Reagent to Label Proteins via Lysine Isopeptide Bonds

Tech ID: 29694 / UC Case 2018-463-0

INVENTORS
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OTHER INFORMATION
KEYWORDS
bioconjugation; isopeptide bonds; Corynebacterium diphtheriae; pilus polymerization; in vitro protein ligation; in vitro peptide ligation

CATEGORIZED AS
▶ Materials & Chemicals
▶ Biological
▶ Medical
▶ Research Tools
▶ Research Tools
▶ Antibodies
▶ Other
▶ Reagents

RELATED CASES
2018-463-0
SUMMARY
Researchers in the UCLA Department of Chemistry and Biochemistry and the University of Texas-Medical Center, Houston Department of Microbiology and Molecular Genetics have modified the *Corynebacterium diphtheriae* (*C. diphtheriae*) sortase enzyme so that it can be used as a bioconjugation reagent in vitro.

BACKGROUND
Sortase enzymes have been used to catalyze various processes in vivo and in vitro, including antibody-drug conjugate construction, protein engineering, and biosensing. In vivo, sortase enzymes catalyze pilus polymerization in many strains of Gram-positive bacteria. Pili are protein polymers expressed on the cell envelope of bacteria and are critical for bacterial virulence. In vitro, bacterial sortase enzymes can be employed to ligate not only their natural protein substrates but also many other peptides and proteins.

The *Staphylococcus aureus* (*S. aureus*) sortase enzyme is a commonly used bioconjugation reagent in vitro, but it preferentially attaches molecules to proteins via a peptide bond as opposed to an isopeptide bond. Isopeptide bonds present numerous advantages over peptide bonds, allowing for more protein sites to be linked and creating more stable linkages with increased resistance to proteolysis. Although the *Corynebacterium diphtheriae* (*C. diphtheriae*) sortase enzyme catalyzes pilus polymerization via the formation of an isopeptide bond in vivo, the wild-type *C. diphtheriae* enzyme is not active in vitro.

INNOVATION
Researchers in the UCLA Department of Chemistry and Biochemistry and the University of Texas-Medical Center, Houston Department of Microbiology and Molecular Genetics have modified the *C. diphtheriae* sortase enzyme so that it can be used as a bioconjugation reagent. Unlike the wild-type *C. diphtheriae* enzyme, the modified enzyme can ligate proteins and peptides in vitro. The modified enzyme enables peptide and protein linkage in high yield via the formation of lysine isopeptide bonds, which are less susceptible to proteolysis and therefore more stable than their peptide bond counterparts. Furthermore, the modified *C. diphtheriae* enzyme can be used in concert with the *S. aureus* sortase enzyme to modify multiple sites on a protein.

APPLICATIONS
- Antibody development
- Antibody-drug conjugates
- Bioconjugation and protein engineering
- Biosensing and biocatalysis
- Selective domain labeling for biophysical studies
- Cell-specific labeling
- Construction of immune-PET (positron emission tomography) reagents for non-invasive imaging
- Lipid modification of proteins
- Targeted therapeutic delivery
- Immobilization of proteins to biacore sensor chips

ADVANTAGES
- Enables peptide and protein linkage via side chain lysine isopeptide bonds in high yield
- Less susceptible to proteolysis than peptide bonds

PATENT STATUS

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ADDITIONAL TECHNOLOGIES BY THESE INVENTORS
- New Method to Increase the Rate of Protein Ligation Catalyzed by the S. Aureus Sortase A Enzyme