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Protein Control of Porphyrin Conformation. Comparison of Resonance Raman Spectra of Heme Proteins with Mesoporphyrin IX Analogues[1]

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Abstract: Resonance Raman spectra are reported for derivatives of iron mesoporphyrin IX, chosen as analogues for the heme group in heme proteins. Attention is focused on high-frequency porphyrin ring modes which are sensitive to the conformation and electronic structure of the heme group. No protein influence on heme conformation is detectable for low-spin Fe(III) or Fe(II) derivatives nor for high-spin Fe(II) derivatives. For high-spin Fe(III) derivatives, a protein influence is detectable in hemoglobin and myoglobin. The porphyrin frequencies suggest a prophyrin conformation more characteristic of Fe(II) than Fe(III). The heme pocket apparently stabilizes the porphyrin conformation appropriate for high-spin Fe(II) and maintains it on oxidation, thereby contributing to the relatively low oxidation potential of deoxyhemoglobin or myoglobin. Axial ligands of increasing π acid strength bound to Fe(II) hemes progressively increase the frequency of the "oxidation state" marker bands. This behavior supports the view that these frequencies are sensitive to the extent of back donation into porphyrin π^* orbitals of iron π electrons, for which the π-acid ligands compete. A derivative thought to contain four-coordinate Fe(II) gives frequencies similar to low-spin Fe(III), consistent with an intermediate spin configuration, with empty $d_{x^2-y^2}$ and half-filled d_{z^2}, d_{xz}, and d_{yz} orbitals. The Raman spectrum of the $(py)_2Fe^{II}$ complex shows vibrational bands of the bound pyridine, enhanced via resonance with a charge-transfer transition at ~475 nm.

The relationship between porphyrin stereochemistry and the biochemical functions of heme proteins continues to be the focus of wide-ranging chemical and physical studies. Previous reports from this laboratory have catalogued the resonance Raman spectra of a number of heme proteins.[2] Observed shifts in porphyrin ring vibrational frequencies have been interpreted[3] in terms of known principles of heme stereochemistry and electronic structure, derived from structural analysis of heme proteins and of metalloporphyrins. In the present study we have examined the resonance Raman spectra of various derivatives of iron mesoporphyrin IX dimethyl ester (abbreviated "MP"), chosen to resemble the prosthetic group of heme proteins in different ligation and oxidation states. The results show that most heme protein resonance Raman spectra are essentially the same as those of the appropriate iron (MP) derivative. In these cases, which include all low-spin hemes as well as five-coordinate high-spin Fe(II) heme, the proteins appear to have negligible influence on the porphyrin conformation. High-spin $Fe^{III}(MP)$ derivatives show spectra appreciably different from those of high-spin Fe(III) hemoglobin or myoglobin derivatives, but they correspond to the spectrum of native horseradish peroxidase, which had previously been thought to be anomalous.[4] The anomaly resides instead in hemoglobin and myoglobin, which apparently constrain the porphyrin to a more highly domed structure. This globin influence on porphyrin conformation may be a factor in stabilizing high-spin Fe(II) heme, which is a requirement for reversible oxygenation.

Experimental Section

Mesoporphyrin IX dimethyl ester was purchased from Sigma Chemical Co. and used without further purification. Dimethylformamide was distilled and stored over molecular sieves. Pyridine (py) was distilled and stored over KOH. Imidazole (Im) and 2-methylimidazole (2-MeIm) were recrystallized from benzene. Other ligands were used as purchased. Carbon tetrachloride, chloroform, and dichloromethane were spectroquality solvents and were used without further purification. D_2SO_4 (99% D) and D_2O were purchased from Stohler Isotope Chemicals and used without further purification.

Mesoporphyrin-*meso*-d_4 IX Dimethyl Ester (MP-d_4). Mesoporphyrin-*meso*-d_4 IX dimethyl ester was prepared by allowing MP to equilibrate in 90% D_2SO_4/D_2O for 12 h.[5] After dilution with H_2O and raising the pH to the isoelectric point with 2 N ammonium hydroxide, the meso deuterated porphyrin was extracted into chloroform. The chloroform layer was washed several times with water to remove acid and allowed to evaporate. Any hydrolyzed porphyrin was re-esterified in H_2SO_4/methanol.[6a] The final product was purified by chromatography on Alumina (Fisher A-540) and recrystallized from chloroform. The visible spectrum agreed with MP and the NMR spectrum showed complete disappearance of the meso protons.[7]

Iron(III) Derivatives. $(X^-)Fe^{III}(MP)$ [X = F, Cl] and their meso-d_4 derivatives were prepared both according to the method of Falk[6b] and the method of Adler et al.[8] Both methods gave similar products as evidenced by their visible[9] and infrared spectra.[9] The final products were purified by chromatography according to the method of Alben et al.[9] and converted to the appropriate halide by heating with the sodium halide in acetic acid. $(Im)_2Fe^{III}(MP)^+$ fluoride and chloride salts, and their meso-d_4 derivatives were prepared according to the methods of Epstein et al.[10] The μ-oxo-bis[$Fe^{III}(MP)$] complex was prepared by shaking a CH_2Cl_2 solution of an iron(III) halide complex with an aqueous solution of 0.01 N sodium hydroxide.

Iron(II) Derivatives. All of the iron(II) derivatives were prepared in a modified Spex spinning cell sealed with a septum. A degassed CH_2Cl_2 solution of an $Fe^{III}(MP)$ halide was overlayered with water containing a small amount of $Na_2S_2O_4$ and an excess of the appropriate ligand. All operations were done in an argon atmosphere. The cell was sealed and shaken vigorously until the CH_2Cl_2 layer turned from brown to red. The CO derivatives were prepared by bubbling CO through the $Fe^{II}(MP)$ solution.

All spectra were recorded on instrumentation reported previously.[11] Concentration of solutions was about 1 mg/ml.

