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## DESCRIPTION

### DRY COMPOSITIONS CONTAINING HYDROPHOBIC AMINO ACID

#### TECHNICAL FIELD

5           The present invention relates to a method for reducing and/or preventing coloration caused by a combined use of saccharide and amino acid. Specifically, the invention provides, as an amino acid usable with a saccharide, an amino acid having an anti-coloring effect  
10 when combined with a saccharide. The invention also provides a method for preventing coloration achieved by using the amino acid.

          The invention further relates to a method for stabilizing a dry composition containing a  
15 pharmacologically active proteinaceous substance. Specifically, the invention relates to a dry composition containing a pharmacologically active proteinaceous substance to which a saccharide and an amino acid are added.

20

#### BACKGROUND ART

          In pharmaceutical compositions, particularly in dry compositions containing a pharmacologically active proteinaceous substance as an active ingredient, sucrose,  
25 glucose, fructose, maltose and like saccharides (Japanese

Unexamined Patent Publications Nos. 1983-92619, 1985-48933 and 1990-138222, etc.), glycine, alanine and like amino acids (Japanese Unexamined Patent Publications No. 1983-146504) have generally been used as stabilizers effective  
5 on the active ingredient.

However, it is generally known that when these saccharides, which are used as stabilizers for pharmacologically active proteinaceous substances, are used with an amino acid, coloration caused by the Maillard  
10 reaction, etc. results. Therefore, pharmaceutical compositions containing a saccharide and an amino acid have the drawback of coloration with the passage of time.

An object of the present invention is to provide an amino acid having an anti-coloring effect for reducing  
15 and/or preventing coloration caused by the combined use of saccharide and amino acid. Specifically, the invention provides an anti-coloring agent containing the amino acid as an essential ingredient suitable for preventing the coloration caused by the change with the passage of time  
20 of a dry composition containing a saccharide, and a method for preventing coloration where the amino acid is used.

Another object of the invention is to provide an amino acid useable as a stabilizer for a dry composition containing a saccharide, particularly, a proteinaceous dry  
25 composition containing a saccharide. More specifically,

the invention provides a dry composition containing a saccharide and the amino acid.

#### DISCLOSURE OF THE INVENTION

5           The inventor has conducted extensive research to achieve the above objects. Consequently, the inventor has found that, in dry compositions containing a saccharide, specifically, dry compositions containing a saccharide and a pharmacologically active proteinaceous substance, use of  
10 specific amino acid enables the significant reduction and/or prevention of coloration attributable to saccharide. He has also found that dry compositions containing a saccharide as a stabilizer, even dry compositions containing a pharmacologically active proteinaceous  
15 substance, can further be improved in stability by adding the specific amino acid. The invention has been accomplished based on the above findings.

          In other words, the invention firstly relates to use of a hydrophobic amino acid preventing the coloration  
20 caused by the combined use of saccharide and amino acid, specifically, the following embodiments are included.

##### 1. Anti-coloring agent

          Item 1. An anti-coloring agent comprising a hydrophobic amino acid as an essential ingredient suitably  
25 preventing coloration of a dry composition containing a

saccharide.

Item 2. The anti-coloring agent according to item 1, wherein the hydrophobic amino acid has a hydropathy index of not less than 2.

5 Item 3. The anti-coloring agent according to item 1, wherein the hydrophobic amino acid has a hydropathy index of not less than 3.8.

Item 4. The anti-coloring agent according to item 1, wherein the hydrophobic amino acid has a  
10 hydropathy index in the range of from 3.8 to 4.5.

Item 5. The anti-coloring agent according to item 1, wherein the hydrophobic amino acid is at least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

15 Item 6. The anti-coloring agent according to item 1, wherein the hydrophobic amino acid content is in the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

20 Item 7. The anti-coloring agent according to item 1, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

Item 8. The anti-coloring agent according to  
25 item 1, wherein the hydrophobic amino acid is used in such

a manner that its concentration becomes in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

Item 9. The anti-coloring agent according to  
5 item 1, wherein the dry composition is a freeze-dried composition comprising a pharmacologically active proteinaceous substance as an active ingredient.

Item 10. The anti-coloring agent according to  
10 item 1, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

Item 11. The anti-coloring agent according to  
15 item 1, wherein the pharmacologically active proteinaceous substance is interferon.

## 2. Method for preventing coloration

Item 12. A method for preventing coloration of  
a dry composition containing a saccharide comprising  
adding a hydrophobic amino acid to the dry composition  
20 containing a saccharide.

Item 13. The method for preventing coloration  
according to item 12, wherein the hydrophobic amino acid  
has a hydropathy index of not less than 2.

Item 14. The method for preventing coloration  
25 according to item 12, wherein the hydrophobic amino acid

has a hydropathy index of not less than 3.8.

