molecules in the body, side effects due to enhanced innate immune response, absence of development of efficient drug delivery system (DDS) and the like.

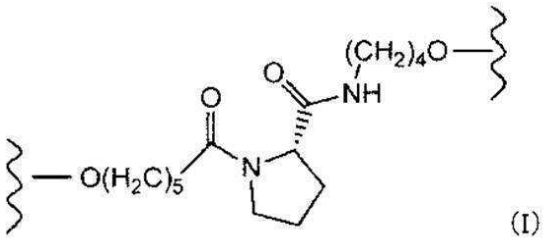
[0005]

In the case of respiratory diseases, pulmonary fibrosis is an effective target disease of nucleic acid medicine, since topical administration of drugs to the lung is possible. To deal with the problems of nucleic acid molecule such as stability in the body and induction of innate immune response, a single-stranded nucleic acid molecule in which the terminals of a double-stranded nucleic acid of siRNA or miRNA are linked by various linkers has been developed (e.g., patent documents 1-5). Hamasaki et al. reported that symptoms were remarkably improved by intratracheal administration of a single-stranded nucleic acid molecule having the following structure carrying a TGF-β1 expression-inhibitory sequence (hereinafter to be also referred to as "PK-0051") to model mice of pulmonary fibrosis and acute lung injury (non-patent document 1).

5'-AGCAGAGUACACACAGCAUAUACC (SEQ ID NO: 1) -P-GGUAUAUGCUGUGUGUACUCUGCUUC (SEQ ID NO: 2) -P-G-3'

(Underline shows a TGF-β1 expression inhibitory sequence. P is a proline derivative linker represented by the following formula (I)).

[0006]



(I)

[Document List]

[Patent documents]

[0007]

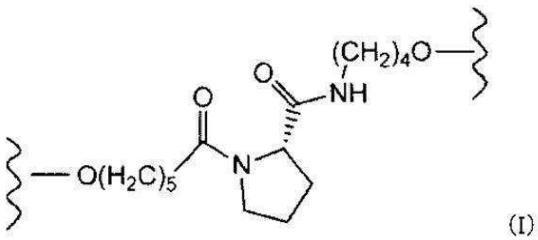
patent document 1: WO 2012/017919

[0011]

Therefore, the present invention is as follows.

[1] A composition comprising a single-stranded nucleic acid molecule consisting of a nucleotide sequence shown by 5′-AGCAGAGUACACACAGCAUAUACC-P-GGUAUAUGCUGUGUGUACUCUGCUUC-P-G-3′ (in the sequence, P is a proline derivative linker represented by the following formula (I))

[0012]



(I)

[0013]

and a buffer, and having the following features:

(a) being in the form of a solution at ambient temperature; and

(b) a content of the nucleic acid molecule after storage at 25°C, relative humidity 60% for 4 weeks, of not less than 80% relative to the content at the time of start of the storage.

[2] The composition of [1], wherein the content of the nucleic acid molecule after storage at 40°C, relative humidity 75% for 4 weeks is not less than 80% relative to the content at the time of start of the storage.

[3] The composition of [1] or [2], wherein the content of the nucleic acid molecule after storage at 60°C for 4 weeks is not less than 60% relative to the content at the time of start of the storage.

[4] The composition of [1], wherein the content of the nucleic acid molecule exposed to the total light exposure 1.2 million Lux•hr is not less than 80% relative to the content at the time of start of the storage.

[5] The composition of any of [1] to [4], wherein the buffer adjusts the pH of the composition to not less than 4.0 and not

[0020]

Table 1

time (min) after injection	mobile phase A (vol%)	mobile phase B (vol%)
0→12	100→60	0→40

flow: 1.0 mL/min

[0021]

ion exchange HPLC

measurement device: LC-20A SHIMAZU HPLC system (AEX-HPLC), manufactured by Shimadzu Corporation

detector: ultraviolet absorption spectrophotometer (measurement wavelength: 260 nm)

column: DNAPac PA-100 (4×250 mm)

column temperature: 80°C

mobile phase A: 25 mM Tris-HCL(pH 8.0), 8 M urea, 10% Acetonitrile

mobile phase B: mobile phase A, 700 mM sodium perchlorate monohydrate

feed of mobile phase: The mixing ratio of mobile phase A and mobile phase B was changed as follows to control concentration gradient.

[0022]

Table 2

time (min) after injection	mobile phase A (vol%)	mobile phase B (vol%)
0→20	90→60	10→40

flow: 1.0 mL/min

[0023]

1. Nucleic acid molecule

The nucleic acid molecule as an active ingredient to be contained in the composition of the present invention is shown by the following nucleotide sequence.

5′-AGCAGAGUACACACAGCAUAUACC-P-GGUAUAUGCUGUGUGUACUCUGCUUC-P-G-3′

generally 0.0001 - 40 wt%, preferably 0.0005 - 20 wt%, further preferably 0.001 - 10 wt%, relative to the whole composition obtained by the method.

[0039]

The present invention is explained in more detail in the following by referring to Examples, which are not to be construed as limitative.

