

influence of a stronger spin-orbit coupling and electronic interaction might reduce the chance of a fluctuational or vibronic process occurring.

There are three absorption bands in the red and near-infrared region at 11 200, 6700, and 3000 cm^{-1} : the highest and lowest carry roughly equal intensity while the middle band is quite weak. Using the tetragonal and rhombic parameters obtained above from an analysis of the Creutz-Taube ion g factors as outlined above, plus a value of $W = 4600 \text{ cm}^{-1}$ and $\lambda = 3000 \text{ cm}^{-1}$, we then calculate the energy levels and relative intensities given in Table IV. The agreement between calculated and observed band positions is very good, and the relative intensities of the bands are well accounted for. It remains to measure the MCD intensities

and g values to complete the analysis. The significance of this fit is that the tetragonal and rhombic parameters are the same as those that account for the properties of the Creutz-Taube ion and are thus truly single-ion properties that are maintained when the ions are coupled. Our conclusion would be that the osmium analogue is definitely delocalized, but there are indications that there is substantial vibronic activity in the Creutz-Taube ion.

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Registry No. Decaammine(μ -pyrazine- N,N')diruthenium(5+), 93503-78-7; decaammine(μ -pyrazine- N,N')diosmium(5+), 85282-23-1.

Resonance Raman Spectroscopy of Metallochlorins: Models for Green Heme Protein Prosthetic Groups

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Abstract: Iron chlorin complexes have been investigated by resonance Raman (RR) spectroscopy as models for the prosthetic groups of green heme proteins. $\text{Fe}^{\text{III}}\text{pPP}$ (pPP = photoporphyrin IX dimethyl ester) and $\text{Fe}^{\text{III}}\text{DC}$ (DC = deuteriochlorin IX dimethyl ester) complexes were examined with 406.7-, 457.9-, 488.0-, 514.5-, and 568.2-nm excitation lines in the high-frequency ($>1000 \text{ cm}^{-1}$) spectral region. The general characteristics of the chlorin spectra have been analyzed and vibrational modes have been assigned in a comparative study with the analogous iron porphyrins, $\text{Fe}^{\text{III}}\text{PP}$ (PP = protoporphyrin IX dimethyl ester) and $\text{Fe}^{\text{III}}\text{DP}$ (DP = deuteroporphyrin IX). Although vibrational frequencies above 1000 cm^{-1} for the spectra of $\text{Fe}^{\text{III}}\text{DC}$ and $\text{Fe}^{\text{III}}\text{pPP}$ complexes were generally similar to those of the analogous porphyrins, major differences were observed in both patterns of relative intensity and in the depolarization ratios. For example, all B_{1g} (depolarized) porphyrin modes become totally symmetric (polarized) vibrational modes in chlorin spectra. At least six new bands are present in metallochlorin spectra. Of particular interest is the observation of new polarized "chlorin" bands flanking the oxidation state marker, ν_4 . These findings reflect a dramatic reduction in molecular symmetry from an effective D_{4h} for metalloporphyrins to an effective $C_2(x)$ for metallochlorins. We also performed a comparative analysis of published M-OEC (OEC = octaethylchlorin) and M-OEP (OEP = octaethylporphyrin) RR spectra to verify that our observations were characteristic of metallochlorins in general. In every case, the M-OEC complexes exhibit RR spectra with a greater number of bands, a new polarized feature in the region of the oxidation-state marker, and a striking increase in the number of polarized Raman bands. In addition, we have found that many characteristic metalloporphyrin vibrational frequencies and assignments, such as for oxidation and spin-state markers and peripheral substituents (i.e., vinyl or carbonyl), are transferable to the study of metallochlorin complexes. Clearly these observations have predictive value and establish testable criteria for identification of metallochlorin prosthetic groups in biological systems such as the green heme proteins.

Heme proteins and heme enzymes are ubiquitous biological molecules involved in oxygen transport and oxygen metabolism. Whereas in heme systems chemical control is generally manifested by variations in axial ligation and the nature of the heme environment, reduction of the porphyrin macrocycle provides an alternate means whereby the reactivity of these macromolecules may be expressed. Examples of such partially saturated systems include dihydroporphyrins, or chlorins; tetrahydroporphyrins, or sirohemes; and the highly reduced macrocycles of vitamin B-12 and cofactor F-430. Metallochlorin complexes, which are the subject of this investigation, occur in chlorophyll pigments and at the active sites of some members of a newly recognized class of proteins known as the green heme proteins. Work in this and other laboratories suggests that the chlorin prosthetic group is found in sulfmyoglobin¹⁻³ and sulfhemoglobin (a major non-functional form of human hemoglobin),^{2b} hemes d and d_1 of

microorganisms,⁴ *Neurospora crassa* catalase,⁵ leukocyte myeloperoxidase,^{3,6,7} and bovine spleen green heme protein.^{3,7} Although a metallochlorin prosthetic group has been implicated for each of these proteins, the exact macrocyclic ring modification(s) and site of ring reduction are still not completely known in most

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of these cases.^{4,8} Furthermore, since some heme proteins which are green in color do not appear to have chlorin prosthetic groups,⁹ general criteria for distinguishing chlorin systems from porphyrin systems would be useful.

The ability of resonance Raman (RR) spectroscopy to provide detailed structural information for heme protein prosthetic groups and porphyrin complexes¹⁰ makes it the method of choice for the study of green heme protein prosthetic group structure. However, no systematic studies of the RR properties of metallochloirins have been undertaken. One paper reports the investigation of metallated octaethylchlorins (OEC),¹¹ and applications to naturally occurring chlorin systems are restricted to a few studies with *Pseudomonas aeruginosa* heme *cd*₁,¹²⁻¹⁴ chlorophylls,^{15,16} and some preliminary work on myeloperoxidase^{6,7} and bovine green heme protein.⁷ Generally, these reports did not utilize the extensive structural and RR spectroscopic data available from porphyrin studies as a foundation for a thorough analysis of the vibrational properties of metallochloirins as is given here.

The work presented herein establishes testable, general characteristics for metallochloirin RR spectra which can readily be extended to the study of green heme protein prosthetic group structure. We have obtained high-frequency (>1000 cm⁻¹)¹⁷ RR spectra of two biologically relevant iron chlorins. Resonance Raman spectra of Fe^{III}pPP (pPP = photoporphyrin IX dimethyl ester) and Fe^{III}DC (DC = deuteriochlorin IX dimethyl ester) complexes were compared to those of the analogous Fe^{III}PP (PP = protoporphyrin IX dimethyl ester) and Fe^{III}DP (DP = deuteroporphyrin IX) complexes. We also performed a comparative analysis of literature M-OEC and M-OEP (OEP = octaethylporphyrin) RR spectra and, in a parallel study,³ we compared the vibrational properties of Fe^{II} and Fe^{III} sulfmyoglobin complexes to those of the native myoglobin species. The RR spectral characteristics of iron chlorin, M-OEC, and sulfmyoglobin complexes are complementary; thus we are confident that RR analysis can be diagnostic for the presence of a chlorin group in a green heme protein.

Experimental Section

Fe^{III}pPP dimethyl ester, isomer 1 (reduced in ring B of the macrocycle), was synthesized and purified according to DiNello and Chang;¹⁸ iron was inserted with use of ferrous bromide in THF and subsequent washing with HCl. Isomers A and B of the Fe^{III}DC dimethyl ester were synthesized and its structure determined according to Clezy and Morell.¹⁹ Raman samples of ClFe^{III}pPP and ClFe^{III}DC were prepared by dissolving the chlorin in freshly distilled CH₂Cl₂, benzene, CCl₄, or acetone, as

Table I. Correlation Table

D _{4h}	(M-OEP)	C ₂ (x)	(M-OEC) ^a
A _{1g}	polarized	A	polarized
B _{1g}	depolarized	A	polarized
A _{2g}	anomalously polarized	B	depolarized
B _{2g}	depolarized	B	depolarized
E _u	Raman forbidden and not observed; IR allowed ^b	{ A + B	{ polarized depolarized

^aOn the basis of the Fe^{II}OEC crystal structure reported by Strauss et al.²⁸ ^bE_u modes are Raman active for iron PP complexes and heme proteins.

indicated in the figure legends. The (Me₂SO)₂Fe^{III} chlorin adducts were prepared by dissolving the chloride complex in freshly distilled Me₂SO; the Me₂SO is known to displace the bound chloride in porphyrin complexes.²⁰ Raman samples of Fe^{III}PP and Fe^{III}DP complexes were prepared similarly. Absorption spectra of the iron chlorin and iron porphyrin complexes were unchanged following laser irradiation. The IR spectrum of ClFe^{III}pPP was obtained on a solid sample in a KBr pellet.

