Labeling Proteins with Small Molecules by Site-Specific Posttranslational Modification

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Site-specific labeling of proteins with small synthetic molecules has been an important approach for the elucidation of protein function, mechanism, and interaction networks. For example, incorporation of site-specific fluorescent probes into proteins allows the detection of protein conformational dynamics and the real-time tracking of protein expression, association, and translocation in the living cell. Incorporation of biotin and other small-molecule affinity labels into proteins provides a high-throughput method for protein microarray fabrication and proteomics studies. Recently, intein-based methods were used to attach a wide range of small molecules including fluorophores, carbohydrates, oligonucleotides, affinity tags, and metal chelators to the C-termini of the target proteins that were expressed as intein fusions. The intein domain was subsequently replaced by a small-molecule cysteine conjugate upon elution from a chitin column. Similarly, human O'-alkylguanine-DNA alkyltransferase (hGAT) has been used for site-specific protein labeling by irreversibly transferring the alkyl group of O'-benzylguanine derivatives to one of its cysteine residues. Although these methods have been shown to be capable of labeling proteins with small molecules, the main drawbacks are that the sizes of intein (454 amino acids) and hGAT (207 amino acids) that need to be fused to the target proteins are too large for many applications. Also, the intein-mediated chemical ligation is a relatively slow process requiring overnight incubation and millimolar concentrations of cysteine derivatives. On the basis of these observations, we believe that a general and efficient method is still needed for site-specific protein labeling by a variety of small molecules. Such a method would use a peptide tag to direct the specific labeling of a target protein in a complex mixture of cellular proteins. The peptide tag should be as small as possible in size and portable to different proteins in order to be generally useful. Here we report such a method in which target proteins are expressed as fusions to a peptide carrier protein (PCP) excised from a nonribosomal peptide synthetase (NRPS). The Sfp phosphopantetheinyl transferase was used to label PCP site-specifically with small molecule—phosphopantetheinyl (Ppant) conjugate (Figure 1). The PCP domain can be as small as 80 amino acids in length, and the labeling reactions were complete within 30 min in the cell lysate with only micromolar concentrations of cysteine-rich domains from NRPS modules EntB (PCP EntB, 98 amino acids) and GrsA (PCP6GrA, 80 amino acids) fused to the N-termini of the target proteins: enhanced green fluorescent protein (EGFP), glutathione-S-transferase (GST) and maltose binding protein (MBP).

For another set of protein fusions, a 15-amino acid linker with the sequence (Ser-Gly-Gly-Gly-Gly)3 was introduced between the PCP and the target proteins: enhanced green fluorescent protein (EGFP), glutathione-S-transferase (GST) and maltose binding protein (MBP). To test this idea, fusion proteins were constructed with the PCP domains from NRPS modules EntB (PCP6EntB, 98 amino acids) and GrsA (PCP6GrA, 80 amino acids) fused to the N-termini of the target proteins: enhanced green fluorescent protein (EGFP), glutathione-S-transferase (GST) and maltose binding protein (MBP). For another set of protein fusions, a 15-amino acid linker with the sequence (Ser-Gly-Gly-Gly-Gly), was introduced between the PCP domain and the target protein and compared to the constructs with no linker inserted. All the fusion proteins were expressed with high yield (20–30 mg/L). The purified fusion proteins were labeled with Ppant biotin in the presence of Sfp and I7e and the site-directed labeling of fusion proteins with biotin was confirmed by enzyme-linked immune assays (ELISA) and Western blotting using streptavidin—horseradish peroxidase conjugate as the probe. To test if the protein labeling can proceed in a high-throughput fashion, the fusion proteins were also expressed in 1 mL cultures in the 96-well deep well plates. After overnight induction with IPTG, deoxycholic acid (DCA) and DNase I were added to initiate cell lysis. Ten microliters of cell lysate was transferred from each well

Figure 1. Sfp-catalyzed biotin labeling of PCP fusion proteins and direct spotting of the labeled protein on avidin glass slides.
Western blotting showed that only the target proteins in the cell cultures in deep well plates, and after in-well cell lysis by DCA, the PCP tag does not interfere with the function of the target protein, and Sfp-catalyzed PCP posttranslational modification and the compatibility of Sfp with various small-molecule probes presented as CoA derivatives denote the generality of the PCP tag for protein labeling. Furthermore, the use of PCP tag for biotin labeling has been shown to be amenable to high-throughput protein microarray preparation and enzymatic screening.

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Supporting Information Available: Results of fusion protein construction, Western blotting, enzymatic assays, and the experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References


9. See Figures S1, S2, and S3 in the Supporting Information.


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