Table I. Resonance Raman Frequencies (cm^{-1}) for Iron Mesoporphyrin IX Dimethyl Ester Complexes[a]

$(X^-)Fe^{III}$-(MP)[b]	$(X^-)Fe^{III}$-(MP-d_4)	$(Im)_2Fe^{III}$-(MP)(X^-)	$(Im)_2Fe^{III}$-(MP-d_4)(X^-)	$[Fe^{III}$-(MP)$]_2O$	$[Fe^{III}$-MP-$d_4)]_2$-O	Fe^{II}-(MP)	(2-MeIm)-Fe^{II}(MP)	$(pip)_2$-Fe^{II}(MP)	Assignment[c]
1632 (dp)	1622 (dp)	1640 (dp)	1637*	1627 (dp)	1622*	1642 (dp)	1606 (dp)	1620 (dp)	$C_\alpha C_m + C_\beta C_\beta$
1588 (p)	1587 (p)	1599 (p)	1596*	1588 (p)	1587*	1596 (p)	1586 (p)	1600 (p)	$C_\beta C_\beta$
1572 (ap)	1561 (ap)	1584 (ap)	1575*	1569 (ap)	1561*	1589 (ap)	1558 (ap)	1583 (ap)	$C_\alpha C_m$
1565 (dp)	1551*	1572 (dp)	1572*	1568 (dp)	1551*	1570 (dp)	1558 (dp)	1537 (dp)	$C_\beta C_\beta$
1495 (p)	1491 (p)	1505 (p)	1503*	1495 (p)	1491*	1506 (p)	1472(p)	1490 (p)	$C_\alpha N$
1409 (dp)	1407 (dp)	1406 (dp)	1405*	1407 (dp)	1407*	1412 (dp)	1406 (dp)	1401 (dp)	$C_\alpha N$
1404 (ap)	1403 (ap)	1403 (ap)	1402*	1403 (ap)	1403*	1408 (ap)	1406 (ap)	1396 (ap)	$C_\alpha N$
1374 (p)	1374 (p)	1375 (p)	1375*	1373 (p)	1372*	1373 (p)	1359 (p)	1358 (p)	$C_\alpha C_\beta + C_\alpha C_m$
1310 (ap)	1211*	1313 (ap)	1223*	1311 (ap)	1209*	1310 (ap)	1312 (ap)	1306 (ap)	$C_\alpha C_\beta + \delta CH$
1225 (dp)	1211*	1223 (dp)	1223*	1222 (dp)	1209*	1224 (dp)	1228 (dp)	1225 (dp)	$C_\alpha C_\beta + \delta CH$
1160** (dp)	1159** (dp)	1160** (dp)	1160*	1159** (dp)	1157*	1159 (dp)	1156 (dp)	1161 (dp)	
1130 (ap)	1129 (ap)	1134	1134*	1133 (ap)	1129*	1131 (ap)	1128 (ap)	1123 (ap)	
1002 (p)		1002 (p)		1000 (p)		997 (p)	993 (p)	1008 (p, br)	
810	805*								
								744 (dp)	
		672*	675*				678 (p)	676 (p)	
				610 (p) (vw)					
581 (p)	579 (p)								Fe–F stretch[d]
				418 (vw)					
377 (vw)							378 (vw)		
345 (vw)		344*	345*				335 (p)		
271*** (vw)		270*	269*				262 (vw)		
							205 (p)		

[a] All data from CH_2Cl_2 solutions except those obtained from KBr pellets, marked with an asterisk; $CHCl_3$ solution, marked with a double asterisk; benzene solution, marked with a triple asterisk. [b] Symbol: X = F, Cl; pip = piperidine; p = polarized, dp = depolarized, ap = anomalously polarized, br = broad, vw = very weak. [c] From ref 3. [d] From ref 24.

Results and Discussion

Tables I and II contain listings of the resonance Raman frequencies of the various derivatives examined, together with the states of polarization of the bands, while Figures 1 and 2 show some representative spectra. Frequencies of the meso-deuterated derivatives are also given in Table I. The deuterium isotope shift reflects the involvement of the meso hydrogens in the vibrations, and will eventually be important in constraining the porphyrin force field, via normal coordinate analysis.[3] In addition to the heme proteins,[11] a number of metalloporphyrins have been studied by Raman spectroscopy,[12] including iron derivatives of octaethylporphyrin.[13] Frequencies of the latter are in accord with the corresponding MP derivatives reported here.

Mesoporphyrin IX dimethyl ester, whose structure is shown in Figure 3, has all eight peripheral substituents joined to the pyrrole rings via saturated carbon atoms (as does octaethylporphyrin). It is a good analogue of the prosthetic group of *c* type cytochromes, in which a terminal hydrogen atom of each of the two ethyl groups of MP is replaced by a sulfur atom of a thioether link to the polypeptide chain. In protoporphyrin IX, which forms the prosthetic group in *b* type cytochromes, hemoglobin, myoglobin, peroxidase, and catalase, the ethyl groups are replaced by vinyl groups. These are conjugated to the π system of the porphyrin ring and induce extra resonance Raman bands.[11] The basic porphyrin vibrational pattern remains unaltered, however. The MP derivatives are, therefore, appropriate models for the protoheme proteins as well, and have the advantage that their Raman spectra are uncluttered by the extra vinyl-induced bands.

Table III compares typical iron MP derivatives with appropriate heme protein derivatives, with respect to the five frequencies, I–V, which have been found to be most diagnostic for the structural concomitants of oxidation and spin state. These correspond to bands A, E, B, C, and F of ref 11 and 14. An additional marker band, D, was originally proposed but apparent variations of this band have since been found to result from its confusion with a nearby vinyl induced mode of protohemes.[3] The comparisons made in Table III are consided separately in the following sections. Figure 4 provides a diagram correlating the positions of bands I, II, IV, and V with oxidation and spin state. This is similar to Figure 4 of ref 4, except that the data for mesoporphyrin analogues have been added, and the examples of ordinary and anomalous high-spin Fe(III) have been reversed (vide infra).

Low-Spin Fe(III). Previous comparisons[2] of heme protein data suggested that for Fe(III) derivatives the porphyrin frequencies are essentially unaffected by the nature of the axial ligands, as long as the axial ligand field is sufficiently strong to maintain a low-spin configuration, for which the iron atom lies in the porphyrin plane. Thus, ferricytochrome *c* and methemoglobin cyanide show very similar frequencies for the structure sensitive bands, although the sixth ligand is a methionine sulfur atom in the former and cyanide ion in the latter. Table III shows that the similarity extends to $(Im)_2$-$Fe^{III}(MP)^+$. There is evidently no significant protein influence on heme structure for low-spin Fe(III) forms.

Low-Spin Fe(II). Table III shows that the structure-sensitive frequencies of $(pip)_2Fe^{II}(MP)$ are essentially the same as those of ferrocytochrome *c* and the cyanide complex of reduced horseradish peroxidase. Again, there is no evidence for protein influence on heme structure.

