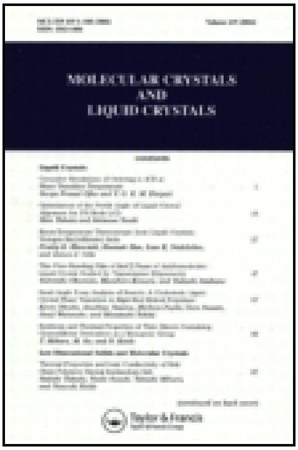

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Transition Frequency and Internal Electric Field for Protoporphyrin-IX in Myoglobin

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TRANSITION FREQUENCY AND INTERNAL ELECTRIC FIELD FOR PROTOPORPHYRIN-IX IN MYOGLOBIN

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Abstract The internal electric field acting on a guest molecule can be determined by measuring the effect of an external electric field on the profile of a photochemical hole. For protoporphyrin-IX in myoglobin we observe a systematic dependence of internal electric field on frequency in the inhomogeneously broadened Q_x band.

INTRODUCTION

Because molecules are made up of charged particles, they necessarily generate electric fields. While overall electrical neutrality requires that these fields vanish at macroscopic distances, at the distances of condensed phase intramolecular separations they can be much larger than the external fields that can be produced in the laboratory. Consequently, the internal electric field that is generated by a given molecular arrangement can be a key determinant of charge transport and trapping. This provides enormous motivation to quantitatively determine internal electric fields, especially in materials that exhibit unusual photoinduced changes in electrical properties[1], and in proteins which facilitate charge separation such as the photosynthetic reaction center[2],

Despite the fundamental importance of the internal electric field, it was only recently opened to direct experimental determination. In studies on all-*trans* 1,3,5,7-octatetraene in n-hexane crystals we have shown how quantum mechanical analysis of measurments of the effect that an external electric field has on the transition energy of a probe molecule can give a quantitative mapping of the internal electric field at the guest at molecular[3] and even atomic[4] resolution. Since the external electric field induced changes in transition energy are rather small (typically of order 0.04 cm^{-1} for 10 kV/cm external fields), the measurements can only be made under high resolution conditions. In our experiments the needed spectroscopic resolution is achieved by low temperature photochemical hole burning techniques. The effect of both external and internal electric fields on transition energy is treated by adding a perturbation term ($-\underline{E}\cdot\underline{\mu}$ in the case of molecular resolution, site energies in the case of atomic resolution) to the Hamiltonian that reproduces the measured excitation energies. In this contribution we report molecular resolution level analysis of experiments on protoporphyrin-IX/myoglobin that uncover an approximately linear relationship between internal field and frequency in the Q_x band.

EXPERIMENTAL

Our experiments were made on highly purified protein in 3:1 by volume glycerol:water glass (optical spectrum in Figure 1, further details in reference 5).

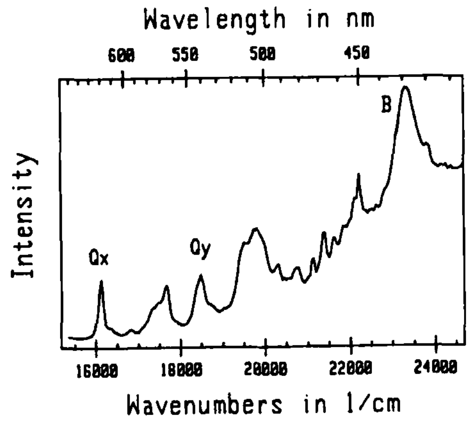


FIGURE 1 Fluorescence excitation spectrum of protoporphyrin-IX myoglobin in 3:1 glycerol:water glass at 77 K. Labels identify the 0-0 bands of transitions to the Q_x state (16140 cm^{-1}), the Q_y state (18462 cm^{-1}) and the B states (B_x at 23282 cm^{-1}, B_y at 23482 cm^{-1}). We have arbitrarily assumed that the unresolved B_x and B_y components are split by 200 cm^{-1} with B_x lower in energy.[6] Because the B state splitting is small compared to the excitation energies, the calculated profiles have no significant dependence on the value chosen for this splitting.

The short lifetime of the excited state of the heme in myoglobin makes it impossible to burn holes that are sufficiently narrow to show the effects of experimentally attainable external electric fields, so the heme was replaced by its free base, protoporphyrin IX. The long lived S_1 state in protoporphyrin IX leads to the formation of narrow holes via the phototautomerization of the inner protons.[7] Apparatus and protocols for the electric field experiments have been described elsewhere.[3]

Hole profiles were measured for a range of external electric fields (1.7 to 12.1 kV/cm) in two geometries (light polarized parallel or perpendicular to the applied field) at 8 positions in the Q_x transition (16051 cm^{-1} to 16192 cm^{-1}). Figure 2 shows typical data.

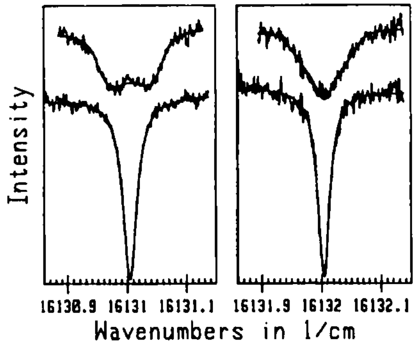


FIGURE 2 Fluorescence excitation scans at 1.2 K of photochemical holes burned into the Q_x 0-0 band of protoporphyrin-IX myoglobin. Laser light polarized parallel to the applied field is shown at the left and laser light polarized perpendicular to the applied field is shown at the right; the lower scans are at zero external field and the upper scans are at 11.05 kV/cm. The smooth line through the noisy data is the profile calculated for local field factor 2.1 and internal field components E_x=29.9 MV/cm and E_y=38.6 MV/cm.

