Claims

1. Isolated polypeptide having protein ligase, preferably cyclase, activity comprising or consisting of
   (i) the amino acid sequence as set forth in SEQ ID NO:1 (VyPAL2);
   (ii) an amino acid sequence that shares at least ~~60, preferably at least 70, more preferably at least 80, most preferably at least 90~~75 % sequence identity with the amino acid sequence set forth in SEQ ID NO:1 over its entire length;
   (iii) an amino acid sequence that shares at least ~~80, preferably at least 90, more preferably at least 95~~95 % sequence homology with the amino acid sequence set forth in SEQ ID NO:1 over its entire length; or
   (iv) a fragment of any one of (i)-(iii) that is at least 150 amino acids in length,
   wherein the amino acid sequence of (ii) and (iii), and the fragment of (iv) comprises:
   (a) the amino acid residue W or Y at the position corresponding to position 195, the amino acid residue I, C, A or V at the position corresponding to position 196, and the amino acid residue T, A or V at the position corresponding to position 197 of SEQ ID NO:1 (LAD1); and
   (b) the amino acid residue A or G at the position corresponding to position 126 and, the amino acid A or P at the position corresponding to position 127 of SEQ ID NO:1 (LAD2), wherein the positions corresponding to position 126 and 127 of SEQ ID NO:1 are not GP.

2. The isolated polypeptide of claim 1, wherein the isolated polypeptide comprises or consists of
   (i) the amino acid sequence set forth in SEQ ID NO:2 (VyPAL2 + N-terminus + Cap remainder) or SEQ ID NO:3 (VyPAL2 proenzyme);
   (ii) an amino acid sequence that shares at least ~~60, preferably at least 70, more preferably at least 80, most preferably at least 90~~75 % sequence identity with the amino acid sequence set forth in SEQ ID NO:2 or 3 over its entire length;
   (iii) an amino acid sequence that shares at least ~~80, preferably at least 90, more preferably at least 95~~95 % sequence homology with the amino acid sequence set forth in SEQ ID NO:2 or 3 over its entire length; or
   (iv) a fragment of any one of (i)-(iii) that is at least 150 amino acids in length,
   wherein the amino acid sequence of (ii) and (iii), and the fragment of (iv) comprises:
   (a) the amino acid residue W or Y at the position corresponding to position 195, the amino acid residue I, C, A or V at the position corresponding to position 196, and the amino acid residue T, A or V at the position corresponding to position 197 of SEQ ID NO:1 (LAD1); and
   (b) the amino acid residue A or G at the position corresponding to position 126 and, the amino acid A or P at the position corresponding to position 127 of SEQ ID NO:1 (LAD2),

wherein the positions corresponding to position 126 and 127 of SEQ ID NO:1 are not GP.

3. The isolated polypeptide of claim 1 or 2, wherein said polypeptide comprises:

(a)

(i) the amino acid residue N at the position corresponding to position 19 of SEQ ID NO:1,

(ii) the amino acid residue H at the position corresponding to position 124 of SEQ ID NO:1,

(iii) the amino acid residue C at the position corresponding to position 166 of SEQ ID NO:1, or combinations thereof;

or

(b)

(i) the amino acid residue A at the position corresponding to position 126 and, ~~optionally,~~ the amino acid ~~A or P, preferably~~ P~~,~~ at the position corresponding to position 127 of SEQ ID NO:1 (LAD2),

~~(ii) the amino acid residue G at the position corresponding to position 126 and the amino acid A at the position corresponding to position 127 of SEQ ID NO:1 (LAD2),~~

~~(iii)~~(ii) the amino acid residue W ~~or Y~~ at the position corresponding to position 195, the amino acid residue I ~~or V~~ at the position corresponding to position 196, and the amino acid residue T~~, A or V~~ at the position corresponding to position 197 of SEQ ID NO:1 (LAD1),

~~(iv)~~(iii) the amino acid residues R at the position corresponding to position 21, H at the position corresponding to position 22, D at the position corresponding to position 123, E at the position corresponding to position 164, S at the position corresponding to position 194, D at the position corresponding to position 215 of SEQ ID NO:1 (S1 pocket),

~~(v)~~(iv) the amino acid residues C at the positions corresponding to positions 199 and 212 of SEQ ID NO:1 (disulfide bridge), or combinations thereof.

4. The isolated polypeptide of any one of claims 1-3, wherein said polypeptide:

(i) is activated by acid treatment at a pH of 5.0 or less, preferably 4.0 or less; and/or

(ii) cyclizes a given peptide with an efficiency of 60 % or more, preferably 80 % or more, preferably at a pH of 5.5 or higher; and/or

(iii) hydrolyzes a given peptide with an efficiency of 20 % or less, preferably 5 % or less, preferably at a pH of 5.5 or higher; and/or

(iv) is glycosylated.

5. A nucleic acid molecule encoding the polypeptide according to any one of claims 1 to 4.

6. A vector comprising the nucleic acid molecule of claim 5.

7. The vector of claim 6, wherein said vector further comprises regulatory elements for controlling expression of said nucleic acid molecule.