For low-spin Fe(II), the nature of the axial ligands can influence the positions of bands I, III, and V. Specifically, π-acid ligands shift these bands to higher frequencies. When the ligand is O_2, as in oxyhemoglobin, the shifts are fully as large as those observed for oxidation to low-spin Fe(III) (see Table III). This was the basis for the suggestion by Yamamoto et al.[15] that the Raman frequencies support Weiss' model[16] of oxyhemoglobin as a superoxide complex of Fe(III). Carbon monoxide also produces shifts to higher frequency in hemoglobin, but they are significantly smaller than the O_2 shifts.[17] The same frequencies are observed in the CO adduct of (py)-Fe^{II}(MP), as shown in Table III. Still smaller shifts are found (Table II) for bisphosphite and bisarylphosphine complexes

Table II. Resonance Raman Frequencies (cm^{-1}) for Six-Coordinate Iron(II) Mesoporphyrin IX Dimethyl Ester Complexes[a]

$(Im)_2$ Fe(PP)[b]	$P[n\text{-}Bu]_3)_2$-Fe(MP)	$(P[Et]_3)_2$-Fe(MP)	$P[Ph][Me]_2)_2$-Fe(MP)	$(P[Me][Ph]_2)_2$-Fe(MP)	$(py)_2$Fe-(MP)	$(\gamma\text{-pic})_2$Fe-(MP)	$(P[OEt]_3)_2$-Fe(MP)	(CO)(py)-Fe(MP)	Assignment[c]
1617	1618	1620	1625	1625	1622 (dp)	1621 1617 (γ-pic)	1629 (dp)	1630	$C_\alpha C_m + C_\beta C_\beta$ Ring stretch (ligand)
					1597 (p) (py)			1599 (py)	Ring stretch (ligand)
1600	1599	1600	1602	1599	1594 (p)		1601 (p)		$C_\beta C_\beta$
1583			1589	1586	1582 (ap)	1583	1590 (ap)	1588	$C_\alpha C_m$
1534	1539	1544	1554	1554	1555 (dp)	1553	1559 (dp)	1567	$C_\beta C_\beta$
1491	1494		1496	1496	1493 (p)	1493	1496	1497	$C_\alpha N$
					1402 (dp)			1409	$C_\alpha N$
1398					1397 (ap)	1401	1405		$C_\alpha N$
1358	1358	1358	1363	1363	1365 (p)	1364	1368	1371	$C_\alpha C_\beta + C_\alpha C_m$
			1308		1310 (ap)	1309		1314	$C_\alpha C_\beta + \delta CH$
					1225 (dp)	1225			$C_\alpha C_\beta + \delta CH$
					1215 (p) (py)	1209 (γ-pic)		1215 (py)	In-plane CH def. (ligand)
1170	1161	1161	1161	1159	1160 (dp)	1161	1160	1164	
	1123	1126	1127	1126	1127 (ap)	1127	1132	1133	
					1046 (p) (py)	1063 (γ-pic)		1043 (py)	Ring breathing (ligand)
					1010 (p) (py)	1025 (γ-pic)		1009 (py)	
		1032	1029						
993			999	998		996			
969			944	940					
	901		910	909					
						817 (γ-pic)			Ring breathing (ligand)
	799		802						
	780		786						
744	741				747	746			
673	667	670	670	670	674	674	677	674	
					634 (py)	543 (γ-pic)		633 (py)	In-plane ring def. (ligand)
	386						381 (vw)		
343	346	347	346	346	349 (p)	348	351	352	
					179 (py)	162 (γ-pic)		180 (py)	
	128	143	170	169			140		

[a] Spectra recorded in CH_2Cl_2. [b] Spectrum recorded in 0.01 N NaOH with $Na_2S_2O_4$ as a reducing agent. Symbols: PP = protoporphyrin IX; $P(n\text{-}Bu)_3$ = tri(*N*-butyl)phosphine; $P(Et)_3$ = triethylphosphine; $P(Me_2Ph)$ = dimethylphenylphosphine; $P(MePh_2)$ = diphenylmethylphosphine; γ-pic = γ-picoline or 4-methylpyridine; $P(OEt)_3$ = triethylphosphite; p = polarized; dp = depolarized; ap = anomalously polarized; vw = very weak. [c] From ref 3. For pyridine and γ-picoline assignments, see Table V.

of Fe^{II}(MP) while for bispiperidine, bisimidazole, and bisalkylphosphine derivatives, the frequencies are slightly below those of the bispyridine derivative. There is a reasonable correlation between the π-acid strength of the axial ligands and bands I, III, and V as shown in Table IV. This correlation supports the previous suggestion[11] that the frequencies of bands I and band III decrease with increasing electron back-donation from iron d_{xz} and d_{yz} orbitals (*z* is the direction perpendicular to the porphyrin plane) to the π^* antibonding porphyrin orbitals. Axial ligands which are π acids compete for the same electrons, thereby increasing the porphyrin frequencies. In this context O_2 is evidently a very effective π acid, and reduces back-donation to the porphyrin ring as much as does oxidation to low-spin Fe(III). Whether on that account the O_2 adduct should be regarded as a superoxide complex of Fe(III) remains problematical and at least partly a matter of semantics.[18-20]

Enhanced Vibrations of Bound Pyridine. Extra Raman bands are observed for $(py)_2$- and $(4\text{-Me(py)})_2Fe^{II}$(MP) which are attributable to vibrations of coordinated pyridine (see Figure 5). The frequencies are compared to those of free pyridine in Table V. These bands appear to be maximally enhanced by excitation at 4765 Å, which falls near a shoulder in the absorption spectrum, assigned to Fe(II) → pyridine charge transfer.[21]

Only two axial ligand modes have previously been observed in heme resonance Raman spectra. One is the Fe-O stretch in oxyhemoglobin found at 567 cm^{-1} by Brunner.[22] Its enhancement is probably due to Fe → O_2 charge transfer transitions which underly the intense porphyrin π-π^* band, as shown by Makinen and Eaton.[23] The other is the Fe-F stretch[24] in $(F^-)Fe^{III}$(MP), discussed below. The present observation of bound pyridine modes gives hope that a search with other laser frequencies, particularly extending into the ultraviolet region, may reveal enhanced vibrations of other axial ligands. Further details of the pyridine enhancement will be published elsewhere.

High-Spin Fe(II). When a CH_2Cl_2 solution of Cl^-- or F^-Fe^{III}(MP) and 2-methylimidazole is shaken with aqueous dithionite, the CH_2Cl_2 layer changes color. Its Raman and absorption spectra are shown in Figures 2 and 6, respectively. The visible spectrum is essentially the same as that reported for high-spin ferrous hemes[25] and heme proteins.[2] The Raman spectrum shows the structure-sensitive bands to be at frequencies which are the same as those observed for high-spin Fe(II) forms in heme proteins (see Table III).

Iron(II) porphyrins normally bind two axial iigands and are

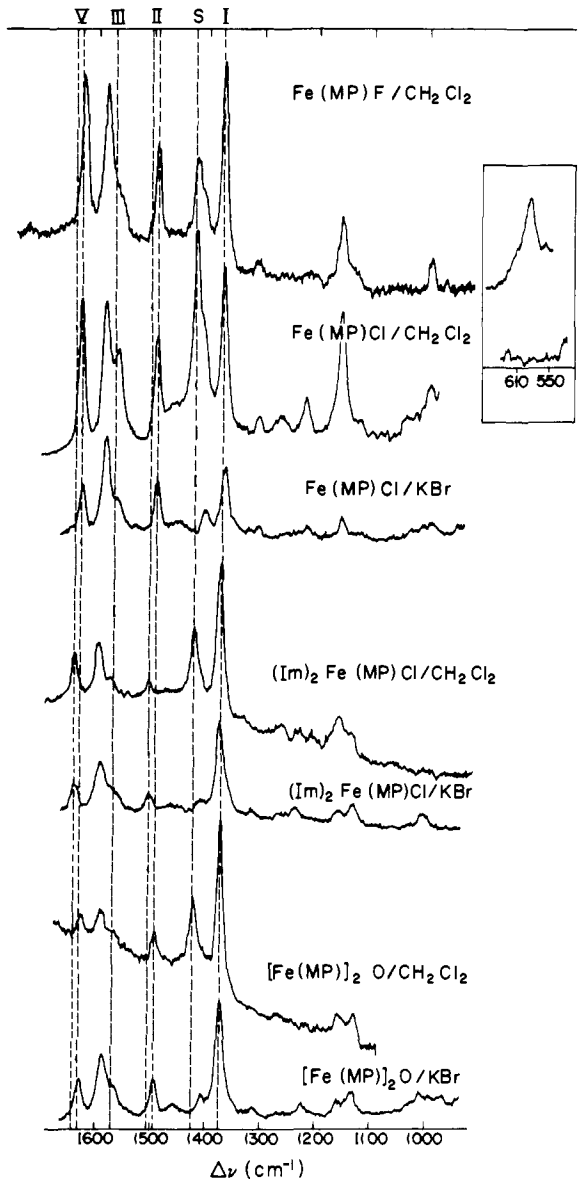

Figure 1. Resonance Raman spectra of Fe^{III}(MP) complexes in CH_2Cl_2 solution and KBr pellets irradiated at 4579 Å. S = CH_2Cl_2 band at 1423 cm^{-1}. Bands I, II, III, and V refer to Table III.

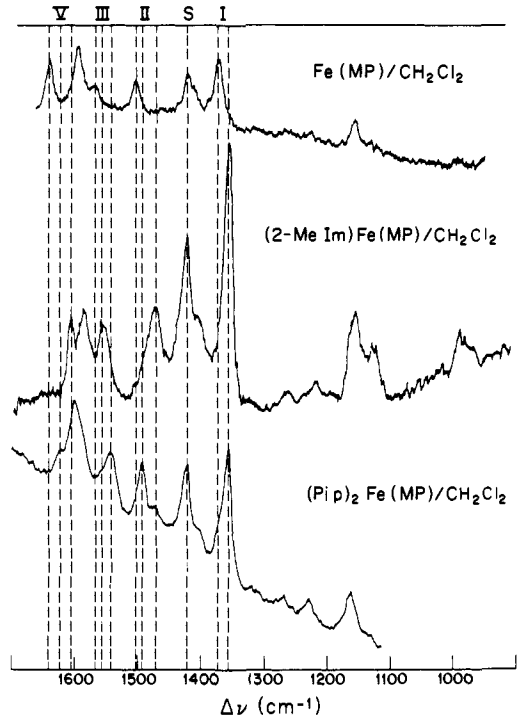

Figure 2. Resonance Raman spectra of Fe^{II}(MP) complexes. Top and bottom spectra were recorded with 4579 Å irradiation while the middle spectrum was recorded with 4765 Å irradiation. S = CH_2Cl_2 band at 1423 cm^{-1}. Bands I, II, III, and V refer to Table III.

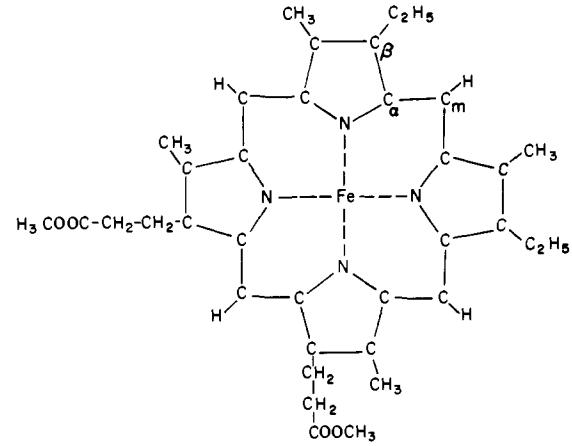

Figure 3. Structure of iron mesoporphyrin IX dimethyl ester.

low spin. 2-Methylimidazole is a sterically hindered ligand, however; the methyl group prevents it from binding simultaneously on both sides of the porphyrin ring. Collman et al.[26] have shown that binding of 2-MeIm to ferrous tetraphenylporphyrin (TPP) produces a high-spin five-coordinate Fe(II) complex, with the iron atom substantially displaced from the porphyrin plane.[27] This complex has been taken to be a model for the five-coordinate heme group of deoxymyoglobin. The present results clearly indicate that a similar complex is formed on binding of 2-MeIm to Fe^{II}(MP). A similar conclusion has been drawn by Wagner and Kassner[25] from the absorption spectrum of a ferrous mesoheme IX complex of 2-MeIm in aqueous solution.[28] The coincidence of the porphyrin vibrational frequencies with those of deoxymyoglobin (Mb) or hemoglobin (Hb) implies that the porphyrin conformation of five-coordinate high-spin Fe(II) heme is the same in solution as in the globin pocket.

