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Metal ion affinity purification of proteins by genetically incorporating metal-chelating amino acids

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ABSTRACT

Affinity tags are efficient tools for protein purification. They allow simple one-step purification of proteins to high purity. However, in some cases the tags cause structural and functional changes in a protein, and need to be removed. Therefore, affinity tags that are readily introduced into proteins with minimal perturbation and have specific affinity for purification are desired. Herein, two metal-chelating amino acids derived from 2,2′-bipyridine and 8-hydroxyquinoline were genetically incorporated into glutathione *S*-transferase (GST) and the mutant proteins were purified by using the metal ion affinity of the unnatural amino acids. The purification of the GST mutants containing 2-amino-3-(8-hydroxyquinolin-3-yl)propanoic acid (HQA) showed that the proteins could be efficiently enriched in Ni–NTA by the metal ion affinity of the unnatural amino acid and purified to excellent purity. This method should be very useful for general protein affinity purification, especially for proteins whose structure or function is affected by affinity tags fused to N- or C-terminals.

1. Introduction

The development of recombinant DNA technology allows large-scale protein expression in host cells, resulting in demand for efficient methods to enrich proteins of interest from crude cell extract. Many methods of protein purification have been developed, including traditional protein precipitation, purification by affinity tags, and chromatographic purification such as ion-exchange chromatography and size exclusion chromatography.[1] The most popular of these methods is protein purification by affinity tags in which a protein is fused with a short peptide, a protein domain, or a whole protein, and purified by specific interaction of the fused tag with an immobilized ligand. Common tags used for the affinity purification include polyhistidines, Strep-tag II (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys), glutathione *S*-transferase (GST), and maltose binding protein (MBP).[2] An important advantage of affinity purification is that it allows simple one-step purification of proteins to high purity. In some cases, however, the fused peptide or protein needs to be removed because it causes a change in protein conformation, a decrease in enzyme activity, or undesired structural flexibility; the removal of affinity tags typically requires a protease and an additional step for separating the target protein from the cleaved tag.[3]

Recently, a genetic method using an amber nonsense codon and corresponding tRNA/aminoacyl-tRNA synthetase (aaRS) pair to incorporate unnatural amino acids into proteins has been developed.[4] With this method, metal-chelating amino acids (Fig. 1) were incorporated into proteins, and a metal-binding site was generated where the unnatural amino acid was incorporated.[5] The genetic incorporation of the metal-chelating amino acids was used to convert the *Escherichia coli* catabolite activator protein (CAP) into a sequence-specific DNA-cleaving protein and to determine X-ray crystal structure of a protein by SAD phasing.[5b,6] In the latter example, the X-ray crystal structure showed that the hydroxyquinoline side chain of HQA could form a stable Zn^{2+}-complex without any other metal-bound side chain around the metal ion.[5b] Here, we incorporate the metal-chelating amino acids, BPA and HQA, into

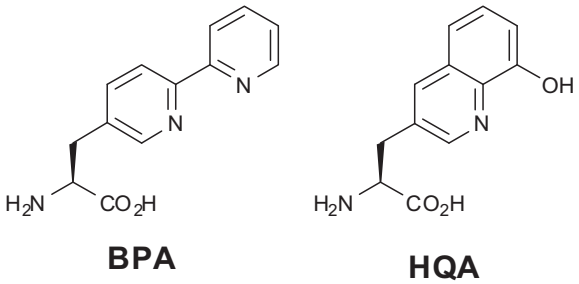

Fig. 1. Structures of metal-chelating amino acids.

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a protein and demonstrate that the mutant protein can be purified by utilizing a commercially available affinity resin.

Among the purification methods using affinity tags, the most common is metal ion affinity purification by the polyhistidine tag (His-tag), in which metal ions, typically Ni^{2+} or Co^{2+}, are immobilized by surface-attached chelators such as nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA) and His-tagged proteins are enriched by interaction with the immobilized metal ions. Although the interaction between the His-tag and the metal ion is not so specific as those of other affinity systems such as FLAG-tag, Strep-tag, GST or MBP, the method has many advantages including stability of immobilized metal ions, high capacity of protein loading, and low costs. Although these advantages have made the His-tag affinity purification method the most popular among the affinity tag purification methods, it has limitations. Due to the short length of the His-tag, typically six histidines (His_6), steric hindrance by the surrounding protein folds can hamper the interaction with immobilized metal ions; extended His-tags (ten histidines long) or two His-tags at both termini of the protein are often used to improve the interaction.[7] However, because even His_6-tags sometimes cause structural or functional perturbation and need to be removed, multiple or extended tags are more likely to result in undesired changes in protein structure and function.[3a,8] Therefore, affinity tags that are readily introduced into proteins with minimal perturbation and have specific affinity for purification are desired.

