Singlet Molecular Oxygen from Superoxide Anion and Sensitized Fluorescence of Organic Molecules

Abstract. *The superoxide anion, O_2^-, evolves singlet molecular oxygen in dimethylsulfoxide solution. Pronounced water quenching of superoxide-sensitized luminescence is indicative of the preferential generation of the $^1\Sigma_g{}^+$ state. Recent identification of O_2^- in the xanthine oxidase system suggests that the generation of singlet oxygen may also occur in enzymatic systems.*

Within the few years since its first identification in a chemical system (*1*, *2*), singlet molecular oxygen has been implicated either by direct or by circumstantial evidence as the active agent in a large number of physical, chemical, and biological systems. I report here the generation of singlet molecular oxygen from potassium superoxide in dimethylsulfoxide (DMSO) solution. The presence of singlet oxygen was detected by the chemical scavenger (*3*) and by the fluorescence sensitization (*2*) techniques. In the luminescence sensitization experiment a pronounced quenching effect of water was observed, as would be expected were $^1\Sigma_g{}^+$ molecular oxygen to be involved. The work reported here indicates that the superoxide ion may be a direct source for $^1\Sigma_g{}^+$ oxygen; peroxides, in contrast, yield $^1\Delta_g$ oxygen.

For the fluorescence sensitization experiment a saturated solution of potassium superoxide (Alfa Inorganics, Inc.) in DMSO (Baker Chemical Company, reagent grade) and a solution of an appropriate fluorescing molecule in DMSO were mixed in a flow apparatus in the dark. The characteristic fluorescence of the dye was then observed. In this manner fluorescence from anthracene, esculin, eosine, and violanthrone was detected.

For the detection of singlet oxygen by chemical means, 2,5-dimethylfuran (City Chemical Corporation, purified by gas chromatography) was used as a scavenger. Five milliliters of 2,5-dimethylfuran were added to 100 ml of DMSO saturated with potassium superoxide. By following the procedure outlined by Foote *et al.* (*4*), I obtained a residue that gave the characteristic peroxide test with potassium iodide.

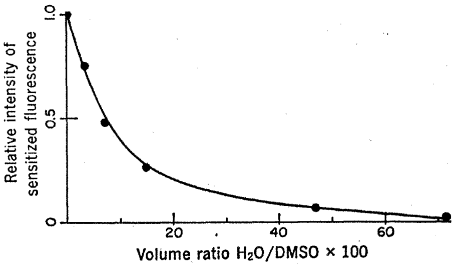

Fig. 1. Water quenching of potassium superoxide–sensitized fluorescence of anthracene in DMSO.

In order to study the effect of water on the sensitized fluorescence intensity, the total light output was recorded with a 1P 28 photomultiplier recorder setup as a function of the percentage of water in the system. A set of solutions was prepared, each containing 10 ml of DMSO saturated with potassium superoxide. Another set of solutions was prepared, each containing 50 ml of $10^{-4}M$ anthracene dissolved in a mixture of DMSO and water; the relative proportions of the components of the solvent mixture were allowed to vary in these solutions. The potassium superoxide solution was added to the anthracene solution, and Fig. 1 shows the result of a study of the quenching of sensitized anthracene fluorescence as a function of the volume of water added.

Potassium superoxide crystal is an ionic lattice of K^+ and O_2^- ions, and from these experiments it is concluded that in the one-electron transfer reaction

$$O_2^- \rightarrow O_2^* + e^-$$

singlet molecular oxygen is generated; however, the detailed nature of this chemical reaction is not yet clearly understood. The energetic requirements of anthracene fluorescence (see Fig. 2) necessitate the generation of the $^1\Sigma_g{}^+$ state. Bader and Ogryzlo (*5*) have shown that this species is sensitive to water quenching, a result which is consistent with the findings reported here. The detailed mechanism of the quenching of the $^1\Sigma_g{}^+$ state by water has not yet been elucidated. A kinetic study of the quenching of sensitized violanthrone fluorescence by water was undertaken to resolve this question but, although violanthrone fluorescence shows a quenching effect similar to that of anthracene fluorescence, the solvent sensitivity of the electronic spectra has complicated the results until now.