8. A host cell comprising the nucleic acid molecule of claim 5, wherein the host cell is preferably an insect cell, more preferably an Sf9 cell.

9. A method for producing a polypeptide of any one of claims 1 to 4, comprising culturing a host cell according to claim 8 under conditions that allow expression of the polypeptide, and isolating said polypeptide from the host cell or culture medium.

10. Use of a polypeptide having ligase or cyclase activity for ligating at least two peptides or cyclizing a peptide, the polypeptide having cyclase activity being the isolated polypeptide of any one of claims 1 to 4.

11. The use of claim 10, wherein the peptide to be cyclized or at least one of the peptides to be ligated comprises~~:~~

~~(a)~~

~~(i)~~ the~~, preferably~~ C-terminal, amino acid sequence $\underline{(X)_oNX^3X^4(X)_r}\underline{(X)_o}\text{~~N/D(X)}_p$, wherein X is any amino acid and o ~~and p are independently from each other integers of~~is at least 2, ~~preferably the amino acid sequence $(X)_oNX^3X^4(X)_r$,~~ wherein X^3 is any amino acid with the exception of P, preferably H, G or S, and X^4 is a hydrophobic or aromatic amino acid, preferably selected from L, I, V, F, C, W, Y and M, and r is 0 or an integer of 1 or more.~~; or~~

~~(ii) the C-terminal amino acid sequence $(X)_oN*/D*$, wherein X is any amino acid, o is an integer of at least 2 and the C-terminal N/D residue is amidated in that the C-terminal carboxy group, preferably the α-carboxy group in case of D, is replaced by an amide group of the formula $-C(O)-N(R')_2$, with R' being any residue;~~

~~and/or~~

~~(b)~~

12. The use of claim 11, wherein the peptide to be cyclized or at least one of the peptides to be ligated comprises the N-terminal amino acid sequence $X^1X^2(X)_q$, wherein X is any amino acid; X^1 is any amino acid with the exception of P; X^2 is any amino acid, but preferably is a hydrophobic amino acid, more preferably V, I or L; and q is 0 or an integer of 1 or more.

~~12.~~13. The use of any one of claims 10~~-12 or 11~~, wherein:

(a) the peptide to be cyclized is the linear precursor form of a cyclic cystine knot polypeptide, a cyclic peptide toxin, a cyclic antimicrobial peptide, a cyclic histatin, or a human or animal cyclic peptide hormone;

and/or

(b) the peptide to be cyclized is 10 or more amino acids in length, or at least one of the peptides to be ligated is 25 or more, preferably 50 or more amino acids in length;

and/or

(c) the peptide to be cyclized comprises or consists of the amino acid set forth in any one of SEQ ID Nos:19 and 21-42, or the amino acid sequence $(X)_nC(X)_nC(X)_nC(X)_nC(X)_nC(X)_nC(X)_nNHV(X)_n$, wherein each n is an integer independently selected from 1 to 6 and X is any amino acid;

and/or

(d) at least one of the peptides to be ligated comprises a detectable marker, preferably a fluorescent marker or biotin.

~~13.~~14. A method for cyclizing a peptide or for ligating at least two peptides, the method comprising incubating said peptide or said at least two peptides with the isolated polypeptide of any one of claims 1 to 4 under conditions that allow cyclization of said peptide or ligation of said peptides.

~~14.~~15. The method of claim ~~13~~14, wherein~~:~~

~~(a)~~ the peptide to be cyclized or at least one peptide to be ligated comprises:

(i) the~~,~~ ~~preferably~~ C-terminal, amino acid sequence $(X)_oNX^3X^4(X)_r$~~$(X)_oN/D(X)_p$~~, wherein X is any amino acid and o ~~and p are independently from each other integers of~~is at least 2, ~~preferably the amino acid sequence $(X)_oNX^3X^4(X)_r$~~, wherein X^3 is any amino acid with the exception of P, preferably H, G or S, and X^4 is a hydrophobic or aromatic amino acid, preferably selected from L, I, V, F, C, W, Y and M, and r is 0 or an integer of 1 or more.~~; or~~

~~(ii) the C-terminal amino acid sequence $(X)_oN^*/D^*$, wherein X is any amino acid, o is an integer of at least 2 and the C-terminal N/D residue is amidated in that the C-terminal carboxy group, preferably the α-carboxy group in case of D, is replaced by an amide group of the formula $-C(O)-N(R')_2$, with R' being any residue;~~

wherein optionally the peptide to be cyclized or the at least one peptide to be ligated is a fusion peptide of a peptide of interest fused N-terminally to the amino acid sequence ~~$N/D(X)_p$, preferably the amino acid sequence~~ $(X)_oNX^3X^4(X)_r$, wherein X^3 is any amino acid with the exception of P, preferably H, G or S, and X^4 is a hydrophobic or aromatic amino acid, preferably selected from L, I, V, F, C, W, Y and M, and r is 0 or an integer of 1 or more.~~;~~

~~and/or~~

16. ~~(b)~~ The method of claim 15, wherein the peptide to be cyclized or at least one peptide to be ligated comprises the N-terminal amino acid sequence $X^1X^2(X)_q$, wherein X is any amino acid; X^1 is any amino acid with the exception of P; X^2 is any amino acid, but preferably is a hydrophobic amino acid, more preferably V, I or L; and q is 0 or an integer of 1 or more.

