

A process of preparing L-methionine directly from L-homoserine

Technical Field

5 The present disclosure belongs to the field of biochemical engineering and specifically relates to a process for direct enzymatic preparation of L-methionine using L-homoserine as a substrate.

Background

10 Methionine is one of the 20 natural amino acids in proteins and a precursor for the synthesis of S-adenosine methionine, which serves as a donor of methyl group in the human body and is involved in various important metabolic processes. Human cannot synthesize methionine and have to obtain it through (for example) food. Methionine is also a feed additive widely used in livestock, poultry, and fish farming.

15 Methionine may be prepared through chemical synthesis. However, chemically synthesized methionine is racemic D/L mixed methionine, while D-methionine is difficult to be utilized in living organisms.

20 Optically pure L-methionine may also be produced through biosynthesis, achieving high bioavailability. Biosynthesis of methionine is usually conducted by introducing an enzyme involved in the methionine biosynthesis metabolic pathway (see Fig. 1) into a host cell (see e.g., CN 105886449 B, CN 106906176 A, CN 103781913 B and CN 114787369 A). However, biosynthesis requires introduction of a large number of exogenous genes into a host cell, requiring complex genetic engineering modulations. Moreover, due to the complex regulatory network controlling the synthetic metabolic pathway of methionine, it is difficult to achieve a high yield.

25 A process is also developed to synthesize methionine by sulfurization and methylation of O-acetyl homoserine using methyl terminated thioether, such as dimethyl disulfide, as source of sulfur and methyl, through fermentation with *Corynebacterium glutamicum* or *Escherichia coli* mutant (see, CN 101223280 B). However, in this process, the methanthiol produced by decomposition of thioether has a strong toxic effect on microorganisms, affecting the growth and hindering the synthesis of methionine.

30 A process for synthesizing L-methionine by catalyzing the reaction of O-succinyl homoserine and sodium methanethiolate using O-succinyl thioltransferase (see CN 113215124 A) and a process for synthesizing L-methionine by catalyzing the reaction of O-acyl homoserine such as O-acetyl homoserine or O-succinyl homoserine with methanthiol

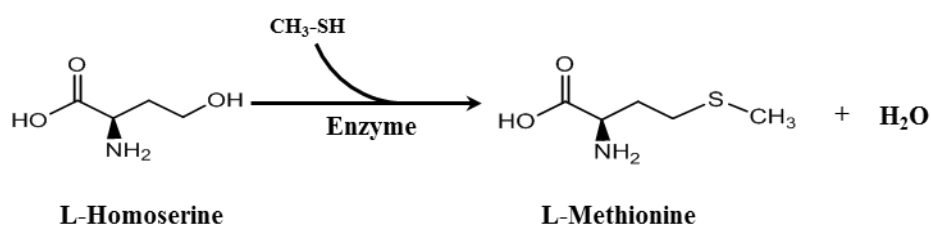
using O-acyl homoserine sulphydrylase (see WO 2008/013432 A1) were also developed.

However, these processes require the production (e.g., through fermentation) of O-acyl homoserine first, which is limited by i) accumulation of O-acyl homoserine; ii) instability of O-acyl homoserine (to acid, base, and heating); iii) inhibition of enzymatic activity by by-product organic acids; and iv) if the pH is adjusted in the reaction process, the production cost, process complexity and product separation difficulty will be increased.

Therefore, there is a need of developing a new process for preparing L-glufosinate, using a stable substrate which is easily obtainable, reducing product inhibition of the reaction, simplifying the process (including production and separation), and reducing costs.

Summary

Surprisingly, the present inventors found that L-homoserine can react with methanethiol under enzymatic catalysis to produce L-methionine and water (as shown in Formula I).



L-homoserine is a compound which is more easily obtainable and stable than O-acyl homoserine, and the reaction of the present disclosure does not produce an organic acid byproduct which inhibits enzymatic activity.

Accordingly, provided is a process for preparing L-methionine, comprising steps of:

a) providing a reaction medium, comprising L-homoserine or a salt, an ester, an amide or an anhydride thereof, methanethiol or a salt thereof, and an enzyme selected from the group consisting of a transferase which transfers an alkyl other than methyl or an aryl, an ammonia lyase, and a lyase which catalyzes the cleavage of a carbon-sulfur bond;

b) incubating the reaction medium to produce a reaction product comprising L-methionine or a salt, an ester, or an amide thereof; and

optionally,

c) recovering the L-methionine or the salt, the ester, or the amide thereof from the reaction medium.

In some embodiments, the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.-, EC 4.3.1.- and EC 4.4.1.-.

In some embodiments, the reaction medium further comprises phosphopyridoxal or a salt

thereof.

In some embodiments, the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.47, EC 2.5.1.48, EC 2.5.1.49, EC 2.5.1.51, EC 2.5.1.52, EC 2.5.1.65, EC 2.5.1.76, EC 2.5.1.113, EC 2.5.1.134, EC 2.5.1.140, EC 2.5.1.144, EC 4.3.1.15, EC 4.3.1.17, EC 4.3.1.18, EC 4.3.1.19, EC 4.4.1.1, EC 4.4.1.2, EC 4.4.1.9, EC 4.4.1.10, EC 4.4.1.11, EC 4.4.1.13, EC 4.4.1.15, EC 4.4.1.16, EC 4.4.1.25, EC 4.4.1.28 and EC 4.4.1.35.

In some embodiments, the enzyme is derived from an organism selected from the group consisting of *Thermus thermophiles*, *Lysinibacillus sphaericus*, *Clostridioides difficile*, *Clostridium novyi*, *Geobacillus stearothermophilus*, *Thermotoga maritima*, *Pseudomonas aeruginosa*, *Nonlabens dokdonensis*, *Leucaena leucocephala*, *Aeropyrum pernix*, *Methanosarcina acetivorans*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli*, *Candida maltosa*, *Streptomyces phaeochromogenes*, *Neurospora crassa*, *Spinacia oleracea*, *Cyanobacteria bacterium*, *Pseudomonas putida*, *Lactococcus lactis*, *Ruegeria pomeroyi*, *Methanococcus maripaludis* and *Camelina sativa*.

In some embodiments, the enzyme is selected from the group consisting of cysteine synthase derived from *Thermus thermophiles* (EC 2.5.1.47 - cysteine synthase), cystathionine γ -synthase derived from *Lysinibacillus sphaericus* (EC 2.5.1.48 - cystathionine gamma-synthase), O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridioides difficile* (EC 2.5.1.49 - O-acetylhomoserine aminocarboxypropyltransferase), O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridium novyi*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Geobacillus stearothermophilus*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Thermotoga maritima*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Pseudomonas aeruginosa*, β pyrazolylalanine synthase derived from *Nonlabens dokdonensis* (EC 2.5.1.51 - beta-pyrazolylalanine synthase), L-mimosine synthase derived from *Leucaena leucocephala* (EC 2.5.1.52 - L-mimosine synthase), O-phosphoserine sulfhydrylase derived from *Aeropyrum pernix* (EC 2.5.1.65 - O-phosphoserine sulfhydrylase), cysteine synthase derived from *Methanosarcina acetivorans* (EC 2.5.1.76 - cystate synthase), [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase derived from *Mycobacterium tuberculosis* (EC 2.5.1.113 - [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase), cystathionine β synthase derived from *Bacillus subtilis* (EC 2.5.1.134 - cystathionine beta-synthase), N-(2-amino-2-carboxyethyl)-L-glutamate synthase derived from *Staphylococcus aureus* (EC

2.5.1.140 - N-(2-amino-2-carboxyethyl)-L-glutamate synthase), S-sulfo-L-cysteine synthase derived from *Pseudomonas fluorescens* (EC 2.5.1.144 - S-sulfo-L-cysteine synthase), diaminopropionate ammonia-lyase derived from *Escherichia coli* (EC 4.3.1.15 - Diaminopropionate ammonia-lyase), L-serine ammonia-lyase derived from *Escherichia coli* 的 (EC 4.3.1.17 - L-serine ammonia-lyase), D-serine ammonia-lyase derived from *Escherichia coli* (EC 4.3.1.18 - D-serine ammonia-lyase), threonine ammonia-lyase derived from *Candida maltose* (EC 4.3.1.19 - threonine ammonia-lyase), cystathionine γ lyase derived from *Streptomyces phaeochromogenes* (EC 4.4.1.1 - cystathionine gamma-lyase), cystathionine γ lyase derived from *Neurospora crassa* (EC 4.4.1.1 - cystathionine gamma-lyase), homocysteine desulfhydrase derived from *Bacillus subtilis* 的 (EC 4.4.1.2 - homocysteine desulfhydrase), L-3-cyanoalanine synthase derived from *Spinacia oleracea* (EC 4.4.1.9 - L-3-cyanoalanine synthase), cysteine lyase derived from *Cyanobacteria bacterium* (EC 4.4.1.10 - cysteine lyase), methionine γ lyase derived from *Pseudomonas putida* (EC 4.4.1.11 - methionine gamma-lyase), cysteine-S-conjugate β lyase derived from *Lactococcus lactis* (EC 4.4.1.13 - cysteine-S-conjugate beta-lyase), D-cysteine desulfhydrase derived from *Escherichia coli* (EC 4.4.1.15 - D-cysteine desulfhydrase), selenocysteine lyase derived from *Escherichia coli* (EC 4.4.1.16 - selenocysteine lyase), L-cysteate sulfo-lyase derived from *Ruegeria pomeroyi* (EC 4.4.1.25 - L-cysteate sulfo-lyase), L-cysteine desulfidase derived from *Methanococcus maripaludis* (EC 4.4.1.28 - L-cysteine desulfidase) and L-cystine β lyase derived from *Camelina sativa* 的 (EC 4.4.1.35 - L-cystine beta-lyase).

In some embodiments, the enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1-31.

In some embodiments, the process is a cell-free process.

