to have only a 10% hyperfine anisotropy in Cu complexes.[10,11] Hyperfine couplings of similar magnitude and anisotropy have been observed for nitrogen couplings in Cu(II)-amide complexes.[25]

A resonance Raman line at 1234 cm^{-1}, characteristic of a simultaneous C-N stretch and N-H bend, is observed in the high pH form of stellacyanin.[26] The intensity of this line grows with increasing pH, and it is in resonance with the 600-nm absorption band, indicating that it arises from the blue Cu site. On the basis of these combined results, we conclude that a nitrogen of an amide ligand is a fourth ligand coordinated to Cu in the high pH perturbed blue form of stellacyanin. One interesting explanation for the appearance of a deprotonated amide coordinated to the blue copper site involves a linkage isomerization of the amide, from carbonyl oxygen to amide nitrogen at high pH as is observed in Cu-amide model chemistry.[27] The amide ligand provides a stronger ligand field than the thioether sulfur bond present in other blue Cu sites, which is consistent with the unique spectroscopic (i.e., rhombic split EPR[28]) and redox properties (i.e., low potential) that distinguish stellacyanin from other blue Cu proteins. This $Cu[N(HIS)]_2S(CYS)N(amide)$ model for the active site in stellacyanin is also consistent with recent molecular modeling predictions.[29] Detailed spectroscopic studies on the low and high pH forms of stellacyanin are presently underway.

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Selective Formation of Molecular Oxygen/Perfluoro Crown Ether Adduct Ions in the Gas Phase

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The abilities of crown ethers to form complexes with alkali cations and organic molecules has generated long-term interest[1-4] in host-guest chemistry.[5] Likewise this interest has extended to the coordination abilities of perfluorinated macrocyclic analogues which exhibit decreased basicities and have different pocket size dimensions. Some biocompatible perfluorinated compounds have high oxygen-carrying capacity[6] and have demonstrated great potential as artificial blood components.[7] From a physiological standpoint, the mechanism of oxygen binding to the fluoro ethers is particularly intriguing but poorly understood. We report herein preliminary results on the selective formation and structural characterization of oxygen/perfluoro crown ether negatively charged complexes in the gas phase.

The perfluoro crown ethers, synthesized via the LaMar direct fluorination procedure,[8,9] were admitted into the source of a triple-quadrupole mass spectrometer via a variable leak valve. The hydro crown ethers were introduced via a direct probe. Argon was introduced into the source manifold at 2-3 × 10^{-6} Torr to aid in the production of thermal electrons for electron-capture negative ionization. The desired reagent gas (CO, CO_2, air for O_2) was added to attain a total manifold pressure of 3.0-9.0 × 10^{-6} Torr, or a source pressure of 1-2 Torr using a chemical-ionization ion volume. The ethers examined included perfluoro-12-crown-4, perfluoro-15-crown-5, perfluoro-18-crown-6, the hydro crown analogues, and an acyclic ether, $CF_3(CF_2)_3(OCF_2CF_2)_2OCF_2(OCF_2CF_2)_2O(CF_2)_3CF_3$.

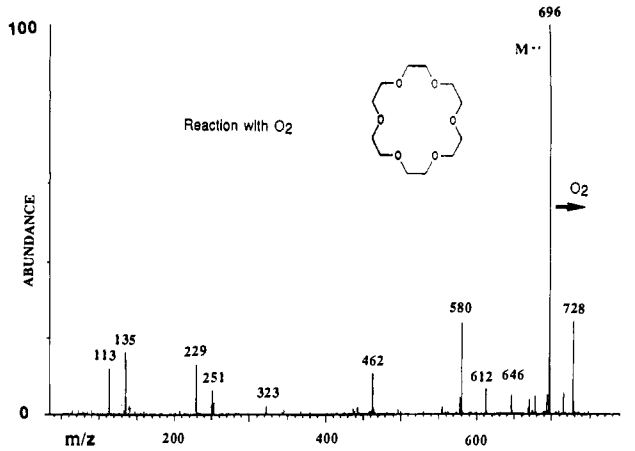

Figure 1. Negative-ionization mass spectrum of perfluoro-18-crown-6 and oxygen.

