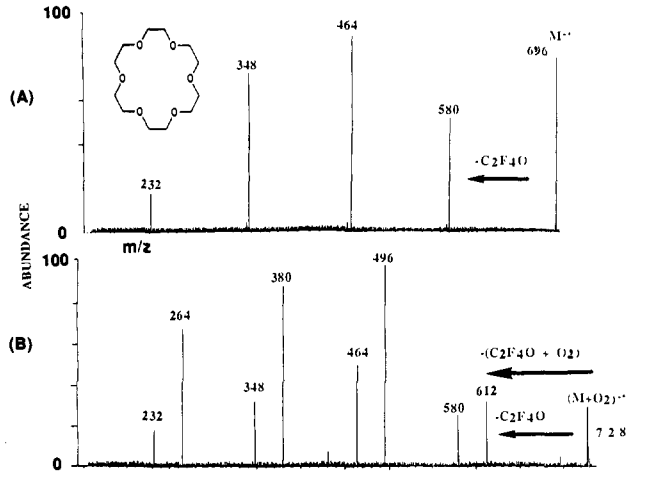

Figure 2. Collisionally activated dissociation mass spectrum at 30-eV kinetic energy of (A) perfluoro-18-crown-6 (m/z 696) and (B) (perfluoro-18-crown-6 + O_2)$^{\bullet-}$ adduct ion (m/z 728).

crown-6 (m/z 696) and its (M + O_2)$^{\bullet-}$ adduct (m/z 728).

The molecular ion, M$^{\bullet-}$, dissociates primarily via a series of losses of $(C_2F_4O)_n$ units (116 amu). The CAD spectrum of the (perfluoro-18-crown-6 + O_2)$^{\bullet-}$ adduct ion shows two series of fragment ions. One is a series of losses of $(C_2F_4O)_n$, analogous to the series of losses observed from the M$^-$ ion, resulting in fragment ions at m/z 264, 380, 446, and 612. This trend indicates that the O_2 is bound to the perfluoro crown ether strongly enough to be retained after the adduct ion is activated, and that this binding interaction presumably must be at least as strong as the C–C and C–O bonds that are cleaved during the dissociative process in which $(C_2F_4O)_n$ units are expelled. The C–C and C–O bond energies for these perfluoro crown ethers have been estimated as 84 and 98 kcal/mol, respectively. Additionally, a series of fragment ions corresponding to loss of $[(C_2F_4O)_n + O_2]$ units is seen at m/z 232, 348, 464, and 580, the same fragment ions produced from CAD of the noncomplexed molecular ion shown in Figure 2A. The nondissociated adduct ion abundances are not the same for the M$^{\bullet-}$ and (M + O_2)$^{\bullet-}$ CAD spectra. This is attributed to variations in experimental conditions and is not necessarily related to differences in adduct ion stabilities.

Simple O_2 loss is not a significant dissociative channel under any collisional activation conditions (for 10–120 eV kinetic energy collisions, the percentage of the total fragment ion abundance due to O_2 loss is 0–5%). This suggests that the O_2–crown ether complex is not a loosely bound adduct, but instead a species in which stronger bonding forces are involved than those associated with weak electrostatic interactions. An adduct species in which O_2 fits in the pocket of the crown ether is possible (enhancing multiple bonding interactions), or a structure in which the O_2 is cradled by four electronegative fluorine atoms is feasible. In general, O–F bonds are not stronger than 50 kcal/mol, so a complex containing a single F–O_2 binding interaction is unlikely. In any case, the inability of the nonfluorinated analogues or the perfluoro acyclic analogues to form (M + O_2)$^{\bullet-}$ adducts underscores the dramatic impact of fluorine substitution and the cyclic nature of the substrate on complexation behavior. Finally, the selectivity of adduct formation between the perfluoro macrocyclic anions and only molecular oxygen (not CO or CO_2) suggests that there may be underlying chemical reasons for this selectivity, not solely topological ones.

Additionally, reactions were performed with a number of reactive *ionic* species, including F^-, Cl^-, OH^-, NH_2^-, and $^-OCH_3$. Only perfluoro crown ether adducts with F^- and $^-OCH_3$ attachment were observed. The selectivity of adduct formation with other small ionic substrates is under further examination.

