product indicates the concurrent presence of the first target and the second target in the biological sample.

Sortase-mediated ligations for the site-specific modification of proteins is reported by Schmohl, L. and Schwarzer, D. (Curr. Opin. Chem. Biol. 22 (2014) 122-128). Race, P.R., et al. (J. Biol. Chem. 284 (2009) 6924-6933) reported the crystal structure of streptococcus pyogenes sortase and implications for sortase mechanism.

Ling and co-workers showed the introduction of a thioester comprising group via a sortase (Ling, J. J. J., et al., J. Am. Chem. Soc. 134 (2012) 10749-10752).

Summary

It has been found that sortase A accepts as nucleophile a polypeptide comprising at its N-terminus a cysteine amino acid residue. The resulting enzymatic conjugation product that is released from the enzyme is a thioester, which can undergo a rearrangement thereafter. It has further been found that site specific, in situ generated C-terminal thioester between the sortase motif and the Sortase itself can be used for native chemical ligation (NCL).

As reported herein is a method for the enzymatic formation/production of a thioester (formation of a new thioester bond between the alpha carbonic acid group of an amino acid and the thiol group of a cysteine) comprising the following step

- incubating

  i) a first polypeptide comprising (optionally within the 100 C-terminal amino acid residues) the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue),

  ii) a second polypeptide that has at its N-terminus a cysteine amino acid residue or is a cysteinyl compound (i.e. a compound that comprises a cysteine amino acid residue with free alpha amino group, e.g. as NH_2 or NH_3^+, and a carboxy group at position 1, which is part of a peptide bond), and

  iii) a third polypeptide that is a sortase A or a catalytically active fragment thereof (i.e. that has sortase A activity),

and thereby producing a thioester.

One aspect as reported herein is a method for the enzymatic formation of a thioester bond comprising the following step

- incubating

i) a first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue),

ii) a second polypeptide that has at its N-terminus a cysteine amino acid residue or a compound that comprises a cysteine amino acid residue with free alpha amino group and a carboxy group at position 1 that is part of a peptide bond, and

iii) a third polypeptide that is a sortase A or a catalytically active fragment thereof,

and thereby forming a thioester bond.

In one embodiment the third polypeptide is derived from Staphylococcus aureus sortase A or Listeria monocytogenes Sortase A.

In one embodiment the method is for the enzymatic conjugation of two polypeptides.

In one embodiment the second polypeptide has at its N-terminus a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues. In one preferred embodiment the second polypeptide has the amino acid sequence CGG, CGGG (SEQ ID NO: 02), CAA or CAAA (SEQ ID NO: 03) at its N-terminus.

In one embodiment the incubating is further in the presence of a thiol additive. In one embodiment the thiol additive is selected from the group consisting of thiophenol, 4-mercaptophenylacetic acid (MPAA), 2-mercaptoethanesulfonate (MESNA) and combinations thereof. In one preferred embodiment the incubating is in the presence of 2-mercaptoethanesulfonate.

In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue). In one embodiment the first polypeptide comprises at its C-terminus the amino acid sequence LPETG (SEQ ID NO: 04).

In one embodiment the first polypeptide and the second polypeptide are independently of each other selected from an antibody variable domain, an antibody heavy chain Fab-fragment, an antibody Fc-region, a tag, and a peptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be any amino acid residue), a linker and a non-sortase motif moiety.

In one embodiment the third polypeptide has the amino acid sequence of SEQ ID NO: 05 or SEQ ID NO: 06.

Detailed Description of the Invention

The current invention is based at least in part on the finding that a sortase A accepts as nucleophile a polypeptide that has a cysteine residue at its N-terminus.