Chemoenzymatic Synthesis of functional proteins for drug delivery system

by

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Abstract

DNA-protein conjugates are promising biomolecules for use in areas ranging from drug delivery system to analysis because of the dual functionalities of DNA and protein. Conjugation requires site-specific and efficient covalent bond formation without impairing the activity of both biomolecules. Herein, we have focused on the use of a microbial transglutaminase (MTG) that catalyzes the cross-linking reaction between a glutamine (Q) residue and a primary amine. In a model bioconjugation, a highly MTG-reactive Q-donor peptide (FYPLQMRG, FQ) was fused to enhanced green fluorescent protein (FQ-EGFP) and a primary amine-clustered DNA aptamer was enzymatically synthesized as a novel acyl-acceptor substrate of MTG, whose combination leads to efficient and convenient preparation of DNA-protein conjugates with high purity. Dual functionality of the obtained DNA-EGFP conjugate was evaluated by discrimination of cancer cells via c-Met receptor targeting ability of the DNA aptamer. The DNA aptamer-EGFP conjugate only showed fluorescence toward cells with c-Met overexpression, indicating the retention of the biochemical properties of the DNA and EGFP in the conjugated form.

We also focused on lipid modification of proteins, which plays a significant role in regulating the cellular environment. Mimicking natural lipidated proteins is a key technique not only for assessing the function of proteins modified with lipids but also to render self-assembly of lipids to a target protein. Especially, self-assembly ability of lipid-protein conjugates has been applied for drug delivery system to anchor the protein on a carrier such as liposomes. However, hydrophobic nature of lipids causes denature of the protein after lipid attachment. Therefore, we propose a facile method of conjugating proteins with lipid-fused peptides under homogeneous physiological conditions by using the MTG reaction. The water-soluble peptide substrates for lipid modification, C14-G₅S-MRHKGS, were newly synthesized, where C14, G₅S, and MRHKGS represent myristic acid, linker peptides composed of G and S, and MTG-reactive K surrounded with basic amino acids, respectively. The MTG-mediated cross-linking reaction between an EGFP fused with LLQG at the C-terminus and C14-G₅S-MRHKGS (5 molar eq) dissolved in a phosphate saline solution resulted in lipid–protein conjugates with yields of 70 to 100%. The anchoring ability of the obtained lipid–protein conjugates to cell membranes was dependent on the number of G residues in the G₅S linker, suggesting that self-assembly and hydrophobicity of the G₅S motif serves to enhance membrane anchoring of lipid-protein conjugates.

Date : 16 November 2018 (Tuesday)
Time : 1630
Venue : Room 4620 (Lifts 31-32)