Resonance Raman Studies of Hemoglobins M: Evidence for Iron–Tyrosine Charge-Transfer Interactions in the Abnormal Subunits of Hb M Boston and Hb M Iwate†

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ABSTRACT: Resonance Raman spectra have been obtained for Hb M Boston [His-E7(58)α→Tyr], Hb M Iwate [His-F8(87)α→Tyr], and Hb M Milwaukee [Val-E11(67)β→Glu]. The abnormal α subunits of Hb M Boston and Hb M Iwate exhibited the porphyrin ν_{10} band at 1628 and 1627 cm^{-1}, respectively, which indicates that the ferric α hemes are five-coordinated in both Hb M Boston and Hb M Iwate. In addition to the porphyrin bands, four extra polarized lines were observed at 1607, 1506, 1278, and 603 cm^{-1} for the α abnormal subunit of Hb M Boston and at 1605, 1506, 1310, and 589 cm^{-1} for that of Hb M Iwate. By comparison with the vibrational spectra of Fe–tyrosine proteins and Fe–phenolate complexes, the 1605–1607- and 1506-cm^{-1} lines are assigned to the phenolate ring vibrations of the heme-coordinated tyrosine, and the 1278-cm^{-1} line of Hb M Boston and the 1310-cm^{-1} line of Hb M Iwate are assigned to the phenolate CO stretching mode. We propose that the 603-cm^{-1} line of Hb M Boston and the 589-cm^{-1} line of Hb M Iwate arise from the Fe–O(tyrosine) stretching mode. These four Raman lines are intensity enhanced upon the excitation around 475–520 nm, probably due to the presence of a charge-transfer interaction between Fe and Tyr. The dissimilarity of the Fe–O and phenolate CO stretching frequencies between Hb M Boston and Hb M Iwate, despite the similarity of frequencies of their porphyrin and phenolate ring modes, suggests that the heme–phenolate bonding angles differ between Hb M Boston and Hb M Iwate although both adopt the five-coordinate form with Tyr as the only axial ligand. The resonance Raman spectra of oxy- and deoxy-Hb M Milwaukee showed no anomaly and can be accounted for by those of the equimolar mixtures of aquomet- and oxy- or deoxy-Hb A.

Hemoglobins M are the class of hemoglobin (Hb)[1] in which heme iron in either the α or β subunit is oxidized in vivo as a result of an amino acid replacement in the heme vicinity. In Hb M Boston [His-E7(58)α→Tyr] and Hb M Saskatoon [His-E7(63)β→Tyr], the distal histidine of the α and β subunits, respectively, and in Hb M Iwate [His-F8(87)α→Tyr] and Hb M Hyde Park [His-F8(92)β→Tyr], the proximal histidine of the α and β subunits, respectively, are replaced by tyrosine. In Hb M Milwaukee [Val-E11(67)β→Glu], valine-E11 of the β subunit, which is located one helical turn apart from the distal histidine, is replaced by glutamic acid. In these Hb M mutants the phenolate group of tyrosine or the carboxyl group of glutamic acid is coordinated to the heme iron of the abnormal subunit, stabilizing the ferric form of the heme and thus not allowing oxygen binding to the abnormal subunit.

The X-ray crystallographic study in deoxy-Hb M Boston by Pulsinelli et al. (1973) demonstrated that the ferric heme iron of the abnormal α subunit is bound to the phenolate oxygen of Tyr-E7 but not to the proximal histidine (His-F8), which normally serves as an axial ligand of the heme iron. The iron is six-coordinated in the abnormal β subunit of Hb M Milwaukee, in which His-F8 and Glu-E11 are bonded to the heme iron (Perutz et al., 1972). For Hb M Iwate, only low-resolution X-ray analysis is available (Greer, 1971), which suggested that the heme iron of the abnormal α subunit might be bonded to both distal histidine (His-E7) and Tyr-F8. Despite the difference in the iron coordination structures, the EPR and optical absorption spectra are similar between Hb M Boston and Hb M Iwate (Shibata et al., 1967).

Resonance Raman spectroscopy provides detailed information about the heme structure of hemoproteins (Spiro, 1975; Felton & Yu, 1978; Kitagawa et al., 1978), and a great deal of experimental data has been accumulated for Hb [for a recent review, see Asher (1981)], although so far there has been no Raman data about the abnormal subunit of the mutant Hb's. In this work we obtained resonance Raman spectra of the M-type mutant Hb's showing that the abnormal subunit of Hb M Boston and Hb M Iwate adopts the five-coordinated structure and that there is a charge-transfer interaction between the heme iron and the coordinated tyrosine with λ_{max} of the CT band around 475–520 nm.

Materials and Methods

Hb M Milwaukee, Hb M Iwate, and Hb M Boston were purified in the carbon monoxy form by ion-exchange chromatography as reported previously (Nagai et al., 1979). The hemoglobin solution was applied to a column of Amberlite IRC-50 equilibrated with 0.1 M sodium phosphate buffer, pH 7.0, and after the pale pink band of Hb A was eluted with the same buffer, Hb M and Hb A_2 were eluted by raising the buffer concentration to 0.5 M. The solution was gel filtered against Tris-HCl buffer (6.4 g/L tris(hydroxymethyl)-

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[1] Abbreviations: Hb, hemoglobin; CT, charge transfer; Fe(PPDBE), protoporphyrin IX dibutyl ester–Fe(III) complex; $OHC_6H_4NO_2$, *p*-nitrophenol; Im, imidazole; IHP, inositol hexaphosphate; HbCO, (carbon monoxy)hemoglobin; EPR, electron paramagnetic resonance.