Resonance Raman spectra of Fe^{III} chlorin and porphyrin complexes were obtained on samples maintained at ~4 °C in standard melting point capillaries with use of either back-scattering from a sample Dewar or a 90° scattering geometry from a Varian variable temperature accessory. Excitation was provided by Spectra-Physics ion lasers (164-05 Ar, 164-01 Kr). The Raman spectrophotometer and computer interface have been reported previously.²¹ Depolarization ratios were calculated from peak heights in spectra obtained by rotation of the polarization incident on the sample fixed in the 90° geometry.²²

Results and Discussion

To characterize the vibrational properties of metallochloirins and, by extension, those of chlorin-containing proteins, we have used the well-defined properties of metalloporphyrins as a foundation. The ferric chlorins, DC and pPP, were chosen for this study not only for their biological relevance as chromophores structurally related to iron PP but also because of extensive previous RR work on the porphyrins DP²³ and PP.^{10,20,24} The absence of conjugated substituents on the macrocycle of DC dictates that any spectral differences between DC and DP complexes must arise from alterations in molecular symmetry and electronic structure induced by reduction of one of its pyrrole rings. Comparison of DC spectral properties with those of the nonbiological M-OEC complexes previously reported¹¹ allows us to separate fundamental chlorin vibrational properties from those of conjugated substituents on an isomeric chlorin such as pPP. Our approach to the investigation of metallochloirins has been dual, combining a comparative analysis of the RR properties of metallochloirin complexes and their porphyrin analogues with a concurrent study of the RR properties of native myoglobin (Mb) and the green heme protein, sulfmyoglobin (SMb), which is suspected to contain a chlorin prosthetic group.³

A. Metallochloirin Molecular Symmetry and Electronic Structure. The effects of a two-equivalent reduction at one of the porphyrin pyrrole rings are quite dramatic. Electronic absorption spectra of metallochloirins (Figure 1) have a strong absorption band at ~600–650 nm which is only 3–5 times less intense than the Soret band, in contrast to the visible bands of porphyrin complexes that are ~10-fold less intense than their Soret bands.^{25,26} Where a metalloporphyrin has planar pyrrole rings

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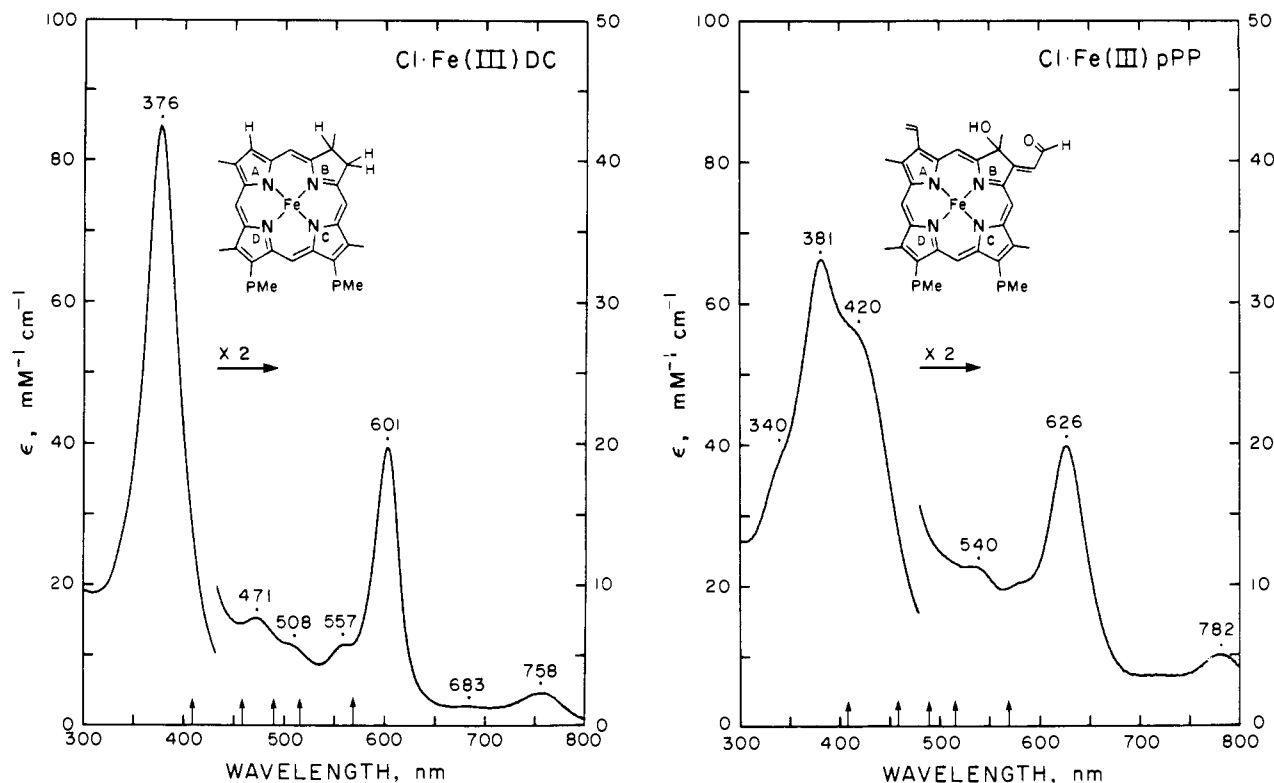


Figure 1. (A, left) Absorption spectrum of $\text{Cl}\cdot\text{Fe}^{\text{III}}\text{DC}$ in CH_2Cl_2 (isomer B shown). (B, right) Absorption spectrum of $\text{Cl}\cdot\text{Fe}^{\text{III}}\text{pPP}$ (isomer 1) in CH_2Cl_2 . RR excitation lines are indicated by arrows.

with all M–N bonds equal in length, a metallochlorin shows loss of planarity at the pyrroline (reduced) ring,^{27,28} and opposite M–N bond pairs are unequal in length.²⁸ An alteration in the patterns of electron density in the chlorin macrocycle leads to increased e^- density at the methine carbons adjacent to the pyrroline ring, that produces acidic, exchangeable methine hydrogens.²⁹ An intramolecular charge transfer toward the reduced site of a chlorin produces an asymmetric charge distribution; moreover, the non-planarity of chlorins results in a dramatic reduction of aromaticity relative to porphyrins.³⁰ It has also been shown that metallochlorins have increased ligand affinity³¹ and faster e^- transfer³¹ and are more easily oxidized relative to metalloporphyrins.^{30–32}

The inner π -conjugation pathways of aromatic metalloporphyrin and -chlorin macrocycles are fundamentally different, as shown in Figure 2. A metalloporphyrin retains x,y equivalence even when conjugating substituents such as vinyl groups (i.e., 2-vinyl and 4-vinyl of PP) are placed cis with respect to the porphyrin ring, thus leading to retention of degeneracy for the x and y electronic transitions and the presence of E_u normal modes (D_{4h} symmetry). In the metallochlorin, there is an *inherent* x,y inequivalence of the macrocyclic π -conjugation pathways,³³ regardless of substituents, resulting in absorption spectra possessing separately allowed Q_x and Q_y visible transitions and occasionally even two Soret transitions²⁵ (see Figure 1B). This x,y inequivalence should also lead to splitting of E_u Raman modes in chlorin

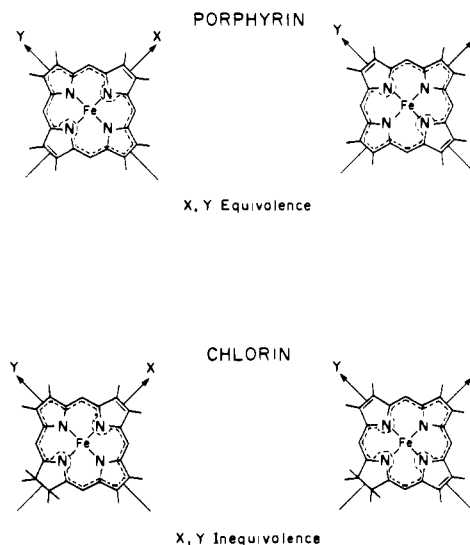


Figure 2. Inner π -conjugation pathways for a metalloporphyrin and a metallochlorin.

IR and RR spectra. Precedence for such splitting occurs both in the work of Choi et al.,³⁴ who observed separate " $E_u(x)$ " and " $E_u(y)$ " components in RR spectra of heme *a* in which trans-conjugated substituents to the porphyrin ring (4-vinyl and 8-formyl) produce x,y inequivalence, and in the RR studies of chlorophylls by Lutz and co-workers.¹⁵

As shown in Table I, the vibrational selection rules and Raman polarization properties for metalloporphyrins and -chlorins are notably dependent on their molecular symmetry. With reference to the crystal structure for $\text{Fe}^{\text{II}}\text{OEC}$,²⁸ we assign an effective $C_2(x)$ molecular symmetry to metallochlorins. This symmetry assignment takes into consideration both the pseudo- S_4 ruffle of the

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Table II. Resonance Raman Frequencies (cm^{-1}) and Depolarization Ratios (ρ) for $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}$ Deuteriochlorin^a

