# Stark-Effect Spectroscopy of the Heme Charge-Transfer Bands of Deoxymyoglobin

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We report the Stark spectra of the Soret, Q, and charge-transfer bands of deoxymyoglobin. The data show that band III has charge-transfer character but that the magnitude of the charge displacement is significantly smaller than expected or than what is observed for band I or even the Soret or Q bands. The data also show that symmetry breaking of iron−protoporphyrin IX in myoglobin produces a larger difference dipole moment in the Soret transition when compared to the dimethyl ester of protoporphyrin IX. The results lend further credence to the hypothesis that the electrostatic environment of the heme pocket in myoglobin is important in modifying the properties of the heme transitions, and they provide a basis for quantitative analysis of the static and time-dependent band shifts observed in response to environmental electrostatic perturbations and ligand binding dynamics.

The electronic absorption of hemes has been investigated for more than 100 years. Ligand and substituent effects have been systematically assigned, and the focus in recent years has shifted toward static and dynamic band shifts that characterize biological function.[1−6] The origins of these band shifts can be roughly divided into specific chemical effects, e.g., changes in ligand or heme substituents, and more global environmental shifts, typically originating in changes in the electrostatic field around the chromophore. The latter effects are Stark shifts due to the interaction between the change in dipole moment ($\Delta\mu$) and polarizability ($\Delta\alpha$) of the chromophore with the electrostatic field of the ordered environment. To understand these electrostatic shifts quantitatively, it is necessary to characterize $\Delta\mu$ and $\Delta\alpha$ for each type of transition, and this can be achieved by measuring the effect of an externally applied electric field, the Stark-effect spectrum, for each transition.[7,8]

The absorption spectra of heme and other metalloporphyrins are dominated by the B (Soret) and Q absorption bands that arise from configuration interaction (CI) of four orbitals, the nearly degenerate pair a_{1u}, a_{2u} HOMO and the doubly degenerate e_{1g} LUMO.[9,10] The result of CI is an intense Soret transition ($\lambda_{max} \approx 435$ nm in deoxymyoglobin, Mb) and a weak Q band ($\lambda_{max} \approx 556$ nm) that gains most of its intensity through vibronic coupling with the Soret band. [11] Although these bands dominate the spectra, there are other less prominent bands which are of interest. These include the four ligand-to-metal charge transfer (CT) and d−d electronic absorption transitions (bands I−IV) which have been assigned in the near-IR region of deoxy-hemoglobin based on single-crystal absorption, CD, and MCD spectroscopy.[12] Band III is relatively narrow and easily resolved, and it has been shown that there are interesting correlations between the band position and ligand rebinding kinetics at cryogenic temperature in Mb. [1−3,13] This band exhibits time-dependent spectral shifts after MbCO photolysis, and this has been taken to be an indicator of protein conformational changes in response to bond breaking and recombination. [4−6,13,14]
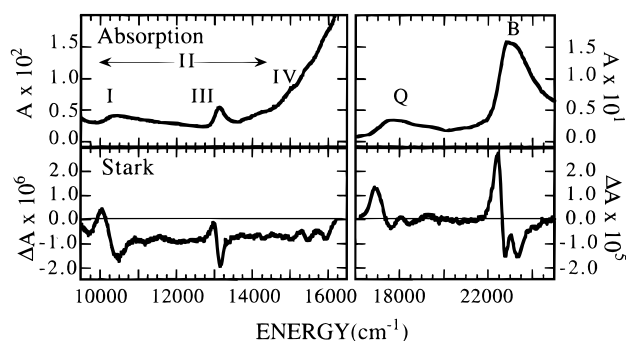
Figure 1. Absorption and Stark spectra for deoxy Mb at 77 K. The Stark spectrum is shown for an applied field of 0.25 MV/cm and $\chi = 90°$ (normal incidence). Note the change in the horizontal scale.

TABLE 1: Difference Dipole Moments $\Delta\mu$ and Polarizabilities $\Delta\alpha$ of Heme Absorption Transitions obtained from the Stark Data on Deoxymyoglobin

transition	$\Delta\mu$ (D)[a]	$Tr\ \Delta\alpha$ (Å³)[a]	ζ_A
B ($\pi-\pi*$)	2.6 ± 0.4	110 ± 25	42°
Q ($\pi-\pi*$)	3.3 ± 0.4	70 ± 15	42°
band I	3.5 ± 0.4	144 ± 20	20°
band III	0.9 ± 0.2	37 ± 9	55°

[a] These values are obtained from the experimental data without considering the effect of the local field correction.[26]

The absorption and Stark spectra of deoxyMb are presented in Figure 1,[15] and the values of parameters extracted from an analysis of the Stark spectra are summarized in Table 1. The Stark spectrum shows clear features for bands I and III, as well as for the Q and B bands; the broad negative base line offset below about 16 000 cm^{-1} may be associated with the Stark effect on band II. The Stark effect for band I is larger than that for band III given its relatively larger line width[16] and weaker absorption intensity, and vibronic structure is visible in the Stark spectrum of band I but not band III. The absorption intensity of band IV is small; the small bands near 15 100 cm^{-1} are reproducible and probably can be attributed to the Stark effect on band IV vibronic bands. Quantitative analysis of band IV

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was not pursued because of the uncertainty of the intensity of this band in the absorption spectrum; however, the Stark effect on band IV appears large relative to that of band III. By measuring ΔA, the change in absorption upon application of field, using polarized light as a function of the experimental angle χ between the polarization direction and the applied electric field, it is possible to obtain the molecular angle ζ_A between the transition moment and $\Delta\mu$.[7,8] The absence of a dependence of ΔA on χ for band III shows that ζ_A is close to the magic angle ($\zeta_A \approx 55°$), while for band I $\zeta_A \approx 20°$ (data not shown).