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aminomethane and 2.54 mL/L concentrated HCl) and then applied to a column of DE-52 (Whatman). First, Hb A_2 was eluted with the Tris-HCl buffer, and second, Hb M was eluted with 0.06 M potassium phosphate buffer. After the Hb solution was gel filtered against 1 mM sodium phosphate buffer, it was stripped of phosphate by passage through a Dintzis column (Nozaki & Tanford, 1967). CO was taken off from Hb in a rotary evaporator under a stream of oxygen and strong illumination of light. Purity of Hb M was examined by isoelectric focusing of the completely oxidized form (Righetti & Drysdale, 1971).

For measurements of Raman spectra, the 300 μM (heme) Hb solution was put into a cylindrical cell that was secured with a rubber septum. Hb was deoxygenated in the Raman cell by repeated evacuation and flushing with N_2 gas. Raman scattering was excited with an Ar/Kr mixed-gas laser (Spectra Physics, Model 164) and recorded with a JEOL-400D Raman spectrometer. The frequency calibration of the spectrometer was performed with indene as a standard (Hendra & Loader, 1968). The uncertainty of the peak frequencies of the individual Raman lines may be as large as ± 1 cm^{-1}. The intensity of Raman lines was determined by drawing an appropriate base line shown in the spectra and measuring the surrounded areas with a Digigrammer (Mutoh Engineering, Model G). The 981-cm^{-1} line of the SO_4^{2-} ion added was used as an internal intensity standard. The relative intensity was not corrected for the absorption by the sample, because the excitation light was introduced so as to graze the front wall of the Raman cell and the scattering from the bottom of the cell was collected.

It is known that the optical absorption spectrum of HbCO is insensitive to the quaternary structure (Perutz et al., 1976). This allowed us to infer the absorption spectra of the abnormal subunit of Hb M from the difference spectra as follows: a 60 μM (heme) solution of (carbon monoxy)-Hb M Boston or Hb M Iwate vs. 30 μM (heme) solution of carbon monoxy β chain of Hb A gives rise to a spectrum of the abnormal α subunit, and the 60 μM solution of (carbon monoxy)-Hb M Milwaukee vs 30 μM solution of carbon monoxy α chain of Hb A gives the spectrum of the abnormal β subunit. Despite extensive efforts, we failed to isolate the abnormal chain due to its instability.

Results

Figure 1 shows the absorption spectra of the CO forms of Hb M Milwaukee, Hb M Boston, and Hb M Iwate used in this study and the spectra of their abnormal subunits obtained as described above. For comparison, the spectrum of aquomet-Hb A is also included at the top. The Q band of the abnormal subunit is located around 495 nm for Hb M Milwaukee, 490 nm for Hb M Boston, and 480 nm for Hb M Iwate, which are appreciably shifted from that of aquomet-Hb A at 500 nm. The CT band characteristic of the ferric high-spin heme proteins is seen at 633, 620, 600, and 590 nm for aquomet-Hb A, Hb M Milwaukee, Hb M Boston, and Hb M Iwate, respectively.

Figure 2 shows the resonance Raman spectra of oxy- and deoxy-Hb M Milwaukee in the presence and absence of IHP. Here, the abnormal β subunit stays always in the met form while the normal α subunit undergoes oxygenation and deoxygenation. The Raman lines of the oxygenated form at 1642 (ν_{10}), 1589 (ν_{19}), and 1505 (ν_3) cm^{-1} [mode numbers are based on Abe et al. (1978)] derive definitely from the normal subunit because they disappear upon deoxygenation. Oxy-Hb M Milwaukee has been shown to be in the low-affinity (T) quaternary structure in the presence of IHP at pH 6.5 but in the high-affinity (R) structure in its absence at pH 9.0 (Perutz et al., 1972; Fung & Ho, 1975).

