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

Summary

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.

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 and at pH 8, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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.

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 and at pH 8, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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.

One aspect as reported herein is use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a clonal population of a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell, wherein said proteolytic enzyme is Lys-C, wherein said

limited digestion is for 35 to 45 minutes at pH 8, wherein said selection is based on the presence of light chain mispairing of the multispecific antibody for each clonal cell population of the recombinant mammalian cell, said presence of light chain mispairing being determined from the mass of fragments obtained by the limited digestion as identified by mass spectrometry, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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,

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.

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 and at pH 8, 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,

wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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,

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.

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 and at pH 8, 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,

wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

As reported herein is 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 a limited time,

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.

One aspect as reported herein is 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 and at pH 8, 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,

wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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.

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 and at pH 8, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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 and at pH 8, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

3. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a clonal population of a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell, wherein said proteolytic enzyme is Lys-C, wherein said limited digestion is for 35 to 45 minutes at pH 8, wherein said selection is based on the presence of light chain mispairing of the multispecific antibody for each clonal cell population of the recombinant mammalian cell, said presence of light chain mispairing being determined from the mass of fragments obtained by the limited digestion as identified by mass spectrometry, and wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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 and at pH 8, 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,

   wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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 and at pH 8, 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,

wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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 and at pH 8, 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,

wherein said multispecific antibody is a bispecific antibody with a VH/VL exchange.

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

11. The method according to any one of claims 4 to 6 or 8 to 10, 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.

12. The use or the method according to any one of claims 1 to 11, wherein the multispecific antibody is a monoclonal multispecific antibody.

13. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody comprises at least two non-peptidically bound light chains.

14. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody comprises at least three non-peptidically bound polypeptides.