Item 15. The method for preventing coloration according to item 12, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

5           Item 16. The method for preventing coloration according to item 12, wherein the hydrophobic amino acid is at least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

10           Item 17. The method for preventing coloration according to item 12, wherein the hydrophobic amino acid is used in such a manner that its concentration becomes in the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

15           Item 18. The method for preventing coloration according to item 12, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

20           Item 19. The method for preventing coloration according to item 12, wherein the hydrophobic amino acid is used in such a manner that its concentration becomes in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

25           Item 20. The method for preventing coloration according to item 12, wherein the dry composition is a

freeze-dried composition comprising a pharmacologically active proteinaceous substance as an active ingredient.

Item 21. The method for preventing coloration according to item 20, wherein the pharmacologically active  
5 proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

Item 22. The method for preventing coloration according to item 12, wherein the pharmacologically active  
10 proteinaceous substance is interferon.

3. Use of hydrophobic amino acid

Item 23. Use of a hydrophobic amino acid for preventing coloration of a dry composition containing a saccharide.

15 Item 24. The use of a hydrophobic amino acid according to item 23, wherein the hydrophobic amino acid has a hydropathy index of not less than 2.

Item 25. The use of a hydrophobic amino acid according to item 23, wherein the hydrophobic amino acid  
20 has a hydropathy index of not less than 3.8.

Item 26. The use of a hydrophobic amino acid according to item 23, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

Item 27. The use of a hydrophobic amino acid  
25 according to item 23, wherein the hydrophobic amino acid

is at least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

Item 28. The use of a hydrophobic amino acid according to item 23, wherein the concentration of the hydrophobic amino acid is in the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

Item 29. The use of a hydrophobic amino acid according to item 23, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

Item 30. The use of a hydrophobic amino acid according to item 23, wherein the concentration of the hydrophobic amino acid is in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

Item 31. The use of a hydrophobic amino acid according to item 23, wherein the dry composition is a freeze-dried composition containing a pharmacologically active proteinaceous substance as an active ingredient.

Item 32. The use of a hydrophobic amino acid according to item 23, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

Item 33. The use of a hydrophobic amino acid according to item 23, wherein the pharmacologically active proteinaceous substance is interferon.

The present invention can effectively reduce or  
5 prevent the coloration caused by the change with the  
passage of time of dry compositions containing a  
saccharide, especially compositions containing a  
saccharide and a pharmacologically active proteinaceous  
substance. Thereby, it can provide dry compositions  
10 excellent in stability.

Secondly, the invention relates to dry  
compositions containing a pharmacologically active  
proteinaceous substance in a stable manner including the  
prevention of coloring.

15 4. Dry compositions

Item 34. A dry composition containing a  
pharmacologically active proteinaceous substance whereto a  
saccharide and a hydrophobic amino acid are added as a  
stabilizer.

20 Item 35. The dry composition containing a  
pharmacologically active proteinaceous substance according  
to item 34, wherein the hydrophobic amino acid has a  
hydropathy index of not less than 2.

Item 36. The dry composition containing a  
25 pharmacologically active proteinaceous substance according

to item 34, wherein the hydrophobic amino acid has a hydropathy index of not less than 3.8.

Item 37. The dry composition containing a pharmacologically active proteinaceous substance according  
5 to item 34, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

Item 38. The dry composition containing a pharmacologically active proteinaceous substance according  
to item 34, wherein the hydrophobic amino acid is at  
10 least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

Item 39. The dry composition containing a pharmacologically active proteinaceous substance according  
to item 34, wherein the concentration of the hydrophobic  
15 amino acid is in the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

Item 40. The dry composition containing a pharmacologically active proteinaceous substance according  
20 to item 34, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

Item 41. The dry composition containing a pharmacologically active proteinaceous substance according  
25 to item 34, wherein the concentration of the hydrophobic

amino acid is in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

Item 42. The dry composition containing a pharmacologically active proteinaceous substance according to item 34, which is a freeze-dried composition.

Item 43. The dry composition containing a pharmacologically active proteinaceous substance according to item 34, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

Item 44. The dry composition containing a pharmacologically active proteinaceous substance according to item 34, wherein the pharmacologically active proteinaceous substance is interferon.

The present invention can provide, by combining a saccharide with a hydrophobic amino acid, dry compositions containing a pharmacologically active proteinaceous substance as an active ingredient in a stable condition even after completion of drying (freeze-drying, etc.) and during storage.

#### BEST MODE FOR CARRYING OUT THE INVENTION

The anti-coloring agent of the invention comprises a hydrophobic amino acid as an essential

ingredient. The anti-coloring agent of the invention significantly prevents coloration with the passage of time, especially when used with a dry composition containing a saccharide.