To this end, we anticipated that the genetic incorporation of metal-chelating amino acids would allow proteins to be purified by metal ion affinity purification (Fig. 2). A recent study concerning the binding of polyhistindines to Ni–NTA showed that the dissociation constant of the hexahistidine peptide to the Ni^{2+} complex was 14 nM.[9] The dissociation constants of the metal ion chelating groups in BPA and HQA for free Ni^{2+} are 91 nM for 2,2′-bipyridine and 0.54 nM for 8-hydroxyquinoline.[10] Based on these data, the binding affinities of the unnatural amino acids to Ni^{2+} were expected to be good enough to enrich a protein of interest on the metal ion site, even though their binding affinities in proteins are dependent upon the steric environment around the unnatural amino acids.

2. Results and discussion

To demonstrate that proteins containing the metal-chelating amino acids can be purified by metal ion affinity purification, BPA and HQA were introduced into glutathione *S*-transferase (GST), which is widely used to generate a fusion protein for protein purification and detection. Based on the X-ray crystal structure of GST, F45 and K112 were chosen to be replaced by the unnatural amino acids because the residues are solvent-exposed and expected to minimize steric repulsion when the unnatural amino acids bind to a metal ion.[11] To incorporate the amino acids, an amber codon was substituted for F45 and K112 by site-directed mutagenesis. The amino acids were prepared according to previously reported methods starting from commercially available compounds, 2-bromo-5-methylpyridine for BPA and *o*-anisidine for HQA.[5b,6] The

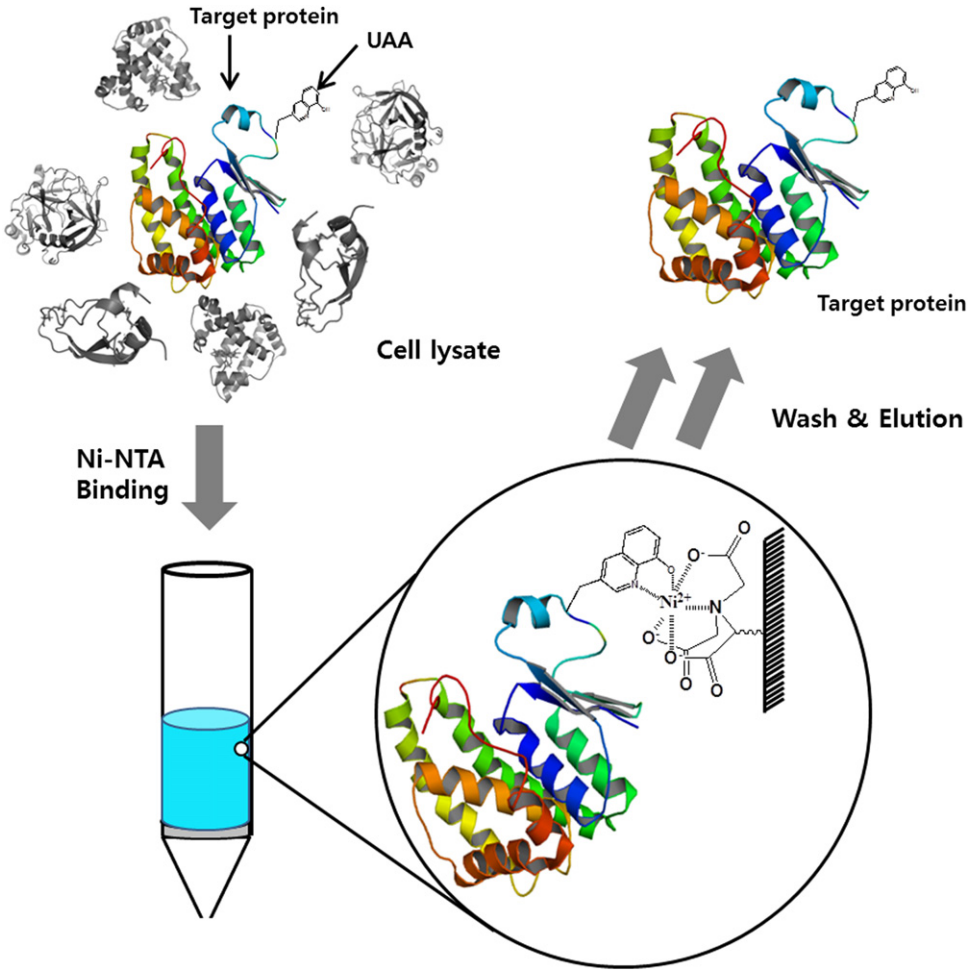

Fig. 2. Protein purification by genetically incorporating a metal-chelating amino acid.