net effect of π-donating ligands on d^0 or d^1 metals is electronically comparable to that of π acid ligands on later transition metals.

Conversion of **1** to **2** involves a net $2e^{-1}$ reduction of the $Cp_2V_2S_4$ subunit. The resultant d^4 Cp_2V_2 fragment is, however, deprived of π-interactions with the sulfur atoms of the dithiolene ligand, thereby inducing the μ-S_2 ligand to reorient so as to function more effectively as a σ-electron donor.

The conversion of an η^1-S_2 to an η^2-S_2 ligand has not been previously observed although several examples of each type exist. This work highlights the ability of the S_2 moiety to function as a facultative ligand and suggests that $\eta^1 \rightleftharpoons \eta^2$ interconversions may be an important facet of the reaction chemistry of other metal sulfide systems.

Acknowledgment. This research was supported by NSF (CHE81-06781). We thank D. M. Giolando for assistance with the preparation of $(i\text{-PrCp})_2V_2S_4$.

Supplementary Material Available: Tables of atomic coordinates, bond angles, bond distances, structure factors, and thermal parameters for **1** and **2** (34 pages). Ordering information is given on any current masthead page.

ESR Study of Cation–Crown Ether Induced Dimerization of a Water-Soluble Porphyrin

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Received May 27, 1983

Water-soluble porphyrins have found use as photocatalysts mediating the photoproduction of H_2 and O_2 in water.[1] For this reason their photophysics and photochemistry is of current interest. It is known that the porphyrins may be present as dimers or higher aggregates in aqueous solution.[2] Evidently this may have a strong effect on the photocatalytic activity. This prompted us to study the conditions that promote aggregation and the structure of the aggregates. During the course of this investigation we found that dimerization in some cases can be strongly promoted by the presence of a cation–crown ether complex. This communication deals with this crown ether induced dimerization.

Figure 1 shows the ESR spectra of photoexcited triplets of tetra(4-sulfonatophenyl)porphyrin (H_2(TPPS), Strem Chemicals) in frozen H_2O/glycerol. The spectra were recorded using field and light modulation with phase-sensitive detection at the modulation frequencies. This detection method takes advantage of the signal enhancement provided by spin polarization.[3] Moreover, it eliminates a strong doublet radical signal. The spin-polarization pattern (cf. Figure 1A) is the same as that found in the spectrum of H_2(TPP).[4] Also, the zero-field-splitting (zfs) parameters of the H_2(TPPS) monomer giving rise to the spectrum shown in Figure 1A (D = 423, E = 81 G) are similar to those of H_2(TPP) in toluene–chloroform (D = 410, E = 84 G). Apparently, the introduction of the sulfonate groups does not have a pronounced effect on the triplet-state characteristics of H_2(TPP).

Previous studies[2] disagree on whether or not H_2(TPPS) forms aggregates. We confirmed earlier findings that the optical absorption bands of H_2(TPPS) exhibit red shifts with increasing concentration and upon addition of cations (K^+, Na^+).[5] The optical absorption data are consistent with the presence of a monomer–dimer equilibrium.

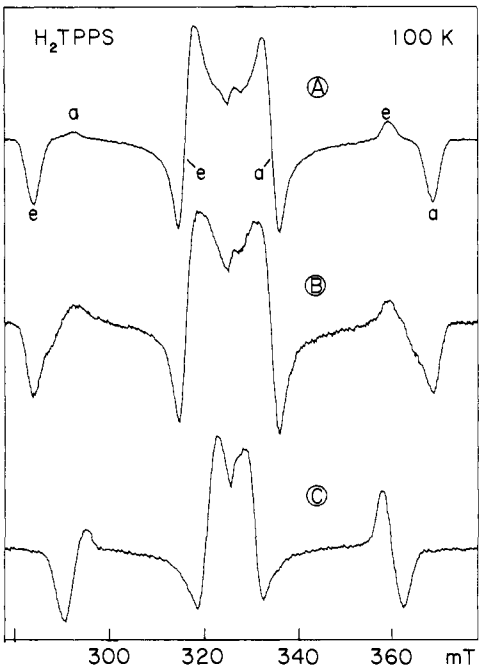

Figure 1. ESR spectra of the photoexcited tripets of H_2(TPPS) in frozen H_2O/glycerol (1:1) at 100 K. Spectra were recorded with a Varian E-9 spectrometer, microwave power 0.5 mW, field modulation 40 G at 100 kHz, 1000-W Xe/Hg light source modulated at 83 Hz. (A) 5×10^{-4} M H_2(TPPS), (B) 5×10^{-4} M H_2(TPPS) with 10^{-1} M KCl, (C) 5×10^{-4} M H_2(TPPS) with 10^{-2} M KCl and 10^{-3} M 18-crown-6. The spectra exhibit enhanced absorption (a) and emission (e) peaks as marked in spectrum A.

