Monitoring the CO and O_2 rebinding to human hemoglobin and its subunits with time-resolved visible and mid-infrared spectroscopy

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Human hemoglobin (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 (CO or O_2). Up to now, there has been 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. In the present study we attempted to answer that question. We used time-resolved laser spectroscopy approach in that study, followed by the kinetic modeling. Picosecond to millisecond time-resolved spectra in the mid-infrared and visible regions were measured at the Central Laser Facility (STFC Rutherford Appleton Laboratories, Harwell, UK). We introduced the kinetic model, which describes the geminate ligand rebinding in the ferrous hemoglobin subunits, ligand migration between the primary and secondary docking site(s), and nonexponential tertiary relaxation within the liganded quaternary structure [1]. Our results reveal significant functional differences in the α and β subunits in both the geminate ligand rebinding and concomitant structural relaxation. 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. We observed the modulation of the ligand rebinding 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.

REFERENCE(S)

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