ever collected for proteins. A Fourier transform of the data over a range of $k = 4$–12 Å^{-1} is presented in Figure 2. This transform shows striking similarity with those for blue copper proteins.[10,11] The features above 2 Å (not corrected for phase shift in the Fourier transform) have been previously suggested to provide evidence for the presence of imidazole ligands.[11] Another obvious feature in this transform is the absence of the large peak at 3.3 Å present in the oxyprotein, which was assigned as a backscattering peak from a copper atom at about 3.6 Å from the absorbing copper.[1,2] The first major peak in the transform was filtered and found to fit two nitrogen (or oxygen) atoms at 1.95 Å. A similar result is obtained for deoxyhemocyanin from *Busycon canaliculatum*.[2] For the obtainment of a more detailed view of the copper coordination sphere, the new technique of "group" curve fitting was applied.[3] The group fitting approach allows more accurate modeling of the phase and amplitude effects of second and third shell atoms of imidazole groups. This technique applied to oxyhemocyanin allowed us to suggest a model for the binding site which consists of two copper(II) atoms separated by 3.55 Å, each bound to two histidines and two oxygen atoms in an approximately square-planar geometry.[1]

Using the imidazole group fitting technique on the EXAFS data for deoxyhemocyanin reveals 2.0 imidazoles at 1.95 Å. The calculated fit, as shown in Figure 1, matches the data extremely well. For investigation of the possibility of a copper scatterer in the copper environment, a copper atom at various distances less than 4.5 Å was fitted to the data. Under no circumstances did copper ever contribute significantly to the overall fit, in most cases giving rise to less than 0.2 atom or a negative number of atoms. Similar results were observed when the same procedures were applied to the half-apo derivative of the protein[12,13] or to the low-temperature data set. Fitting atoms at longer distances becomes fruitless because of the insignificant contribution of scattered waves by long-distance scatterers to the total EXAFS which is dominated by nearest-neighbor scatterers. However, a peak significantly above the noise level was observed at 5.1 Å in the transform (see Figure 2). This peak, Fourier filtered from the *R*-space data, can be fit well with 0.6 copper atom at 5.6 Å. This could be interpreted to mean that, in deoxyhemocyanin, the two copper atoms are separated by a large distance, in contrast to the 3.55-Å distance found in oxyhemocyanin. EXAFS analysis for such a long-distance single-atom scatterer, however, must be taken with extreme caution. It is interesting to note that computer simulations of EPR spectra have calculated Cu(II)–Cu(II) distances to be ca. 6 Å for nitrite-treated hemocyanin.[14]

The EXAFS results presented here for the deoxyhemocyanin suggest that only two ligands are found coordinated to each of the copper sites of the protein. Although such a low coordination number has not previously been found in a metalloprotein or metalloenzyme,[15] this coordination geometry is, nevertheless, quite common among Cu(I) compounds.[16] Crystals of $[Cu(Im)_2]ClO_4$, obtained in a size suitable for powder diffraction, give intensity values comparable to those of linear $[Ag(Im)_2]NO_3$, suggesting that the two structures are essentially isomorphous.[17] A two-coordinate geometry of Cu(I) in a solution of 2:1 complexes with monodentate ligands like NH_3 or imidazole is also strongly supported by the properties of mixed systems containing two different ligands.[16] Hence the copper site in the deoxyhemocyanin can be postulated to consist of two Cu(I) atoms, each coordinated to two histidines, with the facial sides open for attachment of dioxygen. Such an arrangement should also facilitate the removal of dioxygen from the oxyprotein. However, it has been shown that for some Cu(I) models, the low coordination number is sometimes supplemented by one or two additional ligands at considerably larger distances. EXAFS is not very sensitive for detecting a low-Z atom at a large distance from the metal site, and hence the analysis reported herein could have missed a weakly bound ligand at a long copper–ligand distance. Such weak interactions, however, are probably of minor significance in contributing to the ligand field at the copper site.