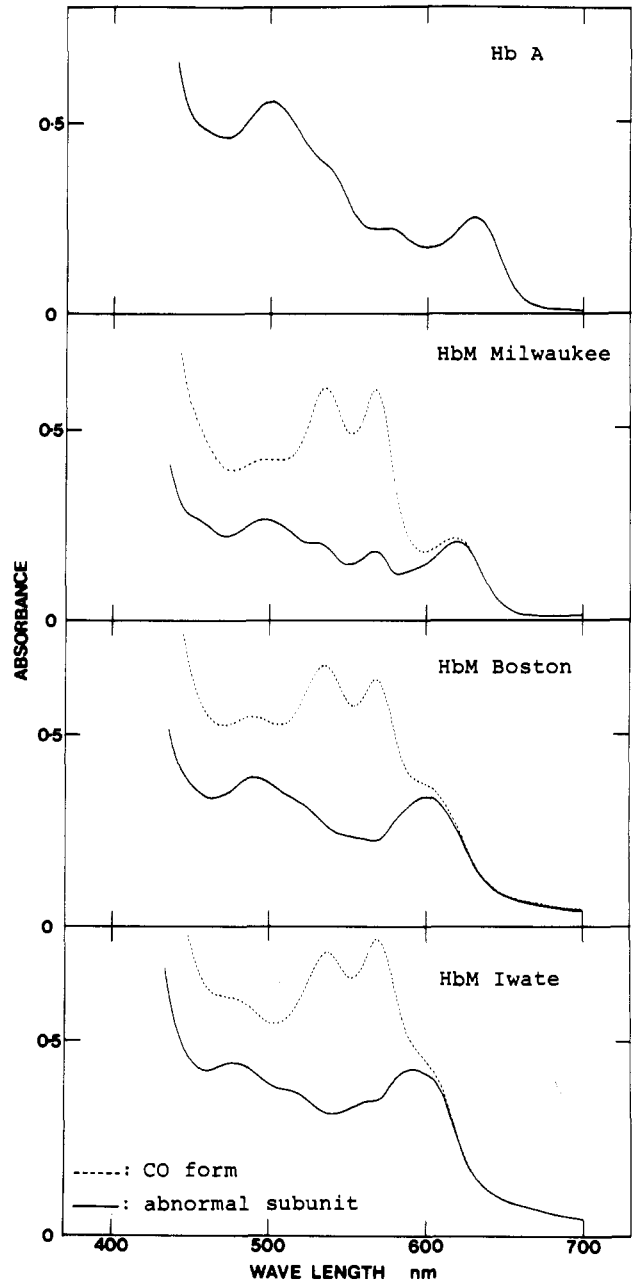

FIGURE 1: Absorption spectra of Hb A, Hb M Milwaukee, Hb M Boston, and Hb M Iwate used in this study. The solid lines denote the difference spectra between the CO form of Hb M and the CO form of the equimolar normal isolated chain of it and thus indicate the spectrum of the abnormal subunit except for Hb A, for which the spectrum of the aquomet form is shown. The broken lines indicate the spectra of the CO form.

Although the Fe–O_2 stretching Raman line at 568 cm^{-1} exhibited no frequency shift between the two quaternary structures, the higher frequency modes gave slight frequency shifts in the oxy form ($\Delta\nu_{10} = -2$, $\Delta\nu_{19} = -4$, $\Delta\nu_3 = -1$ cm^{-1}). This may imply slight expansion of the porphyrin core size in the T structure (Spaulding et al., 1975; Huong & Pommier, 1977; Spiro et al., 1979). The IHP effect is much smaller in deoxy-Hb M Milwaukee, although definite frequency shifts upon switching of a quaternary structure have also been observed for deoxy-Hb A (Shelnutt et al., 1979).

The resonance Raman spectrum of an equimolar mixture of aquomet-Hb A and deoxy-Hb A and that of aquomet-Hb A and oxy-Hb A were measured for comparison with the

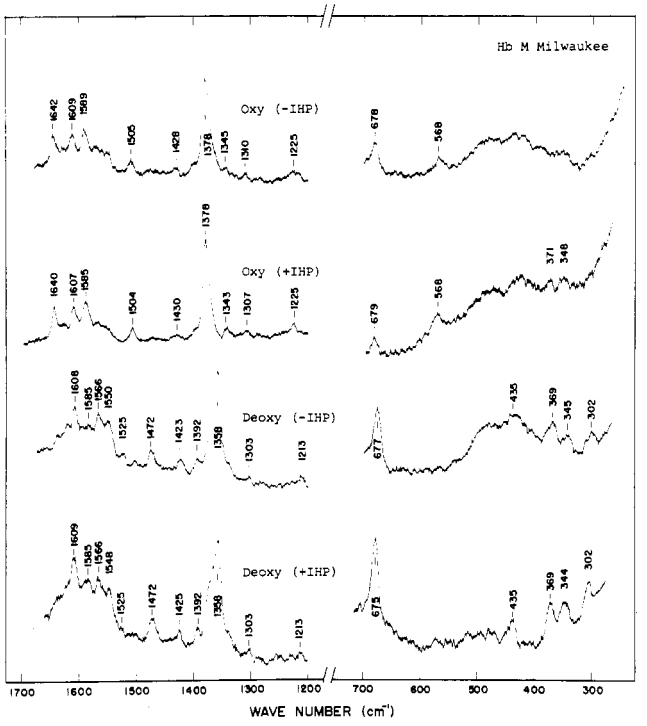

FIGURE 2: Resonance Raman spectra of oxy- and deoxy-Hb M Milwaukee in the presence of IHP at pH 6.5 and in its absence at pH 9.0 excited at 488.0 nm.

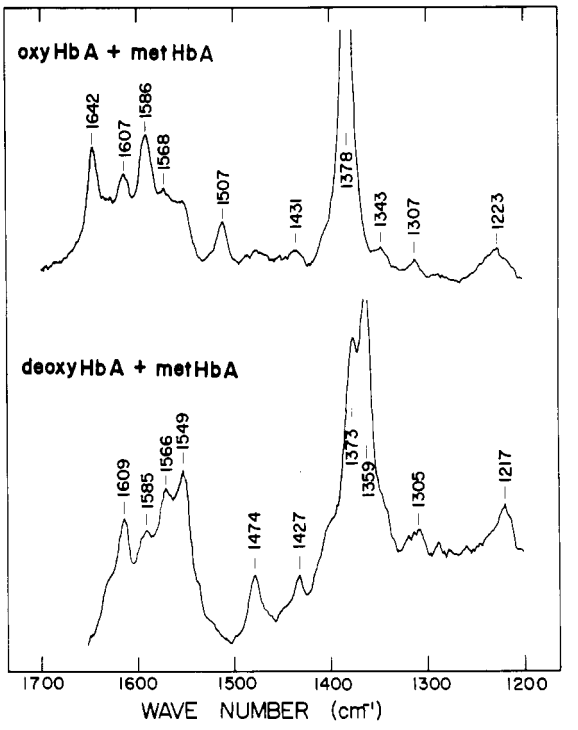

FIGURE 3: Resonance Raman spectrum of the equimolar mixture of met-Hb A and oxy-Hb A (upper) and that of met-Hb A and deoxy-Hb A (lower) excited at 488.0 nm.

