versatility. That is, there are 1000 or more types of ubiquitin ligases in mammal, and ubiquitin ligase plays an important role in recognition of a target protein. Therefore, the complex needs to be individually designed per target protein. Further, the requirement of preparing an individually designed complex per target protein means that the target protein has to be known. Therefore, the technique is difficult to apply, for example, as a tool for identifying a target protein among degraded proteins.

On the other hand, the aforementioned ubiquitin independent technique involving use of a complex having a structure where a molecule capable of binding to a target protein is linked to a hydrophobic tag suffers from low cell membrane permeability and high cytotoxicity due to the hydrophobic tag.

In view of the above circumstances, an object of the present disclosure is to provide a novel protein-degradation inducing tag configured to induce degradation of a target protein, and use thereof.

Means for Solving the Problems

In one aspect, there is provided a protein-degradation inducing molecule, which is a conjugate of at least one protein-degradation inducing tag and at least one protein binding molecule capable of binding to a target protein, the at least one protein-degradation inducing tag being a molecule having a molecular weight of 50 g/mol to 5000 g/mol and having

an affinity with a proteasome without inhibiting degradation of the target protein by the proteasome, the protein-degradation inducing molecule leading the target protein bound to the protein binding molecule to degradation by the proteasome.

In another aspect, there is provided a library of protein-degradation inducing molecules, comprising two or more types of protein-degradation inducing molecules, each of the two or more types of protein-degradation inducing molecules being the protein-degradation inducing molecule as described herein.

In yet another aspect, there is provided a pharmaceutical composition, comprising the protein-degradation inducing molecule as described herein.

In another aspect, there is provided a method of degrading a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule as described herein.

In yet another aspect, there is provided a method of performing functional analysis on a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule as described herein.

In another aspect, there is provided a method of identifying a target protein, the method comprising a step of inducing degradation of a protein using the protein-degradation inducing molecule as described herein, and a step

of identifying a protein subjected to degradation induced by the protein-degradation inducing molecule.

In yet another aspect, there is provided a method of identifying a pathway molecule through a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule as described herein, and a step of identifying a protein showing an altered activity and expression, the protein being different from the target protein.

In another aspect, there is provided a method of screening for a protein-degradation inducing molecule, the method comprising a step of introducing the protein-degradation inducing molecule as described herein, or the library of protein-degradation inducing molecules as described herein into an *in vitro* system including a target protein, and selecting a protein-degradation inducing molecule which has induced degradation of the target protein.

In yet another aspect, there is provided a method of screening for a candidate ingredient for preventing or treating a disease, the method comprising a step of introducing the protein-degradation inducing molecule as described herein, or the library of protein-degradation inducing molecules as described herein into an *in vitro* disease model system, and selecting a protein-degradation inducing molecule which improves a condition of the disease.

In another aspect, there is provided a method of screening for a candidate ingredient for preventing or

treating a disease, the method comprising a step of introducing the protein-degradation inducing molecule as described herein, or the library of protein-degradation inducing molecules as described herein into an *in vitro* disease model system, and extracting a protein-degradation inducing molecule which aggravates a condition of the disease, and selecting a protein subjected to degradation induced by the extracted protein-degradation inducing molecule.

In yet another aspect, there is provided a method of specifying a disease-related protein, the method comprising a step of introducing the protein-degradation inducing molecule as described herein, or the library of protein-degradation inducing molecules as described herein into an *in vitro* disease model system, and extracting a protein-degradation inducing molecule which has altered a condition of a disease, and selecting a protein subjected to degradation induced by the extracted protein-degradation inducing molecule.

Specific means for achieving the above object include the following embodiments.

(1) A protein-degradation inducing tag, which is a molecule having an affinity with a protease without inhibiting degradation of a protein by the protease.