porphyrin assignments ^b		$(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{DC}^c$					chlorin assignments ^d	
mode no.	sym	$(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{DP}^c$ 406.7 nm	406.7 nm	488.0 nm	514.5 nm	568.2 nm	mode no.	sym
ν_{10} C _a C _m	B _{1g}	1608 dp not obsd	1608 p	1608 p	1608 p	1608	ν_{10} C _a C _m	A
ν_{37} C _b C _b	E _u	1582	1574 p	1574 p	1574 p	1574	ν_{37B} C _b C _b	B
ν_{19} C _a C _m	A _{2g}	f	f	f	1563 dp	1563	ν_{37A} C _b C _b	A
ν_2 C _b C _b	A _{1g}	1566 p	1563 p	1563 p	f	f	ν_{19} C _a C _m	B
ν_{11} C _b C _b	B _{1g}	f	1550 p	1550 p	1550 p	1550	ν_2 C _b C _b	A
ν_{38} C _a C _m	E _u	f	1520 p	1520 ^e p	1520 ^e p	1520	ν_{11} C _b C _b	A
ν_3 C _a C _m	A _{1g}	not obsd	1508	1508 ^e dp	1508 ^e dp	1508	ν_{38A} C _a C _m	A
ν_{28} C _a C _m	B _{2g}	1474 p not obsd	1476 p	1476 p	1478 p	1458	ν_{38B} C _a C _m	B
ν_{29} C _a C _b	B _{2g}	1443 dp f	1456 p	f	f	1440	ν_3 C _a C _m	A
ν_4 C _a N	A _{1g}	not obsd	1388 p	1388 p	1388 p	1400	ν_{39A} C _a C _m	A
ν_{21} $\delta(\text{C}_m\text{H})$	A _{2g}	1368 p not obsd	1367 p	1367 p	1367 p	1367	ν_{39B} or ν_{28} C _a C _m	B
$\nu_5 + \nu_9$	A _{1g}	1231 p not obsd	1231 p	1231 p	1231 p	1232	ν_{29} C _a C _b	B
ν_{13} $\delta(\text{C}_m\text{H})$	B _{1g}	f	1195 dp	1195 dp	1195 dp	1195	ν_{12} C _a N	A
ν_{30}	B _{2g}	f	1180 p	1180 p	1180 p	1180	ν_4 C _a N	A
$\nu_6 + \nu_8$	A _{1g}	1130 p	1127 dp	1128 dp	1128 dp	1128	ν_{41} C _a N	A
ν_{22} C _a N	A _{2g}	1127 ap	1095 p	1095 p	1095 p	1095	ν_{21} $\delta(\text{C}_m\text{H})$	B
							ν_{42A} $\delta(\text{C}_m\text{H})$	A
							$\nu_5 + \nu_9$	A
								B
							ν_{43A} C _b -S	A
							ν_{43B} C _b -S	B
							ν_{22} C _a N	B
							ν_{14} C _a C _b	A

^a>1000 cm^{-1} ; $\rho = I_{\perp}/I_{\parallel}$, p = polarized ($\rho \leq 0.5$; see ref 22), dp = depolarized, ap = anomalously polarized. ^bAdapted from ref 20 and 35. ^cThis work. ^dOn the basis of $C_{2v}(x)$ symmetry; see Table I. ^eResolved in I_{\perp}/I_{\parallel} spectra. ^fNo RR band is apparent near this frequency in DP complexes with Soret excitation; however, Fe^{III} PP complexes do have bands in this vicinity (see Table IV) which were used to aid in the assignments for the DC complexes.

pyrrole and pyrroline C_b-C_b bonds and the elongated M-N bond lengths along the 2-fold (*x*) axis bisecting the pyrroline and the *trans*-pyrrole rings. An effective C_{2v} symmetry for metallochlorins, as suggested by Ozaki et al.¹¹ and others,^{7,15a,30} is disallowed by the pseudo-S₄ ruffle of the chlorin macrocycle. Furthermore, both C_{2v} and C_s symmetry can also be eliminated owing to the known optical activity of metallochlorins.²⁹

On the basis of our $C_{2v}(x)$ symmetry assignment for metallochlorins (Table I), it is possible to predict RR properties which should be observed both for metallochlorin complexes and chlorin-containing proteins. These include the following: (a) all B_{1g} (depolarized) porphyrin vibrational modes should become A (polarized) vibrations; (b) A_{2g} (anomalously polarized) and B_{2g} (depolarized) porphyrin modes should become B (depolarized) modes; and (c) E_u (doubly degenerate) porphyrin modes should be split into A and B components. The alteration in molecular symmetry should further result in an increase in the total number of vibrational bands observed as well as an increase in the number of totally symmetric and, hence, polarized Raman bands, relative to the RR spectrum of an analogous porphyrin complex. Finally, the allowed nature of the metallochlorin transition in the visible region suggests that *only* the Franck-Condon scattering mechanism will be operative. Thus, metallochlorin RR spectra are predicted to have a predominance of polarized vibrational bands when *both* visible and Soret excitation are used. In contrast, for porphyrin complexes, depolarized bands predominate with visible excitation arising from vibronically mixed Herzberg-Teller scattering, whereas polarized RR bands predominate with Soret excitation arising from strongly allowed Franck-Condon scattering.¹⁰

B. Resonance Raman Properties of Iron(III) Deuteriochlorin Complexes. At first glance, the RR spectra of $\text{Fe}^{\text{III}}\text{DC}$ complexes (Figures 3 and 4) appear to be quite similar to those of the analogous $\text{Fe}^{\text{III}}\text{DP}$ complexes (Tables II and III) as the frequencies of the band positions are not greatly different. For example, the dominant RR feature in both iron(III)-chlorin and -porphyrin spectra (406.7-nm excitation) is the oxidation-state marker, ν_4 (1367 cm^{-1} for the hexacoordinate Me_2SO adducts and 1370 cm^{-1}

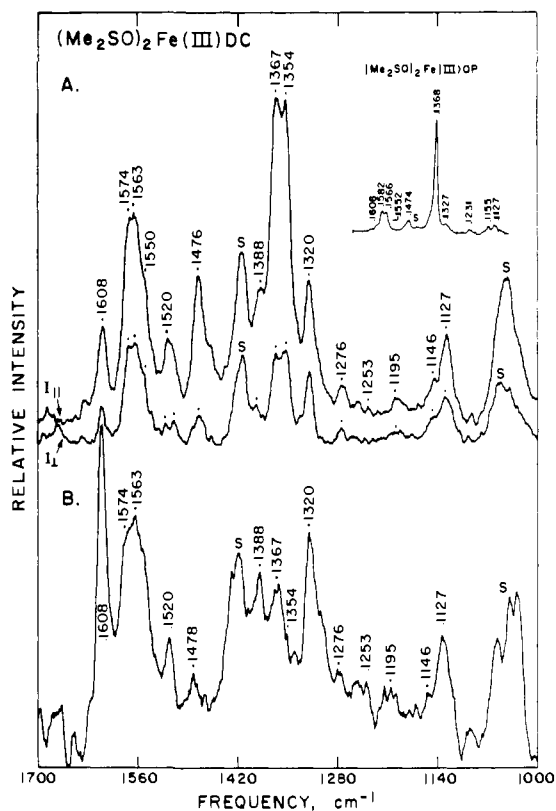


Figure 3. High-frequency RR spectra of $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{DC}$: (A) 406.7-nm excitation, 90° scattering geometry; top, I_{\parallel} , bottom, I_{\perp} ; sample ~ 0.5 mg/mL; conditions laser power 20 mW at sample, slit width 8 cm^{-1} , scanning speed 2 cm^{-1}/s with repetitive scanning (inset: 1 mg/mL $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{DP}$, back-scattering geometry; other conditions as above); (B) 514.5-nm excitation, back-scattering geometry; sample ~ 1.0 mg/mL; conditions laser power 75 mW at sample Dewar, slit width 7.5 cm^{-1} , other conditions as above.

Table III. Resonances Raman Frequencies (cm⁻¹) and Depolarization Ratios (ρ) for ClFe^{III} Deuteriochlorin^a

porphyrin assignments ^b		ClFe ^{III} DP ^c		ClFe ^{III} DC ^c				chlorin assignments ^d	
mode no.	sym	406.7 nm	406.7 nm	457.9 nm	488.0 nm	514.5 nm	568.2 nm	mode no.	sym
ν_{10} C _a C _m	B _{1g}	1629 dp	1631 p	1631	1631 p	1631 p	1631	ν_{10} C _a C _m	A
						1596 dp		?	B
ν_{37} C _b C _b	E _u	<i>e</i>	1588 dp		1588 dp	1588 ^f dp	1588	ν_{37B} C _b C _b	B
ν_2 C _b C _b	A _{1g}	1577 p	1577 p	1577	1577 p	1577 p		ν_2 C _b C _b	A
ν_{19} C _a C _m	A _{2g}	<i>e</i>			1570 dp	1570 ^f dp	1570	ν_{19} C _a C _m	B
ν_{11} C _b C _b	B _{1g}	<i>e</i>	1553 p	1553	1553 p	1553 p		ν_{11} C _b C _m	A
ν_{38} C _a C _m	E _u	1533	1534 p	1534	1534 p	1534 p	1534	ν_{38A} C _a C _m	A
ν_3 C _a C _m	A _{1g}	1484 p	1489 p	1489	1489 p	1489 p	1489	ν_3 C _a C _m	A
ν_{28} C _a C _m	B _{2g}	1463 dp	1462 dp	1462	1462 dp	1462 dp		ν_{28} C _a C _m	B
			1445 ^f dp			1445 ^f dp	1445	ν_{40B} C _a C _b	B
			1442 p		1442 p	1442 p	1442	ν_{40A} C _a C _b	A
ν_{29} C _a C _b	B _{2g}	<i>e</i>	1403 dp	1400	1403 dp	1403	1403	ν_{29} C _a C _b	B
						1398 dp	1398	ν_{20}	B
		not obsd			1384 p	1384 p		ν_{12} C _a N	A
ν_4 C _a N	A _{1g}	1372 p	1370 p	1370	1370 p	1370 p	1370	ν_4 C _a N	A
		not obsd			1355 dp	1355 dp	1355	ν_{41B} C _a N	B
ν_{41} C _a N	E _u	<i>g</i>	1351 p	1351				ν_{41A} C _a N	A
			1326 p	1326	1326 p	1326 p	1326	?	A
			1315 p	1315	1315 p	1315 ^f p		?	A
ν_{21} δ (C _m H)	A _{2g}	<i>e</i>			1309 dp	1310 dp	1309	ν_{21} δ (C _m H)	B
$\nu_5 + \nu_9$	A _{1g}	<i>e</i>	1267 p	1267	1267 p	1267 p		$\nu_5 + \nu_9$	A
			1254 p	1254	1254 p	1254 ^f p	1254	ν_{42A} δ (C _m H)	A
					1240 dp	1240 ^f dp		ν_{42B} δ (C _m H)	B
ν_{13} δ (C _m H)	B _{1g}	1221 dp	1225 p	1225	1225 p	1225 p	1225	ν_{13} δ (C _m H)	A
			1187 dp	1187	1187 dp	1187 dp			B
ν_{30} C _b -S	B _{2g}	<i>e</i>			1155 dp	1155 dp		ν_{30} C _b -S	B
		1149	1143 p		1143 p		1143		A
$\nu_6 + \nu_8$	A _{1g}	1123 p	1126 p		1126 p	1126 p		$\nu_6 + \nu_8$	A
			1111 dp	1115	1115 dp	1115 ^f dp	1118	ν_{44B} C _b -S	B
			1092 p	1092	1092 p	1092 p		ν_{14}	A