EXAFS studies on oxy- and deoxyhemocyanin both indicate the presence of two histidines in the copper coordination sphere. However, the oxygenation process involves several significant changes in the copper site. Upon binding dioxygen, the copper oxidation state changes from Cu(I) to Cu(II), the coordination number increases from 2 to 4, and the Cu–Cu distance is shortened to 3.55 Å. These processes must certainly be accompanied by a substantial rearrangement in the protein structure. Such a conformational change can provide explanations for numerous observations on oxygen binding, such as the dissociation of subunits,[18,19] changes in the 250-nm band in circular dichroism,[18,20] and a slow relaxation time in kinetics studies.[21] A comparative study on four different states of hemocyanin (oxy, deoxy, half-apo, and dimer) will be presented in detail in a forthcoming paper.[12]

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Magnetic Circular Dichroism Studies. 59.[1] Substituent-Induced Sign Variation in the Magnetic Circular Dichroism Spectra of Chlorins

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The occurrence of sign inversions in the magnetic circular dichroism (MCD) of porphyrin derivatives has important analytical applications as well as theoretical implications; however, only a few studies have been made of chlorophylls and chlorin derivatives[2-8] compared to the number dealing with porphyrins[9a]

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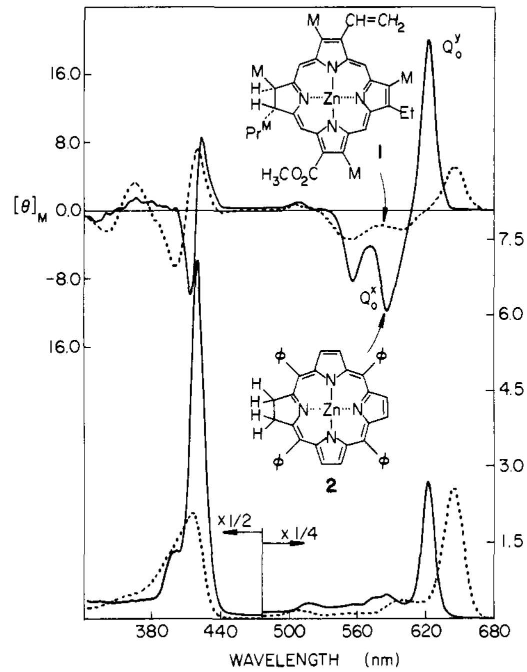

Figure 1. MCD and absorption spectra of zinc tetraphenylchlorin (—) and zinc rhodochlorin dimethyl ester (-----) in benzene.

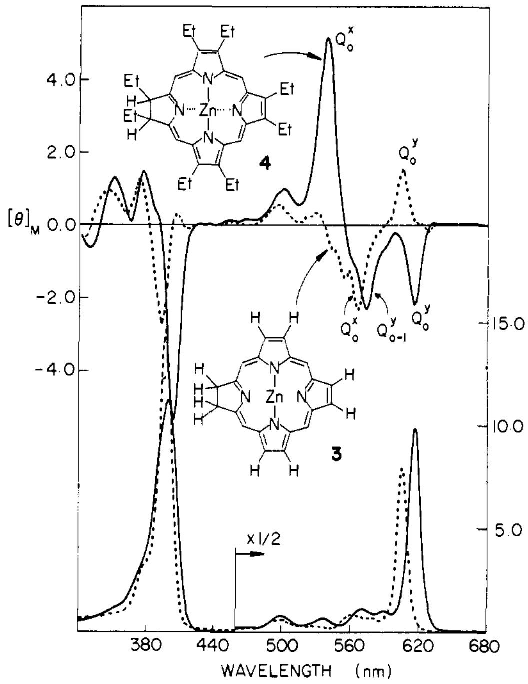

Figure 2. MCD and absorption spectra of zinc octaethylchlorin (—) and zinc chlorin (-----) in benzene.

and heme proteins.[9b] With the exception of tetraphenylchlorin (TPC), almost all of the chlorins investigated[2-6] have had vinyl and/or carbonyl substituents at the pyrrole positions in addition to a meso alkyl group. For all of these compounds the sign pattern of the MCD bands associated with the electronic visible and Soret transitions is inverted (+−+− with increasing energy) as compared

