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Conformational dynamics of human hemoglobin in live red blood cells probed by hydrogen/deuterium exchange based mass spectrometry

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To investigate the mechanism of various cellular events it is crucial to understand the structure-function correlation of biological macromolecules within a live cell. Conventionally, different spectroscopic tools such as NMR, fluorescence, circular dichroism etc. that are used to visualize protein structure are chromophore specific which are identical across all proteins. Therefore structural studies are restricted to purified molecules *in vitro*. However, due to the molecular crowding inside a complex cellular milieu, *in vivo* and *in vitro* environments are radically different. Exploiting permeability of D2O across cell membrane, hydrogen/deuterium exchange (H/DX) was executed inside in side live red blood cells (RBCs) and subsequently the change in conformation dynamics of human hemoglobin associated with its oxygenation in side live RBCs was monitored. Using mass spectrometry, H/DX kinetics of globin polypeptide backbone amide hydrogens of human hemoglobin inside RBC in both oxy and deoxy states was recorded. The obtained kinetic parameters were analyzed using the method of initial rates and the conformational change on deoxy to oxy transition of hemoglobin was explored inside live RBCs. Due to very high abundance of hemoglobin in RBC, it was possible to correlate the observed *in vivo* conformational dynamics with the structure-function correlation of hemoglobin reported *in vitro*. The novelty of present method lies in its applicability to investigate change in conformational dynamics of human hemoglobin associated with its oxygenation in its endogenous environment. Various biological processes like ligand binding, folding and post-translational modification of proteins inside a living cell irrespective of its location and structural complexity.

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