[Examples]

[0040]

Production Example 1 (synthesis of single-stranded nucleic acid molecule)

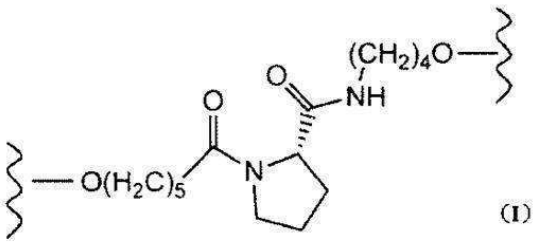
A single-stranded nucleic acid molecule PK-0051 carrying an expression inhibitory sequence of TGF-β1 was synthesized by a nucleic acid synthesizer (trade name: ABI Expedite (registered trademark) 8909 Nucleic Acid Synthesis System, Applied Biosystems) based on the phosphoramidite method. For the aforementioned synthesis, RNA Phosphoramidites (2′-O-TBDMSi, trade name, Samchully Pharm. Co., Ltd.) was used as RNA amidite (hereinafter the same). The aforementioned amidite was deprotected by a conventional method. The synthesized RNA was purified by HPLC. Each RNA after purification was freeze-dried.

As the linker region, L-prolinediamideamidite was used. The underline shows an expression inhibitory sequence of human TGF-β1 gene.

5′-AGCAGAGUACACACAGCAUAUACC-P-GGUAUAUGCUGUGUGUACUCUGCUUC-P-G-3′

(P is a proline derivative linker represented by the following formula (I)).

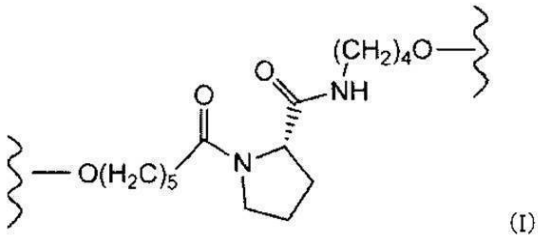
[0041]



(I)

Claims

1. A composition comprising a single-stranded nucleic acid molecule consisting of a nucleotide sequence shown by 5'-AGCAGAGUACACACAGCAUAUACC-P-GGUAUAUGCUGUGUGUACUCUGCUUC-P-G-3' (in the sequence, P is a proline derivative linker represented by the following formula (I))



(I)

and a buffer, and having the following features:

(a) being in the form of a solution at ambient temperature; and

(b) a content of the nucleic acid molecule after storage at 25°C, relative humidity 60% for 4 weeks, of not less than 80% relative to the content at the time of start of the storage,

wherein the buffer adjusts the pH of the composition to not less than 4.6 and not more than 7.0.

2. The composition according to claim 1, wherein the content of the nucleic acid molecule after storage at 40°C, relative humidity 75% for 4 weeks is not less than 80% relative to the content at the time of start of the storage.

3. The composition according to claim 1, wherein the content of the nucleic acid molecule after storage at 60°C for 4 weeks is not less than 60% relative to the content at the time of start of the storage.

4. The composition according to claim 1, wherein the content of the nucleic acid molecule exposed to the total light exposure 1.2 million Lux · hr is not less than 80% relative to the content at the time of start of the storage.

5. The composition according to claim 1, wherein the buffer adjusts the pH of the composition to not less than 5.5 and not more than 6.5.

6. The composition according to claim 1, wherein the buffer comprises one or more buffering agents selected from sodium hydrogen phosphate, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, arginine hydrochloride, sodium citrate, trisodium citrate dihydrate, monosodium L-glutamate, sodium acetate, sodium carbonate, sodium hydrogen carbonate, sodium lactate, monopotassium phosphate, sodium hydroxide, meglumine, glycine, citric acid, and acetic acid.

7. The composition according to claim 1, wherein the buffer comprises citric acid and/or phosphoric acid.

8. The composition according to claim 1, further comprising an isotonicity agent.

9. The composition according to claim 8, wherein the isotonicity agent is one or more selected from D-sorbitol, sodium chloride, glycerol, D-mannitol, potassium chloride, lactitol and sucrose.

10. The composition according to claim 1, which is for the prophylaxis or treatment of pulmonary fibrosis or acute lung injury.

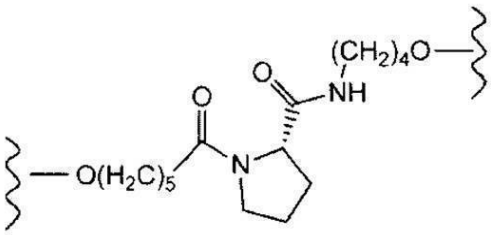
11. A method of producing the composition according to any one of claims 1 to 10, comprising dissolving the aforementioned nucleic acid molecule in a buffer adjusting a pH of the composition to not less than 4.6 and not more than 7.0, and storing the solution at ambient temperature.

12. The method according to claim 11, wherein the buffer comprises citric acid and/or phosphoric acid.

13. The method according to claim 11, wherein the composition is for the prophylaxis or treatment of pulmonary fibrosis or acute lung injury.

14. A method for stabilizing a single-stranded nucleic acid molecule in a composition, comprising dissolving the nucleic acid molecule in a buffer adjusting a pH of the composition to not less than 4.6 and not more than 7.0, and storing the solution at ambient temperature,

wherein the single-stranded nucleic acid molecule consists of a nucleotide sequence shown by 5'-AGCAGAGUACACACAGCAUAUACC-P-GGUAUAUGCUGUGUGUACUCUGCUUC-P-G-3' (in the sequence, P is a proline derivative linker represented by the following formula (I))



(I)

15. The method according to claim 14, wherein the buffer comprises citric acid and/or phosphoric acid.

16. The method according to claim 14, wherein the solution is a composition for the prophylaxis or treatment of pulmonary fibrosis or acute lung injury.

17. The method according to claim 11, wherein the buffer adjusts the pH of the composition to not less than 5.5 and not more than 6.5.

18. The method according to any one of claims 14 to 16, wherein the buffer adjusts the pH of the composition to not less than 5.5 and not more than 6.5.