5           The hydrophobic amino acids to be used in the invention are those having a hydropathy index of about 2 or larger (Jack Kyte and Russel F. Doolittle, "A Simple Method for Displaying the Hydropathic Character of a Protein." *J. Mol. Biol.* 157(1982): 105-132). Any amino  
10 acids having the above property may be used in the invention whether or not they are protein-constituting amino acids. The hydrophobic amino acids may preferably be those having a hydropathy index of about 2.8 or larger such as valine, leucine, isoleucine and phenylalanine; more  
15 preferably those having a hydropathy index of about 3.8 or larger; and even more preferably those having a hydropathy index in the range of from about 3.8 to about 4.5 such as valine, leucine and isoleucine.

          The hydrophobic amino acids to be used in the  
20 invention may be in the form of dipeptides, tripeptides, salts or amides. Examples of the dipeptides of hydrophobic amino acids are leucyl-valine, isoleucyl-valine, isoleucyl-leucine, leucyl-glycine, etc. Examples of the tripeptides of hydrophobic amino acids are  
25 isoleucyl-leucyl-valine, leucyl-glycyl-glycine, etc. The

salts of hydrophobic amino acids include salts thereof with sodium, potassium and like alkali metals or with calcium, magnesium and like alkaline earth metals; adduct salts thereof with phosphoric acid, hydrochloric acid, 5 hydrobromic acid and like inorganic acids or with organic acids such as a sulfonic acid, etc. More specific examples of the salts of hydrophobic amino acids are an L-leucic amide hydrochloride, L-isoleucyl- $\beta$ -naphthylamide hydrobromide, L-valine- $\beta$ -naphthylamide, etc.

10                These hydrophobic amino acids (including those in a form of a salt or amide) may be used singularly or in an arbitrary combination.

                 The anti-coloring agent of the invention targets saccharides generally known to cause coloration with the 15 passage of time due to the Maillard reaction and the like when combined with amino acid. Specifically, the targeted saccharides include those generally used as stabilizers for dry pharmaceutical compositions, particularly pharmaceutical compositions containing a pharmacologically 20 active proteinaceous substance. More specifically, glucose, fructose, maltose and like reducing sugars; sucrose, trehalose, mannitol, dextran 40 and the like are included. These saccharides can be added to a dry composition singularly or in an arbitrary combination. 25 Among those, preferable are sucrose and maltose.

The saccharide content of the dry composition varies depending on the kind of saccharide contained and cannot be generalized. However, a general amount of the saccharide used is 10 wt.% to 90 wt.%, in some cases 20  
5 wt.% to 80 wt.% and in other cases 40 wt.% to 80 wt.% per 100 wt.% of the dry composition. The above proportion is suitably applied when the saccharide used is sucrose.

Content of the hydrophobic amino acid in the dry composition varies depending on the kind of saccharide  
10 contained in the dry composition and cannot be generalized. However, per 100 wt.% of the dry composition, the hydrophobic amino acid can be contained generally about 0.1 wt.% to about 70 wt.%, preferably about 0.25 wt.% to about 60 wt.%, more preferably about 0.5 wt.% to about 50  
15 wt.%, and even more preferably about 1 wt.% to about 50 wt.%.

There is no limitation on the ratio of the hydrophobic amino acid to the saccharide contained in the dry composition. However, the amount of amino acid used  
20 is generally about 0.1 part by weight to about 200 parts by weight, preferably about 0.2 part by weight to about 100 parts by weight and more preferably about 1 part by weight to about 100 parts by weight per 100 parts by weight of the saccharide contained in the dry composition.

25 Dry compositions in which the anti-coloring

agents of the invention are usable include compositions containing a pharmacologically active proteinaceous substance as an active ingredient.

There is no limitation on the pharmacologically active proteinaceous substances to be used as long as they are pharmacologically effective proteins or peptides (including polypeptides). Examples thereof are enzymes, hemoglobin, immunoglobulins, hormones, blood coagulation factors and like proteins, antiviral polypeptides (ex. interferons- $\alpha$ , - $\beta$ , - $\gamma$ , etc.), immunomodulator polypeptides (ex. interleukins-1, 2, 3, 4, 5, 6, 7, 8, etc.), hematinic polypeptides (ex. erythropoietin, granulocyte colony stimulating factor, macrophage-colony stimulating factor and granulocyte-macrophage-colony stimulating factor) and like polypeptides, etc. The proteins and peptides may be contained in the dry composition singularly or in an arbitrary combination. Among these, preferable are interferons.

The above proteins and peptides include those existing in nature, produced by recombinant DNA techniques or chemical syntheses.

There is no limitation on the proportion of the pharmacologically active proteinaceous substance to the dry composition. The amount of the pharmacologically active proteinaceous substance used varies depending on

the kind to be used; however, specific examples of the concentrations thereof are generally not more than 20 wt.%, in some cases 0.00001 wt.% to 10 wt.%, in some cases 0.0001 wt.% to 10 wt.%, in some cases 0.001 wt.% to 10 wt.% and in some cases 0.001 wt.% to 5 wt.%, per 100 wt.% of the dry composition.