The profiles are similar to those recently reported[8]: what is new is our ability to go beyond qualitative statements about the existence of an internal field and determine two of its components which leads to insights about local structure.

INTERNAL FIELDS

The porphin molecule which forms the conjugated core of protoporphyrin-IX is our reference for describing the effect of an external electric field on hole profile. By considering all of the substituents to be part of the environment we take maximum advantage of symmetry and the fact that the fundamental aspects of porphin electronic structure are well understood. As in our initial report on this system[5] we treat the effects of internal and external fields in the point dipole approximation: thus, subject to the uncertainty with which the local field factors are known, the internal field is determined at molecular resolution. The more precise atomic resolution mapping using protocols analogous to those applied to octatetraene in n-hexane[4] is in progress.

In the point dipole approximation the effect of an electric field on the Q_x excitation energy of the D_{2h} symmetry porphin core is described by the Hamiltonian

$$H = \begin{pmatrix} 0 & -E_x\mu_{Qx} & -E_y\mu_{Qy} & -E_x\mu_{Bx} & -E_y\mu_{By} \\ -E_x\mu_{Qx} & W(Q_x) & 0 & 0 & 0 \\ -E_y\mu_{Qy} & 0 & W(Q_y) & 0 & 0 \\ -E_x\mu_{Bx} & 0 & 0 & W(B_x) & 0 \\ -E_y\mu_{By} & 0 & 0 & 0 & W(B_y) \end{pmatrix} \qquad (1)$$

where $W(Q_x)$, $W(Q_y)$, $W(B_x)$ and $W(B_y)$ are the zero field energies of the Q_x, Q_y, B_x and B_y states, respectively, μ_{Qx}, μ_{Qy}, μ_{Bx} and μ_{By} are the corresponding transition dipoles, and the basis is restricted to the π-electron states in the 4-orbital model[9] (Q and B or Soret states). The *x* and *y* components of the electric field at the porphin center are given by the superposition of internal and external fields. As in our previous analysis we assume that in the chromophore reference frame the internal electric field is fixed while the external field is randomly oriented (that is, the internal field is well defined in the molecular frame while the protein molecules are randomly oriented in the laboratory frame). $W(Q_x)$ is chosen so that diagonalization of Eq.1 for zero external field (internal field only) gives the center frequency of the photochemical hole; the other zero field energies were $W(Q_y)$, $W(B_x)$ and $W(B_y)$ equal to 18840 cm^{-1}, 23180 cm^{-1} and 23350 cm^{-1}, respectively. (As would be expected from the smallness of the electric field perturbation relative to the excitation energies, the excitation energy shifts calculated for a given electric field are quite insensitive to the values of the zero field energies). The transition dipoles (μ_{Qx}, μ_{Qy}, μ_{Bx} and μ_{By} equal to 0.102, 0.152, 1.104 and 1.151 Debye, respectively) come from theory[10]. Our choice of 2.1 for the local field factor is discussed elsewhere.[5]

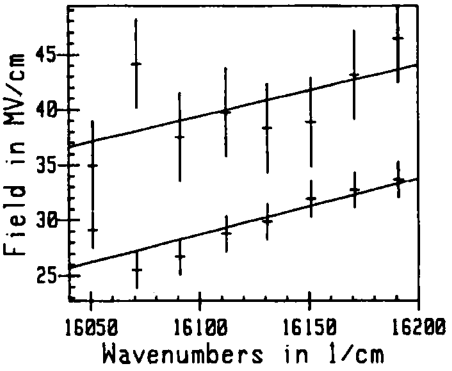
Hole profiles for given internal and external electric fields are calculated in two steps. First, the Q_x excitation energy is calculated by diagonalizing Eq. 1 and weighted by the fourth power of the projection of μ_{Qx} on the light polarization for all relative orientations of laboratory and molecular frames. This profile is then convolved with the zero external electric field hole profile. For a given local field factor the field induced change in a photochemical hole profile is determined by only two adjustable parameters, the x and y components of the internal field. These are sharply determined by fitting profiles for two polarizations (burning and observing light polarized parallel or perpendicular to the external field).

Figure 2 shows the excellent agreement between measured and calculated profiles for data taken near the center of the inhomogeneously broadened Q_x band. With the local field factor 2.1 eight profiles (4 different external fields with light polarized parallel and 4 different external fields with light polarized perpendicular to the applied field) are simultaneously fit at the same quantitative level shown in Figure 2 by the internal field components E_x=29.9 ± 0.3 MV/cm and E_y=38.6 ± 0.7 MV/cm. The same excellent agreement between calculated and measured profiles (7 or 8 data sets per frequency) is obtained for holes burned at other frequencies in the inhomogeneously broadened Q_x band, but the internal electric field values that fit the measured profiles vary systematically with burning frequency (Table 1 and Figure 3).

TABLE 1. Dependence of internal field on position in the inhomogeneous Q_x profile.

Burn Frequency (cm^{-1})	E_x (MV/cm)	E_y (MV/cm)
16051	29.1	35.0
16091	26.7	37.5
16112	28.8	39.7
16131	29.8	38.3
16151	31.8	38.9
16171	32.7	43.1
16191	33.7	46.4

FIGURE 3 Dependence of internal electric fields E_x (lower) and E_y (upper) on frequency in the Q_x band of protoporphyrin-IX in myoglobin.



CONCLUSION

The smooth and systematic dependence of internal electric field on frequency that is observed here implies that the dispersive and electrostatic contributions to the solvent shift of the protoporphyrin-IX Q_x band are significantly correlated. In another contirbution to this volume we show how the assumption of complete correlation can lead to a quantitative accessment of the dispersive and electrostatic shifts.

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