In some embodiments, the reaction medium in step a) comprises a host cell expressing the enzyme. In some embodiments, the process further comprises a step of culturing the host cell.

In some embodiments, in step b), the reaction medium is incubated at 30-40°C, 32-39°C, 34-38°C, 36-38°C or 37°C.

Brief Description of the Drawings

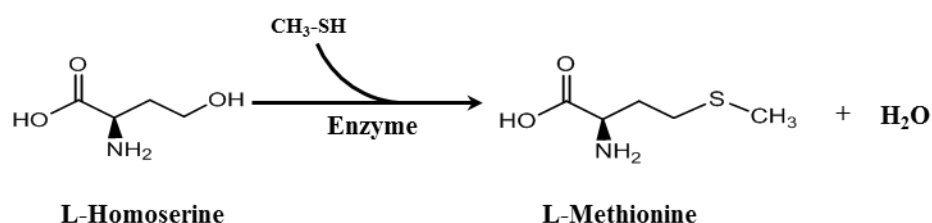
Figure 1 shows a schematic diagram of the biosynthetic pathway of methionine (see, Brewster, J. L., *et al.*, Structures and kinetics of *Thermotoga maritima* MetY reveal new insights into the predominant sulfurylation enzyme of bacterial methionine biosynthesis. The

Detailed Description

Provided is a novel process for preparing L-methionine. Unless otherwise stated, the terms used herein have the general understanding of those skilled in the art.

According to reports in the art, 4-hydroxyl activated L-homoserine (such as O-acyl homoserine) can react with methanthiol under enzymatic catalysis to produce L-methionine.

Surprisingly, the present inventors found that L-homoserine can react with methanthiol under enzymatic catalysis to produce L-methionine and water (as shown in Formula I).



Formula I

L-homoserine is a compound which is easier to obtain and more stable than O-acyl homoserine, and the reaction of Formula I does not produce an organic acid byproduct which inhibits enzymatic activity.

Accordingly, provided is a process for preparing L-methionine comprising steps of:

a) providing a reaction medium, comprising a L-homoserine donor, a methylthio donor, and an enzyme selected from the group consisting of a transferase which transfers an alkyl other than methyl or an aryl, an ammonia lyase, and a lyase which catalyzes the cleavage of a carbon-sulfur bond;

b) incubating the reaction medium to produce a reaction product comprising L-methionine or a salt, an ester, or an amide thereof; and optionally,

c) recovering the L-methionine or a salt, an ester, or an amide thereof from the reaction medium.

I. Substrate and Product

As used herein, “L-homoserine” refers to L-2-amino-4-hydroxy-1-acid, with the molecular formula of C₄H₉NO₃. As used herein, a L-homoserine donor comprises but not limited to L-homoserine or a salt, an ester or an amide thereof, as well as an anhydride formed by L-homoserine with L-homoserine or another acid. The L-homoserine donor also comprises

a derivative with one or two hydrogens substituted on its amino group. The ester of L-homoserine refers to a compound produced by the reaction of the carboxyl group of L-homoserine with an alcohol or a phenol. The amide of L-homoserine refers to a compound produced by the reaction of the carboxyl group of L-homoserine with an amine compound.

5 As used herein, the L-homoserine donor does not comprise a derivative where the hydrogen atom of 4-hydroxyl is substituted by acyl, i.e., O-acyl homoserine.

In step b), L-homoserine is the direct substrate of the enzyme. The process according to the present disclosure does not comprise a step of generating O-acyl-L-homoserine, and O-acyl-L-homoserine is not used in the process.

10 As used herein, "methylthio donor" refers to a compound which can provide methylthio ($\text{CH}_3\text{S}-$), comprising but not limited to methanthiol or a salt thereof, as well as methyl terminated thioether or disulfide, such as dimethyl disulfide. In some embodiments, the methylthio donor is methanthiol or a salt thereof, comprising but not limited to sodium methanethiolate and potassium methanethiolate.

15 II. Enzyme

As mentioned above, the reaction of the present process is conducted under the catalysis of enzymes, i.e., an enzymatic reaction.

20 As used herein, the term "enzyme" refers to a protein or peptide which can specifically catalyze or promote a chemical or biochemical reaction. The enzyme according to the present disclosure may be an intact natural protein or polypeptide or a portion thereof with catalytic activity. The enzyme according to the present disclosure may also be a modified variant of a natural protein or polypeptide. The enzyme according to the present disclosure may be isolated from a living organism, or produced by recombination or synthesis. Those skilled in
25 the art can obtain enzymes through conventional methods.

As used herein, the term "peptide" refers to a chain of at least two amino acids connected by peptide bonds. The term "polypeptide" can be used interchangeably with the term "protein" herein, referring to a chain containing ten or more amino acid residues. All peptide and polypeptide chemical formulae or sequences herein are written from left to right,
30 indicating the direction from the amino terminal to the carboxyl terminal.

The term "amino acid" comprises naturally occurring amino acids and non-naturally occurring amino acids in proteins. The single letter and three letter names of naturally occurring amino acids in proteins are commonly used in the art, see Sambrook, et al. (Molecular Cloning: A Laboratory Manual, 2nd, ed. Cold Spring Harbor Laboratory, Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989).

	Amino Acid	Single letter	Three letter
	Alanine	A	Ala
	Arginine	R	Arg
5	Asparagine	N	Asn
	Aspartate	D	Asp
	Cysteine	C	Cys
	Glutamine	Q	Gln
	Glutamate	E	Glu
10	Glycine	G	Gly
	Histidine	H	His
	Isoleucine	I	Ile
	Ieucine	L	Leu
	Lysine	K	Lys
15	Methionine	M	Met
	Phenylalanine	F	Phe
	Proline	P	Pro
	Serine	S	Ser
	Threonine	T	Thr
20	Tryptophan	W	Trp
	Tyrosine	Y	Tyr
	Valine	V	Val

As used herein, the term “modification” refers to any modification of a peptide composed of the polypeptide or its homologous sequence according to the present disclosure, including but not limited to substitution, deletion, insertion, and/or addition of one or more amino acids.

Some enzymes requires the participation of a coenzyme for the catalysis. The term “coenzyme” is a general term for a type of organic cofactors, which are essential factors for enzyme-catalyzed redox reactions, group transfer, and isomerization reactions. They are involved in the function of transferring electrons, atoms, or groups in enzyme-catalyzed reactions.

The present inventors found that the enzyme as used in the present disclosure only uses phosphopyridoxal as a coenzyme. Accordingly, in some embodiments, the reaction medium further comprises phosphopyridoxal or a salt thereof.

In some embodiments, the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.-, EC 4.3.1.- and EC 4.4.1.-.

In some embodiments, the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.47, EC 2.5.1.48, EC 2.5.1.49, EC 2.5.1.51, EC 2.5.1.52, EC 2.5.1.65, EC 2.5.1.76, EC 2.5.1.113, EC 2.5.1.134, EC 2.5.1.140, EC 2.5.1.144, EC 4.3.1.15, EC 4.3.1.17, EC 4.3.1.18, EC 4.3.1.19, EC 4.4.1.1, EC 4.4.1.2, EC 4.4.1.9, EC 4.4.1.10, EC 4.4.1.11, EC 4.4.1.13, EC 4.4.1.15, EC 4.4.1.16, EC 4.4.1.25, EC 4.4.1.28 and EC 4.4.1.35.

In some embodiments, the enzyme is selected from the group consisting of cysteine synthase (EC 2.5.1.47 - cysteine synthase), cystathionine γ -synthase (EC 2.5.1.48 - cystathionine gamma-synthase), O-acetyl homoserine aminocarboxypropyltransferase (EC 2.5.1.49 - O-acetylhomoserine aminocarboxypropyltransferase), β pyrazolylalanine synthase (EC 2.5.1.51 - beta-pyrazolylalanine synthase), L-mimosine synthase (EC 2.5.1.52 - L-mimosine synthase), O-phosphoserine sulfhydrylase (EC 2.5.1.65 - O-phosphoserine sulfhydrylase), cysteine synthase (EC 2.5.1.76 - cysteine synthase), [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase (EC 2.5.1.113 - [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase), cystathionine β synthase (EC 2.5.1.134 - cystathionine beta-synthase), N-(2-amino-2-carboxyethyl)-L-glutamate synthase (EC 2.5.1.140 - N-(2-amino-2-carboxyethyl)-L-glutamate synthase), S-sulfo-L-cysteine synthase (EC 2.5.1.144 - S-sulfo-L-cysteine synthase), diaminopropionate ammonia-lyase (EC 4.3.1.15 - Diaminopropionate ammonia-lyase), L-serine ammonia-lyase (EC 4.3.1.17 - L-serine ammonia-lyase), D-serine ammonia-lyase (EC 4.3.1.18 - D-serine ammonia-lyase), threonine ammonia-lyase (EC 4.3.1.19 - threonine ammonia-lyase), cystathionine γ lyase (EC 4.4.1.1 - cystathionine gamma-lyase), homocysteine desulfhydrase (EC 4.4.1.2 - homocysteine desulfhydrase), L-3-cyanoalanine synthase (EC 4.4.1.9 - L-3-cyanoalanine synthase), cysteine lyase (EC 4.4.1.10 - cysteine lyase), methionine γ lyase (EC 4.4.1.11 - methionine gamma-lyase), cysteine-S-conjugate β lyase (EC 4.4.1.13 - cysteine-S-conjugate beta-lyase), D-cysteine desulfhydrase (EC 4.4.1.15 - D-cysteine desulfhydrase), selenocysteine lyase (EC 4.4.1.16 - selenocysteine lyase), L-cysteate sulfo-lyase (EC 4.4.1.25 - L-cysteate sulfo-lyase), L-cysteine desulfidase (EC 4.4.1.28 - L-cysteine desulfidase) and L-cystine β lyase (EC 4.4.1.35 - L-cystine beta-lyase).