The dry compositions in which the anti-coloring agents of the invention are used may further contain human serum albumin, a polar amino acid(s) or a salt(s) thereof (ex. an amino acid(s) having a hydropathy index of not larger than 0 or a salt thereof), an inorganic salt(s), gelatin, a surfactant(s), a buffer(s), etc.

The usage of the anti-coloring agents of the invention is not limited as long as they are used in the above dry compositions. Preferably, they are added to a solution containing saccharide(s) and/or pharmacologically active proteinaceous substance(s) prior to the preparation of (i.e., before drying) the dry composition (dried product) or mixed into a solution together with saccharide(s) and/or pharmacologically active proteinaceous substance(s).

There is no limitation on the ratio of the anti-coloring agent to the dry composition. However, per 100 wt.% of the dry composition, hydrophobic amino acid is generally about 0.1 wt.% to about 70 wt.%, preferably

about 0.25 wt.% to about 60 wt.%, more preferably about 0.5 wt.% to about 50 wt.%, and even more preferably about 1 wt.% to about 50 wt.%.

5       The content of hydrophobic amino acid per 100 parts by weight of saccharides contained in the dry composition is generally about 0.1 part by weight to about 200 parts by weight, preferably about 0.2 part by weight to about 100 parts by weight, and more preferably about 1 part by weight to about 100 parts by weight.

10       The invention relates to a method for preventing coloration of dry compositions containing a saccharide comprising use of hydrophobic amino acid. Specific examples of hydrophobic amino acids, saccharides, dry compositions and usage of hydrophobic amino acids are as  
15       described above.

      Furthermore, the invention provides use of hydrophobic amino acid as a stabilizer. The hydrophobic amino acid suitably functions as a stabilizer when combined with a saccharide and is particularly effective  
20       as a stabilizer for dry compositions containing a pharmacologically active proteinaceous substance.

      The invention also relates to a dry composition containing a pharmacologically active proteinaceous substance in which a stabilizer composed of hydrophobic  
25       amino acid and saccharide is used.

The saccharide(s) used in the invention is not limited but may include those generally used or function as stabilizers for dry pharmaceutical compositions, specifically, pharmaceutical compositions containing a pharmacologically active proteinaceous substance. Specific examples are sucrose, maltose, lactose, trehalose and like disaccharides; mannitol, xylitol and like sugar alcohols; dextran 40, dextran 70; chondroitin sulfuric acid and like polysaccharides. These saccharides can be added to the dry compositions singularly or in an arbitrary combination. Among those, preferable are sucrose, maltose, lactose, trehalose and like disaccharides, and more preferable is sucrose.

The saccharide content of the targeted dry composition is not limited and may be generally about 10 wt.% to about 90 wt.%, in some cases about 20 wt.% to about 80 wt.%, and in some other cases about 40 wt.% to about 80 wt.% per 100 wt.% of dry composition. The above proportion is suitably applied when sucrose or like disaccharides is used.

The hydrophobic amino acid content in the dry composition is not limited and may be, per 100 wt.% of dry composition, generally about 0.1 wt.% to about 70 wt.%, preferably about 0.25 wt.% to about 60 wt.%, more preferably about 0.5 wt.% to about 50 wt.%, and even more

preferably about 1 wt.% to about 50 wt.%. The proportion of hydrophobic amino acid to saccharide contained in the dry composition is, per 100 parts by weight of saccharide, generally about 0.1 part by weight to about 200 parts by weight, preferably about 0.2 part by weight to about 100 parts by weight, and more preferably about 1 part by weight to about 100 parts by weight.

#### Examples

10           The present invention will be described below in more detail with reference to Examples. However, the scope of the present invention is not limited to these Examples.

#### Examples 1 to 3

15           In each Example, a mixture was prepared so as to contain 0.25 ml of an interferon- $\alpha$  bulk solution (hereinafter referred to as "IFN- $\alpha$  bulk solution"; titer:  $2 \times 10^7$  IU/ml), 1 mg of Tween 80, 5 mg of sucrose, and 5 mg of an amino acid (isoleucine (Example 1), valine  
20 (Example 2), or leucine (Example 3)) per vial (1 ml capacity). A suitable amount of distilled water for injection was added to dissolve them. The mixture was freeze-dried using a shelf-type lyophilizer (LYOVAC GT-4: LEYBOLD). The resultant freeze-dried composition was  
25 allowed to stand at 70 °C. The extent of coloration and

the titer of the freeze-dried composition were observed with the passage of time, and then the remaining IFN- $\alpha$  activity was estimated by the ratio of the measured IFN- $\alpha$  activity to the IFN- $\alpha$  activity (100%) of the composition immediately after freeze-drying. For comparison, as an amino acid, 5 mg of glycine (Comparative Example 1) or arginine hydrochloride (Comparative Example 2) was used for evaluating the coloration. Furthermore, for evaluating the stability of IFN- $\alpha$ , a test was conducted in the same manner as mentioned above except that a system with no amino acid added was used (Comparative Example 3). The results, including the degree of coloration and the stability of the IFN- $\alpha$  of the compositions obtained in Examples and Comparative Examples, are shown in Table 1 and Table 2.