This conclusion is of considerable interest with respect to current ideas about molecular tension in deoxyhemoglobin.[27,29,30] Using a chemically modified hemoglobin, which can be switched between two quaternary structures, R (relaxed) and T (tense), by the addition of inositol hexaphosphate (IHP), Perutz and co-workers[29] have provided spectroscopic evidence to suggest that the iron atom is under tension with respect to the porphyrin ring in the T form. Hoard and Scheidt[27] have argued that this tension should be accommodated by extra doming of the porphyrin ring. Little and Ibers,[30] on the other hand, have argued against any significant distortion of the heme group in cobalt hemoglobin and, by extension, in iron hemoglobin as well.

Doming of the porphyrin ring is probably the chief contributor to the frequency lowering of Raman bands II, IV, and V in high-spin Fe(II) hemes, as shown by normal coordinate calculations.[3] Extra doming should lower them further. The identical porphyrin ring frequencies found for deoxy-Hb, deoxy-Mb, and (2-MeIm)Fe^{II}(MP) therefore preclude significant extra doming in deoxy-Hb. At the time Hoard and Scheidt made their proposal, the iron to mean heme plane displacement in deoxy-Hb was thought to be about 0.75 Å, some 0.2 Å greater than the corresponding distance in (2-MeIm)Fe^{II}(TPP). New refinement[31] of the deoxy-Hb crystal

Table III. Comparison of Structure and Oxidation State Marker Bands (cm^{-1}) for Iron Mesoporphyrin(IX) Dimethyl Ester with Heme Proteins

	I(p)	II(p)	III(dp)	IV(ap)	V(dp)	Ref
			Low-Spin Fe(III)			
$(Im)_2Fe^{III}(MP)$	1375	1505	1572	1584	1640	This work
$(CN^-)Fe^{III}Hb$	1374	1508	1564	1588	1642	11
Fe^{III}Cyt *c*	1374	1502	1562	1582	1636	11
$(CN^-)Fe^{III}HRP$	1375	1497	1562	1590	1642	4
			Low-Spin Fe(II)			
$(pip)_2Fe^{II}(MP)$	1358	1490	1537	1583	1620	See also Table IV
$(CN^-)Fe^{II}HRP$	1362	1498	1545	1587	1620	4
Fe^{II}Cyt *c*	1362	1493	1548	1584	1620	11
$(CO)(py)Fe^{II}(MP)$	1371	1497	1567	1588	1630	This work
$(CO)Fe^{II}Hb$	1372			1584	1631	17
$(O_2)Fe^{II}Hb$	1377	1506	1564	1586	1640	11
			High-Spin Fe(II)			
$(2\text{-MeIm})Fe^{II}(MP)$	1359	1472	*a*	1558	1606	This work
$Fe^{II}Hb$	1358	1473	*a*	1552	1607	11
$Fe^{II}HRP$	1358	1472	*a*	1553	1605	4
			High-Spin Fe(III)			
$(X^-)Fe^{III}(MP)$	1374	1495	1565	1572	1632	This work
$(H_2O)Fe^{III}HRP$	1375	1500	1550	1575	1630	4
$(F^-)Fe^{III}Hb$	1373	1482	1565	1555	1608	11
			Intermediate-Spin Fe(II)			
$Fe^{II}(MP)$	1373	1506	1570	1589	1642	This work

[a] Obscured by band IV.

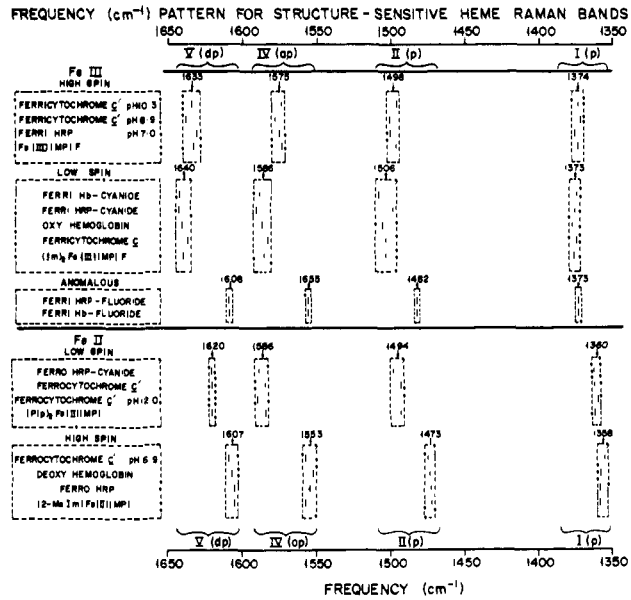

Figure 4. Frequency (cm^{-1}) pattern for structure-sensitive heme Raman bands of various heme proteins and their Fe(MP) analogues: p = polarized; dp = depolarized; ap = anomalously polarized.

structure has reduced the estimate of this displacement to 0.60 Å and has eliminated any significant discrepancy with respect to the TPP model compound.

We conclude from the Raman results that tension in the T form of deoxyhemoglobin is not expressed in any observable distortion of the porphyrin ring. Eisenberger et al.[32a] infer from x-ray absorption studies that the strain energy is not stored in any of the bonds to the iron atom. It may, of course, be expressed elsewhere in the molecule. Hopfield[32b] has suggested that the free energy of cooperativity is stored in many small strains distributed throughout the protein.

High-Spin Fe(III). The low-spin → high-spin transition reduces the frequencies of bands II, IV, and V nearly as much in Fe(III) derivatives of Hb and Mb as in Fe(II) derivatives.[11] For native horseradish peroxidase (HRP)[4] and ferricytochrome *c'* from *Rhodopseudomonas palustris*,[14] however, the corresponding frequency reductions are much less, and have been interpreted as indicating anomalous heme structures in these proteins. The present results, however, show that the anomaly lies instead with Hb and Mb, since all high-spin Fe(III) derivatives of MP give Raman frequencies which are essentially the same as those of native HRP.