~~15.~~17. The use of any one of claims 10 to ~~12~~ 13 or method of claim ~~13~~ 14 or ~~14~~15, wherein the polypeptide having ligase or cyclase activity is immobilized on a solid support, optionally the immobilization is by non-covalent or covalent binding to the solid support, wherein optionally,

(i) the polypeptide having ligase or cyclase activity is glycosylated and the immobilization is facilitated by interaction with a carbohydrate-binding moiety, preferably a concanavalin A moiety or variant thereof, covalently linked to the solid support;

(ii) the polypeptide having ligase or cyclase activity is biotinylated and the immobilization is facilitated by interaction with a biotin-binding moiety, preferably a streptavidin, avidin or neutravidin moiety or variant thereof, covalently linked to the solid support; or

(iii) the polypeptide having ligase or cyclase activity is immobilized on the solid support by reaction with an N-hydroxysuccinimide functional group on the surface of the solid support.

~~16.~~18. A solid support material comprising the isolated polypeptide according to any one of claims 1-4 immobilized thereon.

~~17.~~19. The solid support material of claim ~~16~~18, wherein:

(a) the solid support material comprises a polymer resin, preferably in particulate form, for example agarose; and/or

(b) the isolated polypeptide is immobilized on the solid support material by covalent or non-covalent interactions; and/or

(c) the solid support material is a particulate resin material for chromatography columns; and/or

(d)

(i) the polypeptide having ligase or cyclase activity is glycosylated and the immobilization is facilitated by interaction with a carbohydrate-binding moiety, preferably a concanavalin A moiety or variant thereof, covalently linked to the solid support;

(ii) the polypeptide having ligase or cyclase activity is biotinylated and the immobilization is facilitated by interaction with a biotin-binding moiety, preferably a streptavidin, avidin or neutravidin moiety or variant thereof, covalently linked to the solid support; or

(iii) the polypeptide having ligase or cyclase activity is immobilized on the solid support by reaction with an N-hydroxysuccinimide functional group on the surface of the solid support.

~~18.~~20. A method for increasing the protein ligase activity of a polypeptide having asparaginyl endopeptidase (AEP) activity, the method comprising the steps of:

substituting the amino acid residue at the position corresponding to position 126 of SEQ ID NO:1 with either ~~a small hydrophobic residue or a G residue, preferably~~ an A or a G residue, and

substituting the amino acid residue at the position corresponding to position 127 of SEQ ID NO:1 with either an A or P, such that the amino acid residues at the positions corresponding to positions 126 and 127 of SEQ ID NO:1 are either AP, AA or GP, but not GP

wherein the polypeptide having asparaginyl endopeptidase activity comprises an amino acid sequence that shares at least 60, preferably at least 70, more preferably at least 80, most preferably at least 90 % sequence homology or sequence identity with the amino acid sequence set forth in any one of SEQ ID Nos. 10-14 (VyAEP1-4; VcAEP) over its entire length, and comprises the amino acid residue W or Y at the position corresponding to position 195, the amino acid residue I, C, A or V at the position corresponding to position 196, and the amino acid residue T, A or V at the position corresponding to position 197 of SEQ ID NO:1.

~~19.~~21.A method for producing a polypeptide having protein ligase activity, the method comprising:

(i) providing a polypeptide having asparaginyl endopeptidase (AEP) activity, comprising an amino acid sequence that shares at least 60, preferably at least 70, more preferably at least 80, most preferably at least 90 % sequence homology or sequence identity with the amino acid sequence set forth in any one of SEQ ID Nos. 10-14 (VyAEP1-4; VcAEP) over its entire length, and comprises the amino acid residue W or Y at the position corresponding to position 195, the amino acid residue I, C, A or V at the position corresponding to position 196, and the amino acid residue T, A or V at the position corresponding to position 197 of SEQ ID NO:1; and

(ii) introducing one or more amino acid substitutions into the polypeptide having asparaginyl endopeptidase (AEP) activity, wherein said substitutions comprise substituting the amino acid residue at the position corresponding to position 126 of SEQ ID NO:1 with an A or a G residue and ~~optionally~~ substituting the amino acid residue at the position corresponding to position 127 of SEQ ID NO:1 with either a P or an A residue, such that the amino acid sequence in the positions corresponding to positions 126/127 in SEQ ID NO:1 is either GA, AA or AP, ~~preferably GA or AP~~but not GP~~.~~

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~~20. The method of claim 18 or 19, wherein the polypeptide having asparaginyl endopeptidase activity comprises~~

~~(i) an amino acid sequence that shares at least 60, preferably at least 70, more preferably at least 80, most preferably at least 90 % sequence homology or sequence identity with the amino acid sequence set forth in any one of SEQ ID Nos. 10-14 (VyAEP1-4; VcAEP) over its entire length; and/or~~

~~(ii) an amino acid residue at the position corresponding to position 126 of SEQ ID NO:1 that is neither G nor A~~.