The enzyme according to the present disclosure may be derived from any suitable organism, including prokaryotes and eukaryotes, for example but not limited to archaea, actinomycetes, bacteria, fungi, animals, plants, algae, and yeast. In some embodiments, the enzyme is derived from an organism selected from the group consisting of *Thermus*

thermophiles, *Lysinibacillus sphaericus*), *Clostridioides difficile*, *Clostridium novyi*, *Geobacillus stearothermophilus*, *Thermotoga maritima*, *Pseudomonas aeruginosa*, *Nonlabens dokdonensis*, *Leucaena leucocephala*, *Aeropyrum pernix*, *Methanosarcina acetivorans*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli*, *Candida maltosa*, *Streptomyces phaeochromogenes*, *Neurospora crassa*, *Spinacia oleracea*, *Cyanobacteria bacterium*, *Pseudomonas putida*, *Lactococcus lactis*, *Ruegeria pomeroyi*, *Methanococcus maripaludis* and *Camelina sativa*.

In some embodiments, the enzyme is selected from the group consisting of cysteine synthase derived from *Thermus thermophiles* (EC 2.5.1.47 - cysteine synthase), cystathionine γ -synthase derived from *Lysinibacillus sphaericus* (EC 2.5.1.48 - cystathionine gamma-synthase), O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridioides difficile* (EC 2.5.1.49 - O-acetylhomoserine aminocarboxypropyltransferase), O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridium novyi*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Geobacillus stearothermophilus*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Thermotoga maritima*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Pseudomonas aeruginosa*, β pyrazolylalanine synthase derived from *Nonlabens dokdonensis* (EC 2.5.1.51 - beta-pyrazolylalanine synthase), L-mimosine synthase derived from *Leucaena leucocephala* (EC 2.5.1.52 - L-mimosine synthase), O-phosphoserine sulfhydrylase derived from *Aeropyrum pernix* (EC 2.5.1.65 - O-phosphoserine sulfhydrylase), cysteine synthase derived from *Methanosarcina acetivorans* (EC 2.5.1.76 - cysteate synthase), [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase derived from *Mycobacterium tuberculosis* (EC 2.5.1.113 - [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase), cystathionine β synthase derived from *Bacillus subtilis* (EC 2.5.1.134 - cystathionine beta-synthase), N-(2-amino-2-carboxyethyl)-L-glutamate synthase derived from *Staphylococcus aureus* (EC 2.5.1.140 - N-(2-amino-2-carboxyethyl)-L-glutamate synthase), S-sulfo-L-cysteine synthase derived from *Pseudomonas fluorescens* (EC 2.5.1.144 - S-sulfo-L-cysteine synthase), diaminopropionate ammonia-lyase derived from *Escherichia coli* (EC 4.3.1.15 - Diaminopropionate ammonia-lyase), L-serine ammonia-lyase derived from *Escherichia coli* (EC 4.3.1.17 - L-serine ammonia-lyase), D-serine ammonia-lyase derived from *Escherichia coli* (EC 4.3.1.18 - D-serine ammonia-lyase), threonine ammonia-lyase derived from *Candida maltose* (EC 4.3.1.19 - threonine ammonia-lyase), cystathionine γ lyase derived from *Streptomyces phaeochromogenes* (EC 4.4.1.1 - cystathionine gamma-lyase), cystathionine γ

lyase derived from *Neurospora crassa* (EC 4.4.1.1 - cystathionine gamma-lyase), homocysteine desulphydrase derived from *Bacillus subtilis* (EC 4.4.1.2 - homocysteine desulphydrase), L-3-cyanoalanine synthase derived from *Spinacia oleracea* (EC 4.4.1.9 - L-3-cyanoalanine synthase), cysteine lyase derived from *Cyanobacteria bacterium* (EC 4.4.1.10 - cysteine lyase), methionine γ lyase derived from *Pseudomonas putida* (EC 4.4.1.11 - methionine gamma-lyase), cysteine-S-conjugate β lyase derived from *Lactococcus lactis* (EC 4.4.1.13 - cysteine-S-conjugate beta-lyase), D-cysteine desulphydrase derived from *Escherichia coli* (EC 4.4.1.15 - D-cysteine desulphydrase), selenocysteine lyase derived from *Escherichia coli* (EC 4.4.1.16 - selenocysteine lyase), L-cysteate sulfo-lyase derived from *Ruegeria pomeroyi* (EC 4.4.1.25 - L-cysteate sulfo-lyase), L-cysteine desulfidase derived from *Methanococcus maripaludis* (EC 4.4.1.28 - L-cysteine desulfidase) and L-cystine β lyase derived from *Camelina sativa* (EC 4.4.1.35 - L-cystine beta-lyase). In some embodiments, the enzyme is O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridium novyi*.

In some embodiments, the enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1-31. In some embodiments, the enzyme is a modified variant.

As used herein, the term “variant” refers to a peptide or nucleic acid which has a certain sequence identity compared to the amino acid or nucleotide sequence of a given peptide or nucleic acid.

For the present disclosure, to determine the percentage of identity between two amino acid sequences or two nucleic acid sequences, sequences are aligned for optimal comparison (for example, a gap may be introduced in the first amino acid or nucleic acid sequence for optimal alignment with the second amino acid or nucleic acid sequence). Then the amino acid residues or nucleotides at the corresponding amino acid or nucleotide positions are compared. When the position in the first sequence is occupied by the same amino acid residue or nucleotide in the corresponding position in the second sequence, these molecules are identical at that position. The identity percentage between two sequences is a function of the number of identical positions shared by the sequence (i.e., identity percentage = number of identical positions / total number of positions (i.e., overlapping positions) $\times$ 100). Preferably, these two sequences are of the same length.

Those skilled in the art know that various computer programs may be used to determine the identity between two sequences.

“Amino acid identity percentage” or “amino acid sequence identity percentage” refers to

comparison of amino acids of two polypeptides, and when the optimal comparison is made, the two polypeptides have approximately the specified percentage of identical amino acids. For example, “95% amino acid identity” means that two polypeptides have 95% identical amino acids when the two polypeptides are compared for amino acids at an optimal alignment.

In some embodiments, the enzyme comprises an amino acid sequence having at least 65% or 70%, preferably at least 75% or 80%, more preferably at least 85% or 90%, particularly preferably at least 94%, 95%, 96%, 97%, 98% or 99%, most preferably at least 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 1-31, and the enzyme has the activity of catalyzing the reaction of Formula I.

In some embodiments, the amino acid sequence of the enzyme differs from the amino acid sequence selected from the group consisting of SEQ ID NO: 1-31 in having a substitution, deletion, insertion, and/or addition of one or more amino acids, and the enzyme has the activity of catalyzing the reaction of Formula I. In some embodiments, the amino acid sequence of the enzyme differs from the amino acid sequence selected from the group consisting of SEQ ID NO: 1-31 in having a conserved substitution of one or more amino acids, and the enzyme has the activity of catalyzing the reaction of Formula I. In some embodiments, the amino acid sequence of the enzyme differs from the amino acid sequence selected from the group consisting of SEQ ID NO: 1-31 in having an insertion or a deletion of one or more amino acids, and the enzyme has the activity of catalyzing the reaction of Formula I.

The term “conserved substitution”, also known as substitution with “homologous” amino acid residue, refers to the substitution where an amino acid residue is replaced with another amino acid residue having a similar side chain, such as amino acids with basic side chain (such as lysine, arginine and histidine), amino acids with acidic side chain (such as aspartate, glutamate), amino acids with uncharged polar side chain (such as glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), amino acids with nonpolar side chain (such as alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), amino acids with β -branched side chain (such as threonine, valine, isoleucine) and amino acids with aromatic side chain (such as tyrosine, phenylalanine, tryptophan, histidine).

The conserved amino acid substitution usually has the minimal influence on the activity of the resulted protein. Such substitution is described below. The conserved substitution is the replacement of an amino acid with another amino acid having similar size, hydrophobicity, charge, polarity, spatial characteristics, aromaticity, or the like. When it is desired to finely

regulate the properties of a protein, the substitution is usually conserved.

As used herein, “homologous” amino acid residues refer to amino acid residues with similar chemical properties, which involve hydrophobicity, charge, polarity, spatial characteristics, aromatic characteristics, or the like. Examples of amino acids which are homologous to each other comprise positively charged lysine, arginine, histidine; negatively charged glutamate, aspartate; hydrophobic glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine; polar serine, threonine, cysteine, methionine, tryptophan, tyrosine, asparagine, glutamine; aromatic phenylalanine, tyrosine, tryptophan; serine and threonine with chemically similar side chain groups, or glutamine and asparagine, or leucine and isoleucine.

Examples of conservative amino acid substitutions in proteins include: Ser for Ala, Lys for Arg, Gln or His for Asn, Glu for Asp, Ser for Cys, Asn for Gln, Asp for Glu, Pro for Gly, Asn or Gln for His, Leu or Val for Ile, Ile or Val for Leu, Arg or Gln for Lys, Leu or Ile for Met, Met, Leu or Tyr for Phe, Thr for Ser, Ser for Thr, Tyr for Trp, Trp or Phe for Tyr, and Ile or Leu for Val.

III. Preparation conditions

The enzyme according to the present disclosure can be provided in the form of an isolated polypeptide.

In some embodiments, the process is a cell-free process, that is, the reaction medium in step a) comprises an isolated enzyme. The enzyme may be produced by recombination or synthesis. In some embodiments, the reaction medium is an aqueous medium, preferably an aqueous buffer, for example a phosphate buffer, such as PBS.

In some embodiments, the reaction medium in step a) comprise a host cell expressing the enzyme. In some embodiments, the process according to the present disclosure further comprises a step of culturing the host cell. In some embodiments, the reaction medium comprises a culture medium for culturing the host cell. In some embodiments, the reaction medium is an aqueous medium, preferably an aqueous buffer solution, for example a phosphate buffer solution, such as PBS.

