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Trends in Characterization of PEGylated Proteins by Mass Spectrometry

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Abstract

Characterization of PEGylated proteins in crucial in the biotechnology industry for quality control and formulation purposes. This mini review lists several Mass Spectrometry (MS) methods employed in the past 20 years for PEGylated protein analysis. The trend seems to shift from predominantly qualitative MALDI, to liquid chromatography coupled with MS for quantitative or conformational studies.

Keywords: Mass spectrometry; Proteomics; Proteins; PEGylated proteins

Introduction

PEGylation is a particular crucial step in the formulation of biotherapeutic protein drugs, as the attachment of a Polyethylene Glycol (PEG) leads to increased circulating life of the drug and the dosage interval, improved pharmacokinetic profile, while at the same time it improves the drug solubility and stability to proteolitic enzymes. Attachment of PEGs to proteins occurs on the N-terminus site, carbohydrate, sulfhydryl, and on aminoacids Lys, His, Arg, Tyr, Ser, Thr, Cys, Asp, Glu. The heterogeneity of the PEGylation product, the degree of PEGylation, coupled with the complex protein structure, make the characterization of PEGylated proteins analytically challenging.

MS [1-5] is a robust and accurate analytical tool used widely in characterization of proteins [5-10], protein post-translational modifications [11-15] and protein protein interactions [16,17]. MS is also used in characterization of biotherapeutics (and PEGylated proteins; see below). For decades, MALDI-TOF MS has been employed as the technique of choice for characterization PEGylated proteins in terms of accurate average molecular weight and degree of PEGylation. Table 1 lists several PEGylated proteins reported in publications the past 20 years as analyzed by MALDI. Different types of PEG derivatives were attached to the proteins, ranging in size in ~ 5-50 kDa, mono and heterofunctional, linear of branched, monodisperse or polydisperse. PEGylation derivatization agents include a 12 kDa monomethoxyPEG [18], 20 kDa methoxy-PEG propionaldehyde [19], 20 kDa (mPEG)(2)-Lys-NHS [20], or a monodisperse Boc-PEG-NH2 [21] , 20 kDa and 40 kDa two-branched PEGs , or trimer-structured PEGs with MW of 23.5, 43.5 and 47 kDa [22]. Regardless of the type of PEG used for polymer modification of the protein, MALDI provided excellent information on molecular weight ID, and heterogeneity, specifically on the total amount and distribution of PEG on protein, or site specific information on PEGylation coupling site.

PEGylated Protein	MALDI Results	Reference
Recombinant stem cell factor	Molecular weight	
Superoxide dismutase	Molecular weight, degree of PEGylation, stability	[26]
Superoxide dismutase	Total amount and distribution of coupling PEG to protein	[27]
Recombinant IFN-α2b	PEGylation sites and peptide sequence	[18]
Lysozyme	PEGylation sites	[28]
Ricin A-RTA	PEGylation sites	[22]
Recombinant murine Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Degree of PEGylation	[29]
Recombinant human Granulocyte-Colony Stimulating Factor (rhG-CSF) and consensus interferon (C-IFN)	Molecular weight, ID	[30]
Mono-PEGylated consensus interferon (C-IFN)	Heterogeneity of the PEGylated protein	[31]
Interferon alpha (IFN)	Molecular weight	[32]
Interferon alpha2a, human serum albumin (HSA), coagulation factor VIII , and von Willebrand factor (vWF	Molecular weight and heterogeneity determination	[33]
Type I Ribosome-Inactivating Proteins (RIPs), α-MMC and MAP30	Molecular weight, homogeneicity	[20]

Table 1: Information provided by MALDI for several PEGylated proteins.

Electrospray ionization MS (ESI-MS) has started to gain popularity too in the past decade for analysis of PEGylated proteins. ESI-MS is preferred to MALDI due to automated workflow and reduced sample preparation time. The overlapping protein charge pattern and the polydispersity of the PEgylation derivatives complicate the ESI-MS spectrum, so various techniques have been employed by research groups to reduce these drawbacks. Table 2 lists different liquid chromatography MS methods employed for analysis of several PEGylated proteins, the majority of the work reported in the literature the past seven years.

