amino acids or β-amino acids that the continuous translation introduction is difficult even when natural translation factors are used but the single translation introduction is possible (see Non Patent Literatures 3, 5, and 6).

[0006]

It has also been reported that linear γ-amino acids having a hydroxyl group at the β-position have excellent translation efficiency compared to linear γ-amino acids having no hydroxyl group at the β-position (see Non Patent Literature 13).

[Citation List]

[Patent Literature]

[0007]

[Patent Literature 1] International Publication No. WO 2016/148044

[Patent Literature 2] International Publication No. WO 2019/077887

[Non Patent Literature]

[0008]

[Non Patent Literature 1] Biochemistry 2006, 45, 51, 15541

[Non Patent Literature 2] J. Am. Chem. Soc. 2007, 129, 46, 14458.

[Non Patent Literature 3] J. Am. Chem. Soc. 2013, 135, 5, 1830

[Non Patent Literature 4] Bioorganic & Medicinal Chemistry 21 (2013) 1088

[Non Patent Literature 5] Nucleic Acids Research, 2015, Vol. 43, No. 12, 5687

[Non Patent Literature 6] J. Am. Chem. Soc. 2016, 138, 6, 1962

[Non Patent Literature 7] Nucleic Acids Research, 2017, Vol. 45, No. 22, 12601

[Non Patent Literature 8] J. Am. Chem. Soc. 2018, 140, 12159

[Non Patent Literature 9] Int J Mol Sci. 2019, 20, 522

[Non Patent Literature 10] Nature Chemistry volume 12, 1081

[Non Patent Literature 11] J. Am. Chem. Soc. 2020, 142, 39, 16518

[Non Patent Literature 12] J. Am. Chem. Soc. 2020, 142, 11, 4965

[Non Patent Literature 13] ACS Chem. Biol. 2021, 16, 1325

[Non Patent Literature 14] Cell Chem. Biol. 24, 46-54 (2017)

[Non Patent Literature 15] Nature Communications volume 7, Article number: 11657 (2016)

[Non Patent Literature 16] ACS Chem. Biol. 2021, 16, 1011-1018

[Summary]

[Technical Problem]

[0009]

Accordingly, an object of the present disclosure is to provide a method for producing a peptide that exhibits excellent translation efficiency with respect to the continuous translation introduction of unnatural amino acids.

[Solution to Problem]

[0010]

The present inventors have intensively studied, and, as a result, have found that the translation efficiency in the translation of a peptide having consecutive unnatural amino acids is improved when an amino acid having an oxygen atom at a specific position is translated.

[0011]

The present disclosure is based on the finding and specifically relates to [1] to [123].

[1]

In one aspect, the present disclosure relates to a method for producing a peptide, comprising a step of translating a nucleic acid that comprises a codon encoding a first unnatural amino acid and a codon encoding a second unnatural amino acid which are adjacent to each other, wherein

at least one of the first unnatural amino acid and the second unnatural amino acid is an unnatural amino acid U1, and the unnatural amino acid U1 is an unnatural amino acid that meets the following requirement (A) and meets at least one selected from the group consisting of the following requirements (B) and (C):

(A) a nitrogen atom constituting a main-chain amino group (nitrogen atom N1) and an oxygen atom other than an oxygen atom constituting a main-chain carboxy group (oxygen atom O1) are bound via two atoms to each other;

(B) the oxygen atom O1 constitutes an alkoxy group or an aryloxy group;

(C) the nitrogen atom N1 is bound to a linear or branched C_1-C_6 alkyl group.

[2]

The method according to [1], wherein the unnatural amino acid U1 meets the requirements (A) and (B).

[3]

The method according to [1], wherein the unnatural amino acid U1 meets the requirements (A) and (C).

[4]

The method according to [1], wherein the unnatural amino acid U1 meets the requirements (A), (B) and (C).

