STRUCTURAL DISTRIBUTION AND ROTATIONAL DISORDER IN MYOGLOBIN CRYSTALS

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Spectroscopic measurements provide not only energy level differences but, with appropriate experimental conditions and analysis, also widths of levels. Widths can often be shown to be due primarily to distribution in structure at the spectroscopically active center, such as at the high-spin ferric ion in myoglobin crystals discussed below.

The g-tensor of high-spin ferric ion in heme is highly anisotropic. This property has been used in several laboratories to estimate the disorder in heme normal directions in crystals. For α- and β-chains of horse hemoglobin, 37% of the normals lie outside of a cone of half-angle $\eta_D = 4°$ (Hampton and Brill, 1979); in sperm whale myoglobin, η_D is half this value (Fiamingo, 1980). While rotational disorder corresponding to $\eta_D = 2°$ is the dominant source of line broadening for many orientations of magnetic field, H, it contributes less than a gauss to observed peak-to-trough linewidth when H is parallel to the heme plane. Dipolar interactions are somewhat more effective in producing broadening in the heme plane, but they and misorientation together are of minor influence in comparison with structural distribution.

At 37.5 GHz, Helcke et al. (1968) observed 250% variation in linewidth of electron resonances from the heme plane of aquo myoglobin crystals. In conjunction with measurements of orientation and temperature dependence of power saturation and spin-lattice relaxation time, we have measured the orientation dependence of linewidth at 9.27 GHz (Fiamingo, 1980). These data, together with simulations based upon known hyperfine coupling constants, provide hyperfine component linewidths, the squares of which show a heme plane angular dependence $L + M \cos 2\phi + N \cos(4\phi - \beta)$ with L, M, and N = 159, -22, and -72 G^2, respectively, and $\beta = 122°$ (Fig. 1). Calvo and Bemski (1976) proposed independently fluctuating principal directions and values of the zero-field splitting tensor to

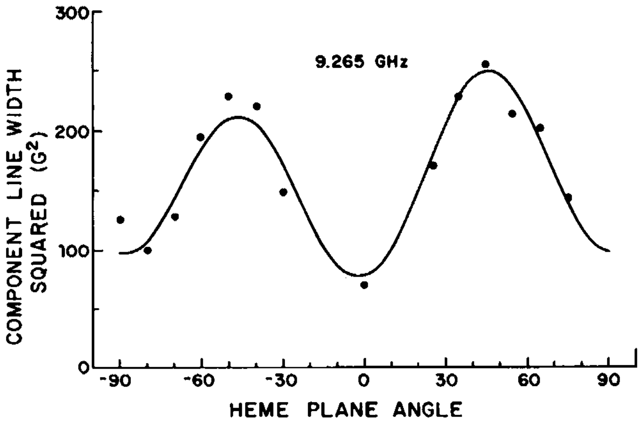

Figure 1 Component linewidth squared. The square of the hyperfine component linewidth (one-half peak-to-trough) is plotted as a function of the heme plane angle. The solid circles are the observed linewidths, converted to component linewidths by a simulation-derived function, and then squared.

explain large heme plane linewidth variation. However, the principal values depend in part upon the same crystal field energies, and hence there is some correlation in the fluctuations. One consequence is the more complex ϕ-dependence found above. We have, therefore, formulated in-plane linewidth orientation dependence upon fluctuations in energy differences and principal x-direction of a four-level center, including concomitant distribution in quartet state admixing. Because average squares of the four fluctuations must be ≥ 0, the five parameters of the model are constrained to usefully small ranges in generating L, M, N, and β. With an angular and frequency independent contribution of 7.5 G, the energy differences and their rms fluctuations are:

	$\Delta(\text{cm}^{-1})$	$\delta\Delta(\text{cm}^{-1})$
${}^4A_2$-${}^6A_1$:	1,940	300 ± 30
${}^4E$-${}^6A_1$:	5,325	300 ± 300
${}^4E_y$-${}^4E_x$:	45	12 ± 12.

The rms fluctuation in principal x-direction is 18°.

Other data, from optical absorption as well as electron paramagnetic resonance, have been analyzed in terms of energy level and structural distributions at the active sites of heme and copper proteins (Muhoberac, 1978; Brill, 1979). These distributions are localized expressions of conformational influence and fluctuation, and appear to be as much a part of the description of a protein and its state as is the average structure. Advantage can be taken of this in detecting and quantifying the effects of chemical and physical agents on proteins. With regard to functional significance, when a reaction is accompanied by change in geometry, structural distribution can reduce the enthalpy of activation (Brill, 1979).

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