Table 1

|                   | Preparation                                               | HP-<br>INDEX<br>of<br>Amino<br>Acid | Coloration<br>(70 °C) |           |           |
|-------------------|-----------------------------------------------------------|-------------------------------------|-----------------------|-----------|-----------|
|                   |                                                           |                                     | Week<br>0             | Week<br>1 | Week<br>2 |
| Examples          | 1. IFN- $\alpha$ + sucrose<br>+ isoleucine                | 4.5                                 | -                     | -         | -         |
|                   | 2. IFN- $\alpha$ + sucrose<br>+ valine                    | 4.2                                 | -                     | -         | -         |
|                   | 3. IFN- $\alpha$ + sucrose<br>+ leucine                   | 3.8                                 | -                     | -         | -         |
| Comp.<br>Examples | 1. IFN- $\alpha$ + sucrose<br>+ glycine                   | -0.4                                | -                     | +         | ++        |
|                   | 2. IFN- $\alpha$ + sucrose<br>+ arginine<br>hydrochloride | -4.5                                | -                     | +         | ++        |

Evaluation of coloration:

- 5
- No coloration was observed
  - ± Slight coloration was observed
  - + Little coloration was observed
  - ++ Much coloration was observed
  - +++ Extreme coloration was observed

Table 2

|                  | Preparation                                | Remaining IFN- $\alpha$<br>Activity (%) |           |           |
|------------------|--------------------------------------------|-----------------------------------------|-----------|-----------|
|                  |                                            | Week<br>0                               | Week<br>1 | Week<br>2 |
| Examples         | 1. IFN- $\alpha$ + sucrose<br>+ isoleucine | 100.0                                   | 71.6      | 51.8      |
|                  | 2. IFN- $\alpha$ + sucrose<br>+ valine     | 100.0                                   | 59.2      | 47.1      |
|                  | 3. IFN- $\alpha$ + sucrose<br>+ leucine    | 100.0                                   | 75.6      | 72.3      |
| Comp.<br>Example | 1. IFN- $\alpha$ + sucrose                 | 100.0                                   | 48.6      | 38.7      |

5

As shown in Table 1, the combined use of sucrose and glycine and the combined use of sucrose and arginine hydrochloride caused coloration of the freeze-dried composition with the passage of time. Using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger prevented coloration of the freeze-dried composition. Furthermore, as shown in Table 2, the stability of IFN- $\alpha$  improved when using sucrose with hydrophobic amino acid having a hydropathy index (HP-INDEX) of 3.8 or larger compared to the stability when using sucrose singularly.

Examples 4 to 6

In each Example, a mixture was prepared so as to contain 0.25 ml of an IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml), 1 mg of Tween 80, 5 mg of maltose, and 5 mg of an amino acid (isoleucine (Example 4), valine (Example 5), or leucine (Example 6)) per vial (1 ml capacity). A suitable amount of distilled water for injection was added to dissolve them. The mixture was freeze-dried using a shelf-type lyophilizer (LYOVAC GT-4: LEYBOLD). The resultant freeze-dried composition was allowed to stand at 70 °C. The extent of coloration of the freeze-dried composition was observed with the passage of time. For comparison, as an amino acid, 5 mg of glycine (Comparative Example 4) or arginine hydrochloride (Comparative Example 5) was used for evaluating the coloration, and the test was conducted in the same manner as mentioned above. The results of the Examples and Comparative Examples are shown in Table 3.

20

25

Table 3

|          | Preparation                                               | HP-<br>INDEX<br>of<br>Amino<br>Acid | Coloration<br>(70 °C) |           |           |
|----------|-----------------------------------------------------------|-------------------------------------|-----------------------|-----------|-----------|
|          |                                                           |                                     | Week<br>0             | Week<br>1 | Week<br>2 |
| Examples | 4. IFN- $\alpha$ + maltose<br>+ isoleucine                | 4.5                                 | -                     | -         | -         |
|          | 5. IFN- $\alpha$ + maltose<br>+ valine                    | 4.2                                 | -                     | -         | -         |
|          | 6. IFN- $\alpha$ + maltose<br>+ leucine                   | 3.8                                 | -                     | -         | -         |
| Comp.    | 3. IFN- $\alpha$ + maltose<br>+ glycine                   | -0.4                                | -                     | ++        | +++       |
| Examples | 4. IFN- $\alpha$ + maltose<br>+ arginine<br>hydrochloride | -4.5                                | -                     | ++        | +++       |

5 Evaluation of coloration:

- No coloration was observed
- ± Slight coloration was observed
- + Little coloration was observed
- ++ Much coloration was observed
- +++ Extreme coloration was observed

10

As shown in Table 3, the combined use of maltose and glycine and the combined use of maltose and arginine hydrochloride caused coloration of the freeze-dried composition with the passage of time. Using maltose with

15

hydrophobic amino acid having a hydropathy index of 3.8 or larger prevented coloration of the freeze-dried composition.

