Conformational stability of proteins conjugated with water-soluble phospholipid polymer from heat-induced denaturation: Effect of the hydrophilicity of the polymer materials

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Introduction: Employment of outstanding chemical and biological activities of the proteins in various bioengineering fields such as medicine and diagnostics, have a great potential utility for developing a specific biomedical devices. However, proteins can be easily deformed by external stress such as heat, light, chemicals, etc; and these kind of denaturation poses a critical problem in biomedical applications. Many studies have been performed to elucidate mechanisms of increasing the stability of proteins or reversing the deactivated activity of denatured proteins. However, even the most successful strategies employed to generate bioconjugate form, such as poly(ethylene glycol) modification (PEGylation) frequently showed periodical drawbacks in activity even though it showed a limited success in increased thermal or chemical stability of proteins. To solve these issues, we develop a bioconjugation process with water-soluble phospholipid polymers.[1)] 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer conjugation is known to preserve protein functionality in much more prolonged period than that of the PEGylated one. Since MPC polymer is known to maintain large amount of free water around MPC units even under the heated condition, we propose that heat-induced conformational change could be effectively suppressed by essential hydrophilic environment formed by the conjugated MPC polymer. In order to verify this hypothesis, the MPC polymers with different compositions of hydrophilic or hydrophobic moiety was synthesized to conjugate the proteins and their effect on thermal-induced conformational changes was investigated.

Method: Three different compositions of random copolymers composed of hydrophilic 2-hydroxyethyl methacrylate (HEMA) and MPC (30, 50, 70 mol %) having a cysteine reactive end functionality was synthesized well controlled atom transfer radical polymerization (ATRP) method. Random copolymers with hydrophobic n-butyl methacrylate (BMA, 30, 50, 70 mol %) was also synthesized by the same method. All the synthesized polymers were then site specifically conjugated with Cys-34 of bovine serum albumin (BSA), and the conformational changes in secondary and tertiary structures during heating-quenching stress (from 25°C to 73°C) was investigated by circular dichroism (CD) and fluorescence study. In order to quantitatively analysis the hydrophobicity of polymers, all the polymer solution was characterized by fluorescence peak shift in 8-anilino-1-naphtalene sulfonic acid. Functionality change was also confirmed by measuring change in hydrolysis catalytic activity of BSA.

Results and Discussions:

1) Synthesis of end functional MPC polymer by ATRP: The sulfhydryl reactive MPC polymers bearing hydrophobic butyl group (PMB) or hydrophilic hydroxyl group(PMH) were synthesized by well-controlled ATRP

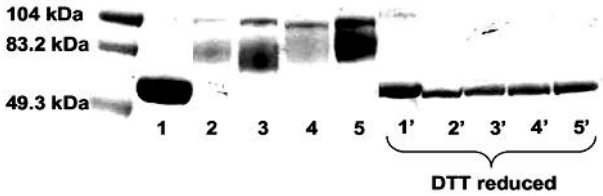

*Figure 1.*SDS-PAGE result of the non conjugated BSA Lan(1), and PHEMA (2), PMH30 (3), PMH50 (4), PMH70 (5) conjugated BSA.

2) Site specific conjugation of polymers with BSA: The representative SDS PAGE result (for PMH series) in *Figure 1* indicates that the site-specific conjugation at the Cys-34 residue was successfully conducted by forming a reversible disulfide bonding.

3) Conformational and functional changes of proteins during thermal stress: The conformational change and its recovery ratio during the heat-quenching process are summarized in *Figure 2*. Clearly, PMB conjugated BSA showed higher denaturation ratio than those of the PMH ones, and even more than native BSA. This result indicates that the hydrophobic group has an adverse effect on conformational change. In the case of the conformational recovery, only 100% MPC polymer conjugated BSA shows the 100% of conformational recovery. Further, the most hydrophobic polymer, i.e. PHEMA, and 30% MPC containing PMB (PMB30) showed the lowest conformational recovery. Similar phenomenon was also observed in the results of the tertiary structural changes. Clearly, The hydrophobic PMB conjugated BSA showed more significantly decrease in the fluorescence intensity which induced by spatial changes in triptophan residue due to the tertiary structural changes. These results indicated that the hydrophilicity of conjugated polymer play an essential role in heat-induced conformation changes in both secondary and tertiary structures. The maintenance of the functionality was also supported by the measurement of the hydrolytic activity of BSA. The results are well corresponding to the CD and fluorescence data.

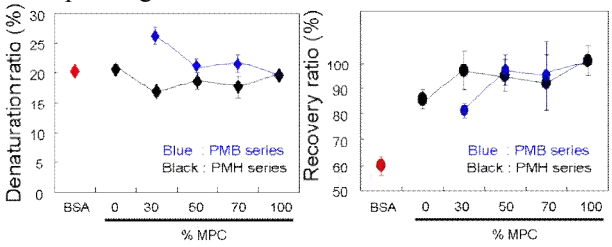

Figure 2. Denaturation and recovery ratio in secondary structure of each samples.

Conclusions: Site specifically conjugated water-soluble phospholipid polymer greatly enhanced the conformational recovery of protein from heat induced denaturation. And essential hydrophilic environment provided by the MPC polymer is thought as a main reason of the conformational stability of proteins.

Refernces:1) Seo J-H et al. Biomaterials 2009, *30*, 4859