A film of the present invention contains a polypeptide derived from spider silk proteins. The decomposition temperature of the film is 240 to 260°C. The film absorbs ultraviolet light having a wavelength of 200 to 300 nm and has a light transmittance of 85% or more at a wavelength of 400 to 780 nm. The film is transparent and colorless in a visible light region.

A method for producing a film of the present invention includes: dissolving a polypeptide derived from spider silk proteins in a dimethyl sulfoxide solvent to prepare a dope; and cast-molding the dope on a surface of a base. Thus, the present invention provides a spider silk protein film that can be formed easily and has favorable stretchability, and a method for producing the same.
FIG. 1
FIG. 4
SPIDER SILK PROTEIN FILM, AND METHOD FOR PRODUCING SAME

TECHNICAL FIELD

[0001] The present invention relates to a spider silk protein film capable of being stretched, and a method for producing the same.

BACKGROUND ART

[0002] Spiders’ threads are also referred to as spider silks, and known to be produced by biosynthetic technologies using natural spider silks as a starting material. Films using spider silk proteins have been proposed in Patent Documents 1-3 below. These disclosures disclose films formed by dissolving spider silk proteins in a hexafluoroisopropanol (HFIP) solvent.

PRIOR ART DOCUMENTS

Patent Documents


DISCLOSURE OF INVENTION

Problem to be Solved by Invention

[0006] However, there has been a major problem that, if a film or resin is used as a substrate in cast molding, the hexafluoroisopropanol (HFIP) solvent proposed in the conventional methods sometimes dissolves the substrate. Further, since HFIP has a boiling point of 59° C. and has low storage stability, it evaporates at the time of cast molding for film formation, which makes it difficult to form films. Because of this, even the characteristics of spider silk protein films themselves have not been clarified.

[0007] In order to solve the above conventional problems, it is an object of the present invention to provide a spider silk protein film that can be formed easily and a method for producing the same that allows easy cast molding without dissolving a substrate made of a film or resin, and to clarify characteristics of the spider silk protein film.

Means for Solving Problem

[0008] A spider silk protein film of the present invention is a film that contains a polypeptide derived from spider silk proteins. The decomposition temperature of the film is 240 to 260° C. The film absorbs ultraviolet light having a wavelength of 200 to 300 nm and has a light transmittance of 85% or more at a wavelength of 400 to 780 nm. The film is transparent and colorless in a visible light region.

[0009] A method for producing a spider silk protein film of the present invention is a method for producing a film that contains a polypeptide derived from spider silk proteins, the method including: dissolving a polypeptide derived from spider silk proteins in a dimethyl sulfoxide solvent to prepare a dope; and cast-molding the dope on a surface of a base.

Effect of the Invention

[0010] The film of the present invention has a decomposition temperature of 240 to 260° C., and has high heat resistance. The film absorbs ultraviolet light having a wavelength of 200 to 300 nm, and has a light transmittance of 85% or more at a wavelength of 400 to 780 nm. The film absorbs ultraviolet light (UV) harmful to the human body, but has a favorable light transmittance at a wavelength of 400 to 780 nm. This film is transparent and colorless in a visible light region. The above characteristics are useful for optical films and the like. Further, by using dimethyl sulfoxide (hereinafter, also referred to as DMSO) as a solvent, the present invention can provide a spider silk protein film that can be formed easily and has favorable storage stability and a method for producing the same that allows easy cast molding without dissolving a substrate made of a film or resin. DMSO has a melting point of 18.4° C. and a boiling point of 189° C., has high storage stability, is less likely to evaporate the time of cast molding and assures high safety, thereby allowing formation of films having a uniform thickness and high transparency.

[0011] Further, such use of DMSO as a solvent not only enhances the stretchability of the spider silk protein film but also allows the use of resin substrates such as a polystