Construction of Polyacrylamide Gel Containing an Engineered Hexameric Hemoprotein and Evaluation of its Mechanical Properties

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Abstract

Stimuli-responsive gels, which change their volumes and properties in response to external stimuli, are attracting attention as unique materials for chemical sensors and actuators. One strategy for constructing these materials is to incorporate functional molecules into the gel as components, so that the microscopic properties of the molecules are reflected in the macroscopic properties of the materials. In particular, materials which respond to light irradiation or specific molecules have been developed by introducing reversible bonds or interactions into the gel as a stimuli-responsive cross-linkage. As a new cross-linker of gel, we have focused on the interaction between heme and heme pocket in hemoproteins. Heme, an iron porphyrin, binds to a protein matrix as a cofactor via reversible interaction. The strength of this interaction depends on the redox state or amino acid residues surrounding heme. Therefore, we hypothesize that the gel containing hemoprotein as a cross-linking unit shows unique mechanical properties modulated by the changes of these factors.

In this study, we employed hexameric tyrosine coordinated heme protein (HTHP) as a cross-linking unit (Figure 1).[1] First, the heme-dependent stabilities of HTHP in each redox state were investigated by titration of denaturants. ΔG values for heme dissociation in Fe^{2+} and Fe^{3+} states were determined to be 25 ± 2 and 57 ± 6 kJ/mol, respectively. This result clearly shows that the reduction of the iron center dramatically decreases the affinity of heme for the protein matrix. Next, mutations around heme were conducted to change the strength of the affinity. Especially, we focused on two arginine residues around heme. These residues could be related to the heme binding through cation-interaction. The R25A and R38A mutants were designed and prepared. The ΔG values of R25A and R38A mutants were determined to be 40 ± 5 and 41 ± 2 kJ/mol, respectively.

Heme derivative tethering an acrylamide group as a reaction site was synthesized and inserted into the apo-form of HTHP to yield reconstituted HTHP (rHTHP). Then, the polyacrylamide gel containing rHTHP as a cross-linker was prepared. A compression test was used to evaluate the redox-responsive mechanical properties of the obtained gels, indicating the elastic modulus decreased with the addition of reductants. This behavior is thought to be caused by changes in the heme-binding for HTHP as a cross-linker. The Young's moduli of gels containing rHTHP and mutants were evaluated by tensile test, showing that the gels containing rHTHP mutants have decreased the Young's moduli relative to the gel containing wild type rHTHP. These findings indicate that HTHP is a potent cross-linker for stimuli-responsive hydrogel.

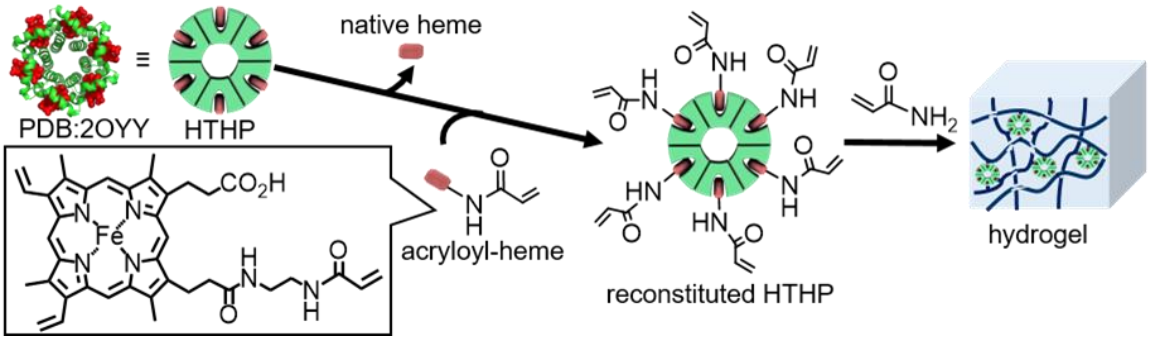

Figure 1. Schematic representation of polyacrylamide gel cross-linked by rHTHP.

[1] H. Dobbek *et al*., *J. Mol. Biol*., **2011**, *368*, 1122-1131.