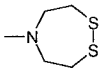
Another subject of the invention is a solid support grafted with a peptide and corresponding to formula (III_1) below:

□-Z-A_1-SEAoff (III_1)

in which A_1 represents a peptide fragment, the C-terminal of which bears a cyclic bis(2-sulphanylethyl)amino group 

called SEAoff, □ represents a solid support and Z represents a linkage group between A_1 and □.

Such a solid support can be used in a polypeptide synthesis kit. Such a polypeptide synthesis kit usually comprises at least one support provided with at least one receiving zone, on or in which at least one grafted solid support (III_1) is placed.

Another subject of the invention is an automated device for peptide synthesis that allows automated implementation of the synthesis method of the invention. Such a device comprises at least one reservoir in which a solid support grafted with a peptide and corresponding to the following formula (III_1) is placed:

□-Z-A_1-SEAoff (III_1)

It also comprises separate reservoirs containing:

- peptides ~~(Ii)~~ (III) $C_i(PG_i)A_{i+1}$SEAoff, which were described above, as well as:
- at least one thiol R-SH, and optionally a reducing agent of the cyclic disulphides
- the coupling activators and
- the washing products.

Such an automated device of this kind also comprises mechanical means for taking and distributing samples of products, as well as data processing means allowing controlled application of these mechanical means.

Another subject of the invention is a solid support grafted with a polypeptide comprising n peptide fragments and at least n-1 amino acids bearing a thiol function and corresponding to formula (IV_n) below:

□-Z-A_1- C_1-A_2-…$C_{i-1}A_i$-…$C_{n-1}A_n$ (IV_n)

Such a solid support can be used for carrying out affinity tests between a polypeptide and another molecule.

For using these grafted solid supports in biological affinity tests, it is generally desirable for the peptide chain A_1- C_1-A_2-…$C_{i-1}A_i$-…$C_{n-1}A_n$ to be folded, so as to have a

It is understood that each of the peptide fragments preferably comprises only amino acid residues selected from the 20 proteinogenic amino acid residues. However, according to a particular embodiment, the peptide fragments A_i in an internal position of the sequence can also comprise one or more non-proteinogenic amino acid residues, and one or more of the peptide fragments A_2 ... A_i ... A_{n-1} can bear one or more modified amino acids, the modification being carried out before the method is implemented. As a non limitative example, the modification of the amino acids can in particular be selected from radicals selected from residues of carboxylic acids (biotin, acetyl group, aminooxyacetic residue, a fluorophore such as tetramethylrhodamine, a chelating agent of metals such as 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), a lipid such as palmitic acid, a polymer, for example alpha-methoxy-omega-carboxy poly(ethylene glycol) or others. It can also be selected from post-translational modifications of proteinogenic amino acids known to a person skilled in the art (methylation, phosphorylation, acetylation, glycosylation, sulphation, hydroxylation, carboxymethylation, bearing suitable protective groups for incorporation in the solid phase, etc.), or optionally a medicinal product (the protein then serving as vector).

The presence of modified amino acids in particular allows the polypeptide to be functionalized with a view to detecting interactions of the polypeptide with other molecules (fluorescence markers etc.), but other types of functionalization can be provided.

In a simple way, a modification can be introduced by using a non-proteinogenic amino acid, in particular a derivative of proteinogenic amino acid, to introduce the modification during the solid-phase synthesis of the fragment in question. By way of example, it is possible to use an Fmoc-L-Lys(biotin)-OH, i.e. a lysine bearing a biotin on its side chain, in order to carry out the synthesis of a fragment. This fragment is then used in the assembly identically to that which uses fragments of proteinogenic amino acids.

The amino acid residues of the polypeptides A_1,...A_i, A_n can optionally be protected temporarily or permanently by protective groups of the functions carried by the side chains, the nature of which varies depending on the reactive functions of the side chains, which are well known to a person skilled in the art.

According to an embodiment of the invention, a peptide fragment A_i comprises between 2 and 600 amino acid residues, preferably between 5 and 100 amino acid residues, more especially preferably between 8 and 50 amino acid residues.

The polypeptide of formula (I~~I~~) can be obtained for example by a usual method of peptide synthesis, in particular a method of solid-phase synthesis. It can also be obtained by means of a previous native ligation.