Addition of cations results in a loss of the monomer ESR signal (Figure 1A) and the appearance of a triplet signal attributed to the dimer. As shown in Figure 1B, with a H_2(TPPS) concentration of 5×10^{-4} M, a K^+ concentration in excess of 10^{-1} M is required for complete dimerization.

Since cations play a role in aggregation, it appeared likely that crown ethers would affect the equilibrium. In fact, we expected that cation–crown ether complexation would inhibit ion-pair formation, driving the equilibrium to the monomer side. Addition of 18-crown-6 (Aldrich) to an aqueous solution of H_2(TPPS) and KCl indeed has a strong effect. However, spectroscopic data show that the equilibrium shifts to the dimer rather than the monomer side. Figure 1C illustrates the pronounced effect on the ESR spectrum. In the absence of K^+ the optical absorption and ESR spectra are virtually unaffected by 18-crown-6 addition. On the other hand, in the presence of 18-crown-6, complete dimerization is attained with a K^+ concentration more than 2 orders of magnitude lower than that required in its absence. It is evident that it is the cation–crown complex that participates in the dimerization reaction. Studies of the effect of 18-crown-6 and K^+ concentration on the equilibrium indicate that the dimer encompasses less than four K^+ 18-crown-6 moieties.

The ESR spectrum establishes that the dimer has a well-defined structure. The change in zfs values (for the dimer D = 362, E = 87 G) is similar to the changes found in the triplet ESR spectra of chlorophylls upon dimerization.[6] Assuming that these changes stem from rapid triplet energy transfer between the dimer constituents (exciton model), they give an insight in the dimer structure.[7] It is noteworthy that the exciton model can account satisfactorily for the pronounced, dimerization-induced change in zfs values. It requires a geometry in which the porphyrin planes make an angle of about 35°. Furthermore, one in-plane principal axis of the zfs tensor in one porphyrin molecule must be roughly parallel to the corresponding axis in the other molecule. For this

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geometry the exciton model predicts a change in kinetics of triplet sublevel population and decay as well.[7] This change should register as a change in optimum phase-angle setting of the lock-in amplifier tuned to the light modulation frequency. In accordance with this prediction, we find that the optimum phase-angle settings for recording of the two triplets differ by about 90°.

Numerous techniques have been used in investigations of the structure and properties of chlorophyll aggregates involved in photosynthesis.[6,7] The evaluation of the data in part must rely on a data base provided by studies of model systems. It appears that H_2(TPPS) could be an ideal model system for the exploration of the effects of dimerization on physical and chemical properties of porphyrin-like structures.

Acknowledgment. Financial support by the Office of Basic Energy Sciences of the Department of Energy under Contract DE-AC-02-81 ER 10911 is gratefully acknowledged.

Registry No. Na^+, 17341-25-2; K^+, 24203-36-9; K^+ 18-crown-6, 31270-13-0; tetra(4-sulfonatophenyl)porphyrin, 39174-47-5.

Structure and Stereochemistry of Tetrahedral Inhibitor Complexes of Papain by Direct NMR Observation

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Received June 10, 1983

Application of ^{13}C NMR spectroscopy under cryoenzymological conditions to the study of the thiol protease papain has revealed structural evidence[4] for the acylenzyme (ES′) in the overall mechanism of eq 1. While it is generally assumed that a tetrahedral intermediate is formed during the acylation and deacylation steps leading from the Michaelis complex (ES) to the products [P_1 (= amine or alcohol) and P_2 (= carboxylic acid)] and the enzyme (E), the evidence for such a labile intermediate is still indirect,[5] although a spectrophotometric study has indicated that a tetrahedral intermediate can be observed at subzero temperature[6] with papain.

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \xrightarrow{k_2} \underset{+\ P_1}{ES'} \xrightarrow{k_3} E + P_2 \qquad (1)$$

Ketonic inhibitors whose functionality mimics the scissile peptide bond have also provided useful models for binding in serine proteases where it has been demonstrated that stabilized covalent tetrahedral species can be characterized by ^{13}C NMR.[7,8] So far, no *peptide* inhibitor has shown evidence of a covalent, tetracoordinated species,[9] nor have productive tetrahedral complexes been observed with thiol proteases by NMR methods.

