Structural characterization of site-specific pegylated r-h-met-G-CSF

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Granulocyte Colony-Stimulating Factor (G-CSF) stimulates the proliferation, differentiation and functional activation of hematopoietic cells of the macrophage and granulocyte lineages. Recombinant human G-CSF is used clinically to treat a variety of hematopoietic disorders, as it can reduce the severity of chemotherapy-induced neutropenia, accelerate hematopoietic recovery following bone marrow transplantation and mobilize blood progenitor cells for transplantation. A limitation of current G-CSF products is the fact that G-CSF has a short circulating half-life and must be administrated to patients by daily injection for optimal effectiveness. Attachment of poly(ethyleneglycol) (PEG) to a protein increases the protein's effective size, thus reducing its clearance rate by the kidney and prolonging its circulating half-life.
The aim of this study was the structural characterization of the pegylated recombinant methionyl-human-G-CSF (r-met-h-G-CSF) expressed in *Escherichia coli* [1]. For the structural study, a combination of mass spectrometric (MS and MS/MS) analyses and conventional methodologies (proteolytic digestion and automated Edman sequencing) were used to determine the molecular mass value of the protein and its pegylated derivative and to identify the site of pegylation.
The mass value of r-met-h-G-CSF obtained by electrospray ionization (ESI) corresponded to 18797±3 Da, which is in agreement with the theoretical one expected for the oxidised met-G-CFS (showing two disulfide bridges), while the mass value of pegylated r-met-h-G-CSF, determined by MALDI-TOF analysis, was centred to 38698 Da, indicating the binding of one PEG molecule to G-CSF.
In order to identify the pegylation site, the r-met-h-G-CSF and its pegylated derivative were subjected to enzymatic digestion by V8 protease and the obtained fragments were analyzed by RP-HPLC-ESI-MS.
The comparison of the two peptide mapping allowed to evidence that 3 fragments present in the V8 digest of met-G-CSF (V9, V10b and V15) were absent in the pegylated V8 digest.
The fragments V9 (125-143), V10b (125-142) and V15 (125-163), all containing the two glutamine residues at position 133 and 135, were not detected in the digestion mixture of pegylated r-met-h-G-CSF. This evidence suggested that the two residues might be possible pegylation sites.
To confirm the pegylation site, the peptide mixture of PEG-G-CSF was separated on 10% SDS-PAGE, and the pegylated peptides, electroblotted on PVDF membrane, were submitted to automated Edman sequencing.
This result allowed establishing that pegylation involved specifically the glutamine residue at position 135.

References

[1] Pozzuolo S., Breme U., Salis B., Taylor G., Tonon, Orsini G. 2008. Efficient bacterial expression of fusion proteins and their selective processing by a recombinant Kex-1 protease. Protein Expression and Purification. 59: 334-341.