HEME-PROTEIN STRUCTURAL INTERACTIONS IN HEMOGLOBIN STUDIED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

James O. Alben, Patrick P. Moh, and George H. Bare, *The Ohio State University, Department of Physiological Chemistry, Columbus, Ohio 43210 U.S.A.*

Fourier transform infrared spectroscopy provides the sensitivity, selectivity, and variety of absorptions required for a probe of molecular structure in biological systems. We have developed these spectroscopic methods and applied them to the study of interactions between heme and protein that permit biological control of oxygen transport in hemoglobin (1, 2). Several absorptions have been identified with specific group vibrations which act as structural probes of known locations within the molecule. The best studied of these are ligands coordinated to the heme iron (3), and the cysteine sulfhydryl groups which may be studied individually because of their different local surroundings (4).

Ligation at heme iron can produce measurable alterations in protein structure that are sensitive to the nature of the ligand. For example, the frequency of the SH vibrational absorption of the α-104 cysteine (at the $\alpha_1\beta_1$ interface) increases in the order, HbCO < HbN_3 < HbNO < [HbCN ≃ HbO_2] < $Hb^+(H_2O)$ < [Hb^+F^- ≃ $Hb^+(HCOO^-)$] << Hb (deoxy) (2, 5). This appears to be caused by change in strength of H-bonding of the α-104 SH group to the peptide carbonyl of α-100 leucine, one turn back in the G-helix, associated with ligand-dependent movement of the heme. This SH frequency shift is much larger with the tetrameric ($\alpha_2\beta_2$) hemoglobin than for the dimeric α-chain derivatives. The hemoglobin quaternary structure accentuates the effects of ligands and "stresses" α-chain tertiary structure (shifts SH frequencies) of ligated and deoxy hemoglobin in opposite directions with respect to isolated α-chains.

The SH vibrational absorption of the β-93 cysteine behaves differently. Although the center frequency does not change with ligation of heme iron, the molar absorptivity decreases in the order HbCO > [HbO_2 ~ HbCN] > $Hb^+(H_2O)$ >> Hb(deoxy). In deoxyhemoglobin, the β-93 SH group is open to exchanging water molecules at the surface of the protein and absorbs too weakly to be observed under present conditions. With ligation of the β-heme, the iron moves toward the mean porphyrin plane, causing rotation of the F-helix, and brings the F-9 cysteine SH into a nonpolar pocket between the F and H helices. The SH vibrational frequency (2,592 cm^{-1}) is too high to allow H-bonding interactions. The relatively high absorptivity in ligated derivatives can be explained by steric constraints, and the broad band width at half-maximum intensity (20 cm^{-1}) is reasonable for large thermal motions at the molecular surface. In contrast with the α-104 cysteine SH group, that at the β-93 position behaves similarly in the $\alpha_2\beta_2$ tetramer and isolated β-chains (β_4). Its absorptivity depends almost exclusively on ligation at the β-heme iron, and appears to be independent of quaternary structure.

Very small, but measurable, effects of protein structure on carbon monoxide coordinated to heme iron have also been observed. The effects of heme-protein interactions are transmitted to coordinated ligands in a manner that is not always simple, but is the sum of ligand field effects, van der Waals and induced dipole interactions with groups surrounding the heme, and steric repulsions toward the heme and coordinated ligand.

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DOES THE GENETIC TYPE OF COLLAGEN DETERMINE FIBRIL STRUCTURE?

Eric Eikenberry, Barbara Brodsky, and Kathleen Cassidy, *College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Department of Biochemistry, Piscataway, New Jersey 08854 U.S.A.*

A number of genetic types of collagen, all triple-helical but with significant variations in their amino acid sequences, have been found and the distribution of these genetic types is tissue specific. For example, tendon is composed only of type I collagen, while cartilage contains largely type II collagen. Skin contains a large amount of type I, but has a significant fraction, ~15%, of type III. Each of these types can form fibrils, but it is not known whether they form distinctive fibril structures that are important in determining tissue organization. We are using x-ray diffraction to analyze a variety of tissues with different collagen genetic types to compare the fibril structures and thus investigate whether genetic type is an important determinant of this structure.

In connective tissues collagen is organized into cylindrical fibrils with the molecules parallel to the fibril axis. The x-ray diffraction pattern of a well-oriented connective tissue specimen, such as a tendon, typically shows a meridional series of Bragg reflections with a 67-nm periodicity, denoted as D, arising from the axial stagger of the molecules and an equatorial pattern dominated by reflections from the intermolecular spacing. The intensities of the meridional reflections contain information on the axial electron density distribution. Comparison of the intensities obtained from different tissues should indicate the degree to which the electron density distributions in the tissues are similar to a resolution of 1.5 nm if, as is typical, 40 orders are observed. Different tissues also contain various kinds and amounts of noncollagenous material, and these components may influence fibril structure or bind regularly to the fibrils, either of which may be expected to influence the meridional intensities. If a set of phases is available, the intensity data can be interpreted directly in terms of electron density and then it may be possible to relate differences in electron density to features of the different genetic types or their organization in tissues.

Tendons consist almost exclusively of type I collagen which is organized into parallel fibrils with diameters ranging from 30 to 400 nm in adult specimens. Wet tendons from a variety of sources consistently show a D periodicity of 67 nm and show the same orders to be strong in their meridional patterns. Among weaker orders there are small differences in the relative intensities, especially in the 14th through 18th orders. Some tendons, such as rat tail tendon,