5 Examples 7 to 9

In each Example, a mixture was prepared so as to contain 0.25 ml of an IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml), 1 mg of Tween 80, 5 mg of trehalose, and 5 mg of an amino acid (isoleucine (Example 7), valine (Example 8),  
10 or leucine (Example 9)) per vial (1 ml capacity). A suitable amount of distilled water for injection was added to dissolve them. The mixture was freeze-dried using a shelf-type lyophilizer (LYOVAC GT-4: LEYBOLD). The resultant freeze-dried composition was allowed to stand at  
15 70 °C. The titer of the freeze-dried composition was observed with the passage of time, and then the remaining IFN- $\alpha$  activity was estimated by the ratio of the measured IFN- $\alpha$  activity to the IFN- $\alpha$  activity (100%) of the composition immediately after freeze-drying. For  
20 comparison, the test was conducted in the same manner as mentioned above except that a system with no amino acid added was used (Comparative Example 6) for evaluating the stability of IFN- $\alpha$ . The results of the Examples and Comparative Example are shown in Table 4.

Table 4

| Preparation      |                                              | Remaining IFN- $\alpha$<br>Activity (%) |           |           |
|------------------|----------------------------------------------|-----------------------------------------|-----------|-----------|
|                  |                                              | Week<br>0                               | Week<br>1 | Week<br>2 |
| Examples         | 7. IFN- $\alpha$ + trehalose<br>+ isoleucine | 100.0                                   | 61.5      | 58.9      |
|                  | 8. IFN- $\alpha$ + trehalose<br>+ valine     | 100.0                                   | 61.5      | 56.9      |
|                  | 9. IFN- $\alpha$ + trehalose<br>+ leucine    | 100.0                                   | 72.7      | 63.0      |
| Comp.<br>Example | 6. IFN- $\alpha$ + trehalose                 | 100.0                                   | 39.2      | 28.4      |

5 As shown in Table 4, the stability of IFN- $\alpha$  improved when using trehalose with hydrophobic amino acid having a hydropathy index (HP-INDEX) of 3.8 or larger compared to the stability when using sucrose singularly.

10 Examples 10 to 12

In each Example, a mixture was prepared so as to contain 0.25 ml of an IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml), 1 mg of Tween 80, 40 mg of sucrose, and 5 mg of an amino acid (isoleucine (Example 10), valine (Example 11),  
15 or leucine (Example 12)) per vial (1 ml capacity). A suitable amount of distilled water for injection was added to dissolve them. The mixture was freeze-dried using a shelf-type lyophilizer (LYOVAC GT-4: LEYBOLD). The

resultant freeze-dried composition was allowed to stand at 70 °C. The extent of coloration of the freeze-dried composition was observed with the passage of time. For comparison, as an amino acid, 5 mg of glycine (Comparative Example 7) or arginine hydrochloride (Comparative Example 8) was used for evaluating the coloration, and the test was conducted in the same manner as mentioned above. The results of the Examples and Comparative Examples are shown in Table 5.

10

Table 5

|          | Preparation                                         | HP-<br>INDEX<br>of<br>Amino<br>Acid | Coloration<br>(70 °C) |           |           |
|----------|-----------------------------------------------------|-------------------------------------|-----------------------|-----------|-----------|
|          |                                                     |                                     | Week<br>0             | Week<br>1 | Week<br>2 |
| Examples | 10. IFN- $\alpha$ + sucrose + isoleucine            | 4.5                                 | -                     | -         | -         |
|          | 11. IFN- $\alpha$ + sucrose + valine                | 4.2                                 | -                     | -         | -         |
|          | 12. IFN- $\alpha$ + sucrose + leucine               | 3.8                                 | -                     | -         | -         |
| Comp.    | 7. IFN- $\alpha$ + sucrose + glycine                | -0.4                                | -                     | +         | ++        |
| Examples | 8. IFN- $\alpha$ + sucrose + arginine hydrochloride | -4.5                                | -                     | +         | ++        |

15

Evaluation of coloration:

- No coloration was observed
- ± Slight coloration was observed
- + Little coloration was observed
- 5 ++ Much coloration was observed
- +++ Extreme coloration was observed

As shown in Table 5, the combined use of sucrose and glycine and the combined use of sucrose and arginine  
10 hydrochloride caused coloration of the freeze-dried composition with the passage of time. Using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger significantly prevented coloration of the freeze-dried composition.