In several instances, qualitative MS methods were easily implemented for quantitative analysis of PEGylated compounds using analytical standards. Surrogate peptides from tryptic dygests were employed by Wu et al. [23] in protein quantification, while isotopically labeled internal standards of de-PEGylated proteins allowed precise and accurate quantification of PEGylated proteins [24,25]. Quantitative analysis of biotherapeutic PEGylated proteins is especially useful in the determination of freely circulating drug in biological fluids. In addition to quantitative and qualitative information on the protein linear structure, MS approaches exist for probing the secondary conformation and the protein dynamics, hydrogen/deuterium exchange MS is a valuable orthogonal MS tool for more in depth exploration of the PEGylated protein structure. Hydrogen/deuterium exchange has been used successfully by the Wei et al. [19] to examine changes in the protein conformation and dynamics induced by the PEGylation process in the structure of the granulocyte colony stimulating factor.

Conclusion

In conclusion, different orthogonal MS approaches are needed

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PEGylated Protein	LC/MS Method	MS Results	Reference
Subtilisin Carlsberg	ESI-MS	Stability, Degree of PEGylation	[34]
Subtilisin and lipase	ESI-MS	Degree of PEGylation	[35]
Recombinant Human Granulocyte-Colony Stimulating Factor (rHuG-CSF)	On-column 2-methoxy-4,5-dihydro-1H-imidazole derivatization of digested PEG rHuG-CSF and subsequent LC/MS investigation	PEGylation site, protein sequence	[36]
rHuG-CSF	Ultra performance liquid chromatography-mass spectrometry-to-the-E (UPLC-MS(E)	Peptide sequence information	[36]
Granulocyte Colony-Stimulating Factor (rh-GCSF)	Gas-phase ion/molecule chemistry combined with ion mobility separation and ESI-TOF-MS	Molecular weight	[37]
IgG4 heavy chain	Post column addition combined with LC/MS -TOF	Molecular weight	[38]
Human granulocyte colony-stimulating factor, human growth hormone, and horse heart apomyoglobin	ESI-MS/MS	PEGylation sites	[21]
PEGylated glucagon and IgG4 antibody light chain	in-source fragmentation (ISF) with CID-MS/MS	PEGylation sites	[39]
Ubiquitin	Ion exchange chromatography and top-down MS consisting of two consecutive fragmentation steps (MS3)	PEGylation dites, isoforms	[40]
PEGylated-Adnectin-1 and PEGylated-Adnectin-2	LC-MS-based detection of multiple peptides following trypsin digestion	Quantification in dried blood spots	[41]
PEGylated scaffold protein	LC-MS/MS	Quantification of the total circulating drug in monkey plasma samples	[42]
Granulocyte colony stimulating factor (G-CSF)	Hydrogen/deuterium exchange mass spectrometry (HDX MS)	Localization of changes in protein structure and conformation induced by PEGylation	[19]
Adnectin	Protein precipitation coupled with trypsin digestion followed by LC-MS/MS	Quantitative toxicity studies in monkey plasma	[43]
Recombinant human granulocyte-colony stimulating factor (PEGylated rhG-CSF or pegfilgrastim)	Electrospray ionization-mass spectrometry (ESI-MS) and direct infusion; charge reduction by the addition of amine.	Molecular weight	[44]

Table 2: Several LC/MS methods employed for PEGylated protein analysis and information derived from the analyses.

to fully characterize PEGylated proteins since PEGylation introduces challenges to the routine MS analysis of a protein, such as increased charging, heterogeneity, stability, and conformational changes.