The preparation of bis(2-sulphanylethyl)amino peptide: Y-A_1-SEAoff (step a1) of the method) can be carried out by the method described by Melnyk, O. et al., Bis(2-

sulphanylethyl)amino native peptide ligation., Org. Lett., 12(22), 5238-41(2010), as well as in FR-2952058. According to the embodiment of this method, the C-terminal amino acid is coupled to a support of polymer resin P grafted with SEA (bis(2-sulphanylethyl)amino) by bringing a support of SEA grafted polymer resin into contact with a halide of amino acid or with an amino acid and an activating agent, preferably selected from PyBOP® (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate), BOP, PyBroP® (bromo-tris-pyrrolidino-phosphonium hexafluorophosphate), or else HATU (2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate methanaminium) and more especially preferably PyBroP and HATU. This embodiment is described in more detail below in the examples of the preparation of the starting peptide fragments. A_1-SEA-P is then functionalized with a group Y and then detached from the polymer P.

The method of the invention comprises the reaction of a functionalized peptide Y-A_1-SEAoff (II_1) with a solid support designated □-Y', □ representing the solid support itself and Y' representing a reactive function capable of reacting with Y to form a group Z according to diagram 1:

□-Y' + Y-A_1-SEAoff ⟶ □-Z-A_1-SEAoff

(II_1) (III_1)

Diagram 1

The solid support is preferably a polymer in the form of insoluble or soluble particles (beads). It is possible for example to use resins based on polystyrene, polyacrylamide, polyethylene glycol, cellulose, polyethylene, polyester, latex, polyamide, polydimethylacrylamide and resins derived therefrom. It is also possible to use a silica gel or glass beads as the solid support.

Advantageously, a solid support is used selected from the synthetic hydrophilic polymers such as the resins PEGA, ChemMatrix ®, SPOCC (superpermeable organic combinatorial chemistry) or natural carbohydrate polymers such as agarose or sepharose.

These resins are grafted with a group Y' comprising a function selected from an azide, an alkyne, ~~preferably a true alkyne or~~ a terminal alkyne, a cyclooctyne, Y is then itself selected from an alkyne, ~~preferably a true alkyne or~~ a terminal alkyne, and an azide.

According to a variant, these resins are grafted with a group Y' that comprises an aldehyde function, Y is H and the N-terminal amino acid of A_1 is selected from a cysteine, a serine or a threonine, or an NH_2 group capable of forming a Schiff base, such as the hydrazinocarbonyl group H2NNHCO, or more generally a derivative of hydrazine or H_2NO, i.e. a derivative of hydroxylamine.

More precisely, the preferred embodiments of the invention have the following:

biological tests, in particular affinity tests of the polypeptides synthesized for molecules of any kind.

Another ~~subject of the invention~~ disclosure of the application consists of an test and/or diagnostic kit comprising at least one support comprising at least one receiving zone, on which at least one solid support grafted with at least one polypeptide comprising n peptide fragments and at least n-1 amino acids bearing a thiol function is placed, the grafted solid support corresponding to formula (IV_n) below:

$\square$-Z-A_1- C_1-A_2-...$C_{i-1}A_i$-...$C_{n-1}A_n$ (IV_n)

Thus, the test kit can be used for screening biologically active molecules or for diagnostic tests. It can be envisaged that the diagnostic kit comprises several separate receiving zones, on which a solid support grafted with identical or different polypeptides is deposited. For example, solid supports grafted with identical or different polypeptides can be placed in the wells of a multiwell plate so as to form a test and/or diagnostic support.

Preferably, for carrying out tests, the polypeptides grafted onto the solid support are brought into contact beforehand with a medium that promotes the formation of a two- and/or three-dimensional structure. For example, following the teaching of E.C.B. Johnson et al., Angew. Chem. Int. Ed. 2006, 45, 3283-3287, the polypeptide can be put in contact with a phosphate buffer of pH 8 and can be subjected to the release of air, or the peptide can be put in contact with an aqueous solution with pH equal to 9. Another useful means for folding is the use of the pair GSH, GSSG (GSH for glutathione, GSSG for oxidized glutathione).

The test and/or diagnostic kit can further comprise means for detecting interaction between the polypeptides and the molecules tested.