In some embodiments, the host cell comprises an expression vector encoding the enzyme. In some embodiments, the host cell is *Escherichia coli*, for example *Escherichia coli* BL21(DE3). In some embodiments, the expression vector is a prokaryotic expression vector, for example pET-29b(+).

In some embodiments, in step b), the reaction medium is incubated at 30-40°C, 32-39°C, 34-38°C, 36-38°C or 37°C.

In some embodiments, the reaction medium has a PH of about 5-about 8, preferably about 6 – about 7.8, for example 7.5.

Examples

5 Through the following examples, those skilled in the art will understand the present disclosure more clearly. It should be noted that the examples are provided for illustrative purpose only rather than limitation to the scope of the present disclosure.

Example 1. Materials and methods

10 Unless otherwise stated, the experimental methods used herein are conventional, and specific gene cloning procedures can be found in Sambrook et al., 1989 above.

i) Reagents:

- Isopropyl- β -D-thiogalactoside (IPTG) was purchased from Beyotime Biotechnology Co., Ltd.

15 - L-homoserine was purchased from Jiangsu Aikon Biomedical Research and Development Co., Ltd.

- L-methionine was purchased from Merck Sigma-Aldrich;

- Sodium methanethiolate, phosphopyridoxal (PLP), chromatographic grade disodium hydrogen phosphate, sodium tetraborate, methanol, and acetonitrile were purchased from
20 Shanghai Aladdin Biochemical Technology Co., Ltd.

- Sodium dihydrogen phosphate, sodium hydroxide, and Triton-X100 were purchased from Sangon Biotechnology (Shanghai) Co., Ltd.

- OPA reagent was purchased from Agilent Technology (China) Co., Ltd

ii) Vectors and strains:

25 - The expression vector used in the example was pET-29b(+), purchased from Sangon Biotechnology (Shanghai) Co., Ltd.

- The host cell used in the example was *Escherichia coli* BL21(DE3), purchased from Tiangen Biochemical Technology (Beijing) Co., Ltd.

iii) Gene clone

30 The nucleic acid sequences encoding each enzyme in Table 1 were provided to Sangon Biotechnology (Shanghai) Co., Ltd. for synthesis, obtaining pET29b(+) expression vectors containing the nucleic acid sequences encoding each enzyme.

Table 1

Enzyme	EC No.	Species	NCBI-Protein ID	Amino Acid Sequence
cysteine synthase	EC 2.5.1.47	<i>Thermus thermophilus</i>	BAD70170	SEQ ID NO: 1
cystathionine γ -synthase	EC 2.5.1.48	<i>Lysinibacillus sphaericus</i>	ACA40889	SEQ ID NO: 2
O-acetyl homoserine aminocarboxypropyltransferase	EC 2.5.1.49	<i>Clostridioides difficile</i>	APU51341	SEQ ID NO: 3
O-acetyl homoserine aminocarboxypropyltransferase	EC 2.5.1.49	<i>Clostridium novyi</i>	WP_011721301	SEQ ID NO: 4
O-acetyl homoserine aminocarboxypropyltransferase	EC 2.5.1.49	<i>Geobacillus stearothermophilus</i>	ALA70660	SEQ ID NO: 5
O-acetyl homoserine aminocarboxypropyltransferase	EC 2.5.1.49	<i>Thermotoga maritima</i>	WP_004080710	SEQ ID NO: 6
O-acetyl homoserine aminocarboxypropyltransferase	EC 2.5.1.49	<i>Pseudomonas aeruginosa</i>	NP_253712	SEQ ID NO: 7
β pyrazolylalanine synthase	EC 2.5.1.51	<i>Nonlabens dokdonensis</i>	BAA05965	SEQ ID NO: 8
L-mimosine synthase	EC 2.5.1.52	<i>Leucaena leucocephala</i>	AHG97874	SEQ ID NO: 9
O-phosphoserine sulfhydrylase	EC 2.5.1.65	<i>Aeropyrum pernix</i>	BAA80586	SEQ ID NO: 10
cysteine synthase	EC 2.5.1.76	<i>Methanosarcina acetivorans</i>	AAM06667	SEQ ID NO: 11
[CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase	EC 2.5.1.113	<i>Mycobacterium tuberculosis</i>	NP_215852	SEQ ID NO: 12
cystathionine β synthase	EC 2.5.1.134	<i>Bacillus subtilis</i>	NP_390604	SEQ ID NO: 13
N-(2-amino-2-carboxyethyl)-L-glutamate synthase	EC 2.5.1.140	<i>Staphylococcus aureus</i>	BAF66332	SEQ ID NO: 14
S-sulfo-L-cysteine synthase	EC 2.5.1.144	<i>Pseudomonas fluorescens</i>	AIG05382	SEQ ID NO: 15
diaminopropionate ammonia-lyase	EC 4.3.1.15	<i>Escherichia coli</i>	BAE76937	SEQ ID NO: 16
L-serine ammonia-lyase	EC 4.3.1.17	<i>Escherichia coli</i>	BAA15621	SEQ ID NO: 17
D-serine ammonia-lyase	EC 4.3.1.18	<i>Escherichia coli</i>	BAA16229	SEQ ID NO: 18
threonine ammonia-lyase	EC 4.3.1.19	<i>Candida maltosa</i>	EMG50701	SEQ ID NO: 19

cystathionine γ lyase	EC 4.4.1.1	<i>Streptomyces phaeochromogenes</i>	WP_266761426	SEQ ID NO: 20
cystathionine γ lyase	EC 4.4.1.1	<i>Neurospora crassa</i>	AF401238_1	SEQ ID NO: 21
homocysteine desulfhydrase	EC 4.4.1.2	<i>Bacillus subtilis</i>	NP_390603	SEQ ID NO: 22
L-3-cyanoalanine synthase	EC 4.4.1.9	<i>Spinacia oleracea</i>	BAA07177.1	SEQ ID NO: 23
cysteine lyase	EC 4.4.1.10	<i>Cyanobacteria bacterium</i>	HAA27123	SEQ ID NO: 24
methionine γ lyase	EC 4.4.1.11	<i>Pseudomonas putida</i>	AAN66932	SEQ ID NO: 25
cysteine-S-conjugate β lyase	EC 4.4.1.13	<i>Lactococcus lactis</i>	ABJ72384	SEQ ID NO: 26
D-cysteine desulfhydrase	EC 4.4.1.15	<i>Escherichia coli</i>	BAA15739	SEQ ID NO: 27
selenocysteine lyase	EC 4.4.1.16	<i>Escherichia coli</i>	BAA15457	SEQ ID NO: 28
L-cysteate sulfo-lyase	EC 4.4.1.25	<i>Ruegeria pomeroyi</i>	AAV97294	SEQ ID NO: 29
L-cysteine desulfidase	EC 4.4.1.28	<i>Methanococcus maripaludis</i>	CAF31024	SEQ ID NO: 30
L-cystine β lyase	EC 4.4.1.35	<i>Camelina sativa</i>	XP_010509309	SEQ ID NO: 31

iv) Preparation of recombinant host cells

The expression vectors were transformed into *Escherichia coli* BL21(DE3) competent cells, respectively, which were spread on LB agar medium (containing 50mg/L kanamycin), incubated overnight at 37°C. Then single colonies were selected and cultured in LB broth (containing 50mg/L kanamycin) and subjected to sequencing to verify the correctness of the synthesized sequences. The verified clones were stored at -80°C for subsequent experiments.

v) Protein expression and preparation of whole cell catalysts:

The stored clones were activated on LB agar medium. Then, the single colonies were inoculated in LB broth (containing 50mg/L kanamycin) and incubated at 37°C for 12 h with shaking. 400 μ L of culture was transferred into 20 mL of fresh LB liquid medium (containing 50mg/L kanamycin) and incubated at 37°C with shaking until the OD600 reached about 0.6. IPTG (final concentration of 0.4mM) was added and incubation was conducted at 25°C for 16 h to induce protein expression.

After incubation, the cultures were centrifuged at 4000g for 10 min at 4°C, the supernatants were discarded, and the *Escherichia coli* cells were collected. The collected *Escherichia coli* cells were resuspended in precooled 15 mL of 50 mM PBS, pH 7.0 to give

Escherichia coli suspension containing the recombinant enzyme as whole cell catalysts.

vi) Preparation of enzymes

7.5 mL of *Escherichia coli* suspension prepared in v) was taken and the *Escherichia coli* cells were sonicated at 4°C. The cell fragmentation solutions were centrifuged at 4°C at 6000g for 15 min to remove the precipitates, so as to obtain the resultant supernatants as crude enzyme solutions containing the recombinant enzymes. The crude enzyme solutions were purified using AKTA protein purification system to obtain the purified enzymes.

vii) Catalyzing reaction with purified enzymes

Sodium methanethiolate and PLP were added to a solution of L-homoserine in PBS (100mM), and the solution was adjusted to pH 7.5 with phosphoric acid. In the solution, the final concentration of L-homoserine was 169.7mM, the final concentration of sodium methanethiolate was 419.74mM, and the final concentration of PLP was 0.2mM. Purified enzyme as prepared in vi) was added to the above solution and the final concentration was 8mg/mL. At 37°C, the system was shaken continuously on an oscillator (220rpm) for 12 h. The sample was taken for detecting the yield of L-methionine using OPA pre-column derivatization high performance liquid chromatography to determine the catalytic activity of the purified enzyme.

viii) Whole cell catalytic reaction

Sodium methanethiolate, PLP, and Triton X-100 were added to a solution of L-homoserine in PBS (100mM) and the solution was adjusted to pH 7.5 with phosphoric acid. In the solution, the final concentration of L-homoserine was 169.7mM, the final concentration of sodium methanethiolate was 419.74mM, the final concentration of PLP was 0.2mM, and the final concentration of Triton X-100 was 0.2%. Cell suspensions prepared in v) were added to the above solutions respectively, and the final concentrations of the whole cells were 25 OD₆₀₀. At 37°C, the system was shaken continuously on an oscillator (220rpm) for 12 h. The sample was taken for detecting the yield of L-methionine using OPA pre-column derivatization high performance liquid chromatography to determine the catalytic activity of the whole cells.