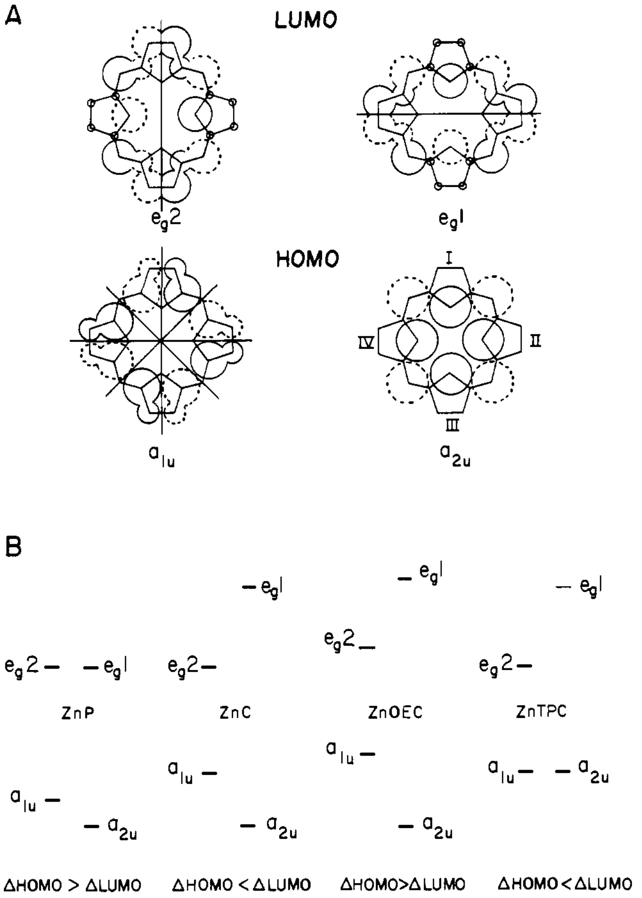

Figure 3. (A) Porphine HOMO and LUMO orbitals redrawn from ref 12. The sizes of the circles are proportional to the atomic orbital coefficients of Seeley. Symmetry nodes are indicated by lines. (B) Representation of the orbital energies adduced for zinc porphine, zinc chlorin, zinc octaethylchlorin, and zinc tetraphenylchlorin in the text. Symmetry labels are appropriate only to the D_{4h} point group and are retained to facilitate tracking of orbital energy level changes in the series. Identical changes in the LUMO's caused by zinc or the four phenyl substituents are not scaled.

to the "normal" (−+−+) sign pattern observed for D_{4h} porphyrins. The inverted pattern is illustrated for zinc rhodochlorin dimethyl ester (ZnRhC, **1**) and ZnTPC (**2**) in Figure 1. This ostensible invariance in the inverted sign pattern reported for a variety of chlorins has led to the tacit assumption that the chlorin perturbation is so traumatic that it alone is responsible for the sign inversions. However, heretofore uncommented upon exceptions occur in the visible MCD spectra[7,8] of two low-spin derivatives of ferrous heme d_1, an iron chlorin,[10] which is thought to have only a single weakly perturbing nonalkyl substituent (vinyl group).

In order to determine whether the normal sign pattern found for heme d_1 is, in fact, the one characteristic for the chlorin chromophore, we have measured the MCD spectra of other weakly perturbed chlorin derivatives. The spectra (Figure 2) of two of these, zinc chlorin (ZnC, **3**) and zinc octaethylchlorin (ZnOEC, **4**) show that ZnOEC, unlike most other chlorin derivatives, does exhibit the −+−+ sign pattern for the electronic bands in both the visible and the Soret regions. On the other hand, the MCD bands of ZnC are inverted in the visible but not the Soret region (a very weak positive band is found, however, at 405 nm).

Michl[11] has recently presented a very useful perimeter model

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which relates the absolute MCD signs of the four lowest energy electronic transitions of systems derivable from a $(4N + 2)$-electron [n]annulene perimeter with their molecular structure. According to this model the signs of the A[11a] or B[11b] terms of such systems are directly related to the relative size of the orbital-energy differences between the two highest occupied (ΔHOMO) and the two lowest unoccupied (ΔLUMO) molecular orbitals. In the event that ΔHOMO − ΔLUMO > 0 a normal (−+−+) sign pattern is predicted, whereas if ΔHOMO − ΔLUMO < 0, then an inverted (+−+−) sign pattern should result.[11d] Michl has applied his model to porphyrins and reduced porphyrins. However, it is evident (Figure 1 and 2) that the MCD sign pattern of chlorins is far more sensitive to substituent effects than had earlier been supposed. Therefore, it is important to examine the model in greater detail.