As seen in Table 1, $|\Delta\mu|$ is surprisingly large for the Soret and Q bands. Interestingly, the Stark effect for the Soret band of Fe−protoporphyrin IX dimethyl ester complexed with 2-methylimidazole has a significantly smaller value of $\Delta\mu = 1.3 \pm 0.2$ D in a glycerol/DMSO glass (66% v/v) (data not shown).[17] This result suggests that the internal field of the protein significantly affects the electronic properties of the heme and is consistent with results of Stark hole burning experiments on a variety of heme proteins.[18−20] Those investigations concluded that the ordered environment of the protein induces a difference dipole in the porphyrin Soret and Q bands.[21]

Typically, CT bands are broad transitions with large values of $\Delta\mu$. Ring-to-metal CT bands in metalloporphyrins, however, would not necessarily be expected to have large values of $\Delta\mu$. In molecules with D_{4h} symmetry, the dipole moment in both the ground and the excited states is rigorously zero, and hence, a CT transition would have $\Delta\mu = 0$. For a C_{4v} metalloporphyrin such as deoxy heme, charge displacement can only occur along the z-axis unless there is symmetry breaking due to the electrostatic environment. A ring-to-metal CT band involves charge displacement from a p orbital at the average position of the plane of the heme to an orbital centered on the iron. If the displacement of the iron is 0.4 Å from the plane of the pyrrole nitrogens,[22,23] then $\Delta\mu \approx 1.9$ D for separation of a full charge over this distance. If significant doming of the heme occurs, the value expected for charge displacement could be larger.

Band I is assigned as a $d_{xz} \rightarrow e_g(\pi^*)$ transition which is polarized along the z-axis, while band III is attributed to the $a_{2u} \rightarrow d_{yz}$ transition which is polarized mostly in the plane of the heme.[11] Band I has a substantial Stark effect ($|\Delta\mu| \approx 3.5$ D) and a small angle $\zeta_A \approx 20°$ consistent with its assignment as a CT band with charge displacement along the z-axis. The assignment for band III is more problematic as it is uncharacteristically narrow for a CT band. The angle $\zeta_A \approx 55°$ for band III is consistent with alignment of $\Delta\mu$ along the z-axis if the transition moment is taken to make an oblique angle with respect to the z-axis, as indicated by polarized single-crystal data.[12] The value of $\Delta\mu \approx 0.9$ D for band III is too large for a d−d transition and indicates that band III has CT character. Band III is an intrinsically weak transition that may borrow at least part of its intensity from vibronic mixing with the Soret band. Vibronic mixing of band III with the Soret band may provide an explanation for both the relatively large $|\Delta\mu|$ of the Soret band and the relatively small $|\Delta\mu|$ of band III by mixing the CT character in the two manifolds. This hypothesis, although consistent with the original assignment of band III as an $a_{2u} \rightarrow d_{yz}$ CT band, indicates that band III has a strikingly different electronic character and dependence on nuclear coordinates than has been evident from absorption spectroscopy.

Time-dependent spectroscopic studies of band III and the Soret band show that both bands undergo spectral shifts following photolysis of MbCO in viscous solvents.[5,24] Significantly *both* the time dependence and magnitude of the change in energy associated with both band shifts are nearly identical. If the time-dependent band shifts were the independent response of these electronic transitions to the same perturbation in the electrostatic field around the heme that accompanies MbCO photolysis, that is electrochromic bandshifts, then the time dependence should be similar. However, the Stark data predicts a greater sensitivity and, hence, larger magnitude of an electrochromic band shift for the Soret band than band III. It is likely that the change in the electrostatic field that accompanies CO photolysis is not symmetric around the heme. We can speculate that if the largest change in the electrostatic field is approximately perpendicular to the heme plane, then the projection of this change on $\Delta\mu$ for band III could be larger than that for the Soret band. This may be testable by comparison with electrostatic calculations or by using other direction-sensitive probes of changes in electrostatic fields such as molecular vibrations.[25]

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(16) When $\Delta\mu$ is the major contributor to the Stark spectrum for an isotropic sample, the Stark (ΔA) line shape is the second derivative of the absorption. Consequently, the magnitude of ΔA depends strongly on the absorption line width.

(17) This agrees well with the small value of $|\Delta\mu|$ for the Soret band of Fe(III) chloride tetraphenyl porphyrin in poly(vinyl alcohol). Davidsson, Å. *Chem. Phys.* **1980**, *45*, 409−414.

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