Initially we found this to be the case for $(X^-)Fe^{III}(MP)$ ($X^- = F^-, Cl^-$) (see Table I). The halides are not altogether satisfactory models for the high-spin Fe(III) derivatives of Hb or Mb, however, since the strong-field ligand, imidazole, always remains coordinated to the latter, and a weak-field ligand (e.g., H_2O or F^-) is bound on the opposite side, away from the direction of displacement of the iron atom with respect to the heme plane. We tried to model these derivatives via coordination of 2-MeIm. (Addition of imidazole to Fe(III) hemes produces the low-spin bisimidazole derivatives.) Addition of a large excess of 2-MeIm to CH_2Cl_2 solutions of $(X^-)Fe^{III}(MP)$, however, failed to influence either the visible ab-

Table IV. Variations of Bands I, III, and V (cm^{-1}) as a Function of the Axial Ligands in $(L)_2Fe^{II}(MP)$

Axial ligands	Band I	Band III	Band V
$(CO)(py)^a$	1371	1567	1630
$(P[OEt]_3)_2$	1368	1559	1629
$(P[Me][Ph]_2)_2$	1363	1554	1625
$(P[Ph][Me]_2)_2$	1363	1554	1625
$(py)_2$	1365	1555	1622
$(\gamma\text{-pic})_2$	1364	1553	1621
$(P[Et_3]_2)$	1358	1544	1620
$(P[n\text{-Bu}]_3)_2$	1358	1539	1618
$(pip)_2$	1358	1537	1620
$(Im)_2{}^b$	1358	1534	1617

[a] For an explanation of the symbols, see Table II. [b] From $(Im)_2Fe(PP)$.

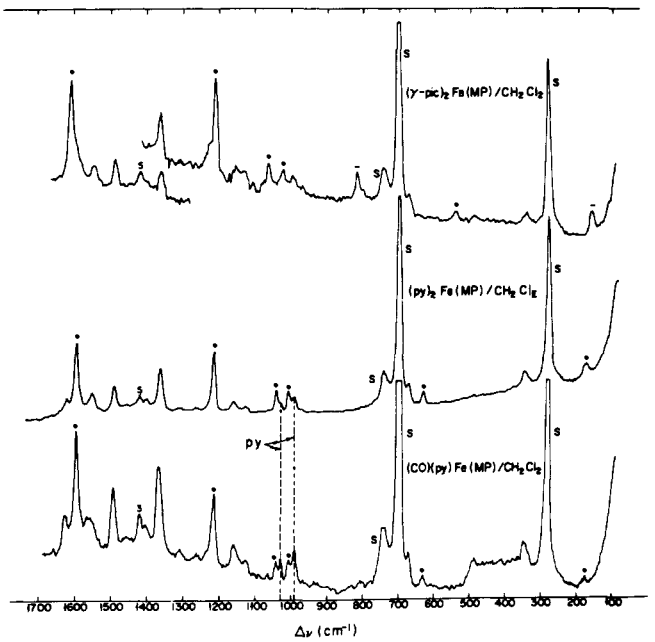

Figure 5. Resonance Raman spectra of $(\gamma\text{-pic})_2Fe(MP)$, $(py)_2Fe(MP)$, and (CO)(py)Fe(MP) irradiated at 4579 Å. S = CH_2Cl_2 bands; py indicates free pyridine: bands marked with an asterisk refer to bound ligand vibrations.

Table V. Resonance Enhanced Raman Bands[a] (cm^{-1}) of Pyridine and γ-Picoline Bound to $Fe^{II}(MP)$ Compared to the Frequencies of the Unbound Ligands

$(py)_2Fe$-(MP)	py	$(\gamma\text{-pic})_2$-Fe(MP)	γ-pic	Assignment[b]
1597	1583	1617	1603	Ring stretch
	1482		1495	Ring stretch
1215	1218	1209	1220	In-plane CH def.
	1068	1063	1042	In-plane CH def.
1046	1030	1025	994	Trigonal ring "breathing"
1010	992	817	801	Totally symmetric ring "breathing"
634	605	543	514	In-plane ring def.
179		162		

[a] All bands are polarized. [b] A_1 vibrations only. Pyridine assignments from F. R. Dollish, W. G. Fately, and F. F. Bentley, "Characteristic Raman Frequencies of Organic Compounds", Wiley-Interscience, New York, N.Y., 1974, pp 264–265; γ-picoline assignments from D. A. Long and W. O. George, *Spectrochim. Acta*, **19**, 1777 (1963).

sorption or Raman spectra of the heme group. Presumably the halide ions are too tightly bound to the heme group in the nonpolar solvent to permit coordination of the sterically hindered 2-MeIm. It is noteworthy that the crystal structure[33] of a six-coordinate, high-spin Mn(III) porphyrin shows the Mn atom to be displaced from the porphyrin plane toward the bound chloride, and away from the bound pyridine.

Addition of sodium tetraphenylborate to CH_2Cl_2/MeOH (10:1) solutions of $(X^-)Fe^{III}(MP)$ did lead to displacement of bound halide, presumably by methanol, with formation of sodium halide. The electronic spectrum shifted perceptibly, as shown in Figure 7. In the case of $(F^-)Fe^{III}(MP)$, the displacement could be monitored by the disappearance of a polarized Raman band at 581 cm^{-1}, which has been assigned to Fe–F stretching on the basis of the ^{54}Fe isotope shift observed in the resonance Raman spectrum of $(F^-)Fe^{III}Et_8por$.[24] Addition of 2-MeIm then leads to coordination, as can be inferred from a substantial change in the visible absorption spectrum, shown in Figure 7. However, the Raman spectrum is quite

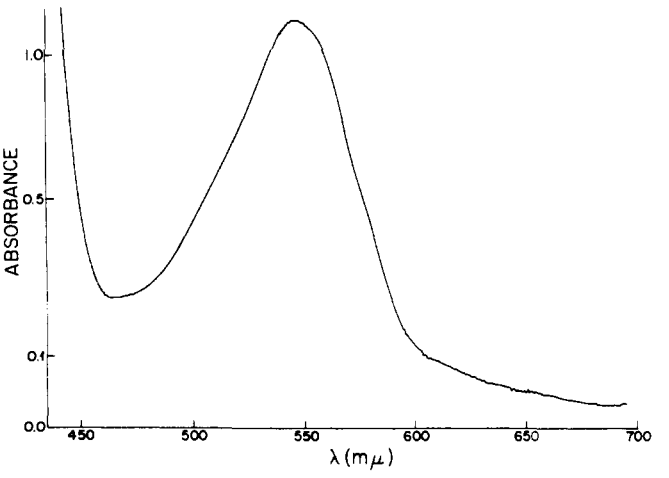

Figure 6. Absorption spectrum of (2-MeIm)$Fe^{II}(MP)$ in CH_2Cl_2 (1 mg/ml).