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Monitoring Oxygen Concentration in Solution by ESR Oximetry Using Lithium Phthalocyanine: Application to Photosynthesis†

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The measurement of dissolved oxygen concentration in solution and tissues has numerous applications to problems in chemistry, biology, and medicine. Polarographic detection via reduction to hydrogen peroxide is the most widely used method, forming the basis for the popular Clarke electrode (Teflon-coated platinum electrode),[1] as well as the more rapid response available in bare metal electrodes.[2] An alternative method for oxygen detection is electron spin resonance oximetry.[3,4] This method relies on ESR detection of line broadening of a stable radical, such as a nitroxide, due to oxygen-induced spin relaxation. The sensitivity of this method is very high, typically $\Delta[O_2] = 10^{-6}$ μmol (for S/N = 1 at 10^{-4} M nitroxide and 1-s response time), and can be extended to the shortest ESR time scale, typically 10^{-7} s.

In this paper, we describe a new application of ESR oximetry in solutions using microcrystals of lithium phthalocyanine (PcLi) as a spin probe. PcLi crystals have been studied by ESR and found to exhibit an extremely sharp line width which is very sensitive to gaseous O_2.[5] Preliminary accounts have begun to appear exploring this dependence for oximetry measurements.[6] Single crystals of PcLi are semiconductors and exhibit extremely narrow ESR line widths even in solution, typically 50 mG in deoxygenated solutions as seen in Figure 1. Upon introduction of air, this broadens by 20-fold (Figure 1). Here, we illustrate the sensitivity and kinetic response of this method by application to oxygen evolution in photosynthetic samples.

The synthesis of PcLi has been reported previously.[7] Spinach PSII membrane fragments were prepared via established procedures.[8] ESR measurements were performed by using a Bruker ESP-300 spectrometer and TE-102 cavity on 10-μL samples held in 1 mm i.d. quartz capillaries. Oxygen was removed from the reaction mixture enzymatically.[9]

PcLi forms tetragonal crystals in which one-dimensional stacks are held together by strong interaction between the planar PcLi molecules along the 4-fold axis.[10] The stacks are far apart, making

† Abbreviations: DCBQ, 1,4-dichlorobenzoquinone; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PcLi, lithium phthalocyanine; PS II, photosystem II.

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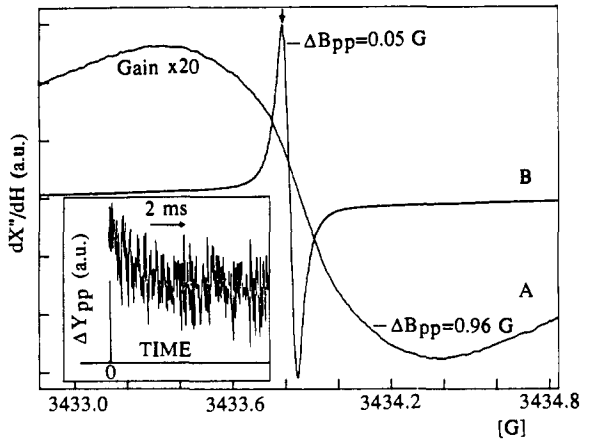

Figure 1. ESR spectra of a buffer solution of PcLi crystals when (A) air exposed and (B) degassed. Arrow indicates ESR position for time-resolved measurement. Inset: Time-resolved changes in PcLi signal amplitude (peak height) related to oxygen evolution in PSII membranes. The trace represents an averaged response after 250 laser pulses separated with 0.2-s dark intervals. Laser pulse at time 0. Time constant: 0.01 ms. For details, see Figure 2.

only weak contact in the lattice and forming channels coaxial with the 4-fold axis into which oxygen freely diffuses without complexation or charge transfer.[5]

In the absence of oxygen, the PcLi ESR signal can be saturated at low microwave power ($P_{1/2}$ = 2.6 mW), while in air-saturated solution, this increases to above our detection limit (>200 mW). This is instantaneous and reversible. Thus the effect arises from the increased spin relaxation rate of the conduction electron spins in PcLi upon collision with oxygen.

The 20-fold broadening of the unsaturated PcLi signal is almost 10 times larger than the broadening observed upon air saturation of the nitroxide molecule perdeuterio[^{15}N]tempone. This increased sensitivity is one of the advantages of the method. The area of the signal is constant at different oxygen concentrations, indicating chemical stability. A plot of ESR signal amplitude at different oxygen concentrations was prepared for calibration (not shown). In the present work, we used nonsaturating microwave power and oxygen concentration changes that were in the linear region of the calibration standards.

