

A novel strategy for the purification of a recombinant protein using ceramic fluorapatite-binding peptides as affinity tags

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Abstract

In recent years, affinity fusion-tag systems have become a popular technique for the purification of recombinant proteins from crude extracts. However, several drawbacks including the high expense and low stability of ligands, their leakage during operation, and difficulties in immobilization, make it important to further develop the method. The present work is concerned with the utilization of a ceramic fluorapatite (CFT)-based chromatographic matrix to overcome these drawbacks. A heptapeptide library exhibiting a range of properties have been synthesized and subjected to ceramic fluorapatite (CFT) chromatography to characterize their retention behavior as a function of pH and composition of the binding buffer. The specific binding and elution behavior demonstrates the possible application of CFT-binding peptides as tags for enhancing the selective recovery of proteins by CFT chromatography. To materialize this strategy, a phage-derived CFT-specific sequence KPRSVSG (Tag1) with/without a consecutive hexalysine sequence, KKKKKKKPRSVSG (Tag2), were fused at the C-terminus of an enhanced green fluorescent protein (eGFP). The resulting gene constructs H-eGFP, H-eGFP-Tag1 and H-eGFP-Tag2 were expressed in *Escherichia coli* strain BL-21, and the clarified cell lysate was applied to the CFT column equilibrated with binding buffer (20-50. mM sodium phosphate, pH 6-8.4). Sodium phosphate (500. mM) or 1. M NaCl in the respective binding buffer was used to elute the fused proteins, and the chromatographic fractions were analyzed by gel electrophoresis. Both the yield and purity were over 90%, demonstrating the potential application of the present strategy. © 2014 The Authors.

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Author keywords

Affinity chromatography; Ceramic fluorapatite; Peptide affinity tags; Peptide synthesis; Production and purification of recombinant proteins; Retention behavior

Indexed keywords

Engineering controlled terms:	Affinity chromatography; Electrophoresis; <i>Escherichia coli</i> ; Peptides; Phosphates; Purification; Recombinant proteins; Sodium compounds
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Engineering uncontrolled terms	Affinity tags; Chromatographic matrix; Enhanced green fluorescent protein; Fluorapatites; Gel electrophoresis; Peptide synthesis; Retention behavior; Selective recovery
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