Example 2. Preparation and detection of MetY (CnMetY) from *Clostridium novyi*

The clone expressing CnMetY (SEQ ID NO: 4) was activated, purified enzyme was prepared, and the amount of L-methionine produced by catalysis of the purified enzyme was determined according to the procedures in Example 1. Upon detection, 12.13g/L of L-methionine was produced by the catalysis of purified enzyme CnMetY.

Example 3. Preparation and detection of whole cells containing sulfhydrylase (MetY) and cystathionine γ lyase (CGL)

Escherichia coli clones expressing CnMetY(SEQ ID NO: 4), CdMetY(SEQ ID NO: 3), GsMetY(SEQ ID NO: 5), TmMetY(SEQ ID NO: 6), PaMetY(SEQ ID NO: 7), NaCGL(SEQ ID NO: 21), SpCGL(SEQ ID NO: 20) were activated, whole cells were prepared, and the amounts of L-methionine produced by catalysis of the whole cells were detected according to the procedures in Example 1. The determined amounts of L-methionine produced by the catalysis of the whole cells were shown in Table 2.

Table 2

Whole cell	SEQ ID NO:	L-methionine yield (g/L)
CdMetY	3	0.51
CnMetY	4	13.81
GsMetY	5	0.47
TmMetY	6	1.07
PaMetY	7	0.11
NaCGL	21	0.17
SpCGL	20	0.39

Example 4. Preparation and detection of whole cells containing enzymes other than sulfhydrylase (MetY) and cystathionine γ lyase (CGL) in Table 1

Escherichia coli clones expressing enzymes other than sulfhydrylase (MetY) and cystathionine γ lyase (CGL) in Table 1 were activated, whole cells were prepared according to the procedures in Example 1, and the amounts of L-methionine produced by catalysis of the whole cells were detected. The catalytic performances of enzymes were evaluated according to the amounts of L-methionine produced. The catalyzing ability of enzymes were shown in Table 3 and presented by ★, more ★ indicating stronger catalyzing ability.

Table 3

Enzyme	EC No.	Species	Amino Acid Sequence	Catalytic Performance
cysteine synthase	EC 2.5.1.47	<i>Thermus thermophilus</i>	SEQ ID NO: 1	★
cystathionine γ -synthase	EC 2.5.1.48	<i>Lysinibacillus sphaericus</i>	SEQ ID NO: 2	★★★
β pyrazolylalanine synthase	EC 2.5.1.51	<i>Nonlabens dokdonensis</i>	SEQ ID NO: 8	★
L-mimosine synthase	EC 2.5.1.52	<i>Leucaena leucocephala</i>	SEQ ID NO: 9	★★

O-phosphoserine sulfhydrylase	EC 2.5.1.65	<i>Aeropyrum pernix</i>	SEQ ID NO: 10	★
cysteine synthase	EC 2.5.1.76	<i>Methanosarcina acetivorans</i>	SEQ ID NO: 11	★★
[CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase	EC 2.5.1.113	<i>Mycobacterium tuberculosis</i>	SEQ ID NO: 12	★
cystathionine β synthase	EC 2.5.1.134	<i>Bacillus subtilis</i>	SEQ ID NO: 13	★
N-(2-amino-2-carboxyethyl)-L-glutamate synthase	EC 2.5.1.140	<i>Staphylococcus aureus</i>	SEQ ID NO: 14	★
S-sulfo-L-cysteine synthase	EC 2.5.1.144	<i>Pseudomonas fluorescens</i>	SEQ ID NO: 15	★
diaminopropionate ammonia-lyase	EC 4.3.1.15	<i>Escherichia coli</i>	SEQ ID NO: 16	★★
L-serine ammonia-lyase	EC 4.3.1.17	<i>Escherichia coli</i>	SEQ ID NO: 17	★★
D-serine ammonia-lyase	EC 4.3.1.18	<i>Escherichia coli</i>	SEQ ID NO: 18	★★
threonine ammonia-lyase	EC 4.3.1.19	<i>Candida maltosa</i>	SEQ ID NO: 19	★
homocysteine desulphydrase	EC 4.4.1.2	<i>Bacillus subtilis</i>	SEQ ID NO: 22	★★★★
L-3-cyanoalanine synthase	EC 4.4.1.9	<i>Spinacia oleracea</i>	SEQ ID NO: 23	★
cysteine lyase	EC 4.4.1.10	<i>Cyanobacteria bacterium</i>	SEQ ID NO: 24	★
methionine γ lyase	EC 4.4.1.11	<i>Pseudomonas putida</i>	SEQ ID NO: 25	★★★★
cysteine-S-conjugate β lyase	EC 4.4.1.13	<i>Lactococcus lactis</i>	SEQ ID NO: 26	★★★
D-cysteine desulphydrase	EC 4.4.1.15	<i>Escherichia coli</i>	SEQ ID NO: 27	★
selenocysteine lyase	EC 4.4.1.16	<i>Escherichia coli</i>	SEQ ID NO: 28	★
L-cysteate sulfo-lyase	EC 4.4.1.25	<i>Ruegeria pomeroyi</i>	SEQ ID NO: 29	★
L-cysteine desulfidase	EC 4.4.1.28	<i>Methanococcus maripaludis</i>	SEQ ID NO: 30	★
L-cystine β lyase	EC 4.4.1.35	<i>Camelina sativa</i>	SEQ ID NO: 31	★

Sequences

SEQ ID NO: 1

MRVEGAIGKTPVVRLAKVVEPDMAEVWVKLEGLNPGGSIKDRPAWYMIKDAEE
5 RGILRPGSGQVIVEPTSGNTGIGLAMIAASRGYRLILTMPAQMSEERKRVLKAFGA
ELVLTDPERRMLAAREEALRLKEELGAFMPDQFKNPANVRAHYETTGPELYEALE
GRIDAFVYGS GTGGTITGVGRYLKERIPHVKVIAVEPARSNVLSGGKMGQHGFQG
MGPGFIPENLDLSLLDGVIVWEEDAFPLARRLAREEGLFLGMSSGGIVWAALQV
ARELGPGKRVACISPDGGWKYLS TPLYAEP

SEQ ID NO: 2

MTLNRSIETKLVQLGNLSDPTTGAVSPPIHLSTAYKHAGIGESTGFDYTRTKNPTRA
LLEAGFADLEGDMGFACSSGMAAIQLVLSIFKPGDELVVPDDLYGGTYRLFNFFQ
ETYNIKPVYSKFESVEQVEALINEHTRALFIETPTNPLMQEFDLQVYAE LAHKHGA
15 LLIVDNTFYTPYFQRPIDLGADIVLHSATKYIGGHNDVLAGIVVAKGTELAERIGFI
HNGSGMVLGAMDSWLLIRGLKTMHLRLKQHDANAKAIAAYLEEEALVTDVLYT
GKGGMLS FRLQKPEWIDPFLRNKLITFAESLGGVESFITYPATQTHADMPYEERV
ERGVCDRLLRFSVGIEEAEDLIADLRQVFDILRKEA

SEQ ID NO: 3

MDNKETICVQGNYPKNGEPRVLPLYQSTTFKYSSIDQLAELFDLKVDGHIYSRIS
NPTIQAFEEKISLLEGGVSSVAVSSGQSANMLAVLNICKSGDSILCSSKVYGGTFNL
LGPSLKKFGIDLISFDLDSSEDEIVELAKENTKVVF AETLANPTLEVIDFEKIANVAK
RINVPFIVDNSLASPVLCNPLKYGANIVTHSTTKYLDGHASSVGGIIVDGGNFNWD
25 NGKFPPELVEPDPTYHGISTYQKFGNAAYATKARVQLLRDYGNC LSPFNAYLTNLN
VETLHLRMRHSENALKIARFLEKHENVDWINYPGLEDNKYYENAKKYLSRGCS
GVLSFGVRGGLENAKKFVEKLQIASLVTHVSDVRTCVIHPASTTHRQLTEEQ LIAS
GVLP SLIRLSVG IENVEDLIADLNQALNF

SEQ ID NO: 4

MNNWKKGTICIQGGYTPKS GEPRVLPYQSTTYKYNDPDEVAALFDLKAEGHMY
SRISNPTVAAFEKIAALEGGVGALAVASGQSASTLSILNVCKSGEHVVAASTLYGG
TYSLFSTTLKKFGIDVTFVDPEATEEEILKNCRENTKAVFGETIGNPGLNVLD FEKF
SNVAKKIKAPFIVDNTIATPYLCNPLKLGANVVVHSATKYIDGHATTVGGVIIDGG
35 NFDWNNGRFPDFTEPDPSYHG IKYTETFGDSAYIVKARVQLLRDLGVCTSPFNAFL
FSLGLETLHLRMRHSDNAIKLGRFLEKHSKVSWSYPLLESHSTYETAKKYL PK
GASGILTFGVKGGVEAGKQFIRNLKLAALVVHLGDARTSVLHPASTTHRQLTEEQ
LSAGVTPDLIRVSVGIEDIEDIEDFDQALNNIK

SEQ ID NO: 5

MSNEQTFRPETLAIHAGQKPD AETGARAVPIYQTSSYVFRDSEHAANLFG LKEEGF
IYTRIMNPTNDVF EKRIA ALEGGIGALALSSGQA AVFYSIINIASAGDEIVSSSIYGG
TYNLF AHTLRKFGITVKFVDPSDPENFERAITDKTKALFAETIGNPKNDVLDIEAVA
DIAHRHAIP LIVDNTVASPYLLRPIEFGADIVVHSATKFIGGHGNSIGGVIVDSGKFD
45 WKGSGKFPEFTEPDPSYHGLVYVD AVGEAAYITKARIQLLRDLGAALSPFNAFLLL
QGLETLHLRMQRHSENALAVAKFLEEEEA VESVNYPGLPSHPSHELAKKYL PNGQ
GAIVTFEIKGGVEAGKKLIDSVKLF SHLANIGDSKSLIHPASTTHEQLSPDEQLSAG
VTPGLVRLSVGTEAIDDLDDLRQAIRQSQT VGVK