The PcLi radical was quite stable in the buffer for at least 2 months. In the PSII membrane mixture, no amplitude change of the ESR signal of PcLi was observed in the dark nor upon illumination until an exogenous electron acceptor (DCBQ) was added to enable reoxidation of the PSII plastoquinone acceptors. No oxygen production was observed in the absence of DCBQ, as monitored polarographically. This shows that the PcLi radical is chemically stable under illumination and in the presence of PSII. This chemical stability is a major advantage over the soluble nitroxide spin probe.

When a series of saturating, single-turnover laser flashes was exposed to dark-adapted PSII membranes, a period-four oscillation in the signal amplitude of PcLi was observed (Figure 2). The changes in the signal amplitude after each flash are plotted in the inset. This is 0 on the first two flashes and maximum on the third, seventh, and 11th flashes, corresponding to the familiar pattern of oxygen formation from the water oxidizing complex.[11] The method described here gives reliable results for oxygen concentrations in the range 0–0.2 mM. The sensitivity of the method is $\Delta[O_2] = 3 \times 10^{-7}$ μmol (for S/N = 1 at 0.5 mg/mL PcLi and 1-s response time). We have been able to use this method effectively for measurement of oxygen in the gas phase, in aqueous suspensions, and in direct contact with nonaqueous membrane samples.

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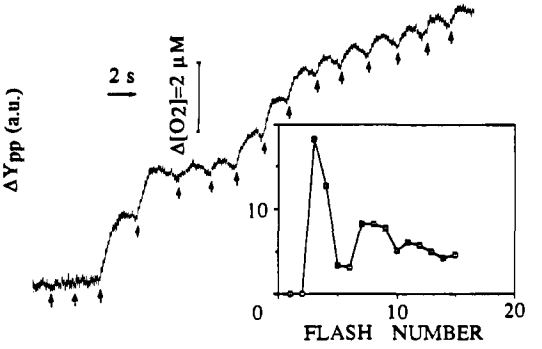

Figure 2. Changes in PcLi ESR signal amplitude (high field derivative peak) after illumination at 300 K of PSII membranes with a series of single-turnover laser flashes separated by 2-s dark intervals. Arrows indicate consecutive laser flashes. PSII membranes were kept in the dark for at least 10 min prior to flash excitation. The reaction medium contained 50 mM Mes-NaOH (pH 6.0), 15 mM $MgCl_2$, 20 mM NaCl, 1 mM DCBQ, 0.5 mg/mL PcLi, and 1 mg/mL chlorophyll as PSII membranes. Excitation source: Nd:YAG laser with a pulse duration of 20 ns, pulse energy of 75 mJ, and λ = 532 nm (MY32, Molectron Corp.). Microwave power: 1 mW. Modulation amplitude: 0.02 G. Time constant: 0.04 s. Inset: plot of the signal amplitude change vs. the flash number.

Time resolution of the signal, as given in Figure 1 (inset), reveals that under repetitive flashes a transient decrease in the PcLi signal can be resolved having a half-life of 1–2 ms. This signal appears only on resonance with the PcLi ESR signal and is abolished by treatments that inactivate oxygen production. These kinetics match those reported for the detection of oxygen at a Pt rate electrode when poised at the reduction potential suitable for oxygen detection.[2,12]

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A Versatile Protecting Group for 1,2-Dicarba-*closo*-dodecaborane(12) and the Structure of a Silylcarborane Derivative

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The incorporation of the icosahedral 1,2-$C_2B_{10}H_{12}$ carborane moiety into ever more complex organic structures has recently become of great importance. This rapid development of carborane chemistry derives from a renewed interest[1a] in the cytotoxic boron–neutron capture reaction $^{10}B(n,\alpha)^7Li$, as the basis of a binary method for cancer therapy (BNCT), and the recent discovery of radiometallacarborane reagents of unprecedented stability suitable for radiomedical application as immunoconjugates.[1b] Unfortunately, the synthesis of very valuable mono-C-substituted 1,2-$C_2B_{10}H_{12}$ species is now based upon the two synthesis methods of limited utility exemplified below.

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