ON A SPECTRALLY WELL-DEFINED AND STABLE SOURCE OF SUPEROXIDE ION, O_2^-

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1. Introduction

It has been shown that superoxide ion, O_2^-, is an endogenic species produced in oxygen-metabolizing organisms and may be related to the toxicity of oxygen and to the bactericidal action in leukocytes [1–10]. This free radical is also produced enzymatically [11], photochemically [12] and chemically [13], but the preparation involves reactive intermediates which would interfere with the chemical and biological reactions of superoxide ion. In order to investigate the chemical and biochemical reactivities of O_2^-, it is necessary to have reliable and simple methods for the preparation of well-defined radical species. The ideal source of O_2^- would have no associated reactive substances and allow spectrophotometric observation of O_2^-. Ionizing radiation of oxygenated water, in particular by the technique of pulse radiolysis, provides high concentrations of O_2^- [14]. However, since O_2^-, being a short-lived species in an aqueous solution, is decomposed immediately after pulse radiolysis, this method could not be used for the study of reactivities of O_2^-. Electrochemical method also provides a well-defined source of O_2^-. Maricle and Hodgson [15] conducted the electrolytic reduction of oxygen in aprotic solvents and established the method of the generation of O_2^- which was identified with ESR spectroscopy. The solution containing O_2^- prepared by electrolysis is stable for several hours at room temperature [11]. Recently, Fee and Hildenbrand [16] prepared O_2^- by electrolysis in some water-miscible solvents and recorded the electronic spectrum, as well as ESR spectrum, of O_2^-. They described that O_2^- displays the electronic spectrum with λ_{max} = 250 nm (ϵ = 2600). They conducted the electrolysis using tetrabutylammonium bromide which shows strong optical absorption in the ultraviolet region, less than 250 nm, as a supporting electrolyte. Then, the absorption maximum observed might result from stray light due to the strong absorption of the electrolyte. In order to define the purity of O_2^-, precise estimation of the spectrophotometric properties is required. Then, we intended to re-examine the electrolytic reduction of oxygen.

The present communication describes the spectral properties of O_2^- prepared by electrolysis.

2. Materials and methods

2.1. *Reagents*

Acetonitrile was purified according to the modification of the method reported by O'Donnel, Ayres and Mann [17]. Tetra-*n*-propylammonium bromide (TPAB) or tetra-*n*-propylammonium perchlorate (TPAP) was used as a supporting electrolyte. TPAB was obtained commercially and was used after recrystallization from ethanolethyl acetate. TPAP was prepared from TPAB and perchloric acid, and was recrystallized from ethanol.

2.2. *Electrolytic procedure*

The electrolysis of oxygen was accomplished according

to the modification of the procedure used by Maricle and Hodgson [15]. For standard two compartment electrochemical cells, a H-type cell was used in which cathodic and anodic chambers were separated from each other by a sintered glass disk. Each electrode was a pool of mercury and has an area of about 7 cm^2. Potential was referred to an aqueous saturated calomel electrode (SCE) which was connected to the cathodic chamber with a double salt-bridge. The electrolytic solution containing 0.1 M TPAB or TPAP was put in each chamber which held 30–40 ml. Oxygen was continuously bubbled through the cathodic chamber. The current was checked by a Hokuto Denko potentiostat–galvanostat HA-111.

The electrolytic reduction of oxygen was made at lower potentials (–90 ~ –0.95 V versus SCE) than –0.98 V versus SCE which was obtained by the cyclic voltammogram of the first reduction process in aereated 0.1 M TPAB or TPAP-acetonitrile solutions on a mercury electrode.

2.3. *Spectroscopic measurements*

The ESR spectrum was measured at room temperature or 77°K on a JEOL-PE-1X spectrometer (X-band) with 100 kHz field modulation. The g-values were obtained by comparison with a Mn^{2+} marker.

The electronic spectrum was recorded at room temperature on a Union Giken SM-401 spectrophotometer.

