Construction of Hydrogel Containing an Engineered Hexameric Hemoprotein and Evaluation of its Redox-responsive Mechanical Properties

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Stimuli-responsive hydrogels exhibit changes in physicochemical properties and/or shapes triggered by external stimuli such as photo irradiation, redox and pH changes. These hydrogels have been receiving widespread attention as new smart biomaterials. Especially, hydrogels having mechanical properties controlled by external stimuli are expected as potent extracellular matrices (ECM) because the importance in cellular behavior has been reported.[1] In this work, we have constructed a metalloprotein-based hydrogel changing the mechanical properties by redox states of the metal center. Toward the redox responsive behavior, heme-heme pocket interaction in hemoprotein was employed as a crosslinker in the hydrogel. Heme *b*, an iron porphyrin complex, is strongly bound into a protein matrix in hemoprotein and the heme-heme pocket interaction depends on the redox of the iron center. Here, hexameric tyrosine-coordinated heme protein (HTHP)[2] was chosen as a cross-linkage unit of a polyacrylamide gel (Fig. 1) due to the expected high affinity of heme and its multimeric structure.

First, the redox-dependent heme-heme pocket interaction of HTHP was investigated though chemical denaturant titration. In the Fe^{3+} state, HTHP shows the highly stable heme-heme pocket interaction, whereas this interaction in the Fe^{2+} state dramatically weakens. Next, a heme derivative tethering an acryloyl group was synthesized to introduce the reaction sites on the HTHP (Fig. 1). The modified heme was inserted into the apo-form of HTHP to obtain the reconstituted protein (rHTHP). Polymerization of acrylamide and rHTHP provided hydrogel. Soaking the hydrogel into a reductant solution promoted reduction of the iron center. The mechanical properties of the hydrogel were evaluated by the compression test, showing decrease of the modulus upon the addition of reductant.

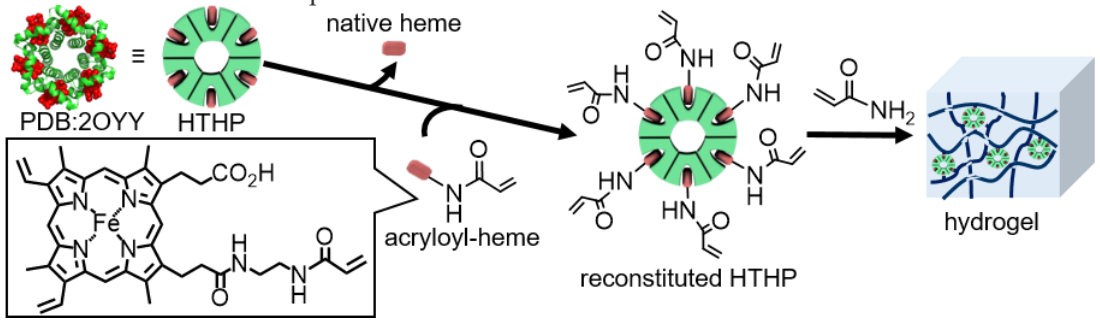

Fig 1. Schematic representation for preparation of hydrogel containing rHTHP as a cross-linkage.

1) L. Wang *et al., Science*, **2005**, *310*, 1139. 2) H. Dobbek *et al*., *J. Mol. Biol*., **2011**, *368*, 1122.