Preparation of a fusion protein of Fc fragment conjugated with EGF

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Fc fragment of an antibody, ca. 25 kD MW, is known to enhance stability of a protein, and thus it is often used as a fusion protein partner to increase the stability of pharmaceutical proteins. In this study, we used a commercially available IdeS protease (FabRICATOR®) to cleave the specific site of rabbit immunoglobulin G (IgG) gamma globulin, and the resulting Fc fragment was separated by Protein A bead. A SPR biosensor study indicated that the Fc fragment showed higher immunobinding ability than the intact antibody. Using rhEGF (epidermal growth factor) as a model protein, we attempted direct chemical conjugation. An activated polyethylene glycol (PEG) was used as a conjugation linker. ButyrALD-PEG-ButyrALD (both ends with aldehyde groups) with average MW of 10 kD, was used to link the N-terminus of Fc and the N-terminus of EGF. SDS-PAGE revealed the conjugation ('Fc-PEG-EGF' fusion protein with ca. 40 kD MW) was successful, and it was recovered by anion exchange and size exclusion chromatography. In conclusion, the chemical conjugation between Fc and EGF seemed to require a linker molecule, and the bifunctional PEG appeared well suited for that purpose.