spectra of deoxy- and oxy-Hb M, respectively. Note that the contribution of the aquomet form to those spectra is generally small except for the ν_4 line around 1378 cm^{-1}, because the Soret band of aquomet-Hb A (λ_{max} = 405 nm) is located further from the excitation wavelength (488.0 nm) than those of deoxy-Hb A (λ_{max} = 430 nm) and oxy-Hb A (415 nm). The spectra of deoxy–met and oxy–met mixtures of Hb A bear a close resemblance to the spectra of deoxy- and oxy-Hb M Milwaukee, respectively. This might imply similarity in the contribution of the met forms to the Raman spectra of Hb M Milwaukee and the Hb A mixture. In the abnormal subunit of Hb M Milwaukee, the γ-carboxyl group of Glu-E11 occupies the sixth coordination position of the heme iron in place of a water molecule in aquomet-Hb A. Since a carboxyl group is a weak ligand like H_2O, the spectral similarity between the abnormal β subunit of Hb M Milwaukee to that of aquomet-Hb A seems reasonable.

The resonance Raman spectra of oxy- and deoxy-Hb M Iwate and Hb M Boston are shown in Figure 4. As the normal β subunits of the oxy and deoxy forms of the Hb M are expected to give rise to Raman spectra essentially similar to those of oxy-Hb A and deoxy-Hb A, respectively, which are roughly approximated by the spectra shown in Figure 3, except for the ν_4 band. Accordingly, the spectral differences between oxy-Hb M and the oxy–met mixture and those between deoxy-Hb M and the deoxy–met mixture would provide information about the contribution of the abnormal subunit. The ν_{10} line of the normal β subunit of oxy-Hb M Iwate (1644 cm^{-1}) is obscured by an intense line of the abnormal α subunit at 1630 cm^{-1}. In the lower frequency region below 500 cm^{-1}, the signal/noise ratios are low, and the contribution of normal subunit seems predominant.

The Raman lines of oxy-Hb M Iwate at 1630, 1308, and 589 cm^{-1} and those of deoxy-Hb M Iwate at 1628, 1507, 1309, and 588 cm^{-1} are noticed to be the extra Raman lines in comparison with the spectra of the oxy–met and deoxy–met mixtures, respectively, and therefore assigned to the abnormal

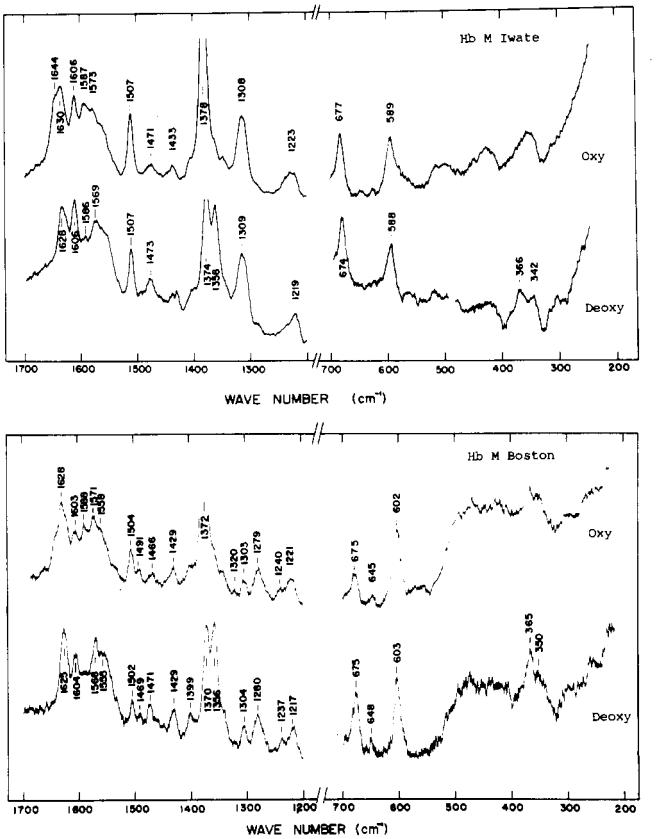

FIGURE 4: Resonance Raman spectra of the oxygenated and deoxygenated forms of Hb M Iwate (upper) and Hb M Boston (lower) excited at 488.0 nm.

α subunit. The little change of their frequencies between the deoxy and oxy forms is also reasonable to assign them to the abnormal subunit. The polarization measurements revealed that all these lines are polarized except for the one at 1628 cm^{-1} (1630 cm^{-1} for the oxy form), which was depolarized and

