Noncovalent Modification of Proteins Using Smart Polymer: Implications in Modulating Enzymatic Activity and Improvement of Thermotorelant Property

Sumon Ganguli[1], Keitaro Yoshimoto[1,2,3], Shunsuke Tomita[1], Hiroshi Sakuma[1], Tsuneyoshi Matsuoka[1], Kentaro Shiraki[1,2] and Yukio Nagasaki[1-6]

[1]Graduate School of Pure and Applied Science, [2]Graduate School of Comprensive Human, [3]Tsukuba Research Center for Interdisciplinary Materials Science (TIMS), [4]Center for Tsukuba Advanced Research Alliance (TARA), [5]Institute of Applied Physics, [6]Satellite Laboratory, International Center for Materials Nanoarchitechtonics (MANA), National Institute of Materials Science. (NIMS), University of Tsukuba, Ten-noudai 1−1−1, Tsukuba, Ibaraki 305-8573, Japan.
Phone: +81-029-863-5749 Fax: +81-029-863-5749 E-mail: sumon@ims.tsukuba.ac.jp

Statement of Purpose: Proteins have evolved to acquire highly specialized biological functions and are ideal for various applications in both biotechnology and medicinal chemistry, although denaturation is one of the major obstacles in protein chemistry. In the field of polymer science, designer molecules such as synthetic polymer as modifiers of protein functions are of enormous interest due to their versatile applications. Such modifications can be achieved covalently and noncovalently. Covalent modifications of a protein with a polymer offer the possibility of irreversibly modifying its biological activity[1]. On the other hand, noncovalent modifications of proteins with synthetic macromolecules offer the possibility of reversible complexation and modulation of its function. In addition, the modification of proteins by polymer improves the stability of proteins under extreme conditions such as high temperatures. The reversible nature of the binding between polymer and protein demonstrate new prospects for protein stabilization and delivery. In the present study, we present a novel technique for the prevention of heat-induced inactivation of lysozyme as well as ribonuclease A (RNase A) by the complex formation with a smart copolymer, poly(*N,N*-diethylaminoethyl methacrylate)-*graft*-poly(ethylene glycol) (PEAMA-*g*-PEG).

Methods: The detail experimental procedures were described in our previous publications[2,3].

Results: We found that PEAMA-*g*-PEG suppressed the enzymatic activity of lysozyme completely without any conformational change of lysozyme indicating the strong interaction. We considered that the complexation was driven by electrostatic interaction between the positively charged amine segment of the PEAMA-*g*-PEG and the negatively-charged active site of lysozyme despite the fact that both are positively-charged. To evaluate the electrostatic interaction between lysozyme and PEAMA-*g*-PEG, an exchange reaction with the polyanion, poly(acrylic acid) (PAAc) was carried out and we observed the recovery of enzymatic activity fully is as shown in the Figure 1. To our surprise, even after heating of lysozyme with PEAMA-*g*-PEG for 20 min at 98 ºC, the addition of PAAc recovered 80% enzymatic activity of lysozyme (Figure 2)[2]. Recently, we also found that PEAMA-*g*-PEG had almost no effect on the enzymatic

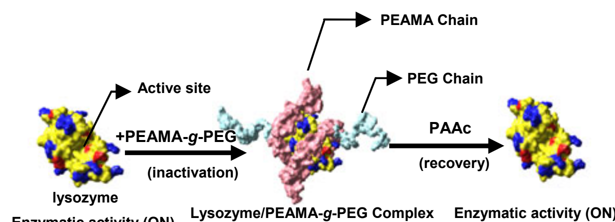

Figure 1: Formation and dissociation of the lysozyme/PEAMA-*g*-PEG complex.

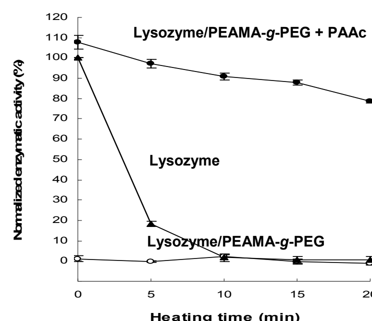

Figure 2: Changes in the enzymatic activity of lysozyme and the lysozyme/PEAMA-*g*-PEG complex as a function of the heating time.

activity of ribonuclease A (RNase A) due to the repulsive force between the positively-charged amine segment of the PEAMA-*g*-PEG and the positively-charged active site of RNase A but effectively prevents the heat-induced irreversible inactivation of RNase A. Circular dichroism (CD) spectral analysis indicating the heat-induced irreversible inactivation was largely suppressed when enzymes complexed with PEAMA-*g*-PEG.

Conclusion: We strongly conclude that modulation of enzymatic activity have performed successfully as well as the thermotorelant property of lysozyme and RNase A was improved effectively based on the complex formation via noncovalent interactions between proteins and PEAMA-*g*-PEG. Binding behavior between polymer and protein characterized by structure retention effects offers new prospects for protein stabilization and delivery.

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