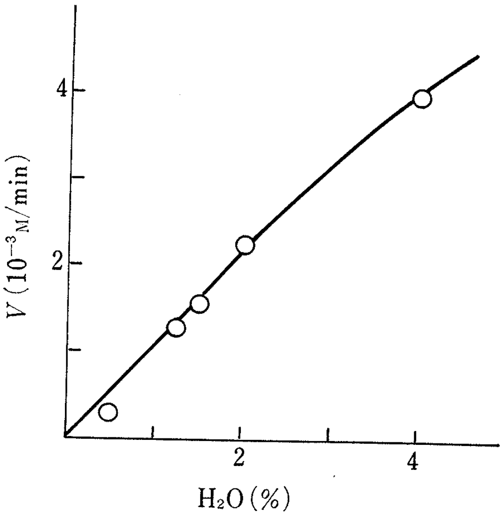

Fig. 1. Relation between the Water Content and the Rate of Decomposition of O_2^-

$[O_2^-]=6.5\times10^{-3}$ M, $T=25°$.

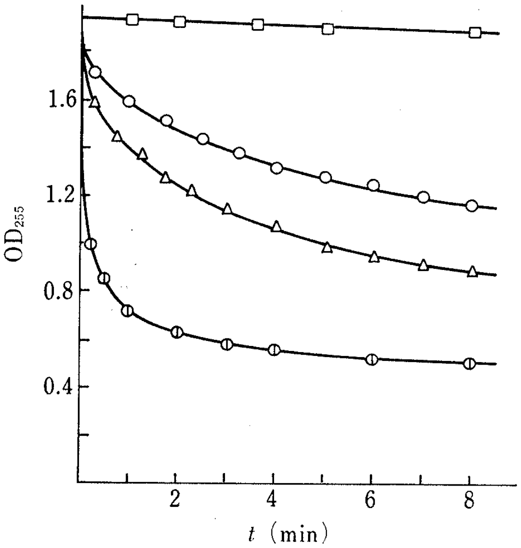

Fig. 2. Time Courses of the Decomposition of O_2^- under Various Conditions

□; absolute acetonitrile,
△; 1.25% aqueous acetonitrile,
⦶; 1.25×10^{-3} M HCl in 1.25% aqueous acetonitrile,
○; 1.25×10^{-2} M KOH in 1.25% aqueous acetonitrile.

The acid-dependent stability of O_2^- is explained by the acid-base equilibrium. O_2^-, which is a Brønsted base, is in equilibrium with HO_2 ($pK_a=4.88$),[15] and the rate of dismutation is second-order in the superoxide. The overall second-order rate constant is relatively small in the reaction between O_2^- and O_2^- (1A: $k<0.3$ M^{-1} s^{-1}). It becomes greater at the pH corresponding to the pK_a of HO_2 (1B: $k=8.9\times10^7$ M^{-1} s^{-1}).[4] The addition of acid, which increases the concentration of HO_2, accelerates the dismutation of the superoxide.

Hydrogen peroxide drastically stimulates the decomposition of O_2^-. The relation between the initial rate of decomposition and the concentration of H_2O_2 is shown in Fig. 3, where the water content was kept constant at 1.25% (v/v) in acetonitrile. The rate increased with increase of H_2O_2 added. The ESR peak height of O_2^- reduced in proportion to the concentration of H_2O_2. It is interesting that, when the peroxide was added at 2×10^{-3} M, the reaction mixture did not consume permanganate after standing for 20 min. H_2O_2, as well as O_2^-, completely disappeared within 20 min. Under the same experimental conditions, the radical adduct of ·OH with a spin trap, 5,5-dimethyl-1-pyrrolin-1-oxide, was identified by ESR spectroscopy.[16] These findings indicate that ·OH is undoubtedly generated from the superoxide.

These results appear to conflict with the observations of previous workers.[9–11] The contradiction may have resulted mainly from the difference in the water content in the reaction mixture. Certainly, we observed that H_2O_2 did not accelerate the decomposition of O_2^- in 5% (v/v) aqueous acetonitrile. In aqueous solutions, the dismutation is more rapid. Whether or not ·OH radical is formed from O_2^- depends on the water content in the reaction medium.

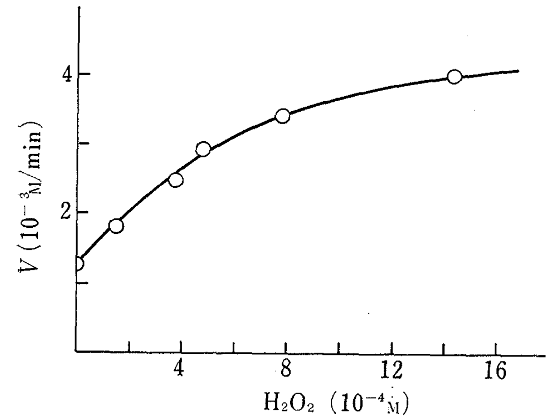

Fig. 3. Effect of H_2O_2 Concentration on the Decomposition of O_2^-

$[O_2^-]=6.5\times10^{-3}$ M, $T=25°$.

References and Notes

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