Dimeric PEGylation of rhEGF by Using Bi-Functional PEG-Aldehyde

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Recombinant human epidermal growth factor (EGF) is a 53-amino acid polypeptide, which exhibits potent mitogenic activities, such as cell proliferation and differentiation of various tissues and cells. EGF binds to the EGF receptor forming a dimerized complex, which is then internalized within cells. The internalized EGF receptor has a tyrosine kinase activity, thus initiating a series of signal transduction cascades. It has been demonstrated that the site of PEGylation was very important and that it had major effects on their biologic activities. In this study, We PEGylated the N-terminus of EGF in a site-specific manner using bi-functional PEG-aldehyde (Mw 3400). We used GPC and IEX chromatography in order to purify dimeric-EGF PEGylates. Various characterization methods, MALDI-TOF techniques were used to reveal that separation of the PEGylates and identification of the dimeric-EGF PEGylates. Tryptic digestion, and MALDI-TOF techniques were used to confirm that the di-EGF PEGylation reaction occurred site specifically at the N-terminal amine group. 2-DE was performed to investigate the changes in isoelectric point of the dimeric-EGF PEGylate. ITC experiment was performed for the thermodynamic analysis between PEG and EGF.