Synthesis of Protein-polymer Conjugates with Controllable Properties

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Statement of Purpose: Protein-polymer conjugation is known to improve protein efficacy by increasing lifetimes in vivo and decreasing immunogenicity. Site-specific conjugation of polymer with protein is an interesting and modern approach for preparing bioconjugates with controllable structure and property. In this work we generated a mutant pyrophosphatase (PPase) with a substituted cysteine residue near the active site for site-specific polymer conjugation. Accurate and reversible control on protein activity was achieved by the site-specific conjugation of poly(2-hydroxyethyl methacrylate) (pHEMA). Moreover, visible light assisted RAFT polymerization of N-isopropylacrylamide (NIPAm) from protein surface at the specific site provides the opportunity for temperature control on protein activity.

Methods: Site-directed mutagenesis was performed to clone mutant *ppa* gene for the generation PPase Cysteine mutant for polymer conjugation. Firstly, atom transfer radical polymerization (ATRP) synthesized pyridyl disulfide-functionalized pHEMA was conjugated to PPase in methanol and PBS buffer (pH 7.4) mixture at room temperature for 30 min. Secondly, the maleimide functional chain transfer agent (CTA) was immobilized to PPase via its R-group to afford PPase-macroCTA and the reversible addition-fragmentation chain transfer (RAFT) polymerization of N-isopropylacrylamide (NIPAm) was carried out in H_2O at 20°C, using (2,4,6-trimethylbenzoyl)phenyl phosphonic acid sodium (TPO-Na) as a photoinitiator under visible light radiation at λ = 420 nm with a mild intensity of 0.2 mWcm^{-2}.

Results: As for the PPase-pHEMA conjugation, SDS-PAGE analysis shows that the pHEMA was site-specifically conjugated to PPase by reversible disulfide bonds (Figure 1a). Activity assays indicate that the enzymatic activity of PPase was almost lost (0.37% of unconjugated PPase) after pHEMA conjugation, and gradually recovered with increasing DTT concentration. Furthermore, reversible regulation of PPase activity was achieved by repeated pHEMA conjugation and DTT reduction (Figure 1b). As for the PPase-pNIPAm conjugation, SDS-PAGE result shows the controllable property of the visible light polymerization of NIPAm from PPase. The molecular weight increased with polymerization time. Meanwhile, this polymerization progress can be easily controlled with light-on/off state (Figure 2a). Activity assays show that the protein activity can be switched by changing the temperature and can also be tuned by changing the molecular weight of conjugates (Figure 2b).

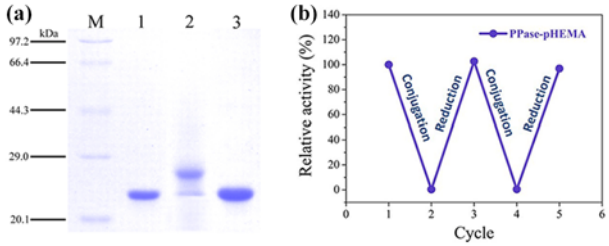

Figure 1. (a) SDS-PAGE analysis of pHEMA conjugation (lane M: molecular weight marker, lane 1: PPase, lane 2: PPase-pHEMA, lane 3: PPC-pHEMA with DTT treatment); (b) Repeatability of PPase activity regulation by pHEMA.

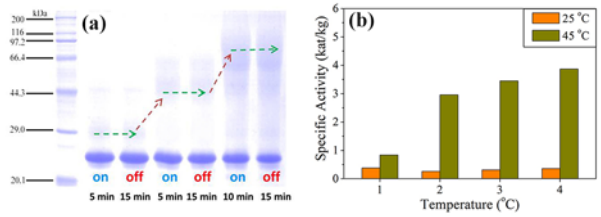

Figure 2. (a) SDS-PAGE result of PPase-pNIPAm conjugates with light-on–off periodically; (b) Specific activity of (1) PPase-macroCTA, (2–4) PPase–pNIPAm conjugates with polymerization time 10min/20min/30min after incubation at 25 or 45°C for 10 min and measured at the same temperature.

Conclusions: In summary, our results provide a general approach for efficient on-demand control of protein property based on site-specific conjugation of polymers. Conjugation of pHEMA to the specific site on protein surface resulted in a clear and reversible decrease of protein activity. In addition, by the fast visible light assisted RAFT polymerization of NIPAm from the protein surface, protein activity is retained and in certain cases provides a conjugate with on-off-switchable properties.

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