(SD2005-272) In Vivo and In Vitro Methods for Site-specific Protein Labeling: Leveraging the CoA Biosynthetic and Protein Modification Pathways

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BACKGROUND

Selective chemical control of biochemical processes within a living cell enables the study and modification of natural biological systems in ways that may not be obtained through *in vitro* experiments. Accordingly, access to promiscuous metabolic pathways has provided a unique chemical entry into small molecule engineering *in vivo.* A method for covalent reporter labeling of carrier proteins using permissive phosphopantetheinyltransferase (PPTase) enzymes and reporter-labeled coenzyme A (CoA) has been commonly used but has been limited to *in vitro* and cell-surface protein labeling, as CoA derivatives have not been shown to penetrate the cell.

TECHNOLOGY DESCRIPTION

Researchers at UC San Diego have patented cell-penetrating analogues of metabolic precursors that can be used ex vivo to site-specifically label proteins. The key feature of this approach is the availability of a viable uptake mechanism, specifically this technology overcomes the obstacle of cell permeability by using labeled metabolic precursors that can be delivered directly into cell culture, which allows for cellular uptake and metabolic conversion into active, labeled CoA derivatives. For example, by reacting pantetheine (or a derivative thereof) with a reporter to form labeled pantetheine. Researchers at UC San Diego have patented phosphorylating the labeled pantetheine to form phosphopantetheine, adenylating the labeled phosphopantetheine to form a labeled dephosphoCoenzyme A, and phosphorylating the 3'-hydrozyl of the labeled dephosphoCoenzyme A to create a labeled coenzyme A analog.

APPLICATIONS

This method may be applicable to natural product pathway manipulation as well as applications in conventional molecular and cellular biology. Furthermore, this method is useful for characterizing biochemical pathways as well as dissecting protein expression, activity, or function in a cell. The significant tolerance to structural modification manifested by the enzymes in this pathway should allow delivery of a wide variety of chemical moieties to ex vivo processes. The reversibility of this labeling approach was demonstrated by the inventors in a subsequent study that used PPTase and acyl carrier proteins (ACP-fusion proteins) for visualization and functionalization studies (Kosa et al 2012, https://www.ncbi.nlm.nih.gov/pubmed/22983458).

ADVANTAGES

- Provides an *in vivo* route to label proteins within the cell using reporter-labeled pantetheine as a cell-permeable precursor to gain access to CoA biosynthetic and proteinmodification pathways. applicable to natural product pathway elucidation, fusion protein localization, and decoding of complex expression patterns.

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OTHER INFORMATION

KEYWORDS

Carrier Proteins, Coenzyme A, Escherichia coli, Fluorescent Dyes, Pantetheine, in vivo and in vitro labeling, cell labeling, protein expression

CATEGORIZED AS

- **Research Tools**
  - Other
  - Screening Assays

RELATED CASES

2005-272-0

▶ The advantage of using a fluorescently tagged pantetheine analog is that it is cell permeable, whereas pre-labeled coenzyme A analogs remain outside the cell. This allows the selective *in vivo* labeling of targeted proteins and fusion constructs.

▶ Can be used to probe natural product producing organisms to identify and manipulate the proteins from biosynthetic systems. Additionally, this technology could be incorporated into a fusion-protein system for *in vivo* reporter labeling of proteins.

STATE OF DEVELOPMENT

In vivo labeling and cellular update studies have been demonstrated in E. coli.

INTELLECTUAL PROPERTY INFO

This technology is available for commercial development and protected by US 7,72,7738 https://www.google.com/patents/US7727738 and US 8,119,364 https://www.google.com/patents/US8119364

RELATED MATERIALS

▶ Kosa NM, Haushalter RW, Smith AR, Burkart MD. Reversible labeling of native and fusion-protein motifs. Nat Methods. 2012 Oct;9(10):981-4 - 10/01/2012

▶ Clarke KM, Mercer AC, La Clair JJ, Burkart MD. In vivo reporter labeling of proteins via metabolic delivery of coenzyme A analogues. J Am Chem Soc. 2005 Aug 17;127(32):11234-5. - 08/17/2005

▶ Mercer AC, La Clair JJ, Burkart MD. Fluorescent multiplex analysis of carrier protein post-translational modification. Chembiochem. 2005 Aug;6(8):1335-7 - 08/06/2005

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	8,119,364	02/21/2012	2005-272
United States Of America	Issued Patent	7,727,738	06/01/2010	2005-272

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