SEQ ID NO: 6

MDWKYGYNTRALHAGYEPPEQATGSRAVPIYQTSSYVFRDSDHAARLFALEEP
GFIYTRIGNPTVSVLEERIAALEEGVGALAVASGQA AITYAILNIAGPGDEIVSGSAL
YGGTYNLF RHTLYKKSGIIVKFVDETD PKNIEEAITEKTKAVYLETIGNPGLTVPDF
EAI AEIAHRHGVPLIVDNTVAPYIFRPFEHGADIVVYSATKFIGGHGTSIGGLIVDSG
55 KFDWTNGKFPPELVEPDPSYHGVS YVETFKAAIYAKCRTQLLRDLGSCMSPFNAF
LFILGLETL SLRMKKHCENALKIVEFLKSHPAVSWVNYPIAEGNKTRENALKYLKE
GYGAIVTFGVKGGKEAGKKFIDSLTLISHLANIGDARTLAIHPASTTHQQLTEEEQL

KTGVTPDMIRLSVGIEDVEDIADLDQALRKSQEG

SEQ ID NO: 7

5 MKLET LAVHAGYSPDPTTRAVAVPIYQTTSYAFDDTQHGADLFDLKVPGNIYTRIM
NPTNDVLEQRVAALEGGVGALAVASGMAAITYAIQTVAEAGDNIVSVAKLYGGTY
NLLAHTLPRIGIQARFAAHDDVAALIDERTKAVFCETIGNPAGNIIDLQALADA
AHRHGVPLIVDNTVATPVLCPFEHGADIVVHSLTKYMGGHGTSIGGIVVDSGKF
DWAANKSRFPLLNTPDPSYHGVITYTEAFGPAAFIGRCRVVPLRNMGAALSPFNAF
10 LILQGLETLALRMRHCDNALAVARYLQQHPQVAWVKYAGLADNPEHALARRYL
GGRPAAILSGIQGGSAAGARFIDALKLVVRLVNIGDAKSLACHPASTTHRQLNAE
ELARAGVSDDMVRLSIGIEHIDDILADLDQALAAAR

SEQ ID NO: 8

15 MSNNIKAYNNVLELIGETPLIKLSQTVASFPGNYYAKAEAFNPGHSSKDRIALHIE
QAEKKGILKPGDTIETTSGNTGFSIAMVSRIKGYDCILAVSSKSSADKIDMLKSMG
AKVYVCPANVSADDPRSYYQVAKRLHDEIKNSIYINQYFNDLNTAHYLTGPEI
WNQTAGEITHLVACSGTGGTISGTARFLKEQNPNIKILGVDAYGSVLKKYHETQEF
DKDEIYPYRIEGLGKNLIPTATDFDVIDSFTKVKDEDAAHMARKISQTEGLFVGYT
20 SGAAQAVKQLVVEGEFDKNSNVVIFPDHGSRYMSKIYNDQWMQEQGFVDSK
STANEQHIEYIK

SEQ ID NO: 9

25 MAVEWGTGIANDVTELVGKTPLVYLNKVVDGCVGRIVAKLEFMEPLSSVKDRIGYS
MIADAEKGLITPGKSVLIEPTSGNTGIGLAFTAAAKGYRLIIVMPASMTLERRMVL
KALGAELVLTDPKIRGAFQKVEEYAKTPNSYVLQQFENPSNPKMHYDTTGPEI
WKATGGKIDALVAGIGTGGTITGSGKFLKEQNPNIKVCAVEPAESPVLVGGQPGSH
GIYGIGAGFIPKVVDVSLLEVVHVTTSEAIETAKLLAVKEGLFVGVSSGAAAAAA
IKVAKRPEMAGKLIVTIFPSCGERYLSSALFESIREESENMTFEP

SEQ ID NO: 10

30 MVFPSPLDFFERFGKPTPLVRSRLQLPNGVRVWLKLEWYNPFSLSVKDRPAVEIISRL
SRRVEKGSVLADATSSNFGVALSAVARLYGYRVRVYLPGAEEFGKLLPRLLGAQV
IVDPEAPSTVHLLPRVMKDSKNEGFVHVNQFYNDANFEAHMRGTAREIFVQSRRG
GLALRGVAGSLGTSGHMSAAAFYLQSVDPISRAVLVQPAQGDSIPGIRRVETGML
35 WINMLDISYTLAEVTL EEAMEAVVEVARSDGLVIGPSGGA AVKALAKKAAEGDLE
PGDYVVVVV PDTGFKYLSLVQNALEGAGDSV

SEQ ID NO: 11

40 MGRFILKCLKCGREYSQEYRLTCENDDSFLRAEYLEKKLELRKQPGIGRFHSHWLP
VQEELTTEAGPITYKSEALARELGLSNLYIGFSGYWPEKGAFIKTCSFKELEAHPTM
QLLKESGGKAIVLASAGNTGRAFAHVSA LTGTDVYIVVPDSGIPKLWLPEEPTDSI
HLISMTPGNDYTDAINLAGRIAKLPGMVPEGGARNVARREGMGTVMLDAAVTIG
KMPDHYFQAVGSGTGGISAW EASLRLREDGRFGSKLPKLQLTQNLPFVPMYNAW
QEGRRDIPEIDMKDAKKRIETYATVLTNRAPPYSVTGGLYDALVDTDGIMYAVS
45 KEEALDAKALFESLEGIDILPPSAVAAA SLLKAVEAGNVGKDDTILLNIAGGGFKRL
KEDFTLFQIEPEITVSNPDVPLEELKL

SEQ ID NO: 12

50 MTRYDSLLQALGNTPLVGLQRLSPRWDDGRDGPHVRLWAKLEDNRNPTGSIKDRPA
VRMIEQAEADGLLRPGATILEPTSGNTGISLAMAARLKGYRLICVMPENTSVERRQ
LLELYGAQIIFSAAEGGSNTAVATAKELAA TNPSWVMYLYQYGNPANTDSHYCGTGP
ELLADLPEITHFVAGLGTGTLMGTGRFLREHVANVKIVAAEPYRGEGVYALRNM
DEGFVPELYDPEILTARYSVGAVDAVRRTREL VHTEGIFAGISTGAVLHAALGVGAG
ALAAGERADIALVVADAGWKYLSTGAYAGSLDDAETALEGQLWA

SEQ ID NO: 13

MTVITDITELIGNTPLLRLKNFDVPEGVAVYAKLEMMNPGGSIKDRLGDMLIRDAL

DSGKVKPGGVIIETAGNTGIGLALSARKYGLKAIFCVPEHFSREKQQIMQALGASI
IHTPRQDGMQGAIQKAIQLETEIENSYCVLQFKNRVNPSTYYKTLGPEMWEALDG
NIHTFVAGAGSGGTFAGTASFLKEKNPAVKTVIVEPVGSILNGGEPHAHKTEGIGM
EFIPDYMDKSHFDEIYTVTDENAFRLVKEAAEKEGLLIGSSSGAALYAAL EEAKKA
5 SAGTNIVTVFPDSSDRYISKQIYEGGI

SEQ ID NO: 14

MVQLHQLFPKHEVFAKLEYMNPGGSMKDRPAKYIIHGIKHGLITENTH LIESTSG
NLGIALAMIAKIKGLKLT CVVDPKISPTNLKIIKSYGANVEMVEEPDAHGGYLMTR
10 IAKVQELLATIDDAYWINQYANELNWQSHYHGAGTEIVETIKQPIDYFVAPVSTTG
SIMGMSRKIKEVHPNAQIVAVDAKGSVIFGDKPINRELPGIGASRVPEILNRSEINQV
IHVDDYQSALGCRKLIDYEGIFAGGSTGSIIAAIEQLITSIEEGATIVTILPDRGDRYL
DLVYSDTWLEKMKS RQGVKSE

SEQ ID NO: 15

MTLQYPTIADCVGNTPLVRLQRMAGNTSNTLLLKLEGN NPAGSVKDRPALSMITR
AELRGQIKPGDTLIEATSGNTGIALAMAAA IKG YKMILIMPDNGSAERKAAMTAY
GAELLLVTPEEGMEGARDLAERMAAEGRGQVLDQFANGDNPEAHYTSTGPEIWR
QTQGTITHFVSSMGTTGTIMGNSRYLKEQNPAIQIVGLQPMEGAAIPGIRRW PQEY
20 LPKIYNAARVDRIIDMAQREAE DTT RRLAREEGIFCGVSSGGAVAGMLRLSAELEN
AVIVAIICDRGDRYLSTGIFDAPN

SEQ ID NO: 16

MSVFLSKIDIADNKKFFNGETSPLFSQSQA K LARQFHQKIAGYRPTPLCALDDL ANL
25 FGVKKILVKDESKRFLN A FKM LGGAYAIAQLLCEKYHLDIETLSFEHLKNAIGEK
MTFATTTDGNHGRGVAAWAAQQLGQNAVIMPKGSAQERVDAILNLGAECIVTDM
NYDDTVRLTMQHAQQHGW EVVQDTAWEGYTKIPTWIMQGYATLADEAVEQMR
EMGVTPTHVLLQAGVGAMAGGVLGYLVDVYSPQNLHSIIVEPDKADCIYRSGVK
GDIVNVGGDMATIMAGLACGEPNPLGWEILRNCATQFISCQDSVAALGMRVLGNP
30 YGNDPRIISGESGAVGLGVLA AVHYHPQRQSLMEKLALNKDAVVLVISTEGD TDV
KHYREV V WEGKHAVAP

