Characterization of a Highly PEGylated Product Related Variant of Commercially Available PEGylated Asparaginase by Higher Order Structural Analysis

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PELICAN™
P. fluorescens expression technology
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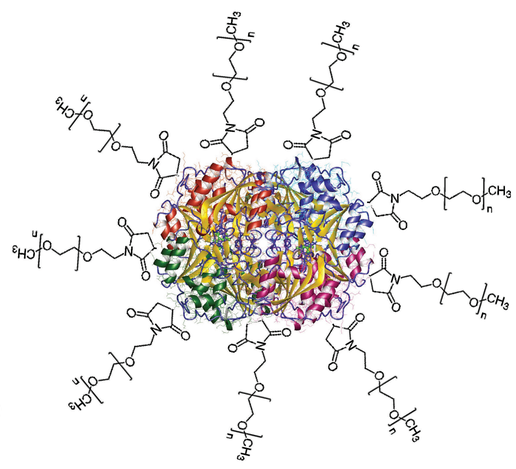
1. INTRODUCTION



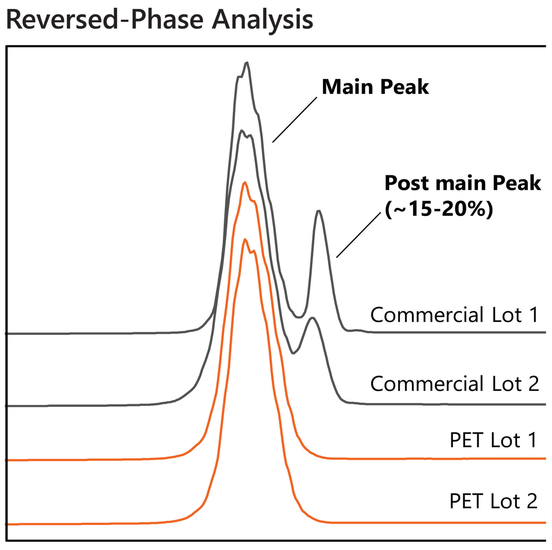
The Pelican Expression Technology™ (PET) platform, a Pseudomonas fluorescens-based protein expression system, is robust and scalable for recombinant protein production. The PET platform is especially well-suited for complex protein production, such as for PEGylated asparaginase, an enzymatic protein therapeutic used as part of a multi-agent chemotherapy regimen to treat acute lymphoblastic leukemia. During the analytical assessment of commercially available PEGylated asparaginase, higher order structure (HOS) analysis identified an unexpected product related variant that was present in the two commercial lots but not in the PEGylated asparaginase produced by the PET platform. A combination of HOS analytical methods enabled identification of this variant and assessment of its impact on enzymatic activity. These results highlight both the development capabilities of the PET platform and the importance of HOS analysis as part of the evaluation of protein therapeutics.

2. BACKGROUND

- Asparaginase is a homotetrameric enzyme comprised of four 34,592 Da monomer subunits
- Tetramer formation is due to non-covalent, mostly hydrophobic interactions at the protein interfaces
- Asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and is used as a cancer treatment for forms of cancer that require extracellular sources of asparagine to be viable
- Tetramer form of the enzyme required for its activity
- PEGylation occurs through a reaction with available lysine residues on the asparaginase protein and improves PK/PD profile of drug
- Analytical methods used to evaluate PEGylated asparaginase in this study:
  RP-UHPLC, SEC, intact mass, MALDI-MS, SEC-MALS, CD and IF, Nessler activity assay

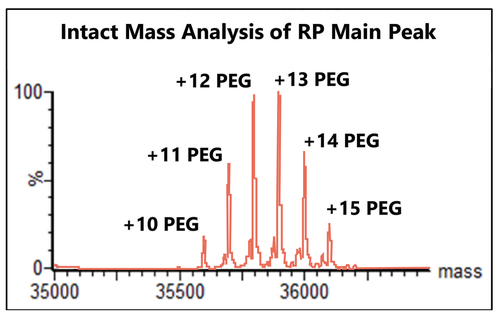
3. RESULTS SUMMARY

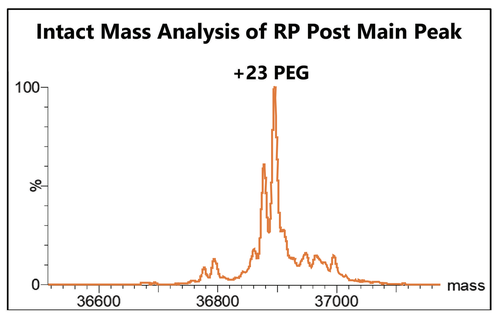
Reversed-Phase Analysis

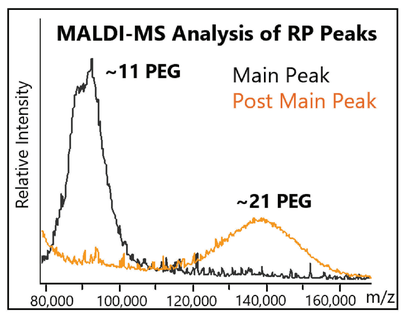


- RP-UHPLC purity analysis of two commercial lots revealed a post main peak product related variant
- The variant population was not observed in two lots of PET product
- The variant population was hypothesized to be a distinct monomeric species

