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Abstract
New immobilized metal ion affinity chromatography (IMAC) matrices containing superparamagnetic nanoparticles and coated with hydrophilic resins are here proposed to improve the purification of polyhistidine (poly-His) tagged proteins. The magnetic chelating resin was prepared by radical polymerization of magnetite (Fe_3O_4), styrene, DVB and GMA-IDA in ethanol/water medium. IDA is immobilized on magnetite and the IDA-magnetite pre-loaded with copper (Cu^{2+}). The IDA-magnetite is then used to bind to test their suitability for the direct recovery of an intracellular, polyhistidine-tagged protein, enhanced green fluorescent protein [EGFP-(His)6], from unclarified E. coli homogenates in a single step. Parameters influencing the purification efficiencies such as pH, ionic strength and imidazole concentration were optimized to achieve improved separation. The optimal selectively was observed in binding buffer (0.2 M NaCl, 0.02 M imidazole, 0.05 M phosphate buffer, pH 8.0), washing buffer (0.4 M NaCl, 0.03 M imidazole, 0.05 M phosphate buffer, pH 6.0), elution buffer (0.50 M imidazole, 0.05 M phosphate buffer, pH 8.0). The high affinity of copper for poly-His makes it effective in the purification of poly-His fusion proteins from a typical expression system. The capacity of Cu(II)-loaded IDA-magnetite for EGFP-(His)6 was 657 μg/g IDA-magnetite, comparable to commercial agarose-based Ni-NTA absorbents. The EGFP was purified from E. coli homogenates in a single step with an overall yield of 70.4 % and purification factor of 12.3. The protocol, starting from the preparation of bacterial lysate and ending with purified proteins takes less than 40 min. Thus, the separation and purification qualities of Cu(II)-loaded IDA-magnetite as well as its ease of use surpass those of commercial agarose-based Ni-NTA absorbents.
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