Li, Y.M., et al. report irreversible site-specific hydrazinolysis of proteins by use of Sortase (Angew. Chem. Int. Ed. Engl. 53 (2014) 2198-2202).

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

Bellucci, J.J., et al. report the use of lysine as nucleophile (Angew. Chem. Int. Ed. Engl. 53 (2014) 1 - 6).

In Uniprot accession number A0A0E1R5I2_LISMN a putative cysteine protease is reported. A sortase A from Listeria monocytogenes comprising 119 residues is reported in Uniprot accession number A9LY59_LISMN.

In WO 2014/183066 a method of conjugating an agent to an animal cell, the method comprising contacting an animal cell with a sortase substrate that comprises a sortase recognition sequence and an agent in the presence of a sortase under conditions suitable for the sortase to conjugate the sortase substrate to an endogenous, non-engineered polypeptide expressed by the animal cell is reported.

A method for producing an antibody Fc-region conjugate, which comprises as first component a recombinant antibody Fc-region and as second component at least one recombinant binding entity that specifically binds to a target, using a sortase A for enzymatic conjugation of the antibody Fc-region to the at least one binding entity is reported in WO 2014/001325.

Site-specific C-terminal internal loop labeling of proteins using sortase-mediated reactions has been reported by Guimaraes, C.P., et. al (Nat. Protoc. 8 (2013) 1787-1799). Nguyen, H.D. and Schumann, W. reported the establishment of an experimental system allowing immobilization of proteins on the surface of Bacillus subtilis cells (J. Biotechnol. 122 (2006) 473–482).

Summary

Herein is reported a novel soluble Sortase A derived from a hitherto unknown Listeria monocytogenes Sortase A with a high activity against the sortase-motif (sortase recognition sequence) LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue).

It has been found that a specifically N-terminally truncated soluble variant of a novel Listeria monocytogenes Sortase A has increased enzymatic activity

compared to other N- or C-terminally truncated variants of the same novel Listeria monocytogenes Sortase A.

As reported herein is a polypeptide comprising the amino acid sequence of SEQ ID NO: 38. In this polypeptide only the amino acid sequence of SEQ ID NO: 38 is/has been obtained from Listeria monocytogenes. In one embodiment the amino acid sequence of SEQ ID NO: 38 is the sole Listeria monocytogenes derived amino acid sequence in the polypeptide.

One aspect as reported herein is a polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domain that has the amino acid sequence of SEQ ID NO: 38 and any of the further domains are polypeptides from different organisms or synthetic/artificial, wherein the domains are conjugated to each other either directly or via a peptidic linker.

In one embodiment the polypeptide comprises additionally a tag at its N- or C-terminus either conjugated directly or via an intervening linker.

In one embodiment the polypeptide is consisting of the amino acid sequence of SEQ ID NO: 38 and the C-terminal tag of SEQ ID NO: 32. In one embodiment the polypeptide is consisting of the amino acid sequence of SEQ ID NO: 38.

In one embodiment the polypeptide is a sortase A.

As reported herein is a (fusion) polypeptide comprising one domain that has the amino acid sequence of SEQ ID NO: 38 and one or more further domains, wherein the domains are conjugated to each other either directly or via a peptidic linker. In one embodiment the domain that has the amino acid sequence of SEQ ID NO: 38 and (each/any of) the further domains are (obtained from) polypeptides from different organisms and/or are synthetic/artificial polypeptides. In one embodiment the domain that has the amino acid sequence of SEQ ID NO: 38 and the one or more further domains are from different organisms/do not exist in this combination in nature.

Another aspect as reported herein is a polypeptide consisting of SEQ ID NO: 38.

As reported herein is the use of a polypeptide as reported herein for the conjugation of a sortase-motif comprising first polypeptide with a second polypeptide comprising i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues.

Another aspect as reported herein is use of a polypeptide as reported herein for the conjugation of a sortase-motif comprising polypeptide with a polypeptide comprising an oligoglycine or oligoalanine or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus.

As reported herein is a method for the (enzymatic) production of a (fusion-)polypeptide 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

  ii) a second polypeptide that comprises i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues, and

  iii) a polypeptide as reported herein,

and thereby producing a (fusion-)polypeptide.

Another aspect as reported herein is a method for the enzymatic production of a polypeptide 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) or LPXTA (SEQ ID NO: 41, wherein X can be any amino acid residue),

ii) a second polypeptide that comprises i) a glycinyl, an alaninyl, or a cysteinyl compound at its N-terminus, or ii) an oligoglycine, or oligoalanine, or a cysteine amino acid residue followed by one to three glycine or alanine amino acid residues at its N-terminus, or iii) a lysine amino acid residue within its 5 N-terminal amino acid residues, and

iii) a polypeptide as defined herein,

and thereby producing a polypeptide.

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

In one embodiment the second polypeptide has at its N-terminus the amino acid sequence GGG, AAA, CGG, CAA, KGG or KAA.

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) or LPXTA (SEQ ID NO: 41, 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) or LPETA (SEQ ID NO: 42) or LPKTG (SEQ ID NO: 43) or LPKTA (SEQ ID NO: 44).

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 or polypeptide, a linker and a non-sortase motif moiety, each first polypeptide comprising the amino acid sequence LPXTG (SEQ ID NO: 01, wherein X can be