Complementary fluorescence sensitization results were found by Legg and Hercules (*6*) when they electrochemically generated O_2^- in DMSO solution, and the fluorescing compound *N*-methylacridone, derived from lucigenin, was luminescent in DMSO but failed to show any luminescence in aqueous solution. McCapra and Hann (*7*) suggested the necessity for *N*-methylacridone chemiluminescence of an "oxetane" intermediate derived from 10,10′-dimethyl-9,9′-biacridylidene in a hydrogen peroxide and sodium hypochlorite reaction mixture. The "oxetane" intermediate was postulated because this system did not exhibit water quenching, although the energetic requirements suggested by our chemiluminescence mechanism (*2*) would require the participation of the water-sensitive $^1\Sigma_g{}^+$ state. In the study reported here the 10,10′-dimethyl-9,9′-biacridylidene system has not been investigated. However, in view of the results of Legg and Hercules, McCapra and Hann's use of pyridine as a solvent may explain their failure to observe a water quenching effect; their result can be attributed to the strong pyridine-water association.

The potassium superoxide–DMSO system provides a direct and controlled source of singlet oxygen which is expected to be very useful experimentally. This system, in contrast to previously explored systems that were found to generate mostly $^1\Delta_g$ oxygen, also provides a possible source for the $^1\Sigma_g{}^+$ state of molecular oxygen. Fridovich and Handler (*8*) suggested that the xanthine oxidase–catalyzed oxidation of xanthine by molecular oxygen involves the superoxide anion, O_2^-, and recent

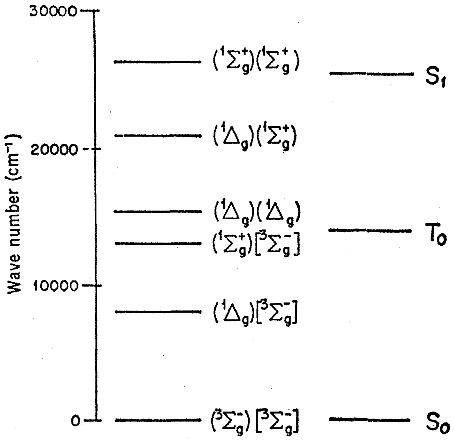

Fig. 2. Energetic disposition of the simultaneous transitions in double molecule states of metastable molecular oxygen and the electronically excited lowest singlet and the triplet states of the anthracene molecule.

experimental evidence, including a direct demonstration with electron paramagnetic resonance spectroscopy (*9*), has shown the presence of a substantial quantity of the O_2^- ion in this system. By analogy, it is expected that singlet molecular oxygen would be generated in this enzymatic system, and this expectation is strongly supported by the observed chemiluminescence of a number of organic molecules present in the xanthine oxidase system (*10*). Since a large number of biological oxidative processes are thought to proceed by way of an "electron transfer" step, the possibility of the involvement of singlet oxygen in such systems needs careful evaluation.

The reaction of potassium superoxide with water is the single most important *chemical* source of oxygen for breathing purposes in hospitals (*11*), mines, submarines (*12*), and space capsules (*13*). Even if only a small fraction of singlet oxygen survives quenching, it could prove to be a serious health hazard.

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References and Notes

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Lunar Gravity over Large Craters from Apollo 12 Tracking Data

Abstract. *The Doppler residuals from the Apollo 12 lunar module radio tracking data indicate large negative accelerations over the craters Ptolemaeus and Albategnius. The mass deficiencies required to produce these accelerations are approximately equivalent to the removal of the surface material to a depth of 1 kilometer over the entire area of these craters. Several other features of the gravity fine structure can also be correlated with topography.*

The ballistic portion of the descent trajectory of the Apollo 12 lunar module presented a unique opportunity to observe strong accelerations due to lunar surface gravity fine structure (less than 5° in diameter). Previous Doppler radio tracking S-band data from the five Lunar Orbiters have permitted estimation of accelerations caused by surface features greater than 10° (300 km) in diameter (*1–3*). Since most of the Lunar Orbiter data were obtained above an altitude of 100 km, the very fine structural features, such as craters, were unresolved. The ballistic portion of the lunar module descent trajectory approached to within 12 km of the lunar surface, and this close approach therefore makes possible the resolution of accelerations caused by features 50 km in diameter.