(2) The protein-degradation inducing tag according to

CLAIMS

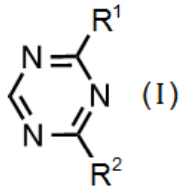
1. A protein-degradation inducing molecule, which is a conjugate of at least one protein-degradation inducing tag and at least one protein binding molecule capable of binding to a target protein, the at least one protein-degradation inducing tag being a molecule having a molecular weight of 50 g/mol to 5000 g/mol and having an affinity with a proteasome without inhibiting degradation of the target protein by the proteasome,

the protein-degradation inducing molecule leading the target protein bound to the protein binding molecule to degradation by the proteasome.

2. The protein-degradation inducing molecule according to claim 1, which is used to be added to a medium in which cells, a tissue, or an organ is cultured, or to be administered orally or parenterally to the living body of eukaryote or prokaryote which has a proteasome.

3. The protein-degradation inducing molecule according to claim 1 or 2, wherein the protein-degradation inducing tag satisfies at least one of the following (c) to (e):

(c) has a structure represented by the formula (I)



(I)

wherein R^1 and R^2 in formula (I) each independently represent a hydrocarbon group having 1 to 20 carbon atoms, an alkyoxy group having 1 to 20 carbon atoms, an aryloxy group having 6 to 20 carbon atoms, a hydroxyl group, a carboxy group, an amino group, or a halogeno group;

(d) has a structure where a proteasome inhibitory activity of aproteasome inhibitor is inactivated; or

(e) is a proteasome activating agent.

4. A library of protein-degradation inducing molecules, comprising two or more types of protein-degradation inducing molecules, each of the two or more types of protein-degradation inducing molecules being the protein-degradation inducing molecule according to any one of claims 1 to 3.

5. A pharmaceutical composition, comprising the protein-degradation inducing molecule according to any one of claims 1 to 3.

6. A method of degrading a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule according to any one of claims 1 to 3.

7. A method of performing functional analysis on a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule according to any one of claims 1 to 3.

8. A method of identifying a target protein, the method comprising a step of inducing degradation of a protein using the protein-degradation inducing molecule according to any one of claims 1 to 3, and

a step of identifying a protein subjected to degradation induced by the protein-degradation inducing molecule.

9. A method of identifying a pathway molecule through a target protein, the method comprising a step of inducing degradation of the target protein using the protein-degradation inducing molecule according to any one of claims 1 to 3, and

a step of identifying a protein showing an altered activity and expression, the protein

being different from the target protein.

10. A method of screening for a protein-degradation inducing molecule, the method comprising a step of introducing the protein-degradation inducing molecule according to any one of claims 1 to 3, or the library of protein-degradation inducing molecules according to claim 4 into an *in vitro* system including a target protein, and selecting a protein-degradation inducing molecule which has induced degradation of the target protein.

11. The method of screening for a protein-degradation inducing molecule according to claim 10, wherein the target protein is a disease causative agent, and the protein-degradation inducing molecule which has induced degradation of the target protein is selected as a candidate ingredient for preventing or treating a disease.

12. A method of screening for a candidate ingredient for preventing or treating a disease, the method comprising a step of introducing the protein-degradation inducing molecule according to any one of claims 1 to 3, or the library of protein-degradation inducing molecules according to claim 4 into an *in vitro* disease model system, and selecting a protein-degradation inducing molecule which improves a condition of the disease.

13. A method of screening for a candidate ingredient for preventing or treating a disease, the method comprising a step of introducing the protein-degradation inducing molecule according to any one of claims 1 to 3, or the library of protein-degradation inducing molecules according to claim 4 into an *in vitro* disease model system, and extracting a protein-degradation inducing molecule which aggravates a condition of the disease, and selecting a protein subjected to degradation induced by the extracted protein-degradation inducing molecule.

14. A method of specifying a disease-related protein, the method comprising a step of introducing the protein-degradation inducing molecule according to any one of claims 1 to 3, or the library of protein-degradation inducing molecules according to claim 4 into an *in vitro* disease model system, and extracting a protein-degradation inducing molecule which has altered a condition of a disease, and selecting a protein subjected to degradation induced by the extracted protein-degradation inducing molecule.