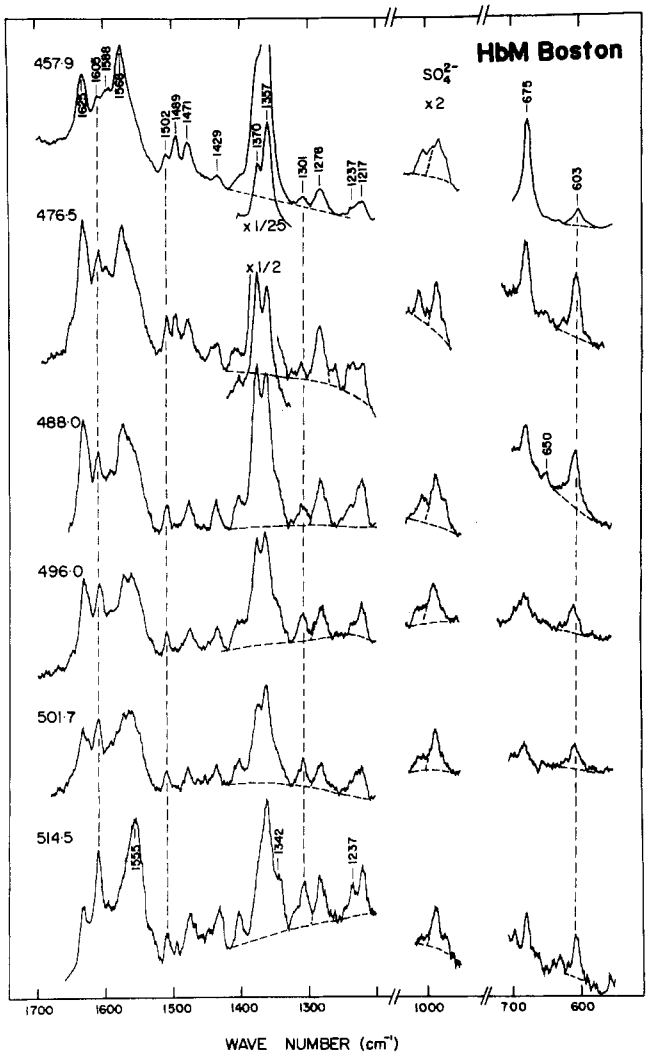

FIGURE 5: Resonance Raman spectra of deoxy-Hb M Boston excited at 457.9, 476.5, 488.0, 496.0, 501.7, and 514.5 nm. The sample contains 2% (w/w) $(NH_4)_2SO_4$, which gives the Raman line marked by SO_4^{2-}. The broken lines depict the base lines and band shape of the overlapped part assumed.

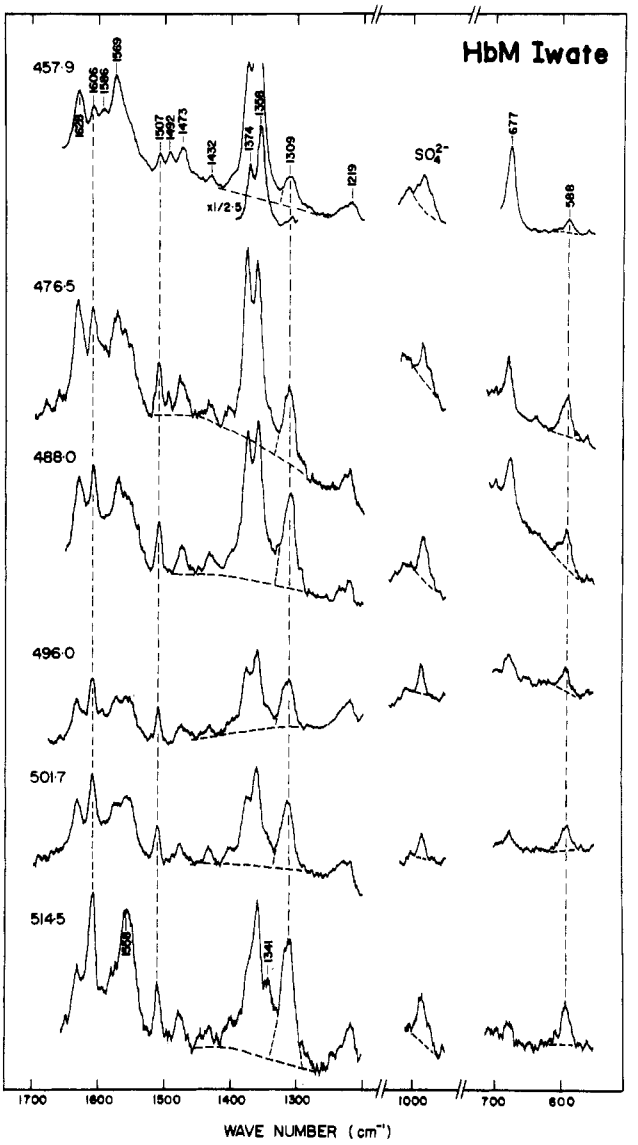

FIGURE 6: Resonance Raman spectra of deoxy-Hb M Iwate excited at 457.9, 476.5, 488.0, 496.0, 501.7, and 514.5 nm. The sample contains 2% (w/w) $(NH_4)_2SO_4$, which gives the Raman line marked by SO_4^{2-}. The implication of the broken lines is the same as in Figure 5.

accordingly assigned to the ν_{10} line of the abnormal α subunit.

One may argue that the 1308-cm^{-1} line of oxy-Hb M Iwate may correspond to the 1307-cm^{-1} line of the oxy–met mixture of Hb A. However, the relative intensity of the line distinctly differs between them. Furthermore, the 1308-cm^{-1} line of deoxy-Hb M Iwate is polarized whereas the 1307-cm^{-1} line of the mixture is anomalously polarized. Probably a weak perpendicular component of the 1308-cm^{-1} line of deoxy-Hb M Iwate (not shown) arises from the underlying ν_{21} mode (Abe et al., 1978), which involves primarily the methine-bridge C–H in-plane deformation mode.

In the same way as for Hb M Iwate, the Raman lines of oxy-Hb M Boston at 1628, 1491, 1279, and 602 cm^{-1} and those of deoxy-Hb M Boston at 1625, 1502, 1489, 1280, and 603 cm^{-1} are assignable to the abnormal α subunit. As only the 1625-cm^{-1} line was depolarized, it was assigned to the ν_{10} mode of the abnormal α subunit. We note that the abnormal α subunit of Hb M Iwate differs from the abnormal α subunit of Hb M Boston regarding the fact that deoxy-Hb M Iwate exhibits two Raman lines at 1309 and 588 cm^{-1} while deoxy-Hb M Boston does at 1278 and 603 cm^{-1}. Their intensity and polarization properties suggest that the origins of these two sets of Raman lines are common between Hb M Iwate and Hb M Boston. In these frequency regions, there are normally no such prominent Raman lines as shown in Figure 4 for ferric heme proteins upon excitation at 488.0 nm. Consequently, the two lines are attributable to vibrations involving the coordinated tyrosine.