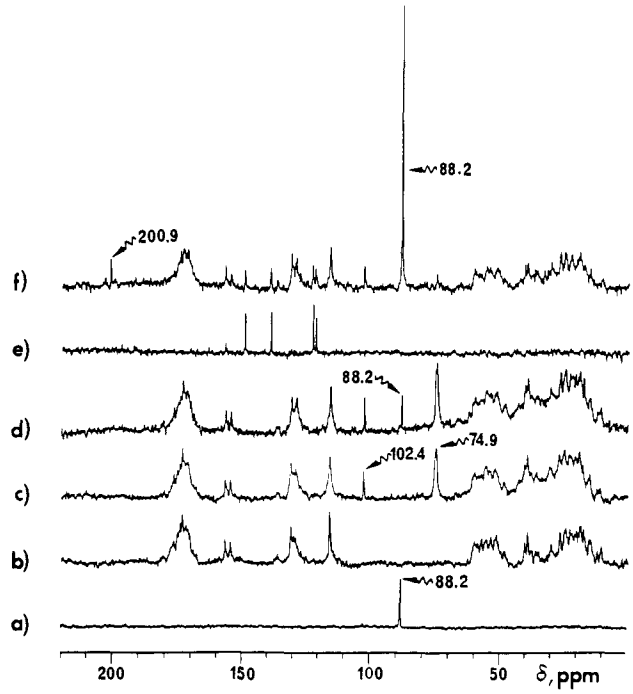
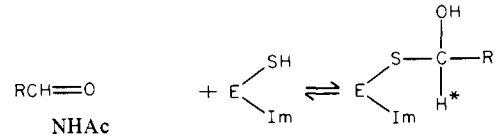


Figure 1. Spectra: 75.47 MHz (^{13}C), proton decoupled at 4–5 W with decoupler power reduced to 0.4 W for 0.2–0.4 s after each acquisition time of 0.2 s, 10-Hz exponential weighting and 8 K time domain data points, 10-μs pulse width (35 μs = 90° pulse). Chemical shifts are relative to Me_4Si. *N*-Acetylphenylalanyl[1-^{13}C]glycinal concentrations; fully active papain concentrations; D_2O, % (v/v); pH; no. of accumulations (all in 10 mM sodium phosphate): (a) 3.41 mM; 0.00 mM; 80; 7.0; 1030. (b) 0.00 mM; 0.61 mM; 26; 7.2; 29 000. (c) 0.69 mM; 0.72 mM; 26; 7.1; 29 000. (d) 1.71 mM; 0.62 mM, 33; 7.1; 32 000. (e) 0.00 mM; 0.00 mM; 33; 7.2; 44 000 plus 2,2-dipyridyl disulfide 1.37 mM. (f) As (d) except pH 4.1 plus 2,2′-dipyridyl disulfide 1.5 mM (see legend Figure 2).

In order to provide the necessary spectroscopic data for the eventual characterization of a tetrahedral adduct of papain, (*N*-acetylphenylalanyl)glycinal (**1**) was selected on the basis of its



1, R = $PhCH_2CHCONHCH_2$
3, R = $PhCONHCH_2$

potent inhibitory properties and the suggestion[10] that hemithioacetal (tetrahedral) structures (as **2**) were formed by addition of the cys-25 thiolate of papain to the aldehyde carbonyl of peptide inhibitors. Using *N*-benzoylamino[1-^{13}C]acetaldehyde (**3**) and papain, Lowe[11] proved, by an ingenious cross-saturation ^1H-NMR experiment, that the magnetization transfer data were in full accord with the presence of a proton (H*) attached to a tetrahedral carbon (τ(H*) 3.81; $^1J(^1H-^{13}C)$ = 183 Hz) although direct observation of a hemithioacetal inhibitor complex (**2**) per se was not possible in this experiment. It was also shown that the aldehyde and not the hydrated form was the true inhibitor of papain.[11] The wide range and diagnostic power of ^{13}C NMR suggested that a complete structural assignment could be made for an aldehyde inhibitor–papain complex, and we now report on the results of such an experiment.

Reaction of [1-^{13}C]aminoacetaldehyde dimethyl acetal (prepared from [1-^{13}C]glycine (90% ^{13}C atom percent)) with *N*-

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