VIBRATIONAL CIRCULAR DICHROISM IS A SENSITIVE PROBE STUDY OF HEME PROTEIN-LIGAND INTERACTIONS

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The molecular basis of the control mechanism determining ligand affinity in heme proteins is often dependent on weak interactions between the ligand and the protein residues in the heme pocket. We demonstrate that vibrational circular dichroism (VCD) of the azide asymmetric stretch of the azide ligand bound to a heme protein is sensitive to the energetically small interactions between the ligand and distal globin amino acid residues E11 and E7. The VCD intensities are reported as anisotropy ratios g, where $g = \Delta A/A_T$, with $\Delta A = A_L - A_R$ and $A_T = \frac{1}{2}(A_L + A_R)$, total absorbance.

We examined the VCD of the asymmetric stretch of covalently bound azide (ca. 2020 cm^{-1}) in detail for the first time using a number of evolutionarily diverse and mutagenic heme proteins. These heme proteins include horse, sperm whale and elephant myoglobins, Chironomus thummi thummi monomeric hemoglobin III, carp and human hemoglobin and mutants of sperm whale (Gly E7) and human (Asn E11) myoglobin. The VCD of sperm whale and horse myoglobin are identical with an anisotropy ratio, g, of ca. 9.7×10^{-4}. The carp and human hemoglobin VCD intensities are reduced by ca. 15% compared to the horse Mb. The elephant Mb which has the distal histidine replaced by a glutamine E7 exhibits a VCD intensity that is ca. 40% less than the horse Mb. The mutant sperm whale Mb which substitutes the E7 histidine with glycine has no observable VCD and has an azide ligand binding affinity that is smaller by an order of magnitude. The same result was obtained for mutant human Mb which has the distal valine E11 replaced by asparagine.

VCD is extremely sensitive to the interations between the azide ligand the E7 and E11 residues of heme proteins. These experiments clearly indicate that these interactions cannot be described simply by the steric interactions in

the heme pocket but must include hydrogen bonding or van der Waals interactions between the lone electron pairs on the azide ligand and the E7 and E11 residues.

The mutant Mb results also indicate that VCD is sensitive to the same energetic interactions that affect the kinetics of ligand binding. Figure 1 shows the FTIR and VCD spectra of various heme proteins while Figure 2 presents a model for the VCD mechanism.

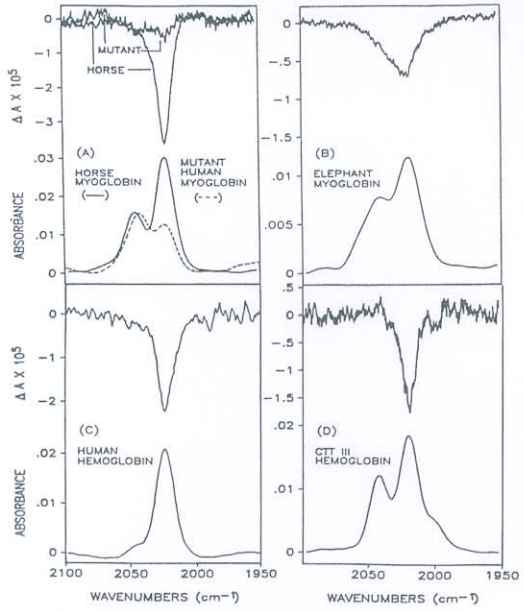

Figure 1. IR absorption (8 cm^{-1} resolution) spectra and VCD (6 cm^{-1} resolution) spectra of: (A) 11.5 mM horse MbN_3 and 7.1 mM mutant (Asn E11) human MbN_3 with 2.7 mM unbound N_3^-; (B) 6.0 mM Elephant MbN_3; (C) 9.0 mM human HbN_3; (D) 6.0 mM CTT III HbN_3. Protein samples were buffered at *ca.* pH 7 with 0.01 M phosphate buffer adjusted to an ionic strength of 0.7 with KCl. All spectra were measured in a 26μm CaF_2 cell and normalized to obtain a low spin band intensity comparable to horse Mb, for ease of comparison.

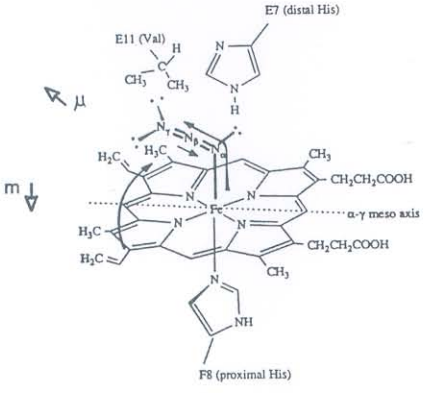

Figure 2. VCD mechanism showing the heme and bound azide ligand interactions with the Val E11 and His E7 in the distal heme pocket. Large arrows show the direction of positive electronic current flow resulting from the N_β motion (small arrow). Approximate directions of the electric (μ) and magnetic (m) dipole transition moments are indicated.