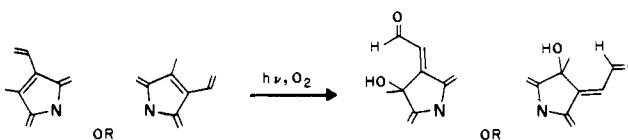
^a >1000 cm⁻¹; $\rho = I_{\perp}/I_{\parallel}$, p = polarized ($\rho \leq 0.5$; see ref 22), dp = depolarized, ap = anomalously polarized. ^b Adapted from ref 20 and 35. ^c This work. ^d On the basis of C_{2v}(x) symmetry. ^e No RR band is apparent near this frequency in DP complexes with Soret excitation; however, Fe^{III}PP complexes do have bands in this vicinity (see Table V) which were used to aid in the assignments for the DC complexes. ^f Resolved in I_⊥/R₁ spectra. ^g 1366 cm⁻¹ (1365-cm⁻¹ IR) Ni^{II}DP, ref 23.

for the pentacoordinate chloride complexes). Other characteristic RR features such as ν_{10} (1608 cm⁻¹ for Me₂SO complexes; 1631 cm⁻¹ for chloride complexes) and ν_3 (1476 cm⁻¹ for Me₂SO complexes; 1489 cm⁻¹ for chloride complexes) are also at similar frequencies in both iron-chlorin and -porphyrin spectra. Thus, the spin states of the iron(III)-chlorin complexes appear to be analogous to those of the iron(III)-porphyrin complexes, suggesting that the RR "spin-state" and "oxidation-state" markers of porphyrins are generally transferable to chlorins.

A closer examination of the Fe^{III}DC spectra reveals an increase in the number of vibrational features, an alteration of relative intensities, and an increase in the number of totally symmetric bands, as compared with the Fe^{III}DP spectra. For example, the 406.7-nm-excitation spectrum of (Me₂SO)₂Fe^{III}DC has at least 10 bands that are absent from the analogous DP spectrum (Table II). Furthermore, both porphyrin B_{1g} and A_{2g} bands have altered polarization properties in the DC spectra, becoming polarized and depolarized features, respectively (Table II). Thus, 13 polarized features occur in the (Me₂SO)₂Fe^{III}DC spectrum in comparison with 5 polarized bands in the DP spectrum (406.7-nm excitation; Table II). In addition, 12 polarized features remain in the 514.5-nm-excitation spectrum of this DC complex, whereas in porphyrin RR spectra, depolarized bands typically predominate upon visible light excitation.¹⁰ The change in depolarization ratio is most clearly evident in the case of ν_{10} which occurs at 1608 cm⁻¹ in both the (Me₂SO)₂Fe^{III}DC and -DP spectra. Given the absence of conjugated substituents to the macrocycle, no obvious assignment other than ν_{10} can be presented for this spectral feature.

The 406.7-nm-excitation RR spectrum of pentacoordinate ClFe^{III}DC has 15 bands not observable in the ClFe^{III}DP spectrum and exhibits 16 polarized RR bands as opposed to 5 polarized bands for the DP complex (Figure 4; Table III). The depolarized ν_{10} of ClFe^{III}DP has become polarized in the -DC spectra (Table III) as have other B_{1g} modes. Anomalously polarized modes of -DP spectra have become depolarized in -DC spectra, and as noted

Scheme I



above for the RR spectra of (Me₂SO)₂Fe^{III}DC, polarized bands also predominate with visible excitation in the ClFe^{III}DC spectra. Our assignments for the vibrational bands of the DC complexes are discussed in detail in section D of this paper.

Comparison of the 488.0-nm-excitation RR spectra of M-OEC¹¹ and M-OEP³⁶ complexes (Figure 5) reveals clear distinctions between the properties of the two types of macrocycles. The band assigned as ν_{10} in the M-OEC spectra is polarized,¹¹ whereas ν_{10} in M-OEP complexes is depolarized. The M-OEC spectra exhibit both a greater total number and more polarized RR features than those of the analogous M-OEP complexes; in addition, they exhibit an altered pattern of relative intensities and a predominance of polarized bands at this excitation wavelength. Another common feature of the RR spectra of the Fe^{III}DC and M-OEC complexes is the presence of one or more "new" polarized bands in the region of the oxidation-state marker, ν_4 .

It is evident that the RR spectral properties of Fe^{III}DC and M-OEC complexes are distinct from those of Fe^{III}DP and M-OEP complexes and reflect a decrease in effective molecular symmetry for the chlorin complexes. Furthermore, a generalized set of chlorin RR properties exists which is consistent with known structural parameters,^{27,28} as well as with the electronic and molecular symmetry properties of chlorins. The vibrational characteristics of metallochlorins outlined above provide criteria which can readily be extended to systems with conjugated substituents such as iron pPP (discussed below), to the chlorophylls, and also to metallochlorin-containing proteins such as *P. aeruginosa* heme *d*₁ and *Escherichia coli* heme *d*.

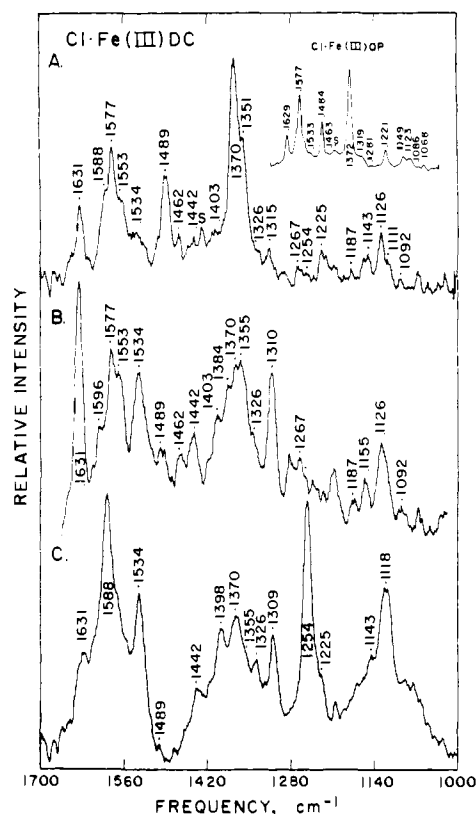


Figure 4. High-frequency RR spectra of $\text{ClFe}^{\text{III}}\text{DC}$: (A) 406.7-nm excitation, back-scattering geometry; sample ~ 1 mg/mL in acetone; conditions, laser power 30 mW at sample Dewar, slit width 5 cm^{-1} , scanning speed $2\text{ cm}^{-1}/\text{s}$ with repetitive scanning (inset: 1 mg/mL $\text{ClFe}^{\text{III}}\text{DP}$ in acetone, other conditions as above); (B) 514.5-nm excitation, back-scattering geometry; sample ~ 1 mg/mL in CH_2Cl_2 ; conditions, laser power 75 mW at sample Dewar, slit width 5 cm^{-1} , other conditions as above; (C) 568.2-nm excitation, back-scattering geometry; sample ~ 1 mg/mL in CH_2Cl_2 ; conditions, laser power 75 mW at sample Dewar, slit width 5 cm^{-1} , other conditions as above.

C. Resonance Raman Properties of Iron(III) Photoporphyrin IX Complexes. The chlorin photoporphyrin IX (pPP) arises from a photochemically induced reduction of the familiar biological prosthetic group protoporphyrin IX (PP).¹⁸ This reaction, which produces two separable isomers (Scheme I), reduces either vinyl-bearing ring A or B of the PP macrocycle and alters the substituents on that ring without affecting the remainder of the macrocycle. The two isomers of $\text{Fe}^{\text{III}}\text{pPP}$, as metallochlorins related to $\text{Fe}^{\text{III}}\text{PP}$, are excellent models for study of the prosthetic group structure of chlorin-containing proteins. They offer the advantages of a clearly defined structure with a known site of reduction, a single conjugated vinyl substituent cis to the reduced ring, which enables us to probe the effect of such substituents on chlorin vibrational properties, and a substituent to the reduced ring having both aldehydic and vinylic character, which may also conjugate with the macrocyclic system.