Figure 1 shows the negative-ionization mass spectrum for the reaction products of perfluoro-18-crown-6 (molecular weight 696) with oxygen. An abundant adduct ion is observed at m/z 728 due to $(M + 32)^{\cdot-}$. This ion is not observed in the absence of oxygen in the source. The $(M + O_2)^{\cdot-}$ adduct ion is observed for the other perfluorinated crown ethers with an abundance of 20-60% relative to the molecular ion. The capability for oxygen adduct formation was also evaluated for the nonfluorinated crown ethers. Adducts with O_2 were *never* observed, either in the positive or negative ionization modes.

Additionally, the abilities of the perfluoro crown ethers to form complexes with CO, CO_2, and Ar, species with sizes and some properties similar to those of O_2, were examined. Adducts with these species were not observed in either the positive or negative ionization modes. Thus, the tendencies of the perfluoro crown ethers to form adducts exhibit striking selectivity for O_2 only. The nonfluorinated ethers do not form adducts with any of these molecules either.

Ion/molecule reactions involving *acyclic* perfluoro ethers were also examined to determine whether the cyclic nature of the crown ethers played a role in the formation of the $(M + O_2)^{\cdot-}$ adducts. The acyclic ether, $CF_3(CF_2)_3(OCF_2CF_2)_2OCF_2(OCF_2CF_2)_2O(CF_2)_3CF_3$, did not form $(M + O_2)^{\cdot-}$ adducts. This suggests that the macrocyclic nature of the perfluoro crown ethers enhances binding to O_2.

In order to elucidate whether the perfluoro crown ether negative *ions* were reacting with neutral O_2 or crown ether *neutrals* were reacting with $O_2^{\cdot-}$, the two substrates were introduced into separate regions of the triple-quadrupole instrument so that selective ion/molecule reactions could be performed. For these experiments, 5 mTorr of O_2 was admitted into the second quadrupole region, which also may serve as a reactive collision chamber. The perfluoro crown ether was ionized in the source, and then the mass-selected molecular ion, $M^{\cdot-}$, was passed through the reaction quadrupole at a kinetic energy of 6 eV. Abundant $(M + O_2)^{\cdot-}$ adducts were observed in the resulting mass spectrum, indicating that crown ether negative *ions* may attach to *neutral* O_2.

Structural details of the perfluoro ether ions, $M^{\cdot-}$, and their O_2 adduct ions, $(M + O_2)^{\cdot-}$, were probed via collisionally activated dissociation (CAD) of the mass-selected parent ions. Argon was used as the target gas at 1.0 mTorr. Figure 2 shows the 30-eV CAD spectra of the molecular ion ($M^{\cdot-}$) of the perfluoro-18-

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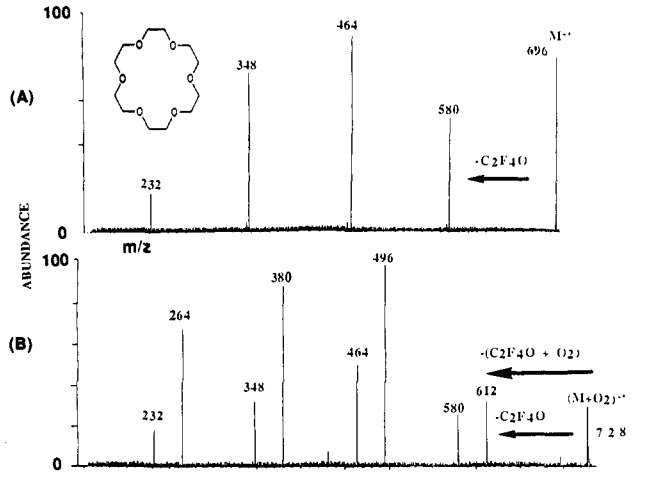

Figure 2. Collisionally activated dissociation mass spectrum at 30-eV kinetic energy of (A) perfluoro-18-crown-6 (m/z 696) and (B) (perfluoro-18-crown-6 + O_2)$^{\bullet-}$ adduct ion (m/z 728).

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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