15

Examples 13 to 15

A freeze-dried composition was obtained in the same manner as in Examples 1 to 3 except that 0.25 ml of IFN- $\beta$  bulk solution (titer:  $2 \times 10^7$  IU/ml) was used  
20 instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that, as Examples 1 to 3, coloration of the freeze-dried  
25 composition can be prevented by using sucrose with

hydrophobic amino acid having a hydropathy index of 3.8 or larger.

Examples 16 to 18

5           A freeze-dried composition was obtained in the same manner as in Examples 10 to 12 except that 0.25 ml of IFN- $\beta$  bulk solution (titer:  $2 \times 10^7$  IU/ml) was used instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was  
10 allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that, as Examples 10 to 12, coloration of the freeze-dried composition can be prevented by using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or  
15 larger.

Examples 19 to 21

          A freeze-dried composition was obtained in the same manner as in Examples 1 to 3 except that 0.25 ml of  
20 IFN- $\gamma$  bulk solution (titer:  $2 \times 10^7$  IU/ml) was used instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that, as  
25 Examples 1 to 3, coloration of the freeze-dried

composition can be prevented by using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger.

5 Examples 22 to 24

A freeze-dried composition was obtained in the same manner as in Examples 10 to 12 except that 0.25 ml of IFN- $\beta$  bulk solution (titer:  $2 \times 10^7$  IU/ml) was used instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that, as Examples 10 to 12, coloration of the freeze-dried composition can be prevented by using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger.

Examples 25 to 27

A freeze-dried composition was obtained in the same manner as in Examples 1 to 3 except that 0.25 ml of erithropoietin bulk solution (titer:  $2 \times 10^7$  IU/ml) was used instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that,

as Examples 1 to 3, coloration of the freeze-dried composition can be prevented by using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger.

5

Examples 28 to 30

A freeze-dried composition was obtained in the same manner as in Examples 10 to 12 except that 0.25 ml of erithropoietin bulk solution (titer:  $2 \times 10^7$  IU/ml) was  
10 used instead of 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml). The resultant freeze-dried composition was allowed to stand at 70 °C, and the coloration thereof was observed with the passage of time. The results show that, as Examples 10 to 12, coloration of the freeze-dried  
15 composition can be prevented by using sucrose with hydrophobic amino acid having a hydropathy index of 3.8 or larger.

Examples 31 to 33

20 One vial (1 ml capacity) of mixture solution was prepared in such a manner that it comprised 0.25 ml of IFN- $\alpha$  bulk solution (titer:  $2 \times 10^7$  IU/ml), 40 mg of sucrose, and 5 mg each of two kinds of amino acid (glycine + isoleucine (Example 31), glycine + valine (Example 32),  
25 or glycine + leucine (Example 33)). A suitable amount of

distilled water for injection was added to dissolve them. Thereafter, freeze-drying was conducted in the same manner as in Examples 10 to 12 obtaining a freeze-dried composition. The resultant freeze-dried composition was  
5 allowed to stand at 70 °C. The extent of coloration of the freeze-dried composition was observed with the passage of time. For comparison, the test was conducted in the same manner as mentioned above except that 5 mg of glycine alone (Comparative Example 7) was used as the amino acid.  
10 The results of the Examples and Comparative Example are shown in Table 6.

Table 6

|                  | Preparation                                              | HP-<br>INDEX<br>of<br>Amino<br>Acid | Coloration<br>(70 °C) |           |           |
|------------------|----------------------------------------------------------|-------------------------------------|-----------------------|-----------|-----------|
|                  |                                                          |                                     | Week<br>0             | Week<br>1 | Week<br>2 |
| Examples         | 31. IFN- $\alpha$ + sucrose<br>+ glycine +<br>isoleucine | 4.5                                 | -                     | -         | -         |
|                  | 32. IFN- $\alpha$ + sucrose<br>+ glycine +<br>valine     | 4.2                                 | -                     | -         | -         |
|                  | 33. IFN- $\alpha$ + sucrose<br>+ glycine +<br>leucine    | 3.8                                 | -                     | -         | -         |
| Comp.<br>Example | 7. IFN- $\alpha$ + sucrose<br>+ glycine                  | -0.4                                | -                     | +         | ++        |

Evaluation of coloration:

- No coloration was observed
- ± Slight coloration was observed
- + Little coloration was observed
- 5 ++ Much coloration was observed
- +++ Extreme coloration was observed

As shown in Table 6, the freeze-dried composition containing sucrose and glycine caused coloration with the passage of time. However, coloration of the freeze-dried composition can significantly prevented by using hydrophobic amino acid having a hydropathy index of 3.8 or larger in addition to sucrose and glycin.

