Design of Clickable Smart Polymers for Enrichment of Dilute Biomarkers

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Abstract

About 25% of the cause of death in the world is an epidemic disease, and 15 million people die every year. Especially, most of the cause of death in the low infrastructure region is due to infectious diseases such as pneumonia, diarrhoeal diseases, HIV, and tuberculosis. In virtually all cases, they could be prevented by appropriate protection. One of the most important and beneficial protection activities is the early detection of diseases. Diagnosis of those diseases during the early stages of infection has been positively linked to a decrease in the mortality and morbidity of the illness. A major challenge is, however, to develop diagnostic tests to meet the needs of the developing world's medical community which does not have access to many of the best medical diagnostic technologies. One of the most simple and in-expensive diagnostic tests is lateral-flow immunochromatographic strip test. Although this diagnostic method has been widely utilized due to the near-patient methods in low-resource settings, the sensitivity levels are poor. Utilizing smart polymer which is respond to external stimuli such as heat and pH for immunoassays, drug delivery, bioseparation, switchable enzyme activity, and affinity precipitation has been explored for more than two decades. Smart polymer-protein conjugates are attractive building block for bioseparation because recognition events occur at the molecular scale and appropriate stimuli rapidly aggregate the conjugates to enable effective isolation for downstream analysis [1-4]. Therefore, the goal of this study is to develop a smart polymer-based diagnostic technology for the purification and enrichment of target biomarkers using smart polymer-antibody conjugates for processing diagnostic samples (Figure 1).

First, *N*-(2-hydroxyisopropyl)acrylamide (HIPAAm) was synthesized and copolymerized with *N*-isopropylacrylamide (NIPAAm). The P(NIPAAm-*co*-HIPAAm) copolymers were then reacted with dibenzylcyclooctyne-acid (DBCO-acid) to immobilize cyclooctyne groups on the side chain (Figure 1a). Since cyclooctyne can readily react with azides under copper-free conditions, it was expected that the designed copolymers would react with azide-biomolecules via click reaction in biological conditions. The cyclooctyne-modified polymer was conjugated to azide-IgG antibody and the successful conjugation with antibody was confirmed by UV-vis spectroscopy and SDS-PAGE analysis. The sharp temperature-responsiveness of the polymer was kept after conjugation with antibody and the transition temperature of conjugates could be easily adjusted near body temperature by polymer designing. The conjugates bound a HIV relevant antigen specifically and with high binding efficiency comparable to native antibodies. Finally, the enrichment of dilute biomarkers was successfully demonstrated using the conjugates (Figure 1b). The proposed bioorthogonal smart polymers will provide a facile method to purify and enrich biomarkers under biological conditions without the requirement of an external power supply.

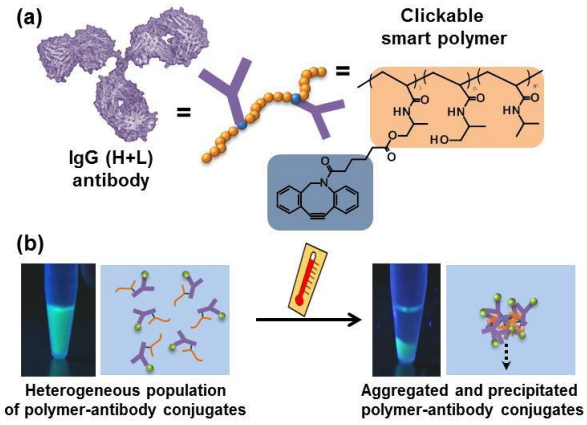

Figure 1. a) Schematic illustration of smart polymer-antibody conjugates. b) The purification and enrichment of target biomarkers using smart polymer-antibody conjugates for processing diagnostic samples.

References

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