In comparing the RR spectral properties of $\text{Ni}^{\text{II}}\text{PP}$ and $\text{Ni}^{\text{II}}\text{OEP}$ complexes, Choi et al.³⁴ noted that "vinyl groups do not alter basic skeletal mode symmetries (ρ ratios) or enhancement patterns". The main effects of the two conjugated vinyl substituents on RR spectra of $\text{Ni}^{\text{II}}\text{PP}$ were to induce new vinyl vibrational modes and to produce RR active E_u modes (only IR active for M-OEP complexes). Thus, the effective symmetry of M-PP complexes as observed by RR spectroscopy remains $\sim D_{4h}$ although the actual molecular symmetry is much lower. Comparison of the RR spectra of 2-vinyl and 4-vinyl $\text{Ni}^{\text{II}}\text{PP}$ ³⁴ to those of 2-acetyl and 4-acetyl nickel(II) diacetylporphyrin²³ also revealed no change in depolarization ratios. These observations indicate that conjugated vinyl and/or carbonyl substituents in a cis disposition have little or no effect on the apparent D_{4h} molecular symmetry of porphyrins or on the observed types of RR active modes (with

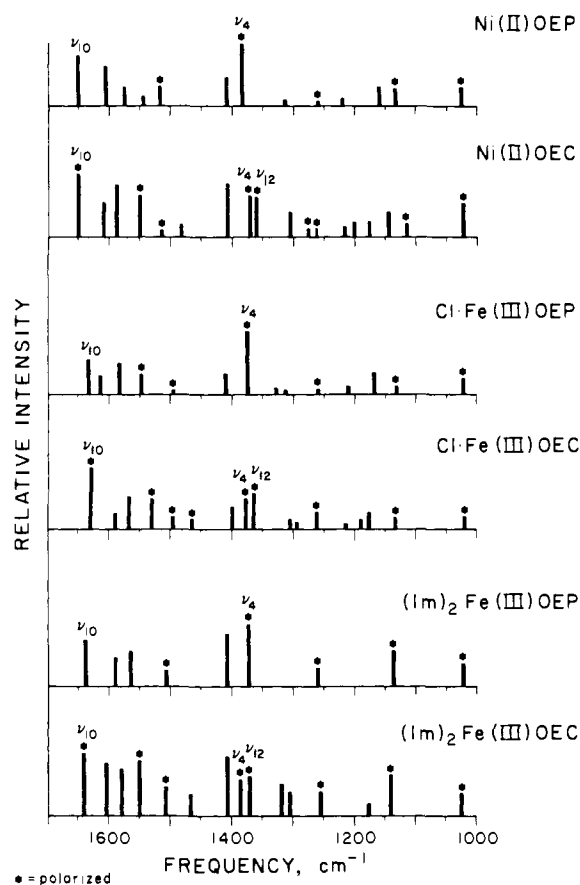


Figure 5. High-frequency RR characteristics of metalloctaethylporphyrins and metalloctaethylchlorins. All spectra were obtained with 488.0-nm excitation. Data for M-OEP complexes adapted from ref 36; data for M-OEC complexes adapted from ref 11. An asterisk indicates polarized (totally symmetric) vibrational bands.

the exception of new vibrational modes of the substituents themselves and the activation of E_u modes).

The above results are important in considering the RR properties of $\text{Fe}^{\text{III}}\text{pPP}$ complexes. The pPP macrocycle has a vinyl substituent cis to a 2'-oxoethylidene (carbonyl- and vinyl-type) substituent on the reduced ring of the macrocycle (see Figure 1B). As discussed above, these substituents alone are not expected to alter the observed depolarization ratios of porphyrin vibrational modes. However, one characteristic of chlorin RR spectra is an alteration of the skeletal mode symmetries relative to those of the analogous porphyrins, reflecting a decreased effective molecular symmetry for the chlorins. Thus, for RR spectra of $\text{Fe}^{\text{III}}\text{pPP}$ complexes, changes in polarization properties of the vibrational bands relative to those of $\text{Fe}^{\text{III}}\text{PP}$ complexes can be expected to be a consequence of the reduced pyrrole ring and cannot logically be attributed to the new 2'-oxoethylidene substituent.

Comparison of RR spectral parameters for $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{pPP}$ and $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{PP}$ complexes (Table IV) reveals many similarities in frequency. However, the RR spectra of $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{pPP}$ (Figure 6) have many new bands, including two flanking the oxidation state marker, not observed in the analogous PP spectra. In addition, the pPP spectra exhibit 11 polarized bands, as compared with 6 polarized features in PP spectra, and both B_{1g} and A_{2g} porphyrin modes have altered polarization properties in the pPP spectra. These general characteristics are consistent with those observed for the $\text{Fe}^{\text{III}}\text{DC}$ and M-OEC complexes. Furthermore, the $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{pPP}$ spectra also have bands at frequencies associated with conjugated substituents in RR spectra of porphyrin complexes: at 1654 cm^{-1} , which we assign as $\nu(\text{C}=\text{O})$ of the 2'-oxoethylidene substituent on the reduced ring B; at 1624 cm^{-1} , assigned as $\nu(\text{C}=\text{C})$ of the vinyl substituent on ring A; and at 1335 cm^{-1} , assigned as a scissors mode [$\delta_s(2)$] of the vinyl substituent. These assignments will be

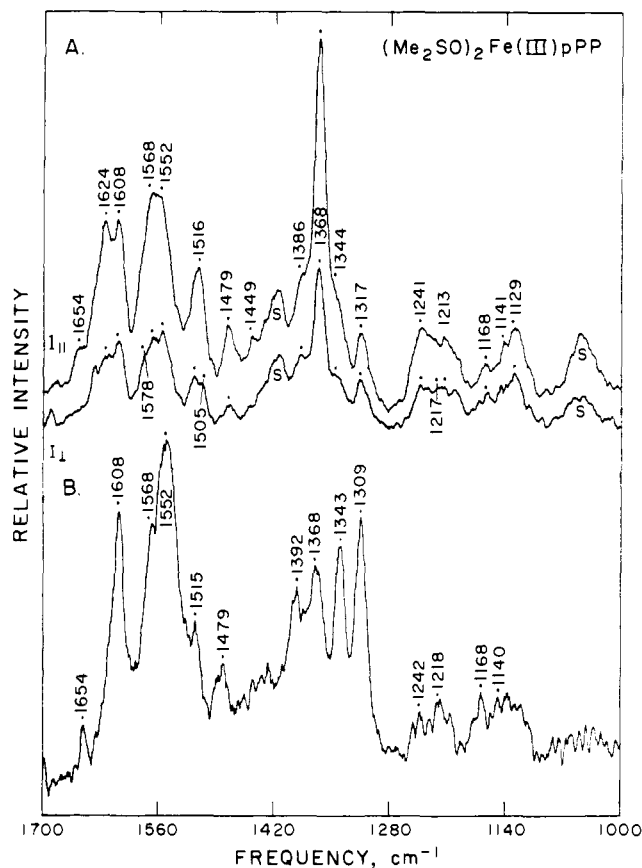


Figure 6. High-frequency RR spectra of (Me₂SO)₂Fe^{III}pPP: (A) 406.7-nm excitation, 90° scattering geometry; top I_{||}, bottom I_⊥; sample ~0.5 mg/mL; conditions, laser power 25 mW at sample, slit width 7.5 cm⁻¹, scanning speed 2 cm⁻¹/s with repetitive scanning; (B) 514.5-nm excitation, back-scattering geometry; sample ~1 mg/mL; conditions, laser power 75 mW at sample Dewar, slit width 7.5 cm⁻¹, other conditions as above.

discussed in more detail in section D.

Although resonance Raman spectra of ClFe^{III}pPP initially suggest a general similarity to the spectrum of ClFe^{III}PP (Figure 7; Table V), a closer study reveals at least 8 bands in the pPP spectra that are absent from the PP spectra. These include an IR-active (1660 cm⁻¹; Figure 8) feature at ~1661 cm⁻¹ that we assign as ν(C=O) of the 2'-oxoethylidene substituent and two new polarized "chlorin" bands flanking the oxidation-state marker. Again, as in the case of Fe^{III}DC and M-OEC complexes, the pPP spectra reveal altered polarization properties relative to those of the analogous PP features. This is particularly noticeable for ν₁₀, a C_aC_m stretching mode that is typically depolarized in porphyrin spectra.¹⁰ Polarized vibrational modes dominate even with visible excitation of the ClFe^{III}pPP complex; again, this is a property not observed for the ClFe^{III}PP complex.

The vibrational characteristics of Fe^{III}pPP complexes are clearly distinct from those of Fe^{III}PP complexes and are highly analogous to those observed for both Fe^{III}DC and M-OEC complexes. These observations enable us to define generalized parameters for the RR spectra of metallochlorin complexes relative to those of analogous metalloporphyrins: (a) an increase in the total number of RR bands; (b) an increase in the total number of polarized (totally symmetric) RR bands; (c) the presence of one or more new polarized "chlorin" bands in the region of the oxidation-state marker, ν₄; (d) altered polarization properties for B_{1g} and A_{2g} modes, a polarized ν₁₀ (>1600 cm⁻¹) being particularly noticeable; (e) slight decreases in frequency for ν₄, the oxidation-state marker; (f) a polarized feature at ~1241 cm⁻¹ that increases dramatically in intensity with increasing excitation wavelengths; and (g) pre-dominance of polarized vibrational modes with visible excitation.

