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Hemoglobin Variant Analysis Using an LTQ-Orbitrap Top-down Platform with ETD, HCD and LTQ-CID

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Novel Aspect

Robust analytical platform with multiple activation techniques for top-down analysis of hemoglobin variants.

Introduction

More than 1200 recognized hemoglobin structural variants exist in the human population, and many of these underlie phenotypic diseases. Additionally, post-translational modifications of hemoglobin which are not observed in gene-based analysis may play an important role in disease. In a recent paper, we described a simple top down methodology based on an LTQ-Orbitrap MS aimed at characterizing point mutations in hemoglobins and transthyretin1. At that time, our approach was limited to LTQ-CID MSMS. In this preliminary communication, we report the use of supplemental activation techniques such as ETD and hexapole HCD for the purpose of hemoglobin variant characterization.

Methods

Whole blood was diluted approximately 1:250 in water and added in a 1: 1 ratio to acetonitrile containing 0.2% formic acid. The samples were introduced into an LTQ-Orbitrap XL mass spectrometer (Thermo-Fisher) using a Triversa NanoMate ESI source (Advion). The fragment ion mass spectra were generated by a variety of activation techniques including LTQ-CID, ETD and HCD as well as nozzle skimmer dissociation (NSD). Fragment ion mass spectra were deconvoluted using Xtract software from Thermo Scientific. The resulting fragment masses were analyzed using BUPID-Topdown (Boston University Protein Identifier-Topdown), a custom-programmed software algorithm written in-house.

Preliminary Data

The basic strategy is to gather a maximum of structural information by obtaining fragmentation data on the intact protein and then breaking it down into complementary ion pairs that can be made to undergo subsequent MSMS as outlined in our previous report1.

Our first experiments consisted of testing the sequence coverage obtained by fragmenting intact protein ions using ETD. The ETD mass spectrum obtained from the beta chain m/z 884.34^{18+} exhibits informative fragmentation in the core region of the protein (residues 58-100) The preliminary results indicate the complementary nature of the data. A similar observation can be made with the alpha chain as the ETD spectrum of the alpha chain m/z 841.24^{18+} showed more complete coverage of the N-terminal (positions 1-30) than that obtained using LTQ-CID. Furthermore, significant fragmentation from positions 50-60 of the core of the sequence was observed in the ETD spectrum and was absent in the LTQ-CID spectrum. We are currently in the process of optimizing these results by using different charge states and varying experimental parameters such as the reaction time of the intact protein ions with the fluoranthene reagent radical anions.

Similar observations of the complementarity of the activation methods were made where NSD (nozzle-skimmer dissociation) was used to generate fragment ions that were subjected to MSMS. The use of HCD further increased to completeness of sequence coverage. For example, HCD of the intact beta chain 17+ charge state provided more complete N-terminal coverage than either LTQ-CID or ETD.

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(1) R. Théberge, G. Infusini, W. Tong, M. E. McComb, C. E. Costello, Int. J. Mass Spectrom. (2010)