A combination of mass spectrometry techniques were utilized in order to characterize the unexpected product related variant in commercial PEGylated asparaginase





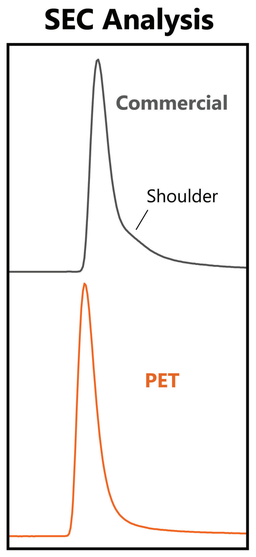


Mass Spectrometry Analysis of RP-UHPLC Populations for Commercial PEGylated Asparaginase

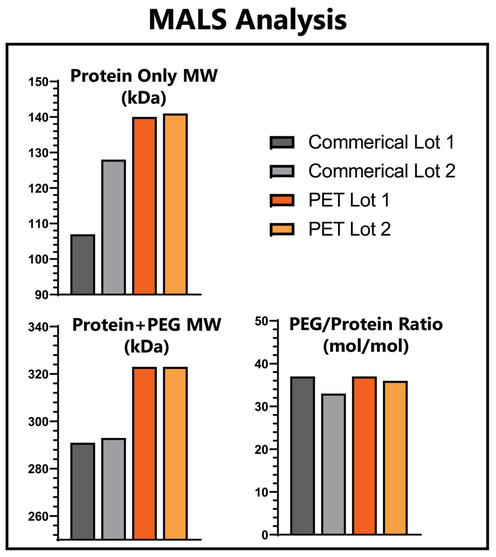
- PEG groups were removed from protein in order to leave a fingerprint for determination of PEG conjugation locations by intact mass spectrometry
- RP main peak revealed to be a monomer subunit containing between 10-15 PEG molecules
- The RP post main peak variant was determined to be a hyper PEGylated monomer subunit containing 23 PEG molecules
- MALDI-MS analysis (without de-PEGylation step) confirmed the identities of the RP populations, and showed the product related variant to have a hyper PEGylated monomer subunit present.

SEC-MALS analysis was performed to investigate whether the variant species self-associated into the tetrameric structure under native conditions.

SEC Analysis

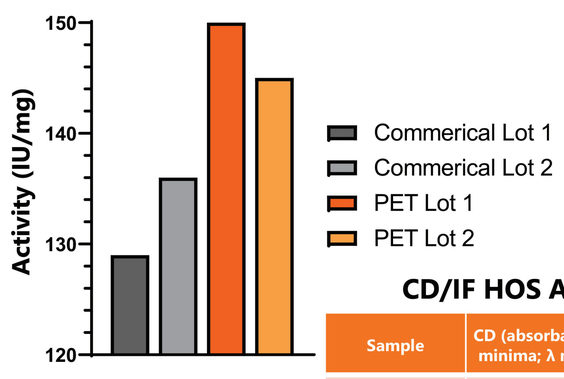


MALS Analysis



- SEC analysis showed a shoulder species of apparent lower hydrodynamic size in commercial drug product that was not observed in PET drug product
- When coupled with MALS analysis, results indicated that the SEC shoulder in commercial drug product was the hyper PEGylated monomeric variant that was not correctly assembled into the tetrameric form
- The hyper PEGylated monomer species exhibited a similar, but slightly smaller hydrodynamic size compared to that of the less PEGylated, correctly formed tetramer

Activity Analysis



CD/IF HOS Analysis

Sample	CD (absorbance minima; λ nm)	IF (absorbance max; λ nm)
Commercial Lot 1	210, 218	319
Commercial Lot 2	209, 218	319
PET Lot 1	210, 217	319
PET Lot 2	209, 218	319

Further HOS analysis was performed to assess the impact of the variant species on enzyme function

- CD/IF analysis showed that the secondary structure of the protein was comparable between the commercial and protein produced by the PELICAN expression technology
  No change to protein folding suggests that hyper PEGylated product variant is incapable of associating into the required tetramer form unit due to increased PEGylation and not because of misfolding of the monomer unit of the protein.
- Enzyme activity observed to be reduced for commercial drug product compared to PET drug product
  Hyper PEGylated monomer variant is unable to catalyze the hydrolysis of asparagine to aspartic acid, as tetrameric state is required for enzyme activity. Since total amount of active enzyme is reduced in the commercial drug product, its enzymatic activity is less than that of PET drug product.

4. CONCLUSION

- Comprehensive HOS analysis of two lots of commercial PEGylated asparaginase revealed an unexpected hyper PEGylated monomer variant
- This product related variant was shown to reduce enzyme activity as the tetrameric structure is required for it to properly function
- This product related variant was not observed in PET produced PEGylated asparaginase
- Observations highlighted both the development capabilities of the PET platform and the importance of HOS analysis as part of the evaluation of protein therapeutics.

REFERENCES & ACKNOWLEDGEMENTS

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