Hemoglobin Variant Analysis Using MALDI-reISD

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Novel Aspect

Top-Down analysis of hemoglobin variants using MALDI-ISD

Introduction – Limit 120 words

More than 1200 recognized hemoglobin structural variants exist in the human population, and many of these underlie phenotypic diseases. Mass spectrometry constitutes a rapid and accurate means for the detection and characterization disease-specific protein variants. In our continuing efforts to develop mass spectrometry based methods to detect and characterize hemoglobin variants, we report preliminary results using MALDI-reISD (in-source decay) for the purpose of hemoglobin variant analysis.

Method – Limit 120 words

Whole blood was diluted approximately 1:250 in water. The sDHB matrix solution was prepared to a concentration of 50 g/L in 50% acetonitrile/water/0.1 formic acid. A saturated solution of 1,5-diaminonaphthalene (1,5-DAN) matrix was prepared in 50% acetonitrile/ water/0.1 formic acid. Analyses were carried out for target spots that were dried down from equal volumes of sample and matrix solutions, using 1 µL of each. MALDI-reISD spectra were acquired on a Bruker UltafleXtreme MALDI-TOF/TOF MS and consisted of signals generated from 12000 to 15000 accumulated laser shots. The resulting fragment masses were analyzed using BioTools and BUPID-Topdown (Boston University Protein Identifier-Topdown), a custom-programmed software algorithm written in-house. Caution: 1,5-DAN is a carcinogen.

Preliminary data – Limit 300 words (299)

The MALDI-reISD spectrum of normal hemoglobin in sDHB exhibited extensive fragmentation consisting mainly of c, z and y-ions describing the N- and C-termini of both the alpha and beta chains. Although both y and z-ion series were present, the signals from the y-ion series were more intense and extensive. The fragmentation roughly covered the first 50 amino acids of the N- and C-termini. In the analysis of a sickle cell sample, a -30 Da peak series was observed for the N-terminal fragmentation of the beta chain, consistent with the presence of the Glu6Val mutation. An alpha chain variant, hemoglobin Westmead (His122Gln), was also analyzed and characterized using MALDI-reISD. We have also obtained data using 1,5-DAN as the matrix and have noted a few significant differences between the spectra recorded with 1,5-DAN and the sDHB data. Generally, 1,5-DAN favors N-terminal fragmentation and generates higher

fragment ion abundances than does sDHB. Direct analyses of hemoglobins from blood samples are well suited for MALDI-ISD since sample purity and quantity are not issues. The “purification” of Hb samples can be performed by simple dilution of whole blood (Hb conc. 124 g/L), whereby the abundances of other blood components become almost insignificant with respect to hemoglobin. Also, sample amount is not an issue and this factor is generally cited as a significant hurdle to applications of MALDI-ISD in proteomics. The main limitation of the technique at this point is the lack of coverage of the core region of the alpha and beta chain sequences. MALDI-reISD offers a simple, fast and potentially high throughput technique for the detection of hemoglobin variants. We are currently working to optimize analysis conditions..

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