The resonance Raman spectra of deoxy-Hb M Iwate and deoxy-Hb M Boston excited at various wavelengths are displayed in Figures 5 and 6, respectively. Both samples contain 2% (w/w) $(NH_4)_2SO_4$, the 981 cm^{-1} line of which serves as the internal intensity standard of Raman lines for variant excitation lines. The polarized ν_7 line (677 cm^{-1} for Hb M Iwate and 675 cm^{-1} for Hb M Boston) was much more strongly resonance enhanced upon excitation at 441.6 nm, but the 588- (Hb M Iwate) and 603-cm^{-1} lines (Hb M Boston) were weakened. Although there exists considerable band overlapping, the base line and the band shape of the overlapped part were assumed to be those drawn with broken lines in Figures 5 and 6, and the integrated intensities of the three bands at 1309, 981, and 588 cm^{-1} for Hb M Iwate and those at 1278, 981, and 603 cm^{-1} for Hb M Boston were measured.

The relative intensities of the two lines of the abnormal subunit to that of the SO_4^{2-} ion thus obtained are plotted

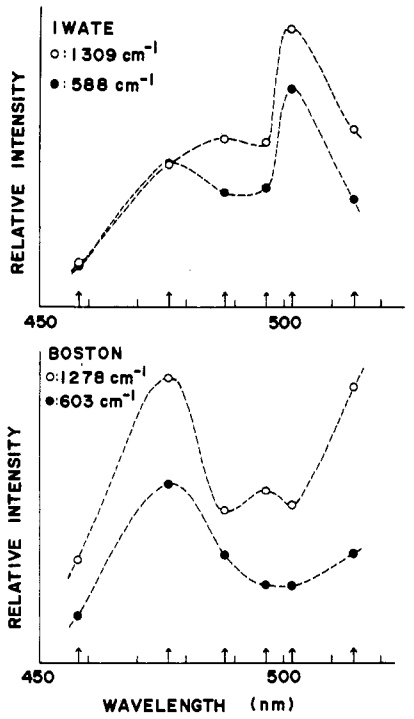

FIGURE 7: Excitation profile of two Raman lines of deoxy-Hb M Iwate (upper) and deoxy-Hb M Boston (lower). The relative integrated intensities of the two lines with regard to the SO_4^{2-} line are plotted against the excitation wavelengths. Broken lines were drawn simply to connect the points smoothly. Each point denotes the average of the five measurements of the integrated intensities.

against the excitation wavelengths in Figure 7. Although large systematic errors may be involved in estimation of the base lines and band shapes, it can be concluded that the 1309- and 588-cm^{-1} lines of Hb M Iwate are intensified upon excitation around 475 and 505 nm and the 1278- and 603-cm^{-1} lines of Hb M Boston are intensified around 475 and >515 nm. The excitation profile of these lines differs from those of the polarized porphyrin modes of various heme proteins (Champion & Albrecht, 1979; Nafie et al., 1973; Strekas et al., 1973).

Discussion

Coordination Structure of the Heme of the Abnormal Subunit. The ν_{10} and ν_{19} frequencies of metalloporphyrin have been demonstrated to have a correlation with a size of the porphyrin core (Spaulding et al., 1975; Huong & Pommier, 1977; Spiro et al., 1979). These two modes arise primarily from the methine-bridge CC stretching vibrations (Abe et al., 1978). When the Fe–N(pyrrole) distances are altered by the interactions between nitrogen and axial ligands (Mashiko et al., 1978), the methine-bridge CC bonds rather than the stiffer pyrrole ring would suffer a distortion, and accordingly, the methine-bridge CC stretching force constant and thus the stretching frequency reflects the axial coordination of the heme iron via the core expansion. In fact, Teraoka & Kitagawa (1980) demonstrated that the ν_{10} frequency is sensitive to the coordination number and the nature of the axial ligands: 1628–1631 and 1615–1625 cm^{-1} for the five- and six-coordinated ferric high-spin compounds.

The present observation showed that the ν_{10} mode of the abnormal α subunits of Hb M Iwate and Hb M Boston is located at 1628–1630 cm^{-1}. Furthermore, we found that the five-coordinated iron porphyrin with a phenolate at the axial position [Fe(III)(PPDBE)($OC_6H_4NO_2$)] and the six-coordinated iron porphyrin with a phenolate and imidazole at the axial positions [Fe(III)(PPDBE)($OC_6H_4NO_2$)(Im)] gave ν_{10} at 1628 and 1642 cm^{-1}, respectively (T. Kitagawa and D. Dolphin, unpublished results). Therefore, we conclude that the abnormal subunits of both Hb M Iwate and Hb M Boston adopt the five-coordinated structure. Our conclusions are consistent with the electron nuclear double resonance study on Hb M Milwaukee, Hb M Hyde Park, and Hb M Iwate (Kankakee) (Feher et al., 1973, and unpublished results quoted therein), which displayed coupling between an unpaired electron of heme and the ^{14}N nucleus of proximal histidine in aquomet-Hb A and Hb M Milwaukee but not in Hb M Iwate and Hb M Hyde Park.