The representations (Figure 3A) of the porphin four-orbital MO's given by Gouterman,[12] which agree qualitatively with the electron density maps computed for magnesium porphine by Spangler et al.,[13] provide a visual basis for estimating the relative magnitudes of the effects of substituents on the energy levels of the HOMO and LUMO orbitals. The work of Meot-Ner and Adler[14] indicates that meso phenyl groups behave as conjugative electron donating ($-E$) substituents with respect to the porphyrin ring. They are effective in splitting the HOMO's, since the ratio $\epsilon_{\max}(Q_{0\text{-}0})/\epsilon_{\max}(Q_{0\text{-}1})$[15] changes from 0.06 to about 1 on going from porphine dianion to tetraphenylporphyrin dianion. Alkyl groups donate electrons to the porphyrin ring[12] but their $-E$ effect[16] is relatively weak, since the visible band ratio for octaethylporphyrin dianion is only 0.5. In addition, an alkyl group is considered to raise the HOMO orbital more than the LUMO's since, although the porphine orbital coefficients at the pyrrole positions (Figure 3A) are larger in the LUMO's than in the HOMO's, the latter are closer in energy to those of the alkyl donor orbitals. Finally, the electron-withdrawing effect of zinc[12] is not strong since the band ratio for zinc porphine is again about 0.5.

With these caveats in mind we summarize the qualitative effects of substituents and ring reduction on the energy levels of the HOMO's and LUMO's of porphine in Figure 3B. Porphine dication (or, equivalently porphine dianion), for which the LUMO's are degenerate by symmetry and the HOMO's accidentally so, is taken as the reference compound. Replacement of the four central protons of porphine dication by zinc lowers the energy of the a_{2u} orbital. ΔHOMO − ΔLUMO > 0 and a normal MCD sign pattern is predicted for ZnP as is observed.[17] Saturation of the pyrrole double bond of ring IV (Figure 3A) reduces the size of the conjugation path, thereby raising the energies of the e_g1 and a_{1u} orbitals but has little effect on the e_g2 and a_{2u} orbitals, since for them the electron density in ring IV is small. The energy shift is greater for e_g1 than for a_{1u} because of the relative difference in the electron densities in the two orbitals at the site of reduction. For ZnC ΔHOMO − ΔLUMO < 0 and an inverted MCD sign pattern is predicted (in agreement with Michl's conclusion[11b] for chlorin dianion) and observed at least for the Q_0^y and Q_0^x bands (Figure 2).[11d]

Relative to ZnC the energy of the a_{1u} orbital of ZnOEC is raised owing to the substitution of the six effective (for this orbital) alkyl perturbers. The energies of both LUMO's are also raised; however, only two alkyl groups perturb the e_g1 orbital, whereas four are effective in raising the energy of the e_g2 orbital. Thus, relative to ZnC ΔLUMO is decreased but ΔHOMO is increased. ΔHOMO − ΔLUMO > 0 and the predicted normal sign pattern is consonant with the experimental spectrum (Figure 2). In ZnTPC the a_{2u} orbital is strongly raised (vide supra) so that now ΔHOMO ~ 0 which is consistent with the inverted sign pattern (Figure 1). In addition, from a comparison of |ΔHOMO − ΔLUMO| for ZnC and ZnTPC one can readily understand the basic cause of the large difference between the values of $[\theta]_M$ for the two chlorins (>8x for the Q_0^y bands). Finally, note that in ZnRhC (Figure 1) a vinyl and a methoxycarbonyl group are situated on rings I and III, respectively. The methoxycarbonyl group, in particular, is strongly electron withdrawing, and substitution of this group for an alkyl in ZnOEC should lower the energies of the e_g2 and a_{1u} orbitals while leaving the e_g1 and a_{2u} orbitals largely unaffected. As a result ΔHOMO − ΔLUMO < 0, thus rationalizing the inverted sign pattern.

We believe that the data and analyses reported here on the effects of substituents on the MCD of reduced porphyrins strongly support the utility and applicability of Michl's model[11] for these systems. In a detailed report[18] we will show that a similar perturbation treatment is required to explain other instances of substituent-induced sign and intensity variations in the MCD of other chlorins, bacteriochlorins, and isobacteriochlorins.

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Photosensitized Electron-Transfer Reactions in Colloidal SiO_2 Systems: Charge Separation at a Solid-Aqueous Interface

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Separation of products formed in photoinduced electron-transfer processes is essential for efficient energy storage.[1,2] Several approaches involving systems such as functionalized micelles,[3,4] liposomes,[5,6] microemulsions,[7] and polyelectrolytes[8,9] have been used as means to assist charge separation. In these processes the thermodynamically favored back reactions of the photoproducts can be retarded, and further utilization of the photochemical energy so stored can make feasible the decomposition of water. Particular attention has been devoted to the photosensitized re-

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