[5]

The method according to any one of [1] to [4], wherein the oxygen atom O1 is present in a substituent substituted with the nitrogen atom N1.

The method according to any one of [1] to [7], wherein the oxygen atom O1, together with a phenyl group, constitutes a corresponding phenoxy group.

[16]

The method according to any one of [1] to [7], wherein the oxygen atom O1 constitutes a hydroxyl group.

[17]

The method according to any one of [1] to [16], wherein the nitrogen atom N1 is substituted with a methyl group or an ethyl group.

[18]

The method according to any one of [1] to [16], wherein the nitrogen atom N1 is substituted with a methyl group.

[19]

The method according to any one of [1] to [16], wherein the nitrogen atom N1 constitutes an unsubstituted amino group.

[20]

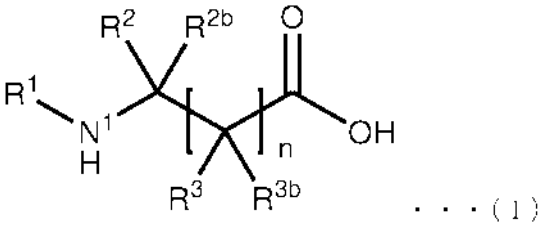
The method according to any one of [1] to [19], wherein the unnatural amino acid U1 is an α-amino acid.

[21]

In another aspect, the present disclosure relates to a method for producing a peptide, comprising a step of translating a nucleic acid that comprises a codon encoding a first unnatural amino acid and a codon encoding a second unnatural amino acid which are adjacent to each other, wherein

at least one of the first unnatural amino acid and the second unnatural amino acid is an unnatural amino acid U1, and the unnatural amino acid U1 is an unnatural amino acid represented by the following formula (I):

[Formula 1]

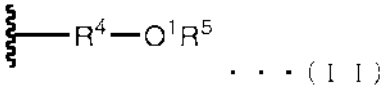


・・・(I)

wherein n is an integer of 0 or more and 2 or less;

R^1 is a group represented by formula (II), a hydrogen atom or a linear or branched C_1-C_6 alkyl group;

[Formula 2]



・・・(II)

The method according to any one of [1] to [20], [48] to [60], and [72] to [81], wherein the unnatural amino acid U2 has, as a side chain, a methyl group or a benzyl group.

[83]

The method according to any one of [1] to [20], [48] to [60], and [72] to [82], wherein the unnatural amino acid U2 has a methyl group as a side chain.

[84]

The method according to any one of [1] to [20], [48] to [60], and [72] to [82], wherein the unnatural amino acid U2 has a benzyl group as a side chain.

[85]

The method according to any one of [1] to [20], [48] to [60], and [72] to [84], wherein the unnatural amino acid U2 is an α-amino acid.

[86]

The method according to any one of [1] to [20], [48] to [60], [72] to [83], and [85], wherein a nitrogen atom constituting a main-chain amino group of the unnatural amino acid U2 is substituted with a methyl group, and the unnatural amino acid U2 is an α-amino acid having a methyl group as a side chain.

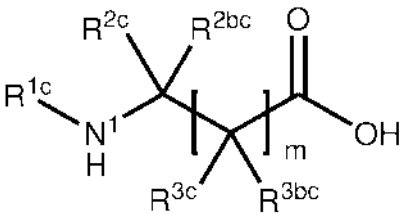
[87]

The method according to any one of [1] to [20], [48] to [60], [72] to [82], and [84] to [85], wherein a nitrogen atom constituting a main-chain amino group of the unnatural amino acid U2 is substituted with a methyl group, and the unnatural amino acid U2 is an α-amino acid having a benzyl group as a side chain.

