

conjunction with LC-MS. Applying limited proteolysis using endoproteinase Lys-C resulted in the predominant cleavage C-terminal of this lysine residue.

Summary

5 It has been found that for the determination of light chain mispairing in a multispecific antibody limited proteolysis of the (multispecific) antibody has to be performed prior to MS analysis as the determination of light chain mispairing in a complete multispecific antibody is hampered by isobaric mass interference.

10 Thus, As reported herein is the use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the analysis of the multispecific antibody's light chain pairing.

One aspect as reported herein is the use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the analysis of the multispecific antibody's light chain pairing, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.

15 As reported herein is the use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the determination of light chain mispairing in the multispecific antibody.

20 One aspect as reported herein is use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the determination of light chain mispairing in the multispecific antibody, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.

As reported herein is the use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell.

25 One aspect as reported herein is use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.

30 In one embodiment the analysis/determination/selection is by using the result of a mass spectrometry of the digested antibody.

As reported herein is a method for the determination of the light chain pairing in a multispecific antibody comprising the following steps:

- a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,
- 5 b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
- c) determining from the results in step b) the light chain pairing of the multispecific antibody.

10 One aspect as reported herein is a method for the determination of the light chain pairing in a multispecific antibody comprising the following steps:

- a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
- b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
- 15 c) determining from the results in step b) the light chain pairing of the multispecific antibody.

As reported herein is a method for the determination of light chain mispairing of a multispecific antibody comprising the following steps:

- a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,
- 5 b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
- c) determining from the results in step b) the light chain pairing of the multispecific antibody and thereby determining light chain mispairing.

10 One aspect as reported herein is a method for the determination of light chain mispairing of a multispecific antibody comprising the following steps:

- a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
- b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
- 15 c) determining from the results in step b) the light chain pairing of the multispecific antibody and thereby determining light chain mispairing.

As reported herein is a method for the selection of a recombinant mammalian cell producing a multispecific antibody comprising the following steps:

- 20 a) individually incubating a sample comprising a multispecific antibody produced by a clonal population of a recombinant mammalian cell of a multitude of recombinant mammalian cells all cells of the multitude producing the same multispecific antibody with a proteolytic enzyme for a limited time,
- b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry,
- 25 c) determining from the results in step b) the presence of light chain mispairing of the multispecific antibody for each clonal cell population, and
- d) selecting based on the results in step c) a recombinant cell producing a multispecific antibody.
- 30

One aspect as reported herein is a method for the selection of a recombinant mammalian cell producing a multispecific antibody comprising the following steps:

- 5 a) individually incubating a sample comprising a multispecific antibody produced by a clonal population of a recombinant mammalian cell of a multitude of recombinant mammalian cells all cells of the multitude producing the same multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
- b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry,
- 10 c) determining from the results in step b) the presence of light chain mispairing of the multispecific antibody for each clonal cell population, and
- d) selecting based on the results in step c) a recombinant cell producing a multispecific antibody.

15 In one embodiment the proteolytic enzyme is selected from the group consisting of Lys-C, plasmin, Asp-N, Arg-C, Glu-C, Ides, pepsin and chymotrypsin.

In one embodiment the multispecific antibody is a bivalent antibody.

In one preferred embodiment the proteolytic enzyme is Lys-C or plasmin.

5 In one embodiment the multispecific antibody is a tri- or tetravalent antibody. In one embodiment the multispecific antibody is a full length antibody comprising two light chains and two heavy chain whereby to the C-termini of the heavy chains a moiety independently of each other selected from the group consisting of no moiety, an Fv fragment, a Fab fragment, a scFv fragment and a scFab fragment is conjugated either directly or via a peptidic linker.

In case the antibody is a tetravalent antibody wherein also binding sites are attached to the C-termini of the heavy chains a double digestion with two proteolytic enzymes might be required.

10 In one embodiment the multispecific antibody is a trivalent or tetravalent multispecific antibody and the method comprises a double digestion by incubating with a mixture of two proteolytic enzymes.

In one embodiment the mixture of proteolytic enzymes is plasmin and Ides. In one embodiment the mixture of proteolytic enzymes is Lys-C and Ides.

