

NON-PATENT DOCUMENT

[0013] Non-Patent Document 1: Iwamoto et. al, Analyst., 2014, 139(3), 576-80.

Non-Patent Document 2: Iwamoto et. al, Anal. Methods, 2015, 21, 9177-
5 9183.

Non-Patent Document 3: Iwamoto et. al, Drug Metabolism and Pharmacokinetics, 2016, 31, 46-50.

Non-Patent Document 4: Iwamoto et. al, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci., 2016, 1023-1024, 9-16.

10 Non-Patent Document 5: Iwamoto et. al, Bioanalysis, 2016, 8(10), 1009-1020.

Non-Patent Document 6: Iwamoto et. al, Biol. Pharm. Bull., 2016, 39(7), 1187-1194.

15 SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0014] As described above, recombinant trypsin exhibits high substrate specificity, and the usability thereof has been confirmed also in the nSMOL method in which an antibody is site-specifically cleaved. However, since recombinant
20 trypsin is expressed with yeast, the production quantity thereof is small. Moreover, since recombinant trypsin needs to be purified by gel filtration chromatography after purification by affinity chromatography, the purification efficiency is low. For these reasons, it is difficult to secure a sufficient supply amount of recombinant trypsin, and moreover, recombinant trypsin is very expensive. Accordingly,
25 recombinant trypsin is largely used for research purposes such as structural analysis of proteins, and industrial use thereof is limited. An objective of the present invention is providing a method for preparing a sample for mass spectrometry for performing quantitation of the concentration of an antibody in blood, etc. using animal-derived trypsin of which a sufficient supply amount can be
30 secured and that is inexpensive.

MEANS FOR SOLVING THE PROBLEMS

[0015] The present disclosure relates to a method for preparing peptide

fragments in which an antibody, which includes a Fc domain immobilized in pores of a porous body, is brought into contact with a protease immobilized on the surfaces of microparticles, to site-specifically cleave the Fab domains of the antibody with the protease. The present disclosure further relates to a kit for preparing peptide
5 fragments used for the above method. The kit of the present disclosure includes microparticles with a protease immobilized on their surfaces.

[0015a] In an aspect, there is provided a method for preparing peptide fragments, the method comprising bringing an antibody, which includes a Fc domain immobilized in pores of a porous body, into contact with protease immobilized on
10 surfaces of microparticles, to site-specifically cleave Fab domains of the antibody with the protease, wherein the protease is animal-derived trypsin, which has chymotrypsin mixed therein, and the chymotrypsin is inactivated, and an amino group in a lysine residue of the trypsin is chemically modified.

[0015b] In another aspect, there is provided a kit for preparing peptide fragments
15 for use in the method as defined herein, wherein the kit comprises microparticles on surfaces of which protease is immobilized, the protease is animal-derived trypsin having chymotrypsin mixed therein, and the chymotrypsin is inactivated, and an amino group in a lysine residue of the trypsin is reductive alkylated.

[0015c] In another aspect, there is provided a method for preparing protease for
20 use in the method as defined herein, comprising the steps of:

inactivating chymotrypsin mixed in animal-derived trypsin by allowing the trypsin to react with a serine protease inhibitor; and

alkylating an amino group in a lysine residue of the trypsin by reductive alkylation using an alkylating agent and a reducing agent,

25 wherein the total amount of the alkylating agent used for the reductive alkylation is 200 mole times or more that of the trypsin.

[0016] The protease immobilized on the surfaces of microparticles is animal-derived trypsin, which has chymotrypsin mixed therein, in which the chymotrypsin is inactivated. The trypsin immobilized on the surfaces of microparticles has a
30 chemically-modified amino group in its lysine residue.

[0017] The chymotrypsin is irreversibly inactivated by allowing N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) to bind to its histidine residue that is

the active center, for example. The amino group in the lysine residue of the trypsin is reductive alkylated, for example.

[0018] The porous body is preferably capable of selectively immobilizing the Fc domain of an antibody in its pores. For example, a porous body coated with protein A and protein G is used.

[0019] The above-described protease can be prepared by altering animal-derived trypsin. The trypsin is altered by being subjected to treatment with a serine protease inhibitor and reductive alkylation, for example. The trypsin after the inactivation of chymotrypsin and the reductive alkylation is preferably purified by affinity chromatography.

[0020] The chymotrypsin is inactivated by allowing the animal-derived trypsin to react with the serine protease inhibitor. As the serine protease inhibitor, one that selectively deactivates chymotrypsin while not deactivating trypsin is preferable, and the above-described TPCK is preferably used.

[0021] By the reductive alkylation using an alkylating agent and a reducing agent, the amino group in the lysine residue of the trypsin is alkylated, suppressing autolysis of trypsin. The total amount of the alkylating agent used for the reductive alkylation is preferably 200 mole times or more that of the trypsin. The total amount of the reducing agent used for the reductive alkylation is also preferably 200 mole times or more that of trypsin. As the alkylating agent, aldehyde is preferable. By using formaldehyde as the alkylating agent, the amino group in the