15

INDUSTRIAL APPLICABILITY

The anti-coloring agent of the invention can significantly reduce and/or prevent the coloration of a dry composition with the passage of time caused by the combined use of saccharide and amino acid. Such an anti-coloring agent is specifically useful for preventing the coloration of a dry composition comprising a saccharide and a pharmacologically active proteinaceous substance. From another point of view, the anti-coloring agent is also useful as a stabilizer for a dry composition

25

containing a saccharide and a pharmacologically active  
proteinaceous substance. Therefore, the invention can  
provide dry compositions containing a pharmacologically  
active proteinaceous substance and an anti-coloring agent  
5 or a stabilizer, i.e., dry compositions containing a  
pharmacologically active proteinaceous substance to which  
a saccharide and an amino acid are added, which exhibits  
excellent stability by significantly preventing the  
coloration and lowered activity observed with the passage  
10 of time.

CLAIMS

1. An anti-coloring agent comprising a hydrophobic amino acid as an essential ingredient suitably preventing coloration of a dry composition containing a  
5 saccharide.

2. The anti-coloring agent according to claim 1, wherein the hydrophobic amino acid has a hydropathy index of not less than 2.  
10

3. The anti-coloring agent according to claim 1, wherein the hydrophobic amino acid has a hydropathy index of not less than 3.8.

15 4. The anti-coloring agent according to claim 1, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

5. The anti-coloring agent according to claim 1,  
20 wherein the hydrophobic amino acid is at least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

6. The anti-coloring agent according to claim 1,  
25 wherein the hydrophobic amino acid content is in the range

of 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

7. The anti-coloring agent according to claim 1,  
5 wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

8. The anti-coloring agent according to claim 1,  
10 wherein the hydrophobic amino acid is used in such a manner that its concentration becomes in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

15 9. The anti-coloring agent according to claim 1, wherein the dry composition is a freeze-dried composition comprising a pharmacologically active proteinaceous substance as an active ingredient.

20 10. The anti-coloring agent according to claim 1, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

11. The anti-coloring agent according to claim 10, wherein the pharmacologically active proteinaceous substance is interferon.

5           12. A method for preventing coloration of a dry composition containing a saccharide comprising adding a hydrophobic amino acid to the dry compositions containing a saccharide.

10           13. The method for preventing coloration according to claim 12, wherein the hydrophobic amino acid has a hydropathy index of not less than 2.

15           14. The method for preventing coloration according to claim 12, wherein the hydrophobic amino acid has a hydropathy index of not less than 3.8.

20           15. The method for preventing coloration according to claim 12, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

25           16. The method for preventing coloration according to claim 12, wherein the hydrophobic amino acid is at least one member selected from the group consisting of valine, leucine, isoleucine and phenylalanine.

17. The method for preventing coloration according to claim 12, wherein the hydrophobic amino acid is used in such a manner that its concentration becomes in  
5 the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

18. The method for preventing coloration  
10 according to claim 12, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

19. The method for preventing coloration  
15 according to claim 12, wherein the hydrophobic amino acid is used in such a manner that its concentration becomes in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

20. The method for preventing coloration according to claim 12, wherein the dry composition is a freeze-dried composition comprising a pharmacologically active proteinaceous substance as an active ingredient.

21. The method for preventing coloration  
25

according to claim 20, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

22. The method for preventing coloration according to claim 12, wherein the pharmacologically active proteinaceous substance is interferon.

10

23. A dry composition containing a pharmacologically active proteinaceous substance whereto a saccharide and a hydrophobic amino acid are added as a stabilizer.

15

24. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the hydrophobic amino acid has a hydropathy index of not less than 2.

20

25. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the hydrophobic amino acid has a hydropathy index of not less than 3.8.

25

26. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the hydrophobic amino acid has a hydropathy index in the range of from 3.8 to 4.5.

5

27. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the hydrophobic amino acid is at least one member selected from the group consisting of  
10 valine, leucine, isoleucine and phenylalanine.

28. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the concentration of the hydrophobic  
15 amino acid is in the range of from 0.1 part by weight to 200 parts by weight per 100 parts by weight of saccharide contained in the dry composition.

29. The dry composition containing a  
20 pharmacologically active proteinaceous substance according to claim 23, wherein the saccharide is at least one member selected from the group consisting of reducing sugars, sucrose, trehalose, mannitol and dextran 40.

25 30. The dry composition containing a

pharmacologically active proteinaceous substance according to claim 23, wherein the concentration of the hydrophobic amino acid is in the range of from 0.1 wt.% to 70 wt.% per 100 wt.% of the total weight of the dry composition.

5

31. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, which is a freeze-dried composition.

10

32. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the pharmacologically active proteinaceous substance is at least one member selected from the group consisting of antiviral polypeptides, immunomodulator polypeptides and hematinic polypeptides.

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33. The dry composition containing a pharmacologically active proteinaceous substance according to claim 23, wherein the pharmacologically active proteinaceous substance is interferon.

20

34. Use of a hydrophobic amino acid for preventing coloration of a dry composition containing a saccharide.

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