

Trends in Characterization of PEGylated Proteins by Mass Spectrometry

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Abstract

Characterization of PEGylated proteins is 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 proteolytic enzymes. Attachment of PEGs to proteins occurs on the N-terminus site, carbohydrate, sulfhydryl, and on amino acids 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 of 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 or 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.

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 digests 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

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- α 2b	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, homogeneity	[20]

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

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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 sites, 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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