3. Results and discussion

The cathodic solution after electrolysis using either TPAB or TPAP as a supporting electrolyte gave no ESR signal at room temperature, but the characteristic ESR spectrum of O_2^- ($g_\perp = 2008, g_\parallel = 2083$) which had been reported by several workers [18–20] was observed at 77°K (fig.1). On the other hand, the cathodic solution gave different electronic spectra depending on the supporting electrolyte used. When TPAB was used as a supporting electrolyte, the spectrum did not show a maximum and could not be measured at the wavelength less than 250 nm. An apparent absorption band may be resulting from rapidly increasing amounts of stray light near the wavelength limits of the spectral measurements. When electrolysis was conducted in TPAP which is optically transmittable

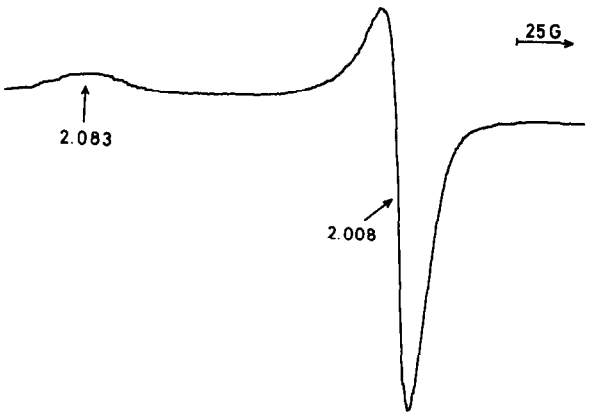

Fig.1. ESR spectrum observed during the electrolytic reduction of oxygen in 0.1 M TPAP-acetonitrile solution. Spectrum was recorded at 77°K. The sample was a cathodic solution after a current of ~ 11 mA was maintained for 9 min.

in the ultraviolet region, the cathodic solution gave an electronic spectrum with λ_{max} = 255 nm as was shown in fig.2.

The signal intensity of ESR spectrum and A_{255} of electronic spectrum increased correspondingly with

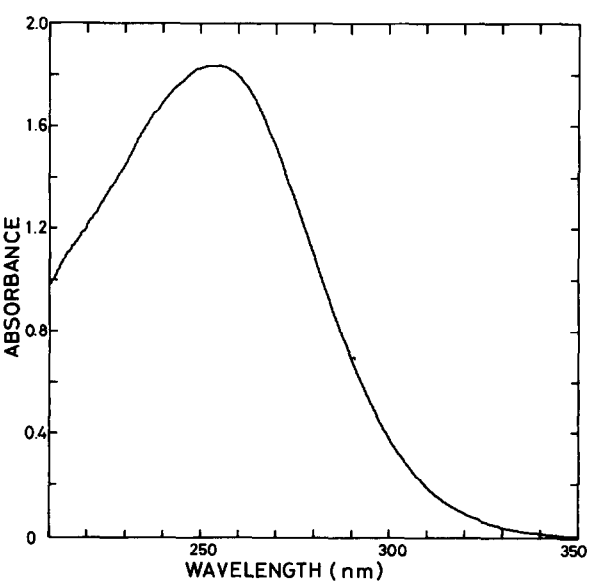

Fig.2. Absorption spectrum observed during the electrolytic reduction of oxygen in 0.1 M TPAP-acetonitrile solution. Spectrum was recorded at room temperature. The sample was a cathodic solution after a current of ~ 11 mA was maintained for 16.5 min. Path length was 5 mm.