SEQ ID NO: 17

MISAFDIFKIGIPSSSHTVGP MNAGKSFIDRLESSGLLTATSHIVVDLYGSLSLTGK
35 GHATDVAIIMGLAGNSPQDVVIDEIPAFIELVTRSGRLPVASGAHIVDFPVAKNIIFHP
EMLPRHENGMRITAWKGQEELLSKTYYSVGGGFIVEEEHFGLSHDVETSVPYDFH
SAGELLMCDYNGLSISGLMMHNELALRSKAEIDAGFARIWQVMHDGIERGMNT
EGVLPGPLNVPRRAVALRRQLVSSDNISNDPMNVIDWINMYALAVSEENAAGGRV
VTAPTNGACGIIPAVLAYYDKFRRPV NERSIARYFLAAGAIGALYKMNASISGAEV
40 GCQGEIGVACSM AAAGLT ELLGGSPAQVCNAAE IAMEHNLGLTCDPVAGQVQIPC
IERNAINAVKAVNAARMAMRRTSAPRVSLDKVIETMYETGKDMNDKYRETSRGG
LAIKVVCG

SEQ ID NO: 18

MENAKMNSLIAQYPLVKDLVALKETTWFNPGTTS LAEGLPYVGLTEQDVQDAHA
45 RLSRFAPYLAKAFPETAATGGIIESELVAIPAMQKRLEKEYQQPISGQLLLKKDSHLP
ISGSIKARGGIYEVLAHA EKLAL EAGLLTDDDYSKLLSPEFKQFFSQYSIAGSTG
NLGLSIGIMSARIGFKVT VHMSADARAWKKAKLRSHGVT VVEYEQDYGVAVEEG
RKA AQSDPNCFFIDDENSRTLFLGYSVAGQRLKAQFAQQGRIVDADNPLFVYLPC
50 GVGGGPGGVAFGLKLAFGDHVHCFFAEP THSPCMLLGVHTGLHDQISVQDIGIDN
LTAADGLAVGRASGFVGRAMERLLDGFYTLSDQTM YDMLGWLAQEEGIRLEPSA
LAGMAGPQRVCASVS YQQMHGFSAEQLRNTTHLVWATGGGMVPEEEMNQYLA
KGR

SEQ ID NO: 19

MSIVRLYTARQLPRYRPLRFVRFNSTQVLS PKQRWTELKDTDFIKDSNGEEQPDYV
KLILTSRVYDVVDDAGSPLTYAINLSHRCGTNVY LKREDLLPVFSFKLRGAYNMIA

HLHSNSPTPVPGVIACSAAGNHAQGVAFSANKLNIPATIVMPTPTPSIKYTNVSRLGS
QVVLYGDDFDSAQECARLSAEQNLIDIPFNHPYVIAGQGTVALEITRQLRLDKL
DAIFVPVGGGLIAGIAVYLKQIAPHVKIIGVETYDADAMYQSLKNHKSTVLDKV
GLFADGTAVKVLGDETWRLAKDLVDEVVLVTTDELCAAIKDIFEDTRSITESGAL
5 SVAGLKRYIEDHSEIDHKGKTYVPVLSGANMNFDRLRVSERAVLGEGREVS LAV
TIPEQPGFAKLQKLINPRAITEFSYRTSSEPTANIFVSFNVIDKKKEKALVINAMTE
AGYKVVDISENELAKSHGRYLVGGKSSIMVMILVKSCVDSISQKDSKKNSNS

SEQ ID NO: 20

10 MTDSVTGAGDGTRAVRAGLPEPVKHEPTLPGPVFAAHFHLPGDPTGPYTYGRDE
NPTWTHLERAIAGELEAPGRDDVETLVFASGMAAISAVLFSQLSAGDTVVL PDDGY
QVPLPLVRAQLEAYGVEVRTAPTGGDAQLDVLDGAKLLWIETPSNPGLDVCDVRR
VEAAHARGCLVAVDNTLATPLGQRPELGADFSVASGTKQLTGHGDILLGYVTGG
DAAAMASVRRWRKIVGAIPGPMEAWLAHRSIATLQLRVDRQNANALVIAEALRD
15 RHEDLGVRYPLPGDP SHKIASQQMRRFGCVVSFTLPTRARADRFLDALRLVDDA
TSFGGVRSTAERRGRWGDAVPEGFIRFSAGAEDPQDLVADVLRALNESSQPTPSE

SEQ ID NO: 21

20 MTASSDHPITTPPRAPSPVHGFGTLAVHAGSPHDPATGAVIEAISLSTTFAQTAVGKP
VGEFEYSRSSNPNRANFEKMVAALEHAKYALAYSSGSATTANILQSLAAGSHVISV
SDVYGGTHRYFTQVAKAHGVKVTFTPEIEVDIRDHITDATKLVWIETPSNPTRLRV
DIRAVATAAHERGILVVVDNTFLSPYVQNPLDHGADIVVHSVTKYINGHSDVVMG
VAAFNSDELYARLSFLQNAIGAVPSAFDSWLAHRGAKTLHLRAREATTNATAIAHA
LEASPLVISVNYPGLESHPHRAIALKQHRNGMGGGMLSFRIHGGHAAAEKFCQYT
25 KIFTLAESLGGVESLCEIPSSMTHAGIPKAQREAVGIFDDLVRISCGVEDAEDLKAD
VLQALERA VADAANGVSNGVNGTH

SEQ ID NO: 22

30 MKKKTLMIHGGITGDEKTGAVSVPIYQVSTYKQPKAGQHTGYEYSRTANPTRTAL
EALVTELESGEAGYAFSSGMAAITAVMMLFNSGDHVVLTDDVYGGTYRVMTKVL
NRLGIESTFVDTSSREEVEKAIRPNTKAIYIETPTNPLLKITDLTLMADI AKKAGVLL
IVDNTFNTPYFQQPLTLGADIVLHSATKYLGGHSDVVGGLVVTASKELGEELHFVQ
NSTGGVLGPQDSWLLMRGIKTLGLRMEAIDQNARKIASFLENHPAVQTLYYPGSS
NHPGHELAKTQGAGFGGMISFDIGSEERVDAFLGNLKLFTIAESLGAVESLISV PAR
35 MTHASIPRERRLELGITDGLIRISVGIEDAEDLLEDIGQALENI

SEQ ID NO: 23

40 MATVSSCLLRRTASRIFKTSLRCFSTTSSSAQTVSGSSPFPFTGTNIKTNVSQ LIGR
TPLVYLSKISEGSGAYIAVKQEMMQPTASVKDRPALAMIEDAEKKGLISPGKTVLIE
PTSGNMGISMAFMAAMKGYKMVLTPSYTSMERRVVMRAFGADLILTD PDKGM
GGTVKKANQLLDSTPDGFMLQQFNNPANTQVHFETTGP EIWEDTQ GKVDIFVMG
IGSGGTVSGVG RYLSQNPVVKIYGVEPAESNILNGGKPGPHLITGNGVGFKPEIL
DMDVMDAVLEVKSDDAVKMARQLALQEGLLVGISSGANTIAALDLAKRPENKG
45 KLIVTIHPSFGERYLSSALFKELREEAENMQPVPVE

SEQ ID NO: 24

50 MSGIYPAPVRLESHRQQFPALANKAYFNYGGQGTLPQASLEAIQQAYEYIQRHGP
SAGVNAWIIIEANKTREAIASELGAPPEAIAITEDVTVCNIA MWGINWQAGDHL
LLTDCEHPSVIATAQEIARRFDVEVSTCPILSTLNQGDPTAVIAQHLRTQTRLVVL SH
ILWNTGQVLPVKEIVEVC RNYSSSSQTIRVLVDAAQSVGCLPLNLTELGADFYAFT
GHKWWCGPEGIGGLYVRPDAWESLNPTFIGWRGIVSNQAGKPTGWKPNAQRFEV
ATSAYPLYAGVRSIAITHQQWGTPQERYQQICQISEYLVQRLSQLDFVKCLRTSPP
QAGLVSFVLTNEHSHQQLVKSLEQQNLMLRTILD PNCVRACVHYFTQHAEIDKLIE
55 AMKKIKSKG

SEQ ID NO: 25

MRDSSHNTGFSTRAIHHGYDPLSHGGALVPPVYQTATYAFPTVEYGAACFAGEEP

GHFYSRISNPTLALLEQRMASLEGGEAGLALASGMGAITSTLWTLRLPGDELIVGR
TLYGCTFAFLHHGIGEFVGVKVRHVDLNDKALKAAISSKTRMIYFETPANPNMQL
VDIAAVVEAVRGHDVHVVDNTYCTPYLQRPLELGADLVVHSATKYLSGHGDIT
AGLVVGRKVLVDRIEGLKDMTGAVLSPHDAALLMRGIKTLALRMDRHCANA
5 QQVAEFLVRQPQVELIHYPGLPSFAQYALAQRMRLPGGMIAFELKGGIEAGRFR
MNALQLFARAVSLGDAESLAQHPASMTSSYTPQERAHHGISEGLVRLSVGLEDV
EDLLADVEQALQACC

SEQ ID NO: 26

10 MTSLKTKVIHGGISTDRTTGAVSVPIYQTSTYKQNGLGKPKKEYEYSRSGNPTRHAL
EELIADLEGGVQGFASFSSGLAGIHAVLSLFSAGDHILADDVYGGTFRLMDKVLT
TGHIYDLVDLSNLEDLKAFAKAEKAVYFETPSNPLKVLDIKEISSIAKAHNALTLV
DNTFATPYLQQPIALGADIVLHSATKYLGGHSDVVAGLVTTNSNELASEIGFLQNSI
GAVLGPQDSWLVRQGIKTLALRMEAHSAQAQIAEFLEASQAVSKVYYPGLVNH
15 EGHEIAKKQMTAFGGMISFELTDENAVKNFVENLRYFTLAESLGGVESLIEVPAVM
THASIPKELREEIGIKDGLIRLSVGVEALEDLTDLKEALEKE

