PICOSECOND TO MILLISECOND TRANSIENT ABSORPTION SPECTROSCOPY OF CARBONMONOXY- AND OXYHEMOGLOBIN IN THE VISIBLE AND MID-INFRARED SPECTRAL REGION

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Human hemoglobin (Hb) is an allosteric protein that transports molecular oxygen (O_2) [1]. Hb is a tetramer consisting of two α and two β subunits. Each subunit contains one identical ferrous heme group which can reversibly bind one ligand molecule (O_2 or carbon monoxide (CO). Hb binds four ligands cooperatively. As tetrameric Hb is liganded, its quaternary structure changes and the ligand affinity increases. At present, there is no definitive agreement on how ligand-induced conformational changes influence individual ligand binding properties of the α and β subunits in the different conformational forms of tetrameric Hb. This information is necessary to understand the molecular mechanism of cooperative oxygenation of Hb as well as the mechanism of ligand transport and tissue oxygenation. The principal aim of this study was to determine how ligand-induced conformational changes influence the individual ligand binding properties of the α and β subunits within Hb.

Taking advantage of the photosensitivity of the chemical bond between the ferrous heme iron and the ligand molecule (O_2 or CO), picosecond to millisecond laser time-resolved spectroscopy in the visible and mid-infrared spectral region was used for kinetic studies of ligand binding and conformational changes following ligand photodissociation in Hb. Valency hybrids of Hb and the isolated Hb chains were used as models for Hb. The valency hybrids were obtained by the method [1]. Time-resolved spectra in the visible (Soret) and mid-infrared region were measured on the ULTRA apparatus [2] at the Central Laser Facility (Didcot, UK). All the experiments were performed in 50 mM Tris buffers, at 19 °C. Excitation wavelength, 543 nm.

A kinetic model for the geminate ligand rebinding in the ferrous hemoglobin subunits, ligand migration between the primary and secondary docking site(s) [3], and nonexponential tertiary relaxation within the liganded quaternary structure, was introduced and discussed. Significant functional non-equivalence of the α and β subunits in both the geminate ligand rebinding and concomitant structural relaxation was revealed. The conformational relaxation following the ligand photodissociation in the α and β subunits was found to decrease the rate constant for the geminate ligand rebinding, this effect being more than one order of magnitude greater for the β subunits than for the α subunits. Evidence was provided for the modulation of the ligand rebinding to the individual α and β subunits within Hb by the intrinsic heme reactivity through a change in proximal constraints upon the relaxation of the tertiary structure on a picosecond to microsecond time scale.

References

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