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Structural Studies of a Circularly Permuted Human Hemoglobin Containing Low $O_2$-affinity Mutations

Rachel Hubbard
Western Washington University

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Structural Studies of a Circularly Permuted Human Hemoglobin Containing Low O₂-affinity Mutations
Rachel Hubbard, P. Clint Spiegel and Spencer Anthony-Cahill
Department of Chemistry, Western Washington University

Abstract
Our research is focused on the production of a hemoglobin-based oxygen carrier (HBOC) that can be used as a therapeutic in the event of acute blood loss. The administration of cell-free hemoglobin is associated with severe adverse effects due to dissociation of the tetrameric α₂β₂ complex into αβ heterodimers. Our approach to designing an effective HBOC is based on a recombinant circularly permuted human hemoglobin in which all of the subunits are linked in a single-chain fashion. This design would prevent the dissociation of the tetramer and allow for the biosynthesis of polymeric hemoglobins of defined mass. Preliminary ligand binding data with our permuted hemoglobin indicates that they prefer the high O₂-affinity R-state conformation over the low O₂-affinity T state. The βN108K and αV96W mutations were introduced to restore T state stability. Preliminary studies of the mutants have shown that while the βN108K mutation improved T state stability, the αV96W mutation displays an unexpected destabilizing effect on the T state. We would like to understand the molecular basis for these surprising results. We intend to determine the X-ray crystal structure of the αV96W mutant as well as the βN108K and αV96W + βN108K double mutant to gain an atomic-level picture of protein structural differences that could explain these results.

Hemoglobin States

Why sc-Hb?

Flash Photolysis

Protein Purification

Ligand Binding Studies

Structural Determination of Permuted Hbs

Future Work/ Research Goals

Acknowledgments

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