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References

1. Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. Nature 422: 198-207.
2. Darie CC (2013) Mass spectrometry and its application in life sciences. Aust J Chem 66: 719-720.
3. Darie CC, De Pascalis L, Mutschler B, Haehnel W (2006) Studies of the Ndh complex and photosystem II from mesophyll and bundle sheath chloroplasts of the C4-type plant *Zea mays*. J Plant Physiol 163: 800-808.
4. Parker CE, Warren MR, Mocanu V (2010) Mass Spectrometry for Proteomics. Frontiers in Neuroscience.
5. Sokolowska I, Ngounou Wetie AG, Woods AG, Darie CC (2013) Applications of mass spectrometry in proteomics. Australian Journal of Chemistry 66: 721-733.
6. Darie CC, Litscher ES, Wassarman PM (2008) Structure, processing, and polymerization of rainbow trout egg vitelline envelope proteins. Applications of Mass Spectrometry in Life Safety, NATO Science for Peace and Security Series A: Chemistry and Biology 23-36.
7. Florian PE, Macovei A, Lazar C, Milac AL, Sokolowska I, et al. (2013) Characterization of the anti-HBV activity of HLP1-23, a human lactoferrin-derived peptide. J Med Virol 85: 780-788.
8. Petrareanu C, Macovei A, Sokolowska I, Woods AG, Lazar C, et al. (2013) Comparative proteomics reveals novel components at the plasma membrane of differentiated HepaRG cells and different distribution in hepatocyte- and biliary-like cells. PLoS One 8: e71859.
9. Roy U, Sokolowska I, Woods AG, Darie CC (2012) Structural investigation of tumor differentiation factor. Biotechnol Appl Biochem 59: 445-450.
10. Spellman DS, Deinhardt K, Darie CC, Chao MV, Neubert TA (2008) Stable isotopic labeling by amino acids in cultured primary neurons: application to brain-derived neurotrophic factor-dependent phosphotyrosine-associated signaling. Mol Cell Proteomics 7: 1067-1076.
11. Aitken A (2005) Identification of post-translational modifications by mass spectrometry. The Proteomics Protocols Handbook 431-437.
12. Sokolowska I, Gawinowicz MA, Ngounou Wetie AG, Darie CC (2012) Disulfide proteomics for identification of extracellular or secreted proteins. Electrophoresis 33: 2527-2536.
13. Sokolowska I, Ngounou Wetie AG, Roy U, Woods AG, Darie CC (2013) Mass spectrometry investigation of glycosylation on the NXS/T sites in recombinant glycoproteins. Biochim Biophys Acta 1834: 1474-1483.
14. Sokolowska I, Ngounou Wetie AG, Woods AG, Darie CC (2012) Automatic determination of disulfide bridges in proteins. J Lab Autom 17: 408-416.
15. Woods AG, Sokolowska I, Darie CC (2012) Identification of consistent alkylation of cysteine-less peptides in a proteomics experiment. Biochem Biophys Res Commun 419: 305-308.
16. Darie CC, Shetty V, Spellman DS, Zhang G, Xu C, et al. (2008) Blue Native PAGE and mass spectrometry analysis of the ephrin stimulation- dependent protein-protein interactions in NG108-EphB2 cells. Applications of Mass Spectrometry in Life Safety, NATO Science for Peace and Security Series A: Chemistry and Biology 3-22.
17. Woods AG, Sokolowska I, Yakubu R, Butkiewicz M, LaFleur M, et al. (2011) Blue native page and mass spectrometry as an approach for the investigation of stable and transient protein-protein interactions. Oxidative Stress: Diagnostics, Prevention, and Therapy 1083: 341-367.
18. Grace M1, Youngster S, Gitlin G, Sydor W, Xie L, et al. (2001) Structural and biologic characterization of pegylated recombinant IFN-alpha2b. J Interferon Cytokine Res 21: 1103-1115.
19. Wei H, Ahn J, Yu YQ, Tymiak A, Engen JR, et al. (2012) Using hydrogen/deuterium exchange mass spectrometry to study conformational changes in granulocyte colony stimulating factor upon PEGylation. J Am Soc Mass Spectrom 23: 498-504.
20. Meng Y, Liu S, Li J, Meng Y, Zhao X (2012) Preparation of an antitumor and antivirus agent: chemical modification of α-MMC and MAP30 from *Momordica Charantia* L. with covalent conjugation of polyethyelene glycol. Int J Nanomedicine 7: 3133-3142.
21. Mero A, Spolaore B, Veronese FM, Fontana A (2009) Transglutaminase-mediated PEGylation of proteins: direct identification of the sites of protein modification by mass spectrometry using a novel monodisperse PEG. Bioconjugate Chem 20: 384-389.

22. Na DH, Youn YS, Lee KC (2004) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for monitoring and optimization of site-specific PEGylation of ricin A-chain. Rapid Commun Mass Spectrom 18: 2185-2189.

23. Wu ST, Ouyang Z, Olah TV, Jemal M (2011) A strategy for liquid chromatography/tandem mass spectrometry based quantitation of pegylated protein drugs in plasma using plasma protein precipitation with water-miscible organic solvents and subsequent trypsin digestion to generate surrogate peptides for detection. Rapid Commun Mass Spectrom 25: 281-290.

24. Du Y, Mehl J, Pristatsky P (2013) MS analysis of biological drugs, proteins, and peptides. Mass Spectrometry for Drug Discovery and Drug Development, John Wiley & Sons, Inc, USA.

25. Watson E, Shah B, DePrince R, Hendren RW, Nelson R (1994) Matrix-assisted laser desorption mass spectrometric analysis of a pegylated recombinant protein. Biotechniques 16: 278-281.

26. Chowdhury SK, Doleman M, Johnston D (1995) Fingerprinting proteins coupled with polymers by mass spectrometry: Investigation of polyethylene glycol-conjugated superoxide dismutase. J Am Soc Mass Spectrom 6: 478-487.

