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A Combinatorial Approach to Regioselective Covalent Modification of Hemoglobin for Antisickling Agents

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Sickle cell disease is characterized by intracellular polymerization of sickle hemoglobin and a decrease in deformability of erythrocytes. Potential exists for antisickling drugs which act through covalent interactions, due to large amount of target protein, hemoglobin. Success in this area requires a systematic way to determine structures with regioselective and specific reactivities for a target receptor.

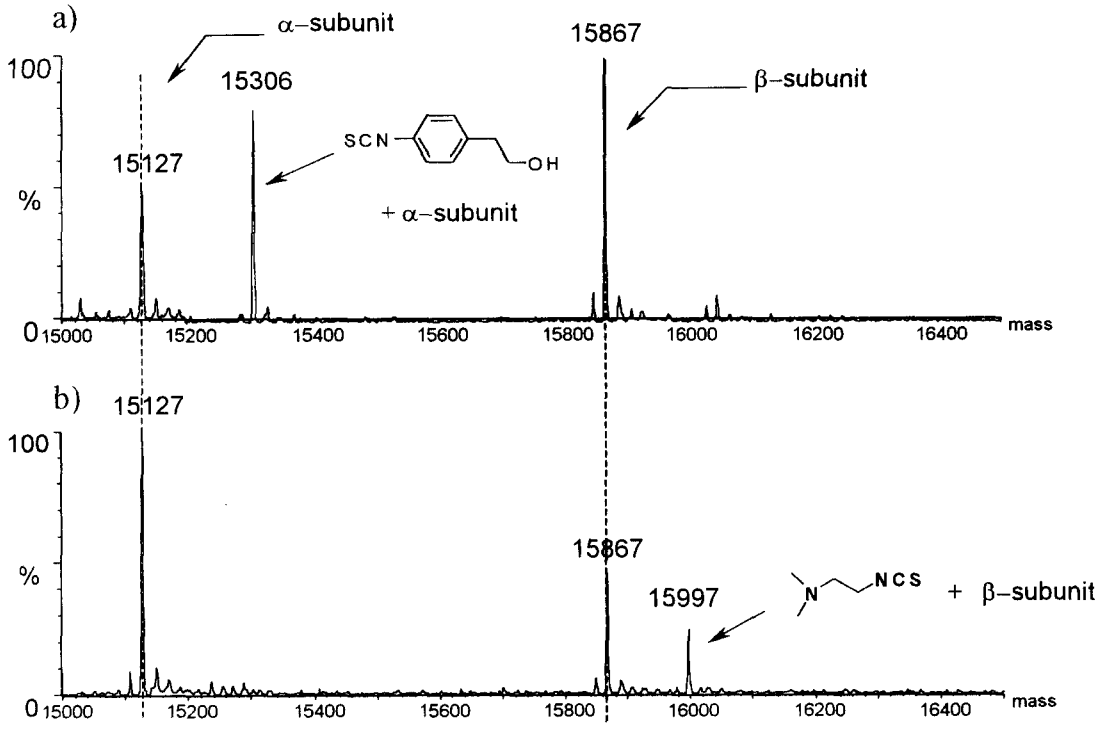

Figure : Product identification from mass shift in HbA mass spectrum processed by maximum entropy deconvolution. a) Modified HbA with aryl isothiocyanate (MW 179.24), b) modified HbA with alkyl isothiocyanate (MW 130.21).

We demonstrate here the ability to direct the site of isothiocyanate reaction on hemoglobin by changing the substituent, and to so affect aggregation activity of sickle cell hemoglobin. Randomly chosen isothiocyanates were prepared and treated with hemoglobin. Electrospray mass spectrum of the modified hemoglobin was used as a high throughput method to define regioselectivity for the α versus β hemoglobin chains. Electrospray mass spectrometry (ESMS) could provide mass shift corresponding isothiocyanate modification without any purification or isolation with minimum amount of hemoglobin. The isothiocyanates showed clear regioselectivity with respect to the α and the β chains, which was determined by the structure of substituent on isothiocyanate. This result suggested that the modification at a specific site on a protein could be achieved by careful design of a structure of substituent. Specific reacting amino acid residues (α N-terminus versus β 93 cysteine) were defined by biochemical or chemical method. The antisickling activities of the isothiocyanates were measured with standard saturation solubility assay (C_{sat}). The degree of the inhibition of HbS polymerization was influenced by the modification site and the structure of the substituent on isothiocyanate. The isothiocyanates modified β 93 cysteine increased oxygen affinity of hemoglobin.

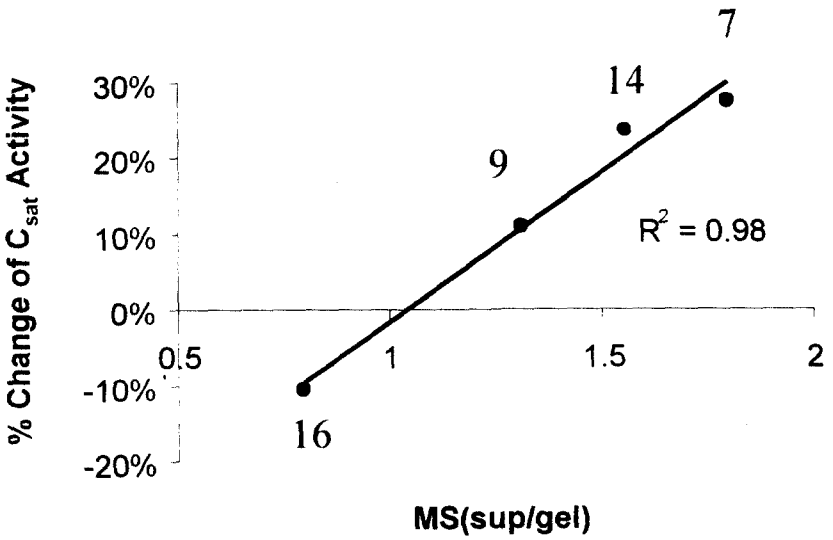

Figure : A plot of ESMS ratio versus % change in C_{sat} activity of 4 isothiocyanates

A homogeneous assay is described for identifying compounds in a combinatorial library that covalently modify hemoglobin and thereby enhance its

solubility. The library assay is based on the hypothesis that HbS modified with an active compound would avoid participating in the polymerization and tend to stay in the supernatant more than the gel. If so, one would expect that the distribution of modified HbS between supernatant and gel would reflect the antisickling activity of the covalent modifier. An exploratory study is presented which demonstrated that the antisickling activity of a family of isothiocyanates, as measured by the standard saturation solubility assay correlated well with the distribution of HbS modified with isothiocyanates between the supernatant and gel measured by electrospray mass spectrometry. The technique has potential for screening libraries capable of covalently modifying other proteins of clinical interest Alzheimers and Huntingtons.