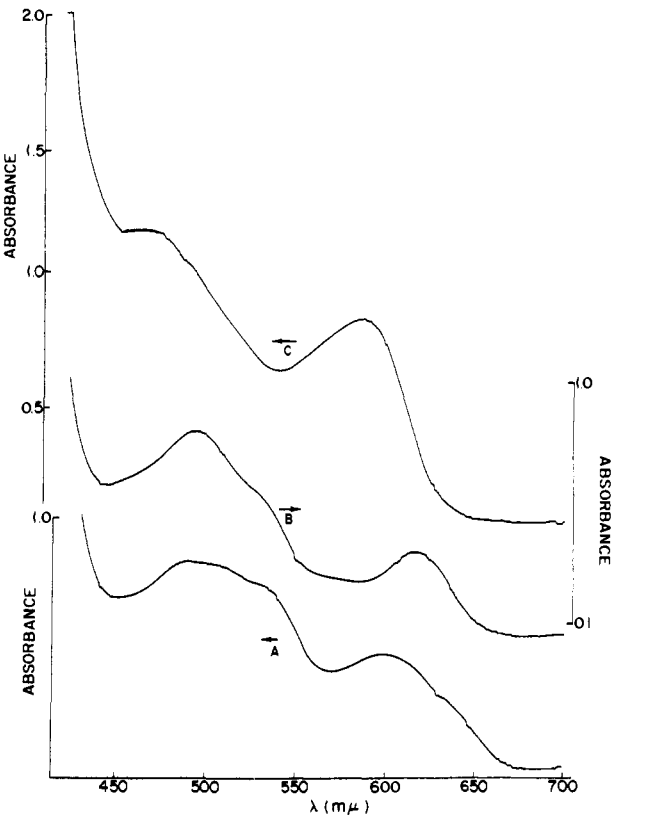

Figure 7. Absorption spectra of: (A)$(F^-)Fe^{III}(MP)$ (1 mg) in CH_2Cl_2 (1 ml)/methanol (0.2 ml); (B) addition of sodium tetraphenylborate (1 mg) to (A); (C) addition of 2-MeIm (5 mg) to (B).

unaltered. These results demonstrate that the Raman frequencies of high-spin ferric hemes are independent of the nature of the axial ligand or ligands. These same frequencies are observed for native HRP[4] and for ferricytochrome c'.[14] Methemoglobin and myoglobin stand out as anomalous.[34] Their lowered frequencies for bands II, IV, and V imply an altered heme structure induced by binding to the globin.

Implications for Heme Structure in Hemoglobin and Myoglobin. As noted above, deoxyhemoglobin and myoglobin display the same Raman frequencies as does (2-MeIm)-$Fe^{II}(MP)$ in CH_2Cl_2, implying the same porphyrin conformation. The one available crystal structure[27] of a high-spin Fe(II) porphyrin, that of (2-MeIm)$Fe^{II}(TPP)$, shows a doming of the porphyrin ring, in the direction of the iron atom displacement; the average distance from the pyrrole nitrogen

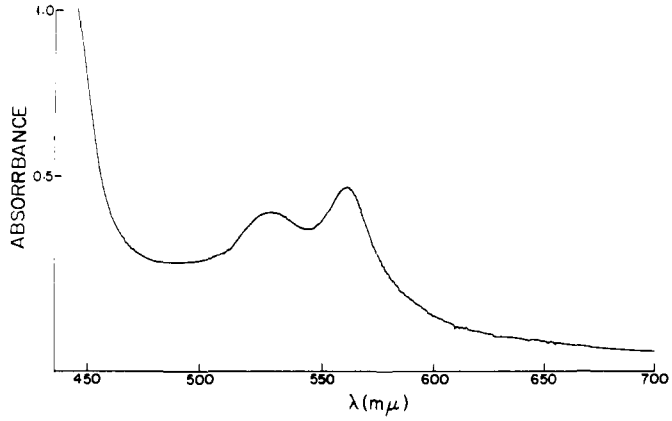

Figure 8. Absorption spectrum of Fe^{II}(MP) in CH_2Cl_2 (1 mg/ml).

atoms to the mean plane of the porphyrin carbon skeleton (P_N···P_C) is 0.13 Å. Crystal structures are available for four high-spin Fe(III) porphyrins.[27,35] For these the doming is appreciably less, with P_N···P_C < 0.05 Å. Bands II, IV, and V in the Raman spectrum have been shown by normal coordinate analysis[3] to be sensitive to the degree of porphyrin doming via the attendant interruption of π conjugation at the methine bridges. The smaller differences in these frequencies between low- and high-spin Fe(III) hemes, vis-a-vis low- and high-spin Fe(II) hemes, may be a reflection of lesser doming in the Fe(III) derivatives. Hoard and Scheidt[27] consider that intermolecular packing is responsible for the greater doming found for (2-MeIm)Fe^{II}(TPP). The Raman data, however, suggest that greater doming for high-spin Fe(II) hemes is maintained in solution.[36]

The frequency differences between low- and high-spin Fe(III) derivatives of Hb and Mb, however, are fully as large as those characteristic of Fe(II). These imply a greater doming for the porphyrin ring than is normal for high-spin Fe(III) hemes, induced by the protein.[38] The extra doming is not linked with hemoglobin quaternary structure. It exists in the R quaternary state, and addition of IHP, which switches aquo-met Hb from R to T, has no influence on the porphyrin frequencies.[40] Moreover, aquo-met Mb displays the same frequencies. Rather, the extra doming must be associated with the tertiary structure of the hemoglobin subunits and of myoglobin. Since low-spin methemoglobin derivatives have porphyrin frequencies characteristic of the normal, planar low-spin heme conformation, there must be a switch in tertiary structure, triggered by the low-spin → high-spin transition, which in turn produces an extra doming of the porphyrin ring.

At first sight it is surprising to find a protein-induced distortion of a prosthetic group in a state of the molecule which is not the physiologically functional state. One rather expects to find distortions of functional active sites which move them along the reaction coordinate.[41] Hemoglobin and myoglobin are not enzymes, however. Their role is simply to bind and release molecular oxygen. It is therefore advantageous to stabilize the binding site, five-coordinate Fe(II) heme, which is otherwise oxidatively unstable. Presumably the heme pocket has evolved for this purpose. At least part of the stability may be associated with the packing of polypeptide side chains about the porphyrin ring in its five-coordinate, domed conformation.