27. Bullock J, Chowdhury S, Johnston D (1996) Characterization of poly(ethylene glycol)-modified superoxide dismutase: comparison of capillary electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry. Anal Chem 68: 3258-3264.

28. Lee H, Park TG (2003) A novel method for identifying PEGylation sites of protein using biotinylated PEG derivatives. J Pharm Sci 92: 97-103.

29. Sainathan SK, Tu L, Bishnupuri KS, Han M, Li A, et al. (2005) PEGylated murine Granulocyte-macrophage colony-stimulating factor: production, purification, and characterization. Protein Expr Purif 44: 94-103.

30. Yun Q, Chen T, Zhang G, Bi J, Ma G, et al. (2005) Novel polyethylene glycol derivative suitable for the preparation of mono-PEGylated protein. Biotechnol Lett 27: 213-217.

31. Yun Q, Xing W, Ma G, Su Z (2006) Preparation and characterization of mono-PEGylated consensus interferon by a novel polyethylene glycol derivative. Journal of Chemical Technology and Biotechnology 81: 776-781.

32. Na DH, Park EJ, Jo YW, Lee KC (2008) Capillary electrophoretic separation of high-molecular-weight poly(ethylene glycol)-modified proteins. Anal Biochem 373: 207-212.

33. Seyfried BK, Siekmann J, Belgacem O, Wenzel RJ, Turecek PL, et al. (2010) MALDI linear TOF mass spectrometry of PEGylated (glyco)proteins. J Mass Spectrom 45: 612-617.

34. Bovara R, Carrea G, Gioacchini AM, Riva S, Secundo F (1997) Activity, stability, and conformation of methoxypoly(ethylene glycol)-subtilisin at different concentrations of water in dioxane. Biotechnol Bioeng 54: 50-57.

35. Gioacchini AM, Carrea G, Secundo F, Baraldini M, Roda A (1997) Electrospray mass spectrometric analysis of polyethylene glycol-protein conjugates. Rapid Communications in Mass Spectrometry 11: 1219-1222.

36. Cindrić M, Cepo T, Galić N, Bukvić-Krajacić M, Tomczyk N, et al. (2007) Structural characterization of PEGylated rHuG-CSF and location of PEG attachment sites. J Pharm Biomed Anal 44: 388-395.

37. Bagal D, Zhang H, Schnier PD (2008) Gas-phase proton-transfer chemistry coupled with TOF mass spectrometry and ion mobility-MS for the facile analysis of poly(ethylene glycols) and PEGylated polypeptide conjugates. Anal Chem 80: 2408-2418.

38. Huang L, Gough PC, Defelippis MR (2009) Characterization of poly(ethylene glycol) and PEGylated products by LC/MS with postcolumn addition of amines. Anal Chem 81: 567-577.

39. Lu X, Gough PC, DeFelippis MR, Huang L (2010) Elucidation of PEGylation site with a combined approach of in-source fragmentation and CID MS/MS. J Am Soc Mass Spectrom 21: 810-818.

40. Abzalimov RR, Frimpong A, Kaltashov IA (2012) Structural characterization of protein–polymer conjugates. I. Assessing heterogeneity of a small PEGylated protein and mapping conjugation sites using ion exchange chromatography and top-down tandem mass spectrometry. International Journal of Mass Spectrometry 312: 135-143.

41. Sleczka BG, D'Arienzo CJ, Tymiak AA, Olah TV (2012) Quantitation of therapeutic proteins following direct trypsin digestion of dried blood spot samples and detection by LC-MS-based bioanalytical methods in drug discovery. Bioanalysis 4: 29-40.

42. Wang SJ, Wu ST, Gokemeijer J, Fura A, Krishna M, et al. (2012) Attribution of the discrepancy between ELISA and LC-MS/MS assay results of a PEGylated scaffold protein in post-dose monkey plasma samples due to the presence of anti-drug antibodies. Anal Bioanal Chem 402: 1229-1239.

43. Dawes ML, Gu H, Wang J, Schuster AE, Haulenbeek J (2013) Development of a validated liquid chromatography tandem mass spectrometry assay for a PEGylated adnectin in cynomolgus monkey plasma using protein precipitation and trypsin digestion. J Chromatogr B Analyt Technol Biomed Life Sci 934: 1-7.

44. Forstenlehner IC, Holzmann J, Scheffler K, Wieder W, Toll H, et al. (2014) A direct-infusion- and HPLC-ESI-Orbitrap-MS approach for the characterization of intact PEGylated proteins. Anal Chem 86: 826-834.

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