Yet another ~~subject of the invention~~disclosure of the application is a method of manufacturing a medicinal product comprising at least:

- manufacturing at least one polypeptide by the method defined above, and
- combining it with a pharmaceutically acceptable support.

In fact, the method of the invention can be applied with the aim of synthesizing polypeptides having therapeutic activity, in particular vaccines. Combining them with a pharmaceutically acceptable support is carried out by a person skilled in the art based on his general knowledge and in relation to the properties of the polypeptide (in particular solubility) and the method of administration selected.

Other features and advantages of the invention will become apparent on reading the following description of a preferred embodiment of the invention, given as an example, and referring to the attached drawing.

FIGURES

Figure 1: general diagrammatic representation of the method of peptide synthesis according to the invention.

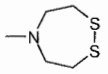
CLAIMS

1. A ~~M~~method of assembling peptide fragments for manufacturing a polypeptide comprising n peptide fragments and at least n-1 amino acids bearing a thiol function, represented by the formula:

$$A_1\text{-}C_1\text{-}A_2\text{-}C_2\text{-}A_3\text{- } \ldots \text{ -}C_{i-1}\text{-}A_i\text{-}\ldots\text{ -}C_{n-1}\text{-}A_n \qquad (I)$$

in which A_1, A_2, A_3, … A_i …, A_n are peptide fragments,
C_1, C_2, C_3 … C_{i-1} … C_{n-1} are amino acid residues bearing a thiol ~~function~~functional group,
n is comprised between 3 and 50,
and
i is any integer between 2 and n, said method involving:

(a1) at least one step of preparing a fragment Y-A_1-SEAoff (II_1) in which A_1 represents a peptide fragment the C-terminal of which bears a cyclic bis(2-sulphanylethyl)amino group



called SEAoff, and

Y is a fragment capable of reacting with a functional group~~function~~ of a solid support so as to form a bond between A_1 and a solid support,

(b) at least one step of reaction of Y-A_1-SEAoff (II_1) with a solid support designated ☐-Y', ☐ representing the solid support itself and Y' representing a reactive functional group~~function~~ capable of reacting with Y to form a group Z according to the diagram:

☐-Y' + Y-A_1-SEAoff ⟶ ☐-Z-A_1-SEAoff

(II_1) (III_1)

(a2) at least one step of preparing a fragment H-C_1(PG_1)-A_2-SEAoff (II_2) in which C_1, A_2 and SEAoff are as defined above and (PG_1) represents H or a protective group of the thiol of the amino acid C_1,

(c1) at least one step of preparing a thioester peptide of formula ~~(III_1')~~ (III'_1) starting from bis(2-sulphanylethyl)amino peptide ☐-Z-A_1-SEAoff (III_1) according to the diagram:

☐-Z-A_1-SEAoff ⟶ ☐-Z-A_1-SR

(III_1) (III'_1)

by the action of a thiol R-SH, ~~optionally~~ in the presence of a reducing agent of the cyclic disulphides, where R ~~can be~~is an alkyl or aryl radical, ~~optionally substituted,~~

(d1) at least one step of condensation of (III'_1) with the peptide fragment (II_2) in the presence of an aromatic thiol ArSH under conditions in which PG_1 is eliminated when it is different from H:

$$\square\text{-Z-}A_1\text{-SR} + \text{H-}C_1(PG_1)\text{-}A_2\text{-SEAoff} \longrightarrow \square\text{-Z-}A_1\text{-}C_1\text{-}A_2\text{-SEAoff}$$

(III'_1) (II_2) (III_2)

(an-1) at least one step of preparing a fragment $C_{n-1}(PG_{n-1})\text{-}A_n$ where (PG_{n-1}) represents H or a protective group of the thiol of the amino acid C_{n-1},

(dn-1) at least one step of condensation of $C_{n-1}(PG_{n-1})\text{-}A_n$ with

$\square\text{-Z-}A_1\text{-}C_1\text{-}A_2\text{-}\ldots C_{i-1}A_i\text{-}\ldots C_{n-2}A_{n-1}\text{SEAoff}$ (III_{n-1})

in the presence of an aromatic thiol ArSH under conditions in which PG_{n-1} is eliminated when it is different from H in order to give:

$\square\text{-Z-}A_1\text{-}C_1\text{-}A_2\text{-}\ldots C_{i-1}A_i\text{-}\ldots C_{n-1}A_n$ (IV_n)

2. The ~~M~~method according to claim 1, which further comprises:

(e) a step of detaching the peptide $A_1\text{-}C_1\text{-}A_2\text{-}\ldots C_{i-1}A_i\text{-}\ldots C_{n-1}A_n$ (I) from the solid support.