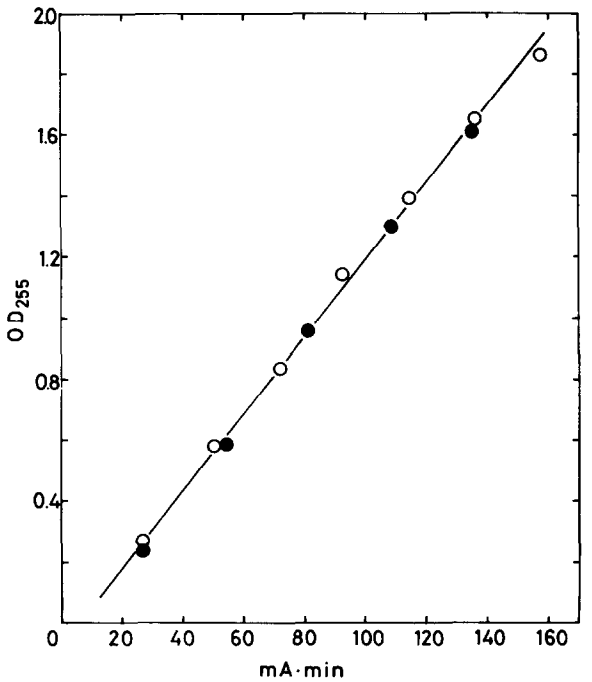

Fig.3. Plot of optical density of superoxide ion at 255 nm versus the amount of electricity. The electrolytic reductions of oxygen were carried out at −0.90 V (○) and −0.95 V (●) versus SCE, respectively. Path length was 5 mm.

the time of electrolysis. When a small amount of water (5% v/v) was added to the cathodic solution, both ESR and electronic spectra decreased and disappeared within 10 min. Those findings indicate that the spectrum in fig.2 is assignable to O_2^-. This is a first demonstration of the electronic spectrum of O_2^- in organic solvents.

The value of A_{255} is related to the amounts of electricity but not to the time of electrolysis, which indicates that O_2^- is not decomposed during electrolysis. The plot of A_{255} against the amounts of electricity gave a straight line irrespective of the applied potentials under the conditions examined (fig.3). The concentration of O_2 reduced electrochemically, C, is expressed by equation (1).

$$C = n \cdot E/F \quad (1)$$

where n is the electrochemical equivalent of O_2 under the experimental conditions, E the total amounts of electricity and F the Faraday constant (96 500 C/equiv.). Since electrolysis was conducted at −0.90 ~ −0.95 V versus SCE, which is lower than the peak potential of reduction of oxygen to O_2^- (−0.98 V versus SCE) and the second-step reduction of oxygen is performed electrochemically at a more negative potential (less than −1.3 V versus SCE) which was observed by cyclic voltammetry of oxygen, it is thought that O_2^- can not be reduced further electrolytically (n = 1). In addition, as stated above O_2^- little undergoes decomposition during electrolysis. Provided that O_2^- is neither reduced nor dismuted under conditions examined, the value of C may be equal to the concentration of O_2^-. When E was 100 A·min, C was calculated as about 2×10^{-3} M from fig.3. An extinction coefficient of O_2^- at 255 nm was calculated to be 1460 $M^{-1} \cdot cm^{-1}$. This value is smaller than those reported by other workers [16,21] as was shown in table 1.

The value reported by Fee and Hildenbrand [16] is unreliable as mentioned above. Rabani and Nielson [21] prepared by pulse radiolysis of an oxygenated water and estimated the concentration of O_2^- from g-values of all primary radical species (e_{aq}^-, H and OH). The difference of ϵ between their and our experiments may be resulted from the different procedures for estimating the concentration of O_2^- and/or from the difference of the solvents employed.

Recently, potassium superoxide, KO_2, which is dis-

Table 1
A comparison of the extinction coefficient of O_2^- generated by several methods

ϵ ($\times 10^3$ $M^{-1} \cdot cm^{-1}$)	λ_{max} (nm)	Method	Ref.
1.97	240–245	Pulse radiolysis[a)]	[21]
2.6 ± 0.3	~250	Electrolysis[b)]	[16]
1.46 ± 0.08	255	Electrolysis[b)]	This work

[a] Measured in H_2O
[b] Measured in acetonitrile

solved in organic solvents with the aid of some crown ethers is used as a source of O_2^- [22–25]. However, the concentration of O_2^- in this preparation is not determined, because crown ether itself shows the strong absorption in the ultraviolet region. The electrolytic reduction of oxygen in 0.1 M TPAP-acetonitrile solution provides a spectrally well-defined preparation which contains considerably high concentration of O_2^- up to about 7×10^{-3} M. Thus, it is suggested that the acetonitrile solution of O_2^- prepared by electrolysis is a good material for studing the biochemical and chemical reactivities.

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