15 In case of an effector silent antibody (L234A, L235A mutations) Ides does not cleave and pepsin has to be chosen.

In one embodiment the mixture of proteolytic enzymes is plasmin and pepsin. In one embodiment the mixture of proteolytic enzymes is Lys-C and pepsin.

In one preferred embodiment the multivalent antibody is a bispecific antibody.

20 In one embodiment the incubating is for up to 60 minutes. In one embodiment the incubating is for 20 to 60 minutes. In one embodiment the incubating is for 35 to 45 minutes.

In one preferred embodiment the incubating is for about 40 minutes.

25 In one embodiment the multispecific antibody that is incubated with the proteolytic enzyme is a deglycosylated multispecific antibody.

In one embodiment the weight ratio of antibody to enzyme is 1:150 to 1: 500. In one embodiment the weight ratio of antibody to enzyme is 1:200 to 1:400. In one embodiment the weight ratio of antibody to enzyme is about 1:200.

In one embodiment the multispecific antibody that is incubated with the proteolytic enzyme has a concentration of from 200 to 600 µg/mL.

In one embodiment step b) is

- 5 b) desalting the incubation mixture of step a) and identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry.

In one embodiment the multispecific antibody is a monoclonal multispecific antibody.

- 10 In one embodiment the multispecific antibody comprises at least two non-peptidically associated light chains.

In one embodiment the multispecific antibody comprises at least three non-peptidically associated polypeptides.

In one embodiment the mass spectrometry is an online determination.

- 15 In one embodiment the mass spectrometry is an offline determination. In one embodiment the offline mass spectrometry is preceded by a desalting step.

Detailed Description of the Invention

DEFINITIONS

- 20 As used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and equivalents thereof known to those skilled in the art, and so forth. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. In contrast thereto reference to "one cell" does not include a plurality of such cells but is limited to a single (isolated) cell.

- 25 It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably.

Further it is also to be noted that the term "comprising" encompasses the term "consisting of" as limitation.

Patent Claims

1. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the analysis of the multispecific antibody's light chain pairing, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.
2. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the determination of light chain mispairing in the multispecific antibody, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.
3. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell, wherein said proteolytic enzyme is Lys-C and wherein said limited digestion is for 35 to 45 minutes.
4. A method for the determination of the light chain pairing in a multispecific antibody comprising the following steps:
 - a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
 - b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
 - c) determining from the results in step b) the light chain pairing of the multispecific antibody.
5. A method for the determination of light chain mispairing of a multispecific antibody comprising the following steps:
 - a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
 - b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and
 - c) determining from the results in step b) the light chain pairing of the multispecific antibody and thereby determining light chain mispairing.

6. A method for the selection of a recombinant mammalian cell producing a multispecific antibody comprising the following steps:
 - a) individually incubating a sample comprising a multispecific antibody produced by a clonal population of a recombinant mammalian cell of a multitude of recombinant mammalian cells all cells of the multitude producing the same multispecific antibody with a proteolytic enzyme for 35 to 45 minutes, wherein said proteolytic enzyme is Lys-C,
 - b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry,
 - c) determining from the results in step b) the presence of light chain mispairing of the multispecific antibody for each clonal cell population, and
 - d) selecting based on the results in step c) a recombinant cell producing a multispecific antibody.
7. The use according to any one of claims 1 to 3, wherein the limited digestion is for 40 minutes.
8. The method according to any one of claims 4 to 6, wherein the incubating is for 40 minutes.
9. The use or the method according to any one of claims 1 to 8, wherein the multispecific antibody that is incubated with the proteolytic enzyme is a deglycosylated multispecific antibody.
10. The use or the method according to any one of claims 1 to 9, wherein the weight ratio of antibody to enzyme is about 1:200.

11. The use or the method according to any one of claims 1 to 10, wherein the multispecific antibody that is incubated with the proteolytic enzyme has a concentration of from 200 to 600 $\mu\text{g/mL}$.
12. The method according to any one of claims 4 to 6 or 8 to 11, wherein step b) is
 - b) desalting the incubation mixture of step a) and identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry.
13. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody is a monoclonal multispecific antibody.
14. The use or the method according to any one of claims 1 to 13, wherein the multispecific antibody comprises at least two non-peptidically bound light chains.
15. The use or the method according to any one of claims 1 to 13, wherein the multispecific antibody comprises at least three non-peptidically bound polypeptides.