Intramolecular Cross-linking of Beta Subunits and PEGylation of Bovine Stroma Free Hemoglobin For Use as a Hemoglobin Based Oxygen Carrier

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Recommended Citation
Salazar, Gil, "Intramolecular Cross-linking of Beta Subunits and PEGylation of Bovine Stroma Free Hemoglobin For Use as a Hemoglobin Based Oxygen Carrier" (2016). Georgia Southern University Research Symposium. 18.
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**MATERIALS & METHOD**

**Methods of Characterization**

- SELC to determine molecular size distribution using running buffer was 50mM MOPS, with flow rate of 5.3ml/min, sample Load of 10ul on a Superose 12 column.
- MALDI-ToF MS to determine molecular weight distribution using a matrix 3,5-dimethoxybenzoic acid (Dimeba), voltement of a 0.1% (v/v) solution of matrix in acetonitrile, 2% (v/v) trifluoroacetic acid (TFA) sample to matrix ratio was 10:1, the sample was injected into a 28 mm stainless steel plate.
- UV-Vis Absorbance Spectroscopy to determine oxidation states and protein concentration
  \[ A = \varepsilon b c \]
  where \( A \) is absorbance, \( \varepsilon \) is molar absorptivity, \( b \) is path length, and \( c \) is molar concentration.

**Materials of Study**

- PEGylated β subunit cross-linked bovine hemoglobin (BPEGXLHb) has the potential to be a functional and stable HBOC.
- Provided a notable degree of conformational stability, but did not provide much.

**Characterization Results**

**SELc Results**

Bovine Hemoglobin content cross-linking with DMAP and PEGylation flow overlay.

**MALDI-ToF MS Results**

UV-Vis Results

SOS-PAGE Results

**Oxidative Stress under H2O2, Study Results**

Initial Kinetics using UV-Vis

- Initial kinetic Comparison of EPR/Vis with different Concentrations of HBOC

**Fe3+ EPR Study at 10K**

Methemoglobin signal (Fe3+)

EPR at 10 K

Superoxide signal

EPR at 40 K

Iron Release Assay Results

**CONCLUSION**

Blood substitutes that are stable and functional been sought after for many years and recently HBOC have been^1 of this interest. Although the intent of the red blood substitute is not completely replace the need for whole blood, it is often misunderstood due to the world substitute. A somewhat realistic term would be ancillary blood substitute. Understanding the purpose of HBOCs we can begin to realize the advantages and limitations of application. From these new methods can be develop to either enhance the advantages or mitigate the limitations. HBOC should be a unique replacement for whole blood, which can temporarily provide similar oxygen carrying capacity, maintain ionic pressure, and provide added insult stress. The main focus of this thesis was the preparation and characterization of bovine β-β subunit cross-linking and PEGylation. Both modifications shown significant stability behavior relative to BSFHb. Although PEGylation did show a marginally stable less than BSFHb under UV-Vis studies and EPR studies shows that BPEGXLHb has a higher attenuation of hydrogen peroxide and produce less concentrations of Fe4+. This slight decrease in stability of BPEGXLHb under UV-Vis is acceptable, due to the gain in vascular retention of the increase MW from the conjugation of the 10 PEG. The data provided evidence that bovine β-β subunit cross-linking prior to PEGylation provided a notable degree of conformational stability, but did not provide much protection for the Fe for the oxidative stress challenge. The evidence shows that PEGylated β-β subunit cross-linked Hb does provide a conformational stability, can attenuate oxidative stress and reduced potential toxicity from ROS. BPEGXLHb also provides improved vascular retention and increase ionic pressure due to the increased MW from the PEG chains. Further modification to increase antioxidant like properties coupled with the wide range of different methods of PEGylation, BPEGXLHb has the potential to be a functional and stable HBOC.

**REFERENCES**


**ACKNOWLEDGEMENTS**

MS-APS Program Director Dr. Michelle McGiboney
My Mentor and Thesis Committee Chair Dr. Li Ma
My Thesis Committee: Dr. Hans-Joerg Schans and Dr. Jonathan Arahamula
Georgia Southern University Department of Physics and Chemistry Chairs: Dr. Clayton Helfer and Dr. John D.Ceasear

**PGEylated B Subunit Intra-Molecular Cross-linked Bovine Hemoglobin Based Oxygen Carrier Blood Substitute**

By Gil Salazar

Hemoglobin based oxygen carriers (HBOC) have the potential characteristics to fill the clinical needs that are not met by normal blood transfusion. The raw material sources for HBOC come from an untapped supply of outdated human or bovine blood, which can eliminate the issue of shortages and blood type complications. This characteristic applicable in treatment of individuals in remote locations or even used in battlefields where emergency hospitals may be for hundreds of miles away. By combining known methods of hemoglobin (Hb) modifications, we were able to create a stable β-β subunit cross-linked bovine hemoglobin (BSFHb) β-β subunits then further modifications on surface residues using SDS molecular weight (SDS-PAGE) and EPR spectroscopy study to show formation of MeHb and Superoxide.Synthesis shows that the combination of methods did create the final product with structural stability as well as attenuate oxidative stress and limit the formation of superoxide.