The Raman evidence points to the heme pocket maintaining the same porphyrin conformation upon oxidation of deoxyhemoglobin or myoglobin to high-spin ferric derivatives. Since this is not the stable conformation for high-spin Fe(III) hemes, it must represent a relatively high-energy state for the heme group. Consistent with this, the reduction potential for aquomethemoglobin or myoglobin, +140 and +50 mV,[42] respectively, is substantially higher than that of native horseradish peroxidase (−270 mV)[42] where high-spin Fe(III) heme appears to have a normal conformation. While previous discussions of globin stabilization of the heme group have focused on dielectric effects and the exclusion of external reactants, the present results suggest that protein stabilization of a porphyrin conformation appropriate for the deoxy form may be a significant factor.

Intermediate-Spin Fe(II). When (F^- or Cl^-)Fe^{III}(MP) in CH_2Cl_2 is shaken with aqueous dithionite in the absence of any ligand, the organic layer develops a new absorption spectrum, shown in Figure 8. An identical spectrum was reported by Brault and Rougee[43] and Momenteau[44] for chlorodeuterohemin reduced in a similar fashion and was interpreted by them to be indicative of intermediate spin iron(II). The Raman bands shift to frequencies which are characteristic of low-spin Fe(III) (see Table III). The new porphyrin species contains Fe(II), however, as demonstrated by the fact that addition of pyridine immediately converts it to $(py)_2Fe^{II}$(MP), readily identifiable by its unique Raman spectrum.

Collman et al.[45] have shown that reduction of (Cl^-)Fe^{III}(TPP) in the absence of ligands generates four coordinate Fe^{II}(TPP), with an intermediate spin state. Its crystal structure shows the iron atom to be in the plane of the four pyrrole nitrogen atoms, and the Fe–N distances are unusually short, 1.972 Å. The iron $d_{x^2-y^2}$ orbital, which points toward the nitrogen atoms, is therefore antibonding and empty, as in low-spin hemes. The d_{z^2} orbital is nonbonding, however, and the six d electrons are distributed among the d_{xy}, d_{xz}, d_{yz}, and d_{z^2} orbitals, giving an intermediate spin state, $S = 2$.

We believe that the reduction product of (Cl^-)Fe^{III}(MP) is likewise a four-coordinate Fe(II) porphyrin, similar to the TPP derivative isolated by Collman et al.[45] For the latter there is some question whether the lowest lying orbital is d_{xy} as suggested by the molecular orbital calculation of Gouterman et al.[18] or d_{z^2} as is more consistent with Mossbauer measurements. If for the present complex the lowest orbital is d_{xy}, then the electronic configuration is the same as for low-spin Fe(III), leaving aside the singly occupied d_{z^2} orbital, which should have minimal influence on the porphyrin ring vibrations. We would then expect a Raman spectrum similar to those of low-spin Fe(III) hemes.

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(36) (a) It is natural to inquire about the source of this difference if it is not intermolecular. Greater doming should be energetically disfavored to the extent that it reduces π conjugation in the porphyrin ring. On the other hand it does provide better orientation of the pyrrole nitrogen lone pairs for bonding to the displaced iron atom. A model of the (2-MeIm)Fe^{II}(TPP) structure shows the pyrrole rings to be nearly colinear with the iron–nitrogen bonds. Orbital following may be less important for high-spin Fe(III) which has a half-filled (spherical) d subshell. It may be significant in this regard that the average Fe–N (pyrrole) distance,[27] 2.086 Å, is nearly the same in (2-MeIm)Fe^{II}(TPP) as in high-spin Fe(III) porphyrins, 2.07 Å (average), although the high-spin octahedral ionic radius is ~0.12 Å less for Fe(III) than for Fe(II).[37] While the ionic radius is not an appropriate comparison for the porphyrin complexes, the similar Fe–N(pyrrole) distances for Fe(II) and Fe(III) do suggest a stronger than usual bond for the former. This line of reasoning must remain speculative, however, pending the availability of further structural data, especially for other high-spin Fe(II) hemes. (b) KBr pellet spectra, taken for (X^-)Fe^{III}(MP) (X = F^-, Cl^-), are essentially the same as the solution spectra (see Figure 1), suggesting no significant structural differences between crystal and solution.
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The Low Temperature Crystal and Molecular Structure of Beryllium Bis(octahydrotriborate), $Be(B_3H_8)_2$

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Abstract: The solid state structure of beryllium bis(octahydrotriborate), $Be(B_3H_8)_2$, consists of two bidentate $B_3H_8^-$ ligands bound to a central beryllium atom by two Be–H–B bridge hydrogen bonds from adjacent boron atoms in each $B_3H_8^-$ unit. Crystals of $Be(B_3H_8)_2$ form in the monoclinic space group $P2_1/n$ with unit cell parameters a = 10.503 (8) Å, b = 7.641 (3) Å, c = 11.399 (9) Å, β = 118.49 (6)°, V = 804 (1) Å^3, and Z = 4. The x-ray crystal structure was solved by direct methods and refined to R_1 = 0.050 and R_2 = 0.061 for 944 independent observed reflections. The molecule has molecular C_2 symmetry with an approximately tetrahedral beryllium atom and $B_3H_8^-$ ligands essentially undistorted from free $B_3H_8^-$ in their internal bonding. $Be(B_3H_8)_2$ is the first reported metal complex having two octahydrotriborate ligands bound to a metal.

Beryllium bis(octahydrotriborate), $Be(B_3H_8)_2$, is a colorless liquid prepared by gently heating an intimate mixture of TlB_3H_8 and $BeCl_2$ in vacuo.[2] Preliminary variable-temperature ^{11}B and ^{1}H NMR studies indicated three basic molecular forms. The two higher temperature forms correspond to partially and completely fluxional molecules, while the low temperature form (observed below −10°) appeared to be static and have C_2 molecular symmetry. In order to confirm the NMR analysis we initiated a low-temperature single-crystal x-ray determination of the static configuration of $Be(B_3H_8)_2$.

Structure Determination

Single-Crystal X-Ray Data. The $Be(B_3H_8)_2$ used was prepared and purified as previously described.[2] Due to the compound's extreme air sensitivity, it was necessary to load and seal the Pyrex x-ray capillaries on the vacuum line.[3] A capillary containing liquid $Be(B_3H_8)_2$ (mp −51°) was placed on a Syntex $P\bar{1}$ four-circle computer-controlled diffractometer equipped with low-temperature accessories, and an irregularly shaped single-crystal was grown at −77 ± 3°. The low crystal-growing temperature was necessary because of the tendency of $Be(B_3H_8)_2$ to supercool. The crystal was subsequently cooled to −175 ± 5° and 15 diffraction maxima were automatically centered