[88]

The method according to any one of [21] to [51] and [61] to [87], wherein the unnatural amino acid U2 is an unnatural amino acid represented by the following formula (VI):

[Formula 16]



・・・(VI)

in the formula (VI),

m is an integer of 0 or more and 2 or less;

R^{1c} is a hydrogen atom or a linear or branched C_1-C_6 alkyl group;

R^{2c} and R^{3c} are each independently a hydrogen atom, a linear or branched C_1-C_6 alkyl group that may be substituted with a 5- to 10-membered aryl group, or a 5- to 10-membered aryl group that may be substituted with a linear or branched C_1-C_6 alkyl group;

R^{2bc} and R^{3bc} are each independently a hydrogen atom or a linear or branched C_1-C_4 alkyl group;

R^{2c} and R^{2bc} may form a ring together with a carbon atom to which they are bound; and

a nitrogen atom to which R^{1c} is bound, R^{1c}, a carbon atom to which R^{2c} is bound, and R^{2c} may together form a ring.

[89]

The method according to any one of [21] to [51] and [61] to [88], wherein R^{1c} is a linear or branched C_1-C_6 alkyl group.

[90]

The method according to any one of [21] to [51] and [62] to [89], wherein R^{1c} is a methyl group or an ethyl group.

[91]

The method according to any one of [21] to [51] and [62] to [90], wherein R^{1c} is a methyl group.

[92]

The method according to any one of [21] to [51] and [62] to [91], wherein R^{2c} is a linear or branched C_1-C_6 alkyl group that may be substituted with a 5- to 10-membered aryl group, or a 5- to 10-membered aryl group that may be substituted with a linear or branched C_1-C_6 alkyl group.

[93]

The method according to any one of [21] to [51] and [62] to [92], wherein R^{2c} is a linear or branched C_1-C_4 alkyl group or a 5- to 10-membered aryl group substituted with a linear C_1-C_3 alkyl group.

[94]

The method according to any one of [21] to [51] and [62] to [93], wherein R^{2c} is a methyl group or a benzyl group.

[95]

The method according to any one of [21] to [51] and [62] to [94], wherein R^{2c} is a methyl group.

[96]

The method according to any one of [21] to [51] and [62] to [94], wherein R^{2c} is a benzyl group.

[97]

The method according to any one of [21] to [51] and [62] to [94], wherein m is 0.

[98]

The method according to any one of [21] to [51], [62] to [95], and [97], wherein R^{1c} and R^{2c} each are a methyl group and m is 0.

The method according to [109] or [110], wherein the cell-free translation system is a translation system reconstituted with a factor derived from E. coli.

[112]

The method according to any one of [109] to [111], wherein the cell-free translation system comprises a ribosome derived from E. coli.

[113]

The method according to any one of [109] to [112], wherein the cell-free translation system comprises a first tRNA to which a first unnatural amino acid is bound and a second tRNA to which a second unnatural amino acid is bound.

[114]

The method according to [113], wherein the first tRNA comprises an anticodon that is complementary to a codon encoding the first unnatural amino acid, and the second tRNA comprises an anticodon that is complementary to a codon encoding the second unnatural amino acid.

[115]

The method according to [113] or [114], wherein the first tRNA and the second tRNA each are an artificial tRNA.

[116]

The method according to [113] or [114], wherein the first tRNA and the second tRNA each are an transcribed tRNA.

[117]

The method according to any one of [113] to [115], wherein the first tRNA and the second tRNA each comprise a body derived from a naturally occurring tRNA.

[118]

The method according to any one of [113] to [117], further comprising a step of preparing the tRNA by a pCpA method.

[119]

The method according to any one of [1] to [118], wherein the nucleic acid is mRNA.

[120]

In another aspect, the present disclosure relates to a method for producing a peptide complex, comprising the method as disclosed herein. In one example, there is disclosed a method for producing a peptide complex, comprising the method according to any one of [1] to [119].

[121]

The method according to [120], wherein the peptide complex is a peptide-nucleic acid complex.

