Chemoselective Chemistry of Catechol : N-terminal Amine Specific Reaction in Peptide and Proteins

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Introduction: PEGylation – covalent attachment of poly(ethylene glycol) (PEG) to proteins or peptides - have been used for increasing therapeutic effect of pharmaceutical proteins. Merits of PEGylation include physical protection of the proteins against enzymatic proteolysis, significant increase of in vivo half-life and solubility of PEGylated drugs(1). However, original activities of bilogical drugs were often decreased when PEG is conjugated. This is primarily due to lack of selectivity of functional groups in the existing PEGylated chemistries. Thus, to maintain original activity of PEGylated molecules, it is critical to develop a method that can modify a specific amino acid residue of proteins, in other words, site-specific or chemoselective PEGylation.

Herein, we describe a novel N-terminal selective PEGylation method utilizing chemistry of catechol. Catechol is a side chain of the unusual amino acid called 3,4-dihydroxy-L-phenylalanine(DOPA) found in the proteins of adhesive pads and threads of mussels(2). It has been known that catechol is oxidized to form catechol quinone that is reactive to primary amine groups (3-4). The PEGylation reaction occurs in a mild neutral condition, which is suitable for maintaining biological activities of PEGylated proteins. This PEGylation strategy is generally applicable to a wide variety of proteins. For all tested pharmaceutical proteins of Granulocyte Colony Stimulating Factor (G-CSF), basic Fibroblast Growth Factor (bFGF), Lysozyme and Erythropoietin (EPO) chemoselective N-terminal PEGylation was observed. Not only large proteins, but small size peptide, Hinge-3, showed N-terminal selective PEGylation. The PEGylated proteins exhibited increased in vivo pharmacokinetics compared to un-PEGylated proteins.

Materials and Methods: mPEG-AM was reacted with HCA. Thus, catechol modified PEG product which is mPEG-CT was obtained. At neutral or slightly acidic buffer condition (PBS 10mM pH 6.5) and 4℃ environment, peptide , hinge-3, was covalently coupled with mPEG-CT. The PEGylation site was verified by tryptic digestion method with RP-HPLC and MALDI-ToF mass spectrometry.

Proteins were reacted with mPEG-CT under the similar condition as peptide. But exact pH values were little bit different among the proteins.

After EPO PEGylation, in vivo stability and activity test was experienced by intravenous administration to mouse.

Results: Catechol-PEG was prepared by a reaction between methoxy-PEG-amine group and 3,4-dihydroxycinnamic acid in DMF for 12 hrs (Figure 1a).

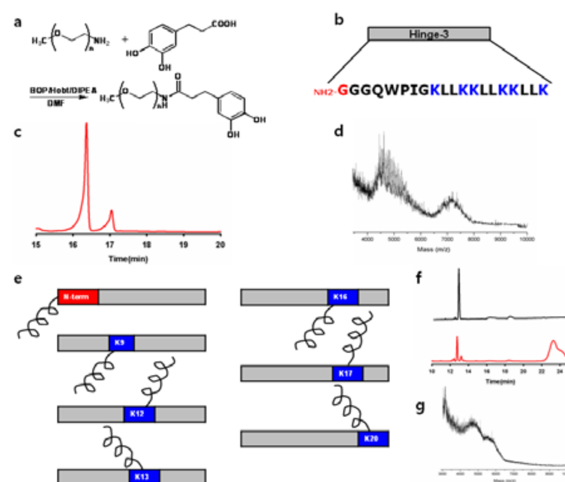

Figure 1. N-terminal PEGylation : peptide (hinge-3)

Hinge-3 (Figure 1b) has several potential PEGylation sites, lysine and N-terminus. However, under the pH 6.5 and 4• condition, only monoPEGylated hinge-3 was detected by RP-HPLC (Figure 1c). At 16.5 min, hinge-3 was shown and monoPEGylated hinge-3 was detected at 17min. MALDI-ToF analysis verified the mass of monoPEGylated hinge-3 (~ 7238 Da) (Figure 1d). To verify the PEGylation site, tryptic digestion test was performed. There are seven monoPEGylated hinge-3 candidates (Figure 1e). Tryptic digestion followed by RP-HPLC showed that PEG-T1 fragment (mPEG-GGGQWPIGK) was eluted at 23.5 min (Figure 1f red). MALDI-ToF analysis of the eluted conjugate showed the mass around 5883 m/z, confirming that the eluted fragment was PEG-T1 (Figure 1g). This result clearly demonstrated that the catechol selectively reacts with N-terminal amine group of a peptide. Furthermore, PEGylation utilizing pharmaceutical proteins such as G-CSF, lysozyme, EPO, and bFGF showed N-terminal specific PEGylation.

References :

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