A Chemoenzymatic Approach for Synthesizing Hemoglobin Oligomers

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Abstract

Oligomerized hemoglobin molecules have been shown to decrease previously observed deleterious effects from treatment with cell-free hemoglobin. To create these constructs, a method was developed which employs the site specific ligation reaction of the sortase A enzyme from S. aureus. Previously developed Hb mutants (acpβ) were further modified by adding either the sortase recognition sequence, LPXTG, to the C-terminus of the α-subunit (s-acpβ), or a tetrasulényl motif to the N-terminus (n-acpβ). Three types of sortase mediated ligation (SMAL) will be employed in this study. First, we will attempt to ligate Hb subunits directly, using a mixture of s-acpβ (subunits) and n-acpβ (nucleophile). The second method will be two-part, first appending either an azide or a strained cyclooctyne to the C- and N-termini of the subunits. Using the well established method of Huisgen cycloaddition, the Hb molecules can then be brought together via their chemoenzymatically appended functional groups. To begin with, we will be using doubly modified Hb, and so our products will be a mixture of oligomeric states. In the future, we will use singly modified monomers to begin and cap our oligomers, as well as alternating the addition of azide of cyclooctyne functionalized subunits to better control the oligomeric states of our products.

Methods: Hb Modification and Click Handle Synthesis

Methods: Functionalizing Hb Monomers

Methods: Hb Monomers

Background: Hb Mutants

Oligomeric constructs of native Hb have been shown to attenuate the adverse effects of intravenous transfusion of cell free Hb. Here, an octameric Hb construct is shown to attenuate the hypertension response – attributed to its decreased ability to permeate through blood vessels and scavenge nitric oxide.

Background: MASML

Metal assisted sortase mediated ligation has been shown to double yields when ligation synthetic peptides to full sized proteins. The figure to the right depict reactions in which GPP acted as either substrate or nucleophile, and the reconstructed MS spectra showing the increase in product yield when treated with 200 µM Ni²⁺.

Future Work: Dendrimeric Hb

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