[122]

A method for producing a peptide complex, comprising:

Claims

[Claim 1]

A method for producing a peptide, comprising a step of translating a nucleic acid that comprises a codon encoding a first unnatural amino acid and a codon encoding a second unnatural amino acid which are adjacent to each other, wherein

at least one of the first unnatural amino acid and the second unnatural amino acid is an unnatural amino acid U1, and the unnatural amino acid U1 is an unnatural amino acid that meets the following requirement (A) and meets at least one selected from the group consisting of the following requirements (B) and (C):

(A) a nitrogen atom constituting a main-chain amino group (nitrogen atom N1) and an oxygen atom other than an oxygen atom constituting a main-chain carboxy group (oxygen atom O1) are bound via two atoms to each other;

(B) the oxygen atom O1 constitutes an alkoxy group or an aryloxy group;

(C) the nitrogen atom N1 is bound to a linear or branched C_1-C_6 alkyl group.

[Claim 2]

The method according to claim 1, wherein the oxygen atom O1, together with a linear or branched C_1-C_6 alkyl group, a C_3-C_{10} cycloalkyl group, a C_6-C_{10} aryl group, a C_7-C_{12} arylalkyl group, or a C_7-C_{12} alkylaryl group, constitutes a corresponding C_1-C_6 alkoxy group, a corresponding C_3-C_{10} cycloalkoxy group, a corresponding C_6-C_{10} aryloxy group, a corresponding C_7-C_{12} arylalkoxy group, or a corresponding C_7-C_{12} alkylaryloxy group.

[Claim 3]

The method according to claim 1 or 2, wherein the nitrogen atom N1 is substituted with a methyl group or an ethyl group.

[Claim 4]

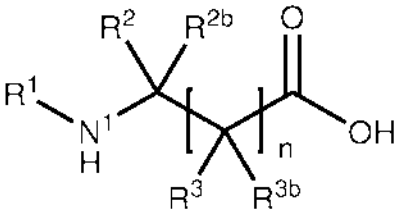
The method according to any one of claims 1 to 3, wherein the unnatural amino acid U1 is an α-amino acid.

[Claim 5]

A method for producing a peptide, comprising a step of translating a nucleic acid that comprises a codon encoding a first unnatural amino acid and a codon encoding a second unnatural amino acid which are adjacent to each other, wherein

at least one of the first unnatural amino acid and the second unnatural amino acid is an unnatural amino acid U1, and the unnatural amino acid U1 is an unnatural amino acid represented by the following formula (I):

[Formula 1]

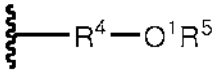


. . . (I)

wherein n is an integer of 0 or more and 2 or less;

R^1 is a group represented by formula (II), a hydrogen atom or a linear or branched C_1-C_6 alkyl group;

[Formula 2]



. . . (I I)

R^2 and R^3 are each independently (a) a group represented by the formula (II), (b) a hydrogen atom, or (c) a linear or branched C_1-C_4 alkyl group;

R^{2b} and R^{3b} are each independently a hydrogen atom or a linear or branched C_1-C_4 alkyl group;

R^2 and R^{2b} or R^3 and R^{3b} may form a ring together with a carbon atom to which they are bound;

any one or more of R^1, R^2, and R^3 is a group represented by the formula (II);

N^1 (nitrogen atom N1) in the formula (I) and O^1 (oxygen atom O1) in the formula (II) are bound via two atoms to each other;

when R^1 is a substituent represented by the formula (II), R^4 and R^2 may form a ring together with a carbon atom to which R^2 is bound and N^1;

when a plurality of R^3 is present, they are each independently the same as or different from each other;

when a plurality of R^{3b} is present, they are each independently the same as or different from each other;

in the formula (II),

[Formula 3]



represents a binding position with N^1;

R^4 is a C_1-C_2 alkylene group;

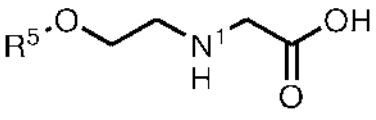
R^5 is a hydrogen atom, a linear or branched C_1-C_6 alkyl group, a C_3-C_{10} cycloalkyl group, a C_6-C_{10} aryl group, a C_7-C_{12} arylalkyl group, or a C_7-C_{12} alkylaryl group; and

when R^2 is a substituent represented by the formula (II), at least one of R^1 in the formula (I) and R^5 in the formula (II) is not a hydrogen atom.

