Proline/Alanine Tails as Ionization Enhancement Tags in Native Mass Spectrometry

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Summary

❖**This study aimed to study the impact of Proline/Alanine-tag on l-asparaginase protein dynamics.**

❖**Our current analysis demonstrates that the PA-tail fragment did not change the core protein dynamics using TRESI-HDX-MS to provide identical stability with untagged l-asparaginase.**

❖**Novel Aspects: In this work, we propose PASylation® as a new tool for ionization enhancement through chain ejection.**

Introduction

❖ Asparaginase (ASNase) is an enzyme used for the treatment of acute lymphoblastic leukemia (ALL) and is an integral component of treatment protocols with long-term survival rates in children that have progressively increased to about 90%.

❖ We are presently carrying out native mass spectrometry, ion mobility and hydrogen deuterium exchange on a 'PASylated' version of this protein, which incorporates a Proline/Alanine 'tail in order to enhance longevity in vivo using PASylation technology[1].

❖While characterizing structural features of this protein that confer resistance to degradation in vivo, we noted that this species exhibits a unique electrospray ionization mechanism that appears to be driven primarily by chain ejection, even for the relatively large, folded tetramer.

❖In this work, we explore the role of a long-disordered tail in protein ionization, including the possibility that PA(S)tails may serve as 'ionization enhancement tags' for proteins that are inefficiently ionized via electrospray.

❖ Hydrogen-Deuterium Exchange (HDX) coupled to MS is widely used to study protein dynamics

❖ Continuous time-resolved Electrospray ionization TRESI-HDX-MS technique is used to characterize protein structural transitions in relatively ordered regions of proteins[2].

Experimental Methods

❖PASylated ASNase was acquired from Jazz pharmaceuticals. These batches were treated using Amicone Ultra 0.5 mL Centrifugal Filters with a 30 KDa cutoff.

❖Conformational dynamic studies using Continuous TRESI-HDX-MS.

❖Solvents were delivered using injection pumps which introduce the protein samples and D_2O into the system.

❖Flow rates were 2 uL/min for the protein carrier buffer (100 mM ammonium acetate), 2 uL/min for the D_2O carrier buffer (100% H_2O), and 16 uL/min for the acid quench (10% acetic acid, pH 2.5)

❖ Analytes were subjected to native mass spectrometry on a Waters G2S ion mobility mass spectrometer by direct infusion ESI.

❖Data were collected on a Waters Synapt G2-Si and processed with Mass Spec Studio 1.0

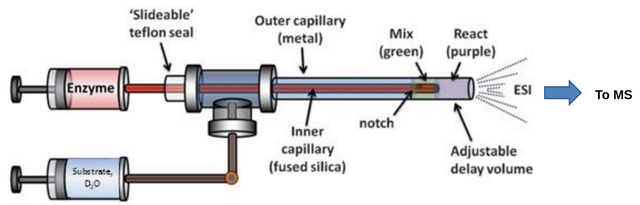

Figure 1. The continuous pullback device TRESI-HDX Coupled to Mass Spec utilized to perform automated data collection.

Results and Discussion

PA Tag Ionizes By Chain Ejection Model (CEM)

❖The untagged protein exhibits a typical 'native' mass spectrum for a large multimeric protein, corresponding to relatively low charge and a relatively narrow distribution associated with charge residue ionization.

❖ The population is more than 90% tetrameric, based on the relative peak intensities of the tetramer vs. the monomer. The peaks associated with the monomer also appear to ionize via a charge residue mechanism, which is consistent with a folded protein.

❖Interestingly, the PA tail, added as a PASylation tag to improve in vivo stability of this protein, radically changes the native ESI mass spectrum.

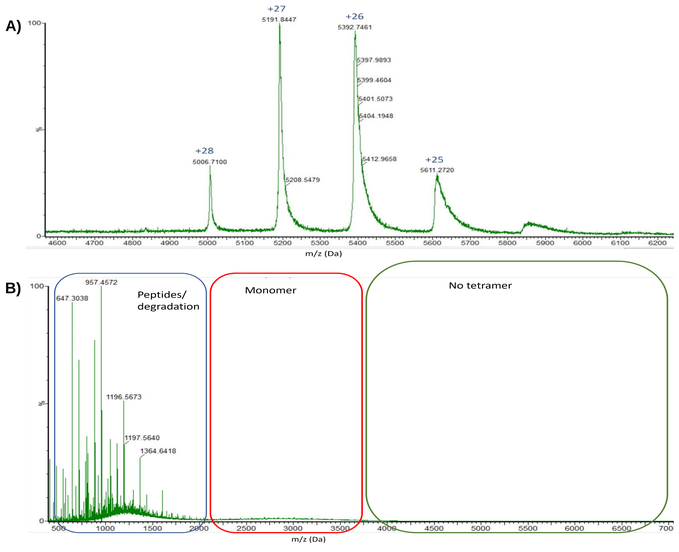

Figure 2. Mass spectrum for L-asparaginase protein samples. A) Untagged L-asparaginase, four dominant charge states are observed, with +27 being the most intense. The spectrum matches the expected protein size, Mwt=140 KDa. B) PA-tagged L-asparaginase ionized in different mechanisms, and its structure unfolds during electrospray conditions.

❖In particular, the tagged protein exhibits a mixture of intense, highly charged monomeric, dimeric, trimeric and tetrameric species.

❖This suggests that the PASylated protein has undergone ionization primarily by chain ejection rather than charge residue, which would imply that the ionization mechanism is dominated by the unstructured PA 'tail', in line with previous observations made with isolated PAS sequences[3].

PA Tagged Protein Show Similar Dynamics As Untagged Protein

❖Here we implemented HDX-MS to obtain three timepoints to reveal regions of l-asparaginase samples.

❖The protein samples were quenched and digested before MS analysis so that the deuterium exchanged information was locked to a specific peptide.

❖Subtracting protein deuterium uptake of PA-tagged and Untagged samples, showed the same percentage of deuterium and, thus, similar dynamics.

❖Interestingly, PA-tag attachment did not induce any significant decreases or increases in deuterium uptake in the core region.

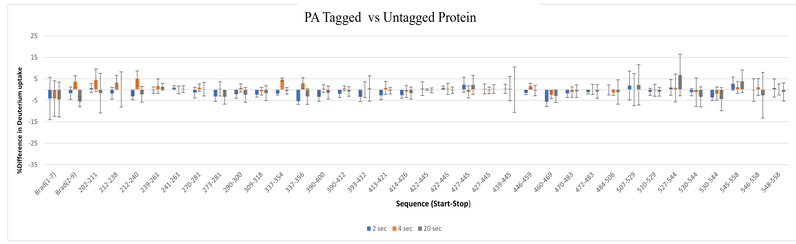

Figure 3..TRESI-HDX-MS analysis of PA-tagged and untagged l-asparaginase protein samples. Show the same deuterium uptake in tagged and untagged samples. Error bars are 2σ significance threshold.

Conclusions and Future Work

❖PA-tagged proteins are ionizing via CEM model

❖PA tag did not impact core protein dynamics.

❖Two questions that we will explore: (1) What is the minimum length of unstructured 'tail' required for a 'native' protein to favor chain ejection over charge residue and (2) can PA tails be used as 'ionization enhancement tags' to facilitate detection of large or otherwise 'difficult to ionize' protein complexes?

References

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