May 18th, 9:00 AM - 12:00 PM

Structural Studies to Understand the Effects of N108K T-State Stabilizing Mutation in Circularly Permuted Hemoglobin

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Abstract

The primary goal of our project is to develop a functional hemoglobin based oxygen carrier (HBOC) for clinical care. In order to accomplish this, we are working on designing a single chain hemoglobin (sHb) that can serve as the building block of a monodisperse polymeric HBOC. The sHb is formed from novel covalent fusions between the two α chains and two circularly permuted β chains. The initial constructs of non-covalently linked α and cp-β showed decreased stability and low T-state affinity (Asmundson, A.L., et al., 2009, Biochemistry 48, 5436-5446). To counter these effects, two point mutations have been included to increase T-state affinity (Tsai et. al., 1999, Biochemistry 38, 8751-8761). The first mutation, N108K, shows greater T-state affinity, however the second mutation, V96W, shows decreased T-state affinity. I am conducting X-ray crystallography trials of the βN108K mutant in order to understand the structural basis for this unexpected effect on the T-state stability, and to inform future protein engineering of the HBOC candidates.

Introduction

Figure 1. Single chain α-cpβ structure obtained from x-ray crystallography (Sigurjonsson, L., and Spiegel, P.C. unpublished).

Figure 2. Full photolysis of CO from HbA and α-cpβ (A) and partial photolysis of CO from HbA and α-cpβ (B). Experiments were conducted in CO-saturated 0.1M potassium phosphate and 1mM EDTA, pH 7.0 at 20 C, (Asmundson, et. al., 2009).

Figure 3. Cells morphology. Poor morphology seen in Lane 1 shows N108K and Lane 2 shows V96W sample after IMAC, lane 3 shows N108K after SEC.


Figure 5. α-cpβ (sHb) strands. α strand is blue, α in red, linkers in green, and βN108K in purple (Murphy, M., and Spiegel, P.C. unpublished).

Figure 6. Full photolysis of 1 atm of CO from HbA, α-cpβ, α-cpβ SMT, α-cpβ N108K, α-cpβ V96W (A) and full photolysis of 92.8 µM. (Apperson, J., 2013, WWU Master’s Thesis).

Figure 7. Electrospray Ionization Mass Spectrometry (ESI-MS) data. Raw data (A) and deconvoluted to show the alpha and beta peaks (B).

Figure 8. SDS-PAGE of different purification states. Lane 1 shows a V96W sample after IMAC, lane 2 shows N108K after Mono Q, and Lane 3 shows N108K after SEC.

Crystallization

Purification

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Yield</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Post IMAC</td>
<td>35 mg/L</td>
<td></td>
</tr>
<tr>
<td>Post MonoQ</td>
<td>10 mg/L</td>
<td></td>
</tr>
<tr>
<td>Post SEC</td>
<td>4 mg/L</td>
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</tbody>
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