3. The ~~M~~method according to claim 1 or claim 2, in which the solid support $\square$ is selected from ~~resins, in particular from~~ resins based on polystyrene, polyacrylamide, polyethylene glycol, cellulose, polyethylene, polyester, latex, polyamide, polydimethylacrylamide, synthetic or natural hydrophilic polymers, glass beads, silica gels.

4. The ~~M~~method according to any one of claims 1 to 3, in which $C_1,\ldots C_i\ldots C_n$ are cysteines.

5. The ~~M~~method according to any one of claims 1 to 4, in which $PG_1,\ldots PG_i\ldots PG_n$ are tert-butyl sulphenyl groups.

6. The ~~M~~method according to any one of claims 1 to 5, in which:

Y' comprises a function selected from an azide and Y is selected from the groups comprising an alkyne functional group~~function~~, or

Y' comprises an alkyne functional group~~function~~ and Y is selected from the groups comprising an azide functional group~~function~~ or

Y' comprises an aldehyde functional group~~function~~, Y is H and the N-terminal amino acid of A_1 is selected from a cysteine, a serine or a threonine, or

Y' comprises an aldehyde functional group~~function~~, Y comprises an NH_2 group capable of forming a Schiff base.

7. The ~~M~~method according to any one of claims 1 to 6, in which R is selected from an alkyl radical comprising 1 to 12 carbon atoms, linear or branched, ~~optionally substituted,~~ or C6-C12 aryl~~, optionally substituted~~.

8. The ~~M~~method according to any one of claims 1 to 7, in which, for every i = 2,...n-2, the method comprises

(ci) at least one step of conversion of

□-Z-A_1-C_1- ...$C_{i-1}A_i$SEAoff (III_i)

to

□-Z-A_1- C_1- ...C_{i-1}-A_iSR (III'_i)

by the action of a thiol R-SH, optionally in the presence of a reducing agent of cyclic disulphides,

(di) at least one step of condensation of C_i(PG_i)A_{i+1}SEAoff where (PG_i) represents H or a protective group of the thiol of the amino acid C_i, with

□-Z-A_1- C_1- ...$C_{i-1}A_i$-SR (III'_i)

in order to give

□-Z-A_1-C_1- ...C_iA_{i+1}SEAoff (III_{i+1}), wherein steps (ci) and (di) are carried out before step dn-1).

9. The ~~M~~method according to ~~any one of~~ claims ~~1 to~~ 8, in which, for every i = 2,...n-2, the steps di) of the method are carried out under conditions in which PG_i is removed selectively in situ, without affecting SEAoff and the solvent of the reaction is an aqueous buffer, with pH between 4 and 9, containing an aromatic thiol.

10. Method of manufacturing a medicinal product comprising at least:

- manufacturing at least one polypeptide by the method according to any one of claims 1 to 9, and

- combining it with a pharmaceutically acceptable support.

11. Solid support grafted with a peptide and corresponding to the following formula (III1):

□-Z-A1-SEAoff (III1)

in which A1 represents a peptide fragment, the C-terminal of which bears a cyclic bis(2-sulphanylethyl)amino group

called SEAoff, □ represents a solid support and Z represents a linkage group between A1 and □.

12. Polypeptide synthesis kit comprising at least one support provided with at least one receiving zone, on or in which at least one grafted solid support according to claim 11 is placed.

13. Automated device for synthesis of peptides comprising at least one reservoir in which a solid support grafted with a peptide and corresponding to the following formula (III1) is placed:

□-Z-A1-SEAoff (III1)

separate reservoirs containing:

- peptides (IIi) Ci(PG i)Ai+1SEAoff, as well as:

- at least one thiol R SH, and optionally a reducing agent of the cyclic disulphides

- coupling activators and

- washing products,

mechanical means for taking and distributing samples of products, as well as data processing means allowing controlled application of these mechanical means.