The ferric hemes in both Hb M Iwate and Hb M Boston are high spin, but Ainscough et al. (1978) found that further coordination of 1-methylimidazole to the high-spin phenolate–heme complex gives rise to a low-spin ferric complex. If both His-E7 and Tyr-F8 were coordinated to the heme iron in Hb M Iwate as suggested by the low-resolution X-ray study (Greer, 1971), the heme in the abnormal α subunit of Hb M Iwate would not exist as a high-spin compound.

Charge-Transfer Interaction between Fe(III) and Tyrosine. When tyrosine forms a CT complex with an Fe(III) ion, usually four internal vibrations of the phenolate group around 1600, 1500, 1270, and 1170 cm^{-1} gain resonance enhancement of Raman intensity upon excitation in the CT band. This was first found for serum transferrin (Tomimatsu et al., 1973, 1976; Gaber et al., 1974) and later widely for non-heme-iron dioxygenases (Tatsuno et al., 1978; Keyes et al., 1978; Felton et al., 1978; Que & Heistand, 1979; Bull et al., 1979).

The spectra of Hb M Iwate shown in Figure 4 display three Raman lines at 1606, 1507, and 1308 cm^{-1}. The similar lines were also observed for Hb M Boston (1603, 1504, and 1279 cm^{-1}). The ν_3 line of the oxygenated hemes is usually observed around 1505 cm^{-1} but it is shifted to 1470 cm^{-1} upon deoxygenation (Spiro & Burke, 1976; Kitagawa et al., 1976). The ν_3 line of the five-coordinated ferric heme is mostly observed around 1490 cm^{-1} and is more intensified upon excitation at shorter wavelengths. The 1492-cm^{-1} line of deoxy-Hb M Iwate exhibited such a trend whereas the 1507-cm^{-1} line is most intensified around 500 nm. Therefore, the 1492-cm^{-1} line of deoxy-Hb M Iwate and the 1489-cm^{-1} line of deoxy-Hb M Boston are assignable to the porphyrin ν_3 mode, in agreement with the frequency categorization for the five-coordinated ferric high-spin complexes (Spiro et al., 1979), whereas the 1507-cm^{-1} line of deoxy-Hb M Iwate and the 1502-cm^{-1} line of deoxy-Hb M Boston are presumably associated with the internal vibrations of the coordinated tyrosine.

The ν_{10} line of deoxyheme usually appears around 1605 cm^{-1} as does the peripheral vinyl stretching mode of the porphyrin (Adar, 1975, 1977; Spiro & Strekas, 1974; Tsubaki et al., 1980; Callahan & Babcock, 1981). The ν_{10} line should be depolarized, but the vinyl stretching mode would be polarized. Therefore, even if there appeared the Raman line of the phenolate internal vibration around the 1605-cm^{-1} region, it would be extremely difficult to assign it exclusively to the phenolate vibration. In the polarization measurements, the parallel component of the 1606-cm^{-1} line of deoxy-Hb M Iwate and the 1605-cm^{-1} line of deoxy-Hb M Boston is stronger than what is expected for the depolarized mode. The excess parallel component may be attributed to a vibrational mode of the bound phenolate of tyrosine.

As shown in Figure 3, both met–oxy and met–deoxy mixtures of Hb A displayed no prominent Raman lines between 1230 and 1300 cm^{-1}. On the other hand, iron-coordinated tyrosine is known to exhibit a prominent Raman line due to the CO stretching vibration of phenolate around 1270 cm^{-1}

(Gaber et al., 1974). Accordingly, the 1278-cm^{-1} line of Hb M Boston presumably arises from the CO stretching mode of the coordinated tyrosine. In analogy, the polarized component of the 1308-cm^{-1} line of Hb M Iwate is assigned to the same vibrational mode, although a small contribution of the porphyrin ν_{21} mode to an intensity of this line might be involved. Hence, it is concluded that the CO stretching frequency of phenolate significantly differs between Hb M Boston and Hb M Iwate.

The Raman lines of Hb M Boston at 603 cm^{-1} and of Hb M Iwate at 589 cm^{-1} are not assignable to a porphyrin mode, because in this frequency region no line of such intensity had been observed for any spin state of heme proteins. In regard to the tyrosine-coordinated non-heme-iron proteins, two Raman lines are reported around 524 and 592 cm^{-1} for protocatechuate 3,4-dioxygenase (Bull et al., 1979; Felton et al., 1978), although the corresponding lines were extremely weak for transferrin (Tomimatsu et al., 1976). The two lines may be assigned to the ν_{6a} and ν_{6b} modes of phenol, which are observed at 532 and 617 cm^{-1} for free phenol (Green, 1961), and if this were the case, the 603-cm^{-1} line of Hb M Boston and the 589-cm^{-1} line of Hb M Iwate might be assigned to ν_{6b} of phenolate. Although this possibility cannot be completely ruled out, we think it less likely because of the following reasons: (1) The frequencies significantly differ between Hb M Boston and Hb M Iwate, but other frequencies of the phenolate ring modes around 1500–1600 cm^{-1} show little difference between them. (2) The Raman line due to ν_{6a} is not found for Hb M Iwate and Hb M Boston despite the fact that the ν_{6a} line is more intense than the ν_{6b} line for protocatechuate 3,4-dioxygenase.

