Rapid assessment of protein structural heterogeneity using native LC/MS

Wenhua Yang and Igor A. Kaltashov

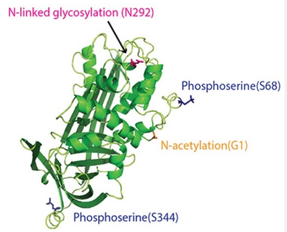
University of Massachusetts/Chemistry Department, Amherst, MA

Wenhua Yang

Introduction

The vast majority of proteins exhibit significant heterogeneity due to post-translational modifications (PTMs), which have a profound effect on protein behavior *in vivo* and its physical and chemical properties. Exhaustive de-novo cataloging and characterization of all PTMs within a given protein is usually a huge undertaking.

However, in many applications the emphasis is placed on detection and quantitation of modifications that are known to occur within a particular system. Ovalbumin (OVA) is a glycoprotein with some PTMs. We use it as a model system to evaluate the utility of on-line ion exchange chromatography/ MS for this task.



PTMs of ovalbumin

Goals

- To systematically analyze the PTMs of ovalbumin;
- To rapidly assess ovalbumin structural heterogeneity using on-line ion exchange chromatography/MS.

Instrumentation and Methods

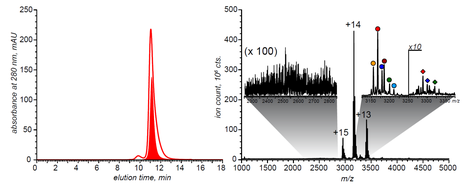
The protein stock solution was prepared by dissolving SEC-purified and lyophilized OVA in 50 mM ammonium acetate and stored at 4°C. Ion exchange chromatography (IXC)/MS measurements were carried out using a ProPac SAX-10 column (Thermo Fisher Scientific, Waltham, MA) and the mass spectrometer (a SolariX 7, Bruker Daltonics, Billerica, MA, FTICR) with ESI source.

A linear gradient of 0 to 70 % mobile phase A over 35 min (A: 50 mM ammonium acetate, B: 500 mM ammonium acetate) was used and final flow rate to FTICR was 25 µL/min.

Flow chart of OVA heterogeneity assessment using IXC/MS

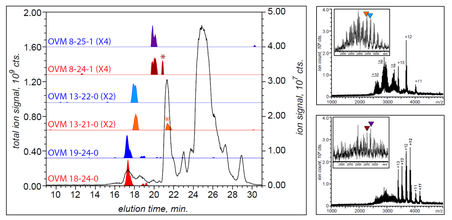
Results

- Out of the twenty-one major proteoforms detected by MS alone, twelve are charge variants that are expected to have different retention characteristics on IXC.



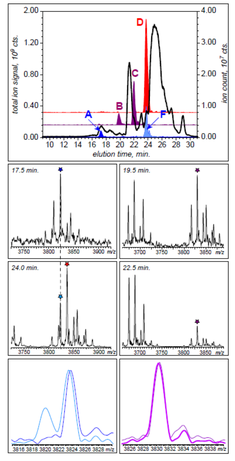
SEC purification of OVA and the native ESI mass spectrum of the collected SEC fraction

- A magnified view of the ionic signal in the m/z region 2200-2900 reveals the presence of low-abundance ionic species.

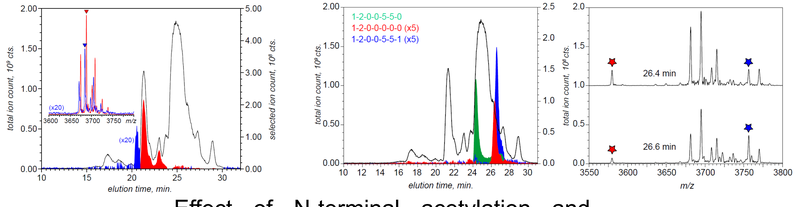


Extracted ion chromatograms for several representative glycoforms of ovomucoid (OVM) detected in the OVA sample.

- OVM and OVA were co-eluted in IXC.
- Eighty-four different OVM glycoforms were detected in the OVA sample.
- Four phosphorylation types were detected and analyzed in IXC-MS.
- Isobaric mono-phosphorylated species could be properly differentiated in IXC-MS.

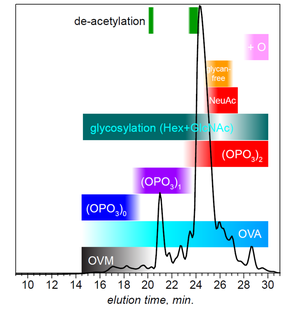


Influence of phosphorylation on OVA elution in IXC.



Effect of N-terminal acetylation and glycosylation of OVA on its elution in IXC.

- Most OVA proteoforms (129 out of a total of 138) appear to be acetylated.
- The low-abundance carbohydrate-free (a-glycosylated) forms were found in OVA sample.
- The presence of an acidic NeuAc unit within the carbohydrate chain results in a significant increase of the retention time.
- Decreasing the total number of either Hex or GlcNAc residues within the carbohydrate chain by a single saccharide unit results in a slightly enhanced retention.



Annotated IXC chromatogram of the SEC-purified OVA

- Over a hundred and thirty different OVA proteoforms were identified.
- The PTMs and heterogeneity of ovalbumin were systematically analyzed using on-line ion exchange chromatography/MS.

Conclusions and Future Direction

- Over a hundred and thirty different ovalbumin proteoforms were identified based on their elution times and/or masses, in addition to eighty-four different glycoforms of ovomucoid, a low-level protein impurity that completely escaped detection by MS alone.
- The analysis is fast, occurs in a single LC/MS run, and does not require any chemical/enzymatic (pre)treatment of the protein sample.
- Online IXC/MS is ideally suited for situations where rapid assessment of the protein quality is required e.g. to evaluate the influence of a production process change on PTMs of a therapeutic protein or to control allergenic PTMs in food processing.

References and Acknowledgments

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2. K. Muneeruddin, C. E. Bobst, R. Frenkel, D. Houde, I. Turyan, Z. Sosic and I. A. Kaltashov, Analyst, 2017, 142, 336-344.

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