SEQ ID NO: 27

20 MPLHNLTRFPRLEFIGAPTPLEYLPRFSDYLGREIFIKRDDVTPMAMGGNKLRLKLE
FLAADALREGADTLITAGAIQSNHVRQTAATAVAAGLGLHCVALLNPITTAENYLT
NGNRLLLDLNFNTQIEMCDALTDPNALQLEELATRVEAQGFRPYVIPVGGSNALGAL
GYVESALEIAQQCEGAVNISSVVVASGSAGTHAGLAVGLEHLMPESELIGVTVSRS
VADQLPKVVNLQQAIAKELELTASAEILLWDDYFAPGYGVPNDEGMEAVKLLARL
EGILLDPVYTGKAMAGLIDGISQKRFKDEGPILFIHTGGAPALFAYHPHV

SEQ ID NO: 28

25 MIFSVDKVRADFPVLSREVNGLPLAYLDSAASAQKPSQVIDAEAEFYRHGYAAVH
RGIHTLSAQATEKMENVRKRASLFINARSAEELVFVRGTTEGINLVANSWGNNSNR
AGDNIISQMEHHANIVPWQMLCARVGAELRVIPLNPDGTLQLETLPTLFDEKTRL
30 LAITHVSNVLGTENPLAEMITLAHQHGAKVLVDGAQAVMHHPVDVQALDCDFY
VFSGHKLYGPTGIGILYVKEALLQEMPPWEGGSMIATVSLSEGTTWTKAPWRFE
AGTPNTGGIIGLGALEYVSALGLNNIAEYEQNLMHYALSQLESVPDLTLYGPQNR
LGVIAFNLGKHHAYDVGSFLDNYGIAVRTGHHCAMPLMAYYNVPAMCRASLAM
YNTHEEVDRLVTGLQRIHRLLG

SEQ ID NO: 29

35 MHLARYPRRFIAHLPTPLERLDRLTAELGGPEIWIKRDDCTGLSTGGNKTRKLEFL
MAEAELOQADMVMTQGATQSNHARQTAFAAKLGMDCHILLEDRTGSNNANY
NNNGNVLLDHLHGATTEKRPGSGLDMNAEMEKVAEKFRADGRKVYTIPTGGGSNP
40 TGALGYVNCAFEMLNQFNERGLKVDHIVHATGSAGTQAGLITGLQAMNAQIPLL
GIGVRAPKPKQEENVYNLACATAEKLGCPCGVVAREDDVANTDYVGEYGIPTESG
LEAIRMFAELEAILLDPVYSAKGAAGFIDLIRKGFHKKGERVVFLHTGGAVALFGY
DNAFDYSGRWVA

SEQ ID NO: 30

45 MDDYKRILITKILKNEVTEALGCTEVGLIGYAVSLCNISDPFSIEKIELTLNNGSFKN
AYAVGVPNTKKYGILPAVVGGLLGDHKNKLLVFNGIKYSQKLEDFIKERLKRIVINS
PLYCGVKIKDNSGNTFESLIKDNHLNVVIPKINNKLISEINGSEKEEYKNLELLDFLE
YIDEIPEEIIQLVEKTIYTNNNLIKGDFLNFNDCLSNMVNKTTSACNTRMIGENMP
50 AMSVAKSGNMGIMATLPIIAYDYSNEQNQEKLKILLSVLVTIYATYKSSYLSSMC
GCVSKGGMGAVIGLCYYKNGKNIKLDSAARTFTANLPGIICDGGKVGKALKLAS
GCFAAYSSLFVDISYENGIVGKNFKECVENISEISKIMGDLDSDIVKIMSKKEI

SEQ ID NO: 31

55 MANDRSVDWKFSGSEAAKEASAASLGTYSSKLFALCDPLGKPIPPRSKSAETSHT
AEKAVVEAVLCGTGNAYAPSLGLPVARSAVAEYLNRLPKKLTADDVFMVVGK
QAIELTVDILAKPKANVLLPKPGFPWDVVRTIYKNLEVRKYDFIPEQNYEIDFDSV

RALVDENTFAIFIINPHNPNGNTYSEVHLKKLAELARELRIMVVSDEVFRWTVFGS
NPFVPMGKFSSIVPVVTLGSISKGWIVPGWRTGWVALHDLDGVFKCTKVLTAAKQ
FLVINSKPPTVIQAALPTILENTPEEFFHQRQKFLKEKADFAYSKLKEIPTLTCLYP
EACTFLWTELNLSRFADIKDDEEFCEKLATEENLVLLPGIAFGLKNWARHSIDMEIS
5 TLEDALGRLKSFCERHSIIIEVPLKDVNGVK

10

Claims

1. A process for preparing L-methionine, comprising steps of:

a) providing a reaction medium, comprising L-homoserine or a salt, an ester, an amide or an anhydride thereof, a methylthio donor, and an enzyme selected from the group consisting of a transferase which transfers alkyl other than methyl or aryl, ammonia lyase, and a lyase which catalyzes the cleavage of a carbon-sulfur bond;

b) incubating the reaction medium to produce a reaction product comprising L-methionine or a salt, an ester, or an amide thereof; and

optionally,

c) recovering the L-methionine or the salt, the ester, or the amide thereof from the reaction medium.

2. The process according to claim 1, wherein

the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.-, EC 4.3.1.- and EC 4.4.1.-.

3. The process according to claim 1 or 2, wherein

the reaction medium further comprises phosphopyridoxal or a salt thereof.

4. The process according to any one of claims 1-3, wherein

the enzyme is selected from the group consisting of enzymes with the EC numbers of EC 2.5.1.47, EC 2.5.1.48, EC 2.5.1.49, EC 2.5.1.51, EC 2.5.1.52, EC 2.5.1.65, EC 2.5.1.76, EC 2.5.1.113, EC 2.5.1.134, EC 2.5.1.140, EC 2.5.1.144, EC 4.3.1.15, EC 4.3.1.17, EC 4.3.1.18, EC 4.3.1.19, EC 4.4.1.1, EC 4.4.1.2, EC 4.4.1.9, EC 4.4.1.10, EC 4.4.1.11, EC 4.4.1.13, EC 4.4.1.15, EC 4.4.1.16, EC 4.4.1.25, EC 4.4.1.28 and EC 4.4.1.35.

5. The process according to any one of claims 1-4, wherein

the enzyme is derived from an organism selected from the group consisting of *Thermus thermophiles*, *Lysinibacillus sphaericus*, *Clostridioides difficile*, *Clostridium novyi*, *Geobacillus stearothermophilus*, *Thermotoga maritima*, *Pseudomonas aeruginosa*, *Nonlabens dokdonensis*, *Leucaena leucocephala*, *Aeropyrum pernix*, *Methanosarcina acetivorans*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli*, *Candida maltosa*, *Streptomyces phaeochromogenes*,

Neurospora crassa, *Spinacia oleracea*, *Cyanobacteria bacterium*, *Pseudomonas putida*, *Lactococcus lactis*, *Ruegeria pomeroyi*, *Methanococcus maripaludis* and *Camelina sativa*.

6. The process according to any one of claims 1-5, wherein

the enzyme is selected from the group consisting of cysteine synthase derived from *Thermus thermophilus*, cystathionine γ -synthase derived from *Lysinibacillus sphaericus*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridioides difficile*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Clostridium novyi*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Geobacillus stearothermophilus*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Thermotoga maritima*, O-acetyl homoserine aminocarboxypropyltransferase derived from *Pseudomonas aeruginosa*, β pyrazolylalanine synthase derived from *Nonlabens dokdonensis*, L-mimosine synthase derived from *Leucaena leucocephala*, O-phosphoserine sulfhydrylase derived from *Aeropyrum pernix*, cysteine synthase derived from *Methanosarcina acetivorans*, [CysO sulfur-carrier protein]-thiocarboxylate-dependent cysteine synthase derived from *Mycobacterium tuberculosis*, cystathionine β synthase derived from *Bacillus subtilis*, N-(2-amino-2-carboxyethyl)-L-glutamate synthase derived from *Staphylococcus aureus*, S-sulfo-L-cysteine synthase derived from *Pseudomonas fluorescens*, diamminopropionate ammonia-lyase derived from *Escherichia coli*, L-serine ammonia-lyase derived from *Escherichia coli*, D-serine ammonia-lyase derived from *Escherichia coli*, threonine ammonia-lyase derived from *Candida maltosa*, cystathionine γ lyase derived from *Streptomyces phaeochromogenes*, cystathionine γ lyase derived from *Neurospora crassa*, homocysteine desulphydrase derived from *Bacillus subtilis*, L-3-cyanoalanine synthase derived from *Spinacia oleracea*, cysteine lyase derived from *Cyanobacteria bacterium*, methionine γ lyase derived from *Pseudomonas putida*, cysteine-S-conjugate β lyase derived from *Lactococcus lactis*, D-cysteine desulphydrase derived from *Escherichia coli*, selenocysteine lyase derived from *Escherichia coli*, L-cysteate sulfo-lyase derived from *Ruegeria pomeroyi*, L-cysteine desulfidase derived from *Methanococcus maripaludis* and L-cystine β lyase derived from *Camelina sativa*.

7. The process according to any one of claims 1-6, wherein

the enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1-31.

8. The process according to any one of claims 1-7, wherein the process is a cell-free process.
9. The process according to any one of claims 1-7, wherein the reaction medium in step a) comprises a host cell expressing the enzyme.
10. The process according to claim 9, wherein the process further comprises a step of culturing the host cell.
11. The process according to any one of claims 1-10, wherein in step b), the reaction medium is incubated at 30-40°C, 32-39°C, 34-38°C, 36-38°C or 37°C.