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Construction of aptamer based system for purification of lysozyme from egg white

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Recently, several types of polymer brushes with different functional groups have been prepared, such as poly(glicidylmethacrylate), p(GMA), grafted and amine modified resin, p(GMA) grafted polystyrene resin with tertiary amine, p(GMA) grafted and sulphonate groups modified beads, etc., have been shown to have high adsorption capacities [1-2]. These polymer brushes were grafted onto various materials surfaces using surface-initiated atom transfer radical polymerization (SI-ATRP), which has played a significant role in the modification of surface properties [2]. Magnetic separation using magnetic polymeric support is a quick and easy method for the reliable capture of the target protein from crude biological fluids. The magnetic carriers could be conveniently and quickly separated from the medium under magnetic field. Recently, there has been increased interest in the use of magnetic carriers in protein purification [2]. Aptamer ligands have been used in the area of affinity chromatography over traditional antibody and small molecule ligands [3]. Aptamer ligands are selected for their specific binding to their target molecules. Aptamers are of research interests for affinity chromatographic system, and biosensor construction.

We present here a simple single-step lysozyme purification system from diluted whole chicken egg white. For this purpose, magnetic particles (MP's) were synthesized via magnetic co-precipitation reaction. Then, MP's were coated with (3-aminopropyl)triethoxysilane (APTES) and the pendant $–NH_2$ groups subsequently modified with bromo-acetyl-bromide to produce Br ended MP's. SI-ATRP of GMA from the MP's-Br surface was carried out to produce the fibrous polymer chain grafted MP's-g-p(GMA), and epoxy groups of the MP's were used for simultaneous immobilization of lysozyme spesific aptamer. The preparation steps of MP's-g-p(GMA)-aptamer were characterized by SEM, TEM, ATR-FTIR, BET and analytical methods. The separation performance and selectivity of the MP's-g-p(GMA)-aptamer system for lysozyme adsorption from aqueous medium were evaluated. The separation capacity of the MP's-g-p(GMA)-aptamer to lysozyme was examined using lysozyme solution and diluted egg white solution. The optimum condition of lysozyme adsorption on the MP's-g-p(GMA)-aptamer was determined as the initial lysozyme concentration at 1.0 mg mL^{-1} and at pH 7.0 with adsorption time 30 min. The maximum lysozyme adsorption pH was obtained at pH 7.0 and adsorption capacity of the MP's-g-p(GMA)-aptamer was found to be 73.6 mg lysozyme/g support. The high surface area of the fibrous polymer grafted magnetic particules with lysozyme specific aptamer exhibited high binding capacity to lysozyme, and resistance to binding of nonspecific adsorption to other tested protein such as egg white albumin, ovalbumin, and hemoglobin. When the MP's-g-p(GMA)-aptamer were applied to purification of lysozyme from diluted chicken egg white, lysozyme could be purified to single-band purity according to SDS-PAGE. The reusability studies showed that, about 83% of the initial adsorption capacity of the MP's-g-p(GMA)-aptamer could be maintained after 10 cycles of uses.

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3. *Ozalp V.C., Bayramoglu G., Erdem Z., Arica M.Y.* // Pathogen detection in complex samples by quartz crystal microbalance sensor coupled to aptamer functionalized core–shell type magnetic separation. Anal Chim Acta.-2015.-**853**, N 3.-P. 533-540.