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

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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) which 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 hemoglobins indicates that they prefer the high O₂-affinity R-state conformation over the low O₂-affinity T state. The αV96W and αV96W mutations were introduced to restore T state stability. Preliminary studies of the mutants have shown that while the αN108K 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 αV96W + αN108K 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

X-ray Crystallography

Full Photolysis

Partial Photolysis

1. IMAC (Immobilized Metal ion Affinity Chromatography)
2. Mono Q (Strong Anion Exchanger)
3. SEC (Size Exclusion Chromatography)

Future Work/ Research Goals
- Obtain crystal structures of αcpβ + V96W + αN108K
- αcpβ + V96W
- αcpβ + αN108K
- Gain understanding of anomalous effects of V96W on T state structure
- Establish reliable bioreactor fermentation protocol to increase yields

Acknowledgments

Future Work/ Research Goals

X-ray Crystallography

Functional characterization of the permutations show a significant reduction in protein stability. Structural models are essential to guide protein engineering efforts aimed at increasing stability and optimizing function. X-ray crystallography was employed to obtain atomic resolution electron density maps of a cpβ and sca-cpβ which were then used to refine models of the proteins and reveal structural changes due to the modifications.

Circular Permutation

The Approach

Protein Yields

<table>
<thead>
<tr>
<th>Protein</th>
<th>sc-Hb (mg/L)</th>
<th>αcpβ + N108K (mg/L)</th>
<th>αcpβ + 6M (mg/L)</th>
<th>HUG (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>αcpβ + V96W</td>
<td>21.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>αcpβ + αN108K</td>
<td>2.7</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sc-Hb + αN108K</td>
<td>13.3</td>
<td>1.2</td>
<td></td>
<td>120</td>
</tr>
</tbody>
</table>

Structural Determination of Permuted Hbs

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