Unfortunately, the Apollo 10 and 11

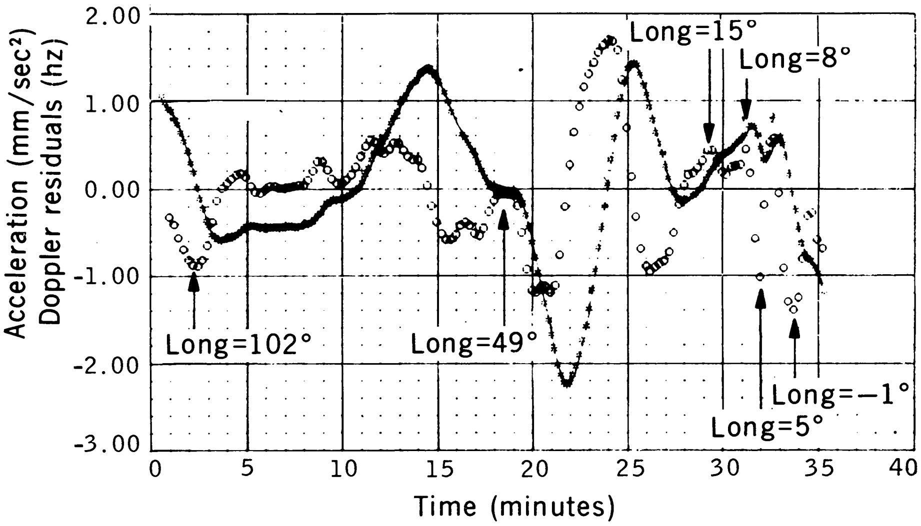

Fig. 1. Earthward velocity residuals (asterisks), and resulting accelerations (circles), after a triaxial model orbit determination.

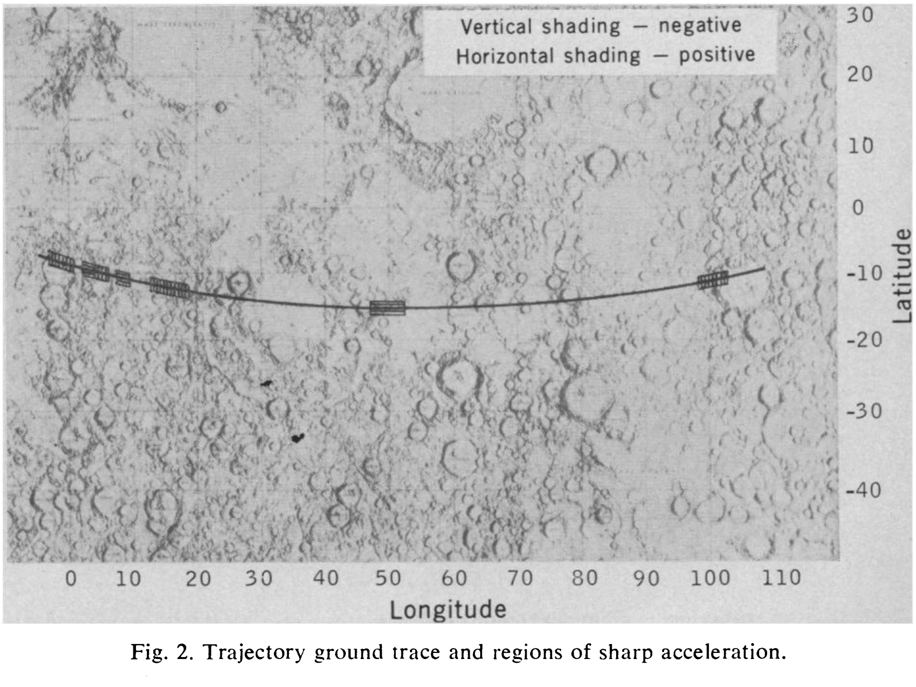

Fig. 2. Trajectory ground trace and regions of sharp acceleration.