D. Assignment of Fe^{III}DC and Fe^{III}pPP Vibrational Bands. On the basis of the observed frequencies and polarization properties

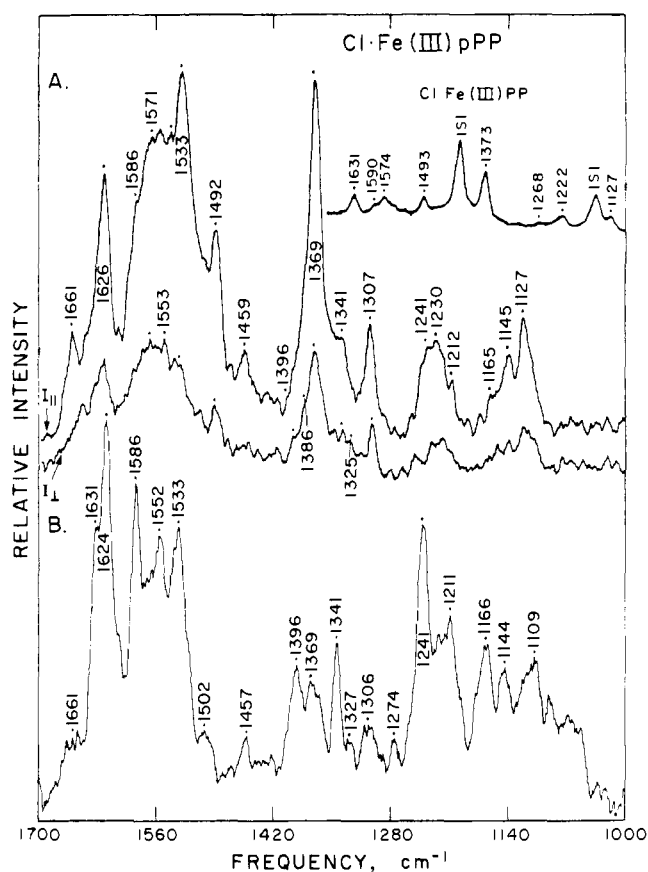


Figure 7. High-frequency RR spectra of ClFe^{III}pPP: (A) 406.7-nm excitation, 90° scattering geometry; top I_{||}, bottom I_⊥; sample ~0.5 mg/mL in CCl₄; conditions, laser power 20 mW at sample, slit width 7.5 cm⁻¹, scanning speed 2 cm⁻¹/s with repetitive scanning (inset: ClFe^{III}PP, 1 mg/mL in CH₂Cl₂, 406.7-nm excitation, back-scattering geometry, other conditions as above); (B) 568.2-nm excitation, back-scattering geometry; sample ~1 mg/mL in CCl₄; conditions, laser power 75 mW at sample Dewar, slit width 3 cm⁻¹, other conditions as above.

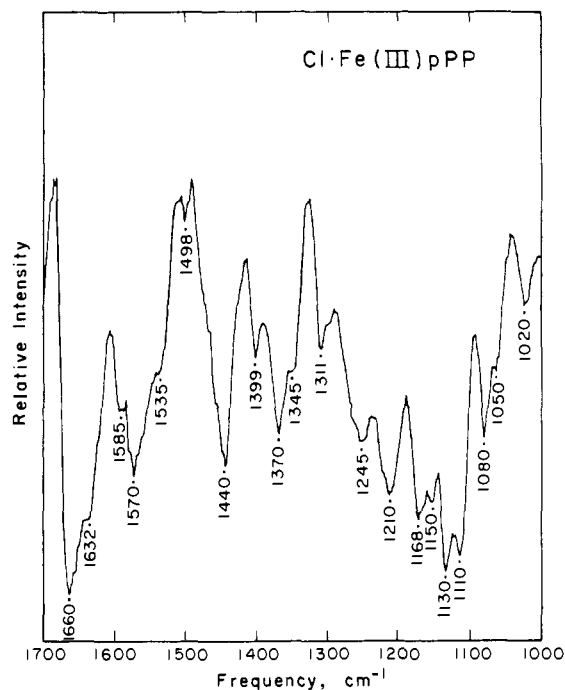


Figure 8. High-frequency IR spectrum of ClFe^{III}pPP.

of the DC and pPP vibrational bands and our assignment of an effective C_{2v}(x) symmetry for metallochlorins (Table I), we have assigned the Raman bands of the iron(III) chlorins using the mode

Table IV. Resonance Raman Frequencies (cm^{-1}) and Depolarization Ratios (ρ) for $(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}$ Photoporphyrin^a

porphyrin assignments ^b		$(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{PP}^d$				chlorin assignments ^e	
mode no.	sym	$(\text{Me}_2\text{SO})_2\text{Fe}^{\text{III}}\text{PP}^c$	406.7 nm	514.5 nm	568.2 nm	mode no.	sym
		not obsd	1654 p	1654 p	1654	$\nu(\text{C}=\text{O})$	A
		not obsd	1638 dp			?	B
$\nu(\text{C}=\text{C})$		1621 p	1624 p	not obsd	not obsd	$\nu(\text{C}=\text{C})$	
ν_{10}	B_{1g}	1610 dp	1608 p	1608 p	1608	ν_{10}	A
ν_{37}	E_u	1580	1578 dp			ν_{37B}	B
		not obsd	1568 p	1568 p	1568	ν_{37A}	A
ν_2	A_{1g}	1559 p			1558	ν_2	A
ν_{19}	A_{2g}	1560 ap	1552 dp	1552 dp	1552	ν_{19}	B
ν_{11}	B_{1g}	1545 dp	not obsd	not obsd	not obsd		
ν_{38}	E_u	1518	1516 p	1516 p	1517	ν_{38A}	A
		not obsd	1505 dp	1505 ^f dp		ν_{38B}	B
ν_3	A_{1g}	1480 p	1479 p	1479 p		ν_3	A
ν_{28}	B_{2g}	1447 dp	1449 dp	1450 dp	1450	ν_{28}	B
$\delta_s(\text{=CH}_2)_{(1)}$		1429 dp	not obsd	not obsd	not obsd		
ν_{29}	B_{2g}	1392 dp	not obsd	not obsd	1396	ν_{29}	B
ν_{20}	A_{2g}	1389 ap	not obsd	1392 dp	not obsd	ν_{20}	B
		not obsd		1386 ^f p	1386	ν_{12}	A
ν_4	A_{1g}	1370 p	1368 p	1368 p	1368	ν_4	A
		not obsd	1344 p	1343 p	1343	ν_{41A}	A
$\delta_s(\text{=CH}_2)_{(2)}$		<i>g</i>		1335 ^f dp		$\delta_s(\text{=CH}_2)_{(2)}$	
ν_{21}	A_{2g}	1313 ap	1317 dp	1317 ^f dp	1315	ν_{21}	B
				1309 ^f p			
$\delta_s(\text{CH}=\text{)}$		1311 dp					
$\nu_5 + \nu_9$	A_{1g}	1239 p	not obsd	not obsd	not obsd		
		not obsd	1241 p	1242 p	1243	ν_{42a}	A
			1217 dp	1218 dp	1217	ν_{42B}	B
ν_{13}	B_{1g}	1225 dp					
			1213 dp	1213 ^f dp			
ν_{30}	B_{2g}	1170 dp	1168 dp	1168 dp	1169	ν_{30}	B
			1141 p	1140 p			
			1129 dp		1129		
$\nu_6 + \nu_8$	A_{1g}	1130 p		1118 dp	1117		

^a $>1000 \text{ cm}^{-1}$; $\rho = I_{\perp}/I_{\parallel}$, p = polarized ($\rho \leq 0.6$; see ref 22), dp = depolarized, ap = anomalously polarized. ^b Adapted from ref 20 and 35. ^c Reference 20, data obtained at several excitation wavelengths. ^d This work. ^e On the basis of $C_2(x)$ symmetry. ^f Resolved in I_{\perp}/I_{\parallel} spectra. ^g $\sim 1340 \text{ cm}^{-1}$, ap, in $\text{ClFe}^{\text{III}}\text{PP}$ (see Table V).

Table V. Resonance Raman Frequencies (cm^{-1}) and Depolarization Ratios (ρ) for ClFe^{III} Photoporphyrin^a

porphyrin assignments ^b		$\text{ClFe}^{\text{III}}\text{PP}^d$						chlorin assignments ^e	
mode no.	sym	$\text{ClFe}^{\text{III}}\text{PP}^c$	406.7 nm	457.9 nm	488.0 nm	514.5 nm	568.2 nm	mode no.	sym
		not obsd	1661 p	1660 p	1660	1662	1661 p	$\nu(\text{C}=\text{O})$	
		1626 p					1631	$\nu(\text{C}=\text{C})$	
ν_{10}	B_{1g}	1626 dp	1626 p	1625 p	1626	1626	1624 p	ν_{10}	A
ν_{37}	E_u	1591	1586 p	1585 p	1585	1586	1586 p	ν_{37A}	A
ν_2	A_{1g}	1570 p	1571 p	1571 p	1570	not obsd	not obsd	ν_2	A
ν_{19}	A_{2g}	1571 ap	not obsd	not obsd	1558	1558	1558 ^f dp	ν_{19}	B
ν_{11}	B_{1g}	1553 dp	1553 p	1553 p	1554	1553	1552 p	ν_{11}	A
ν_{38}	E_u	1533	1533 p	1533 p	1533	1533	1533 p	ν_{38A}	A
		not obsd	1504	1504 dp	1503	1503	1502	ν_{38B}	B
ν_3	A_{1g}	1491 p	1492 p	1493		1494	1491 ^f p	ν_3	A
ν_{39}	E_u	not obsd	1459 p	1459 p	1459		1457 p	ν_{39A}	A
ν_{28}	B_{2g}	1453 dp	not obsd	not obsd	not obsd	not obsd	not obsd		
$\delta_s(\text{=CH}_2)_{(1)}$		1435 p	not obsd	not obsd	not obsd	not obsd	not obsd		
ν_{29}	B_{2g}	1403 dp	1396 dp	1395 dp	1395	1395	1396 dp	ν_{29} or ν_{40B}	B
ν_{20}	A_{2g}	1403 ap							
ν_{12}	B_{1g}	not obsd	1386 p	1388 p	1386	1386	1386 ^f p	ν_{12}	A
ν_4	A_{1g}	1373 p	1369 p	1369 p	1369	1369	1369 p	ν_4	A
ν_{41}	E_u	not obsd	1341 p	1341 p	1340	1341 p	1341 p	ν_{41A}	A
$\delta_s(\text{=CH}_2)_{(2)}$		1340 ap	1325	1326	1325	1327	1327 dp	$\delta_s(\text{=CH}_2)_{(2)}$	
ν_{21}	A_{2g}	1309 ap	1307	1307 dp	1305	1306	1306 dp	ν_{21}	B
$\delta(\text{CH}=\text{)}$		1308 dp							
ν_{42}	E_u	not obsd			1272	1274	1274 dp	ν_{42B}	B
$\nu_5 + \nu_9$	A_{1g}	1260 p	not obsd	not obsd	not obsd	not obsd	not obsd		
		not obsd	1241 p	1241 p	1240	1241	1241 p	ν_{42A}	A
ν_{13}	B_{1g}	1228 dp	1230 p						
		not obsd	1212 p	1212 p	1212	1212	1211 p		
ν_{30}	B_{2g}	1170 dp	1165 dp	1165	1164	1165	1166 dp	ν_{30}	B
ν_{43}	E_u	not obsd	1145 p			1144 p	1145 p	ν_{43A}	A
$\nu_6 + \nu_8$	A_{1g}	1130 p	1127 p	1127	1127			$\nu_6 + \nu_8$	A
ν_{22}	A_{2g}	1127 ap	not obsd	not obsd	not obsd	not obsd	not obsd		