[Claim 6]

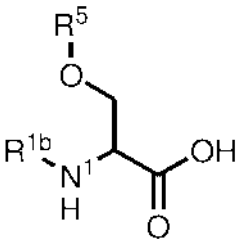
The method according to claim 5, wherein the unnatural amino acid U1 is an unnatural amino acid represented by the following formula (III), (IV) or (V):

[Formula 4]



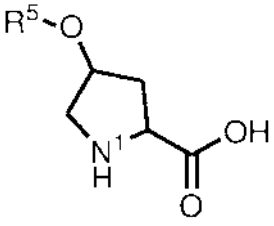
・・・(III)

[Formula 5]



・・・(IV)

[Formula 6]



・・・(V)

in the formulas (III), (IV) and (V), R^{1b} is a hydrogen atom or a linear or branched C_1-C_6 alkyl, and R^5 has the same meaning as R^5 in the formula (II).

[Claim 7]

The method according to claim 5 or 6, wherein R^5 is a linear or branched C_1-C_6 alkyl group, a C_6-C_{10} aryl group, or a C_7-C_{12} arylalkyl group.

[Claim 8]

The method according to any one of claims 5 to 7, wherein R^1 and R^{1b} each are a methyl group or an ethyl group.

[Claim 9]

The method according to any one of claims 5 to 8, wherein n is 0.

[Claim 10]

The method according to any one of claims 1 to 9, wherein the first unnatural amino acid is the unnatural amino acid U1, and the second unnatural amino acid is an unnatural amino acid that is different from the unnatural amino acid U1 (unnatural amino acid U2).

[Claim 11]

The method according to any one of claims 1 to 10, wherein a codon encoding the first unnatural amino acid is translated before a codon encoding the second unnatural amino acid.

[Claim 12]

The method according to any one of claims 1 to 11, wherein the nitrogen atom N1 and the oxygen atom O1 are bound via two carbon atoms to each other.

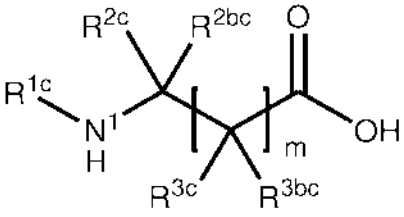
[Claim 13]

The method according to any one of claims 1 to 12, wherein the unnatural amino acid U1 does not comprise a heteroatom other than a nitrogen atom and an oxygen atom.

[Claim 14]

The method according to claim 10, wherein the unnatural amino acid U2 is an unnatural amino acid represented by the following formula (VI):

[Formula 7]



... (VI)

in the formula (VI),

m is an integer of 0 or more and 2 or less;

R^{1c} is a hydrogen atom or a linear or branched C_1-C_6 alkyl group;

R^{2c} and R^{3c} are each independently a hydrogen atom, a linear or branched C_1-C_6 alkyl group that may be substituted with a 5- to 10-membered aryl group, or a 5- to 10-membered aryl group that may be substituted with a linear or branched C_1-C_6 alkyl group;

R^{2bc} and R^{3bc} are each independently a hydrogen atom or a linear or branched C_1-C_4 alkyl group;

R^{2c} and R^{2bc} may form a ring together with a carbon atom to which they are bound; and

a nitrogen atom to which R^{1c} is bound, R^{1c}, a carbon atom to which R^{2c} is bound, and R^{2c} may together form a ring.

[Claim 15]

A method for producing a peptide complex, comprising the method according to any one of claims 1 to 14.