

Peptide Tags for a Dual Affinity Fusion System

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Fusion protein systems can facilitate the purification of proteins produced by recombinant DNA technology (1-3). In these systems, a tag is fused to a target protein. The affinity of the tag for a specific ligand is exploited for the purification of the fusion protein. Here, we demonstrate that during affinity purification many truncated polypeptides carrying the affinity tag are copurified with the full-length fusion protein. We also show that use of a dual affinity fusion system allows isolation of homogeneous fusion protein. Finally, we describe short peptides that can serve as effective tags in a dual affinity fusion system.

We recently developed a new fusion protein system in which the S-peptide of ribonuclease A (RNase A) was employed as an affinity tag (4). S-peptide (residues 1-20) and S-protein (residues 21-124) are the enzymatically inactive products of the limited digestion of RNase A by subtilisin. S-peptide binds S-protein with high affinity to form RNase S, which has full enzymatic activity. This specific interaction allowed the facile purification and detection of a fusion protein in which a modified S-peptide (D14N S15) was attached at the N-terminus of β -galactosidase. One-step affinity chromatography under nondenaturing conditions yielded fusion protein that was >95% pure. Nevertheless, an extremely sensitive gel assay—zymogram electrophoresis—revealed that many truncated polypeptides carrying the D14N S15 tag were copurified with the full-length fusion protein. This result was not unexpected, as the heterologous production of proteins in *Escherichia coli* often generates such truncated proteins, which are likely to be the products of proteolytic degradation.

In a dual affinity fusion system, a different tag is attached to each end of the target protein. Affinity purification using each of these tags eliminates contamination from truncated polypeptides that contain only one tag. This approach has been described with protein tags (5-7). Proteins are undesirable tags, however, because their large size is likely to perturb properties of a target protein. Here, we describe the use of peptides as tags for a dual affinity fusion system.

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Materials and methods. For peptide tags, we chose S-peptide (D14N S15; 15 residues) (4) and polyhistidine (His₆; 6 residues) (8). As a target protein we chose β -galactosidase. The DNA fragment encoding D14N S15- β -galactosidase in plasmid pSG919 (4) was amplified by the PCR and inserted into plasmid pET29b+ (Novagen; Madison, WI) to yield expression vector pSGT12. In this vector, the phage T7 RNA polymerase promoter controls the production of a fusion protein in which β -galactosidase is sandwiched between D14N S15 and His₆ (Fig. 1). Protein production, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), zymogram electrophoresis, and β -galactosidase assays were performed as described (4,9).

Results. The D14N S15- β -galactosidase-His₆ fusion protein was purified from *E. coli* extract by column chromatography on S-protein-Sepharose resin or Ni²⁺ chelation resin or both (Table 1). Each of these procedures yielded >95% pure protein as judged by SDS-PAGE (Fig. 2, top). The chromatography products were also analyzed for the presence of a D14N S15 tag by zymogram electrophoresis (Fig. 2, bottom). Both the unpurified sample (Fig. 2, bottom, lane 3) and the sample purified by S-protein affinity chromatography (Fig. 2, bottom, lane 4) contained several truncated polypeptides along with full-length fusion protein. A similar ensemble of polypeptides was likely copurified by Ni²⁺ chelation chromatography. Unfortunately, polypeptides cannot be detected by zymogram electrophoresis if they have lost their D14N S15 tag. Thus, only the full-length fusion protein was observed by zymogram electrophoresis after Ni²⁺ chelation chromatography (Fig. 2, bottom, lane 5). No significant amount of truncated polypeptides was observed by zymogram electrophoresis after purification by S-protein affinity chromatography followed by Ni²⁺ chelation chromatography (Fig. 2, bottom, lane 6).

Discussion. We have demonstrated that chromatography based on the affinity of only one tag for a ligand may not yield homogeneous (that is, >99% pure) protein. Rather, the use of two tags, each at a different terminus of the target protein, may be necessary to purify a fusion protein to homogeneity. The two tags used here, D14N S15 and His₆, add only 21 residues to the



FIG. 1. Fusion protein with a peptide tag at each terminus, and the dual affinities used in its purification. In this work, the target protein was β -galactosidase.

TABLE 1

Purification of D14N S15- β -Galactosidase-His₆ Protein from 50-ml Culture of *E. coli* Strain BL21(DE3)/pSGT12

Purification step	Total catalytic activity (10 ⁶ units) ^a	Total protein (mg)	Specific catalytic activity (10 ⁶ units/mg) ^a	Purification factor	Yield (%)
Crude extract	2.1	37	0.56	1.0	100
S-protein affinity	1.5	6.8	2.2	3.9	71
Ni ²⁺ chelation	1.1	5.5	2.0	3.6	52
S-protein affinity + Ni ²⁺ chelation	1.1	5.0	2.2	3.9	52

^a Units refer to β -galactosidase units (9).

target protein and allow >99% pure protein to be obtained with >50% yield under nondenaturing conditions. The D14N S15 tag can also be used to detect the

fusion protein in a sensitive gel assay. The use of dual affinity fusion systems such as that described here is likely to be of particular use to researchers (such as protein crystallographers) who demand that their proteins be >99% pure.

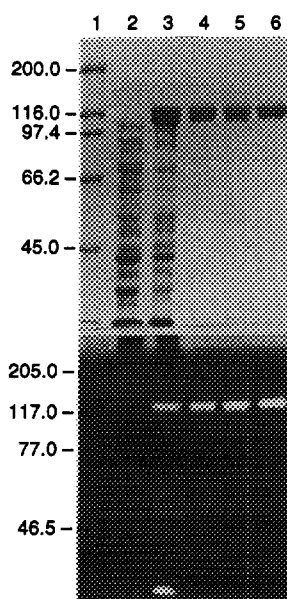


FIG. 2. Analysis of the purity of D14N S15- β -galactosidase-His₆. Proteins from *E. coli* BL21(DE3)/pSGT12 were analyzed by staining with Coomassie brilliant blue after SDS-PAGE (top) or by zymogram electrophoresis (bottom). Lane 1, mol wt markers (kDa); lane 2, total protein from uninduced cells; lane 3, soluble protein from IPTG-induced cells; lane 4, protein purified by S-protein affinity chromatography; lane 5, protein purified by Ni²⁺ chelation chromatography; lane 6, protein purified by both S-protein affinity chromatography and Ni²⁺ chelation chromatography.

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