^a $>1000 \text{ cm}^{-1}$; $\rho = I_{\perp}/I_{\parallel}$, p = polarized ($\rho \leq 0.6$; see ref 22), dp = depolarized, ap = anomalously polarized. ^b Adapted from ref 20 and 35. ^c Reference 20, data obtained at several excitation wavelengths. ^d This work. ^e On the basis of $C_2(x)$ symmetry. ^f Resolved in I_{\perp}/I_{\parallel} spectra.

numbering system of Abe et al.³⁵ Structural and electronic modifications of a metallochlorin relative to a metalloporphyrin could, of course, alter the specific contributions to a given vibrational mode.

1. Macrocylic Substituents. As stated previously, the Fe^{III}DC complexes have no conjugated substituents that could be expected to contribute to the high-frequency RR spectra. This fact has been very useful in our assignment of the Fe^{III}pPP vibrational bands and has enabled us to separate substituent vibrations from those of the macrocycle itself. Both the Me₂SO and chloride adducts of Fe^{III}pPP have a vibrational feature above 1650 cm⁻¹ that is absent from the Fe^{III}DC spectra. We have assigned this IR active band (Figure 8) as $\nu(\text{C}=\text{O})$ of the 2'-oxoethylidene substituent, noting that it is in agreement with reported frequencies for $\nu(\text{C}=\text{O})$ in heme *a* complexes³⁴ and for the formyl substituent of chlorophyll *b*.^{15a} Observation of this feature in the RR and IR spectra of Fe^{III}pPP complexes indicates that the substituent on the reduced ring is in conjugation with the remainder of the macrocycle.

The Fe^{III}pPP complexes also have a conjugated vinyl substituent on ring A of the macrocycle which is cis to the reduced ring. Choi and co-workers^{20,24} assigned vibrational modes arising from vinylic substituents. These include $\nu(\text{C}=\text{C})$ at $\sim 1621\text{--}1626\text{ cm}^{-1}$ for high-spin Fe^{III}PP complexes; $\delta_s(1)$ at $\sim 1429\text{--}1435\text{ cm}^{-1}$ and $\delta_s(2)$ at $\sim 1340\text{ cm}^{-1}$ (vinyl scissors modes); and $\delta(\text{CH}=\text{C})$ at $\sim 1308\text{--}1311\text{ cm}^{-1}$.^{20,24} Of these bands, only $\nu(\text{C}=\text{C})$ is polarized and is thus observable with Soret and near-Soret excitation in porphyrins; the other vinyl modes are depolarized or anomalously polarized and are observable with visible excitation.^{20,24} In the case of monovinyl heme *a* complexes, only one of the two vinyl scissors modes was observable, $\delta_s(2)$ at $\sim 1335\text{--}1340\text{ cm}^{-1}$.³⁴ Examination of the (Me₂SO)₂Fe^{III}pPP spectra (Figure 6; Table IV) reveals bands at 1624 and 1335 cm⁻¹ that are absent from the (Me₂SO)₂Fe^{III}DC spectra (Figure 3; Table II). We assign the 1624-cm⁻¹ feature in the (Me₂SO)₂Fe^{III}pPP spectra as $\nu(\text{C}=\text{C})$ and the feature at $\sim 1335\text{ cm}^{-1}$ as the second vinyl scissors mode, $\delta_s(2)$. No evidence of $\delta_s(1)$ or $\delta(\text{CH}=\text{C})$ is seen in the (Me₂SO)₂Fe^{III}pPP spectra. The $\delta(\text{CH}=\text{C})$ mode cannot be assigned to the 1309-cm⁻¹-polarized band (Table IV) since (Me₂SO)₂Fe^{III}DC spectra (Table II) also have a polarized band at 1308 cm⁻¹.

For the ClFe^{III}pPP spectra, $\nu(\text{C}=\text{C})$ is not clearly observable. There is an intense, polarized band at $\sim 1626\text{ cm}^{-1}$ which persists in spectra obtained with different excitation frequencies (Table V). However, we assign this band as ν_{10} , which has become polarized in the metallochlorin complexes because of the decreased effective molecular symmetry, as in the ClFe^{III}DC spectra ($\sim 1631\text{ cm}^{-1}$) and the ClFe^{III}OEC spectra ($\sim 1629\text{ cm}^{-1}$).¹¹ Since $\nu(\text{C}=\text{C})$ was observable in 406.7-nm-excitation spectra of (Me₂SO)₂Fe^{III}pPP (Figure 6; Table IV) along with a polarized ν_{10} at $\sim 1608\text{ cm}^{-1}$, we suggest that $\nu(\text{C}=\text{C})$ in the ClFe^{III}pPP complexes corresponds with the shoulder at 1631 cm⁻¹ (1632 cm⁻¹ IR; Figure 8) and is thus hidden by the intense ν_{10} at $\sim 1626\text{ cm}^{-1}$. As in the (Me₂SO)₂Fe^{III}pPP spectra, the ClFe^{III}pPP spectra give no evidence of either $\delta_s(1)$ or $\delta(\text{CH}=\text{C})$; only $\delta_s(2)$, although weak, is repeatedly observable at $\sim 1325\text{ cm}^{-1}$ (Table V).

2. Porphyrin A_{1g} and B_{1g} Modes. As expected for an effective C₂(x) symmetry, all of the A_{1g} vibrational modes of Fe^{III}PP and Fe^{III}DP complexes remain polarized in the Fe^{III}pPP and Fe^{III}DC spectra (Tables II–V). The features assigned as ν_2 and ν_3 in the pPP and DC spectra are close in frequency to the analogous porphyrin modes. However, in both the DC and pPP spectra, the frequency of ν_4 is slightly decreased relative to the corresponding DP and PP feature. From our analysis, the oxidation state markers of *P. aeruginosa* heme *d*₁,^{12,13} myeloperoxidase,^{7,8} bovine spleen green heme protein,⁸ sulfmyoglobin,³ and M-OEC chlorins¹¹ are also all decreased in frequency relative to those of iron porphyrin complexes. As discussed by Carey,³⁷ RR peak positions are a

property solely of the electronic ground state of the molecule under investigation, while RR peak intensities are related to the excited electronic state of the molecule. Thus, our observation of altered band positions for metallochlorin RR spectra reflects differences in the ground electronic states of chlorins relative to porphyrins. Furthermore, as clearly shown in Figures 5 and 7, the relative intensities of the metallochlorin RR bands also differ from those of the analogous metalloporphyrin, indicating differences between the excited electronic states of chlorins and porphyrins.

The DC and pPP spectra (Tables II–V) have vibrational bands that correspond quite well in frequency to the B_{1g} vibrational modes of DP and PP complexes. These include ν_{10} , ν_{11} , and in some cases ν_{13} . However, while each of these bands is depolarized in the DP and PP spectra, the corresponding band is strongly polarized in the DC and pPP spectra. This observation reflects the alteration of molecular symmetry in the iron chlorins relative to that of iron porphyrin complexes and, furthermore, is in agreement with the symmetry properties we predicted for a metallochlorin (Table I). Of additional interest is the observation of the new strongly polarized "chlorin band", ν_{12} , at $\sim 1386\text{ cm}^{-1}$ in both the DC and pPP spectra (Figures 3, 4, 6, and 7; Tables II–V). The $\sim 1386\text{ cm}^{-1}$ feature cannot be assigned as ν_{20} ($\sim 1400\text{ cm}^{-1}$, A_{2g} in porphyrins¹⁰) since an A_{2g} mode would be expected to become depolarized for the effective C₂(x) symmetry of metallochlorins (Table II). This is not the first observation of ν_{12} in metallochlorin RR spectra; Ozaki et al.¹¹ assigned ν_{12} in their study of M-OEC complexes as a $\nu(\text{C}_a\text{N})$ mode based on its frequency shift with ¹⁵N substitution. A second new "chlorin" band, ν_{41A} , is discussed in section 3, below.

3. Porphyrin E_u (Doubly Degenerate) Modes. The loss of x,y equivalence in a metallochlorin (Figure 2) should result in splitting of degenerate E_u porphyrin modes into two components which differ in their polarization properties (Table I). This phenomenon is observed in the (Me₂SO)₂Fe^{III}DC spectra for 9 bands which we have assigned in Table II, and it is also apparent in the Cl-Fe^{III}DC spectra (Table III). For the (Me₂SO)₂Fe^{III}pPP and ClFe^{III}pPP spectra, ν_{37A} and ν_{37B} , ν_{38A} and ν_{38B} , ν_{40A} , ν_{41A} , ν_{42A} and ν_{42B} , and ν_{43A} are identifiable (Tables IV and V). In each case, the polarized component is clearly observable, whereas the depolarized component is either absent or weak, as expected if a Franck–Condon scattering mechanism is dominant. The $\sim 1240\text{ cm}^{-1}$ -polarized component of ν_{42} is noteworthy since a strong feature in this spectral region is not typically observed in heme proteins or porphyrin complexes. Furthermore, a strong band at $\sim 1250\text{ cm}^{-1}$ occurs in the IR spectrum of ClFe^{III}pPP (Figure 8). This band is also clearly evident in the RR spectra of M-OEC complexes¹¹ as well as in the RR spectra of SMb³ and *P. aeruginosa* heme *d*₁.^{12,13} and in spectra of the highly reduced nickel tetrapyrrole, F-430.³⁸ Also, ν_{42A} increases in intensity with increasing excitation wavelengths in the DC and pPP spectra (Figures 3–6) as well as in the RR spectra of SMb complexes.³ The assignment of ν_{42A} as a methine carbon–hydrogen deformation mode [$\delta(\text{C}_m\text{H})$] might appear to be inconsistent with its intensity in DC and pPP spectra since such modes are generally only weakly Raman active. However, it is known that the electron density at the methine carbons adjacent to the pyrroline ring of chlorins is greatly increased relative to that of porphyrins;²⁹ such a phenomenon might possibly enhance the Raman activity of ν_{42} .

The strongly polarized "chlorin" band at $\sim 1340\text{ cm}^{-1}$ in the pPP spectra ($\sim 1345\text{ cm}^{-1}$ IR; Figure 8) and $\sim 1350\text{ cm}^{-1}$ in the DC spectra (Figures 3–6, Tables II–V) has been assigned as ν_{41A} , a vibrational stretching mode of C_a–N bonds. We can be confident that this band is a real feature of the DC and pPP spectra based both on its reproducibility and on the identity of the electronic absorption spectra of our DC and pPP samples before and after the Raman experiment. Willems and Bocian²³ have also assigned an IR and Raman active weak band near ν_4 in a series of acetyl-

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and formyl-substituted nickel deuteroporphyrins as ν_{41} (E_u). A strong, clearly polarized band which we assign as ν_{41A} is also a feature of the RR spectra of Fe^{III}SMb and Fe^{III}SMb,³ MPO,^{6,7} and BGHP.⁷

4. Porphyrin B_{2g} and A_{2g} Modes. For the Fe^{III}DC and Fe^{III}pPP complexes, we predict that both B_{2g} and A_{2g} porphyrin modes should become B-type chlorin modes (Table I). This phenomenon is observed in the RR spectra of Fe^{III}DC complexes for the porphyrin A_{2g} modes ν_{19} and ν_{21} (Tables II and III); however, ν_{20} is not observed. For the Fe^{III}pPP complexes, again ν_{19} and ν_{21} are observable as depolarized bands in the appropriate frequency region although ν_{20} is not observed.

The porphyrin B_{2g} modes ν_{28} , ν_{29} , and ν_{30} are seen in the Fe^{III}DC spectra (Tables II and III). The vibrational band corresponding to ν_{30} occurs in $(Me_2SO)_2Fe^{III}pPP$ and $ClFe^{III}pPP$ spectra. Both ν_{28} and ν_{29} are present in $(Me_2SO)_2Fe^{III}pPP$ spectra, but only ν_{29} occurs in the $ClFe^{III}pPP$ spectra (Tables IV and V). These A_{2g} and B_{2g} porphyrin modes are generally only weak features (B, depolarized) in the chlorin spectra, owing to the dominant Franck-Condon scattering mechanism.

In summary, the assignments for the Fe^{III}DC and Fe^{III}pPP vibrational bands (Tables II-V) concur with our predictions for a metallochlorin having an effective $C_2(x)$ symmetry. Both the disparities between metallochlorin and metalloporphyrin RR properties and the many general similarities between Fe^{III}DC, Fe^{III}pPP, and M-OEC RR properties indicate that it is indeed possible to distinguish between porphyrins and chlorins by RR spectroscopy alone. Furthermore, we have presented a set of identifying characteristics for metallochlorin RR spectra that provide new structural information when applied to the green heme proteins.

E. Application to Green Heme Systems. Observation of vibrational modes arising from conjugated substituents to the macrocycle in metallochlorins is of particular interest. First of all, it establishes that many experimental observations and assignments derived from the study of metalloporphyrins can be successfully extended to the study of metallochlorins. Second, it has implications for structural assignments of the prosthetic groups in the green heme proteins. The chlorin pPP has a single vinyl substituent on ring A (3-vinyl) of its macrocycle, while heme *a* has a single vinyl substituent on ring B (4-vinyl). However, for both systems, only one of the two vinyl scissors modes, $\delta_s(2)$, was observable. This finding suggests that the observation of $\delta_s(2)$ alone is consistent with a single vinyl substituent, rather than being related to the macrocyclic position of vinylic substitution. Furthermore, it also suggests that if both vinyl scissors modes, $\delta_s(1)$ and $\delta_s(2)$, are observed, then *two* vinyl substituents are present.

We have applied these results to the RR spectra of sulfmyoglobin (SMb), myeloperoxidase (MPO), bovine spleen green heme protein (BGHP), and *P. aeruginosa* heme *d*₁.³ The RR spectra of all four green heme proteins exhibit the general characteristics which we have defined as typical of metallochlorins. Heme *d*₁ is known to be an iron chlorin,⁴ and iron chlorin prosthetic groups have been suggested for SMb,¹⁻³ MPO,^{3,6,7} and BGHP.^{3,7} For all four of these proteins, no evidence of $\nu(C=C) > 1600\text{ cm}^{-1}$ is available.^{3,6,7,12,13} We suggest that such a vinyl mode, if present, would most likely be obscured by the strongly polarized ν_{10} feature, as noted for $ClFe^{III}pPP$ (see section D.1.). The SMb spectra have

a distinct feature at 1425 cm^{-1} , in the region assigned by Choi et al.^{20,24} to $\delta_s(1)$, and a weak feature at $\sim 1335\text{ cm}^{-1}$, in the region of $\delta_s(2)$.³ Thus, given that the SMb prosthetic group is known to be derived from the iron PP of myoglobin,^{1,3} we suggest the presence of at least one, and probably both, vinyl substituents on the iron chlorin moiety of SMb. If correct, retention of vinylic conjugation to the SMb prosthetic group implies that the site of ring reduction must be on either of the propionic acid bearing rings. This proposal is supported by preliminary NMR results.³⁹

For both MPO and BGHP, Babcock et al.⁷ used the absence of $\nu(C=C)$ above 1600 cm^{-1} as evidence against vinylic substituents to the covalently attached metallochlorin prosthetic groups. However, as discussed above, $\nu(C=C)$ could easily be hidden under the strongly polarized ν_{10} feature. Furthermore, in the RR spectra of MPO reported by Babcock et al.⁷ and by Sibbett and Hurst,⁶ clearly observable features occur at ~ 1425 and $\sim 1335\text{ cm}^{-1}$, in the regions assigned to vinyl scissors modes.^{20,24} Analogous bands occur in the RR spectra of BGHP.⁷ Thus, given the transferability of metalloporphyrin vinylic modes to metallochlorins, we strongly suggest that both MPO and BGHP have two vinyl substituents in conjugation with their chlorin prosthetic groups. This hypothesis has two important implications for the MPO and BGHP macrocycles. It indicates that the site of reduction on the MPO and BGHP metallochlorin is not on the vinyl-bearing rings since these are in conjugation. It also indicates that the site of covalent linkage of these metallochlorins (believed to be structurally similar⁷) to the protein is most likely not at the typical vinyl group positions 2 and 4, as with cytochrome *c*, but through the substituents on rings C and D. The recent report by Schultz et al.,⁴⁰ suggesting that the MPO prosthetic group is attached to the protein via the propionic acid side chains, supports our analysis.

For *P. aeruginosa* heme *d*₁, the macrocyclic structure has not been completely defined, although alcohol, vinyl, carboxylate, and α -keto substituents have all been suggested.^{5,9,13,41} Examination of the heme *d*₁ RR spectra for vinyl substituents using our analysis of chlorin vinyl vibrations suggests the absence of vinyl groups on the heme *d*₁ macrocycle. This finding is consistent with the recent structural analysis of heme *d*₁ by Timkovich et al.⁹

Although chlorophylls *a* and *b* both have vinyl substituents at the 2-position, Lutz and co-workers observed no bands corresponding to $\nu(C=C)$ and suggested that the vinyls are oriented out of plane.¹⁵ However, their RR data do exhibit a weak feature at $\sim 1335\text{ cm}^{-1}$ in both chl *a* and *b* spectra that is absent in the 2-ethyl chl *d* spectrum; this feature might easily correspond to the vinyl scissors mode, $\delta_s(2)$, as discussed above for other chlorin systems. Further work will be required to clarify this ambiguity.

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