We propose that these are due to the Fe–O(phenolate) stretching mode. The $Fe-O_2$ stretching vibration of the oxygenated heme was observed around 570 cm^{-1} for oxy-Hb A (Brunner, 1974; Nagai et al., 1980) and oxygenated iron–porphyrin complexes without protein (Burke et al., 1978; Hori & Kitagawa, 1980). Therefore, with regard to the frequencies, the two modes of Hb M Iwate (589 cm^{-1}) and Hb M Boston (603 cm^{-1}) are assignable to the Fe(III)–O^-(phenolate) stretching mode. If greater donation of electrons from phenolate to the heme iron strengthened the Fe(III)–O^- bond on the one hand and weakened the phenolate CO bond on the other, the higher Fe(III)–O^- stretching mode would lead to the lower CO stretching frequency, in agreement with the trend of the frequency differences between Hb M Boston and Hb M Iwate. The excitation profiles of the two modes shown in Figure 7 suggested that a CT band exists around 475 and 505 nm for Hb M Iwate and around 475 and >510 nm for Hb M Boston, although λ_{max} cannot be determined definitely from the present study due to a possible systematic error involved in the intensity estimation.

It is worth noting that the Fe(III)(PPDBE)($OC_6H_4NO_2$) complexes, which gave visible absorption and EPR spectra similar to those of Hb M Boston and Hb M Iwate (Ainscough et al., 1978), exhibited neither the phenolate internal modes nor the Fe(III)–O^- stretching vibration upon excitation between 441 and 515 nm (T. Kitagawa and D. Dolphin, unpublished results). One may speculate that the phenolate of the model compounds assumes the perpendicular coordination to the heme plane, whereas the phenolates of Hb M Boston and Hb M Iwate are forced to tilt because of some restriction by a protein conformation. In fact, the C_1C_4 axis of the coordinated phenolate of Hb M Boston is tilted to the heme plane (Pulsinelli et al., 1973). If the tilted geometry allowed direct interaction between the π orbitals of phenolate and that of porphyrin, the resonance enhancement of phenolate modes only for the protein-supported phenolate would be understandable. Then, the differences in the frequencies of the particular two modes between Hb M Boston and Hb M Iwate may imply a difference in the tilting angle of the phenolate with regard to the heme plane.

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Registry No. Fe, 7439-89-6; tyrosine, 60-18-4; Hb M Boston, 39340-61-9; Hb M Iwate, 9035-03-4; Hb M Milwaukee, 9035-05-6; oxy-Hb M Milwaukee, 37294-38-5; oxy-Hb M Iwate, 84416-55-7; oxy-Hb M Boston, 84416-54-6; methemoglobin A, 12646-21-8; oxy-Hb A, 9062-91-3; Hb A, 9034-51-9; HbCO A, 9072-24-6; HbCO M Milwaukee, 37294-36-3; HbCO M Boston, 84416-53-5; HbCO M Iwate, 63799-52-0.

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Dynamics of an Interfacial Methylene in Dimyristoylphosphatidylcholine Vesicles Using Carbon-13 Spin Relaxation†

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ABSTRACT: The dynamic and conformational properties of the 2-methylene on the *sn*-2 chain of dimyristoylphosphatidylcholine have been investigated in small unilamellar vesicles. An analysis of the spin relaxation of a proton-coupled ^{13}C nucleus has been used to provide the additional information necessary to propose a specific geometry for motion. The results suggest a model with three motions in addition to vesicle tumbling: (1) a slow axial rotation of the entire molecule about the bilayer normal ($\tau \simeq 2 \times 10^{-8}$ s); (2) torsional oscillations about C–C bonds on a very fast time scale; and (3) rapid jumps ($\tau = 6 \times 10^{-10}$ s) between two conformers having approximate gauche$^+$ and gauche$^-$ conformations about the C_2–C_3 bond of the *sn*-2 chain. The proposed conformations are compared to those previously predicted on the basis of crystal structures, spectroscopic data, and energy-minimization calculations.

The dynamic state of the lipid component of a biological membrane has been shown to affect a number of functional properties including transport (Chapman, 1975), immunochemistry (Parse et al., 1978), and enzyme activity (Moore et al., 1981). While it is often possible to establish correlations between dynamics and function in terms of rather poorly defined concepts, such as "fluidity", it is clear that an understanding of the precise way in which dynamics and function are linked in an anisotropic system such as a lipid bilayer will require more detailed pictures of motional properties.

Numerous spectroscopic techniques have now been applied to help define membrane motional properties (Grell, 1981). Among the more fruitful approaches has been a study of nuclear magnetic resonance (NMR) spin-relaxation times and line shapes (Chan et al., 1981; Jacobs & Oldfield, 1981; Seelig & Seelig, 1980). Several specific models for motion of lipid hydrocarbon chains have resulted (Pace & Chan, 1982; Gent & Prestegard, 1977; London & Avitable, 1977; Edholm, 1981). However, because of the limited number of parameters measured in conventional NMR studies, assignment of motional correlation times to specific chain motions is difficult and often based as much on physical intuition as experimental data. Recently, it has been shown that by studying the coupled spin relaxation of a ^{13}C-labeled lipid methylene group ($^{13}CH_2$), sufficient additional parameters can be measured to begin to assign correlation times to specific motions (Fuson & Prestegard, 1983). The recent work focused on a fatty acid dissolved in a lipid bilayer, and results are perhaps most relevant for lipid acyl chain motions in regions relatively unconstrained by lipid backbone geometry and interfacial effects.

The reason that ^1H-coupled ^{13}C spin-relaxation studies of a methylene group offer sensitivity to additional geometric and dynamic parameters is closely linked to an ability to separate autocorrelation and cross-correlation spectral densities. Most ^{13}C spin-relaxation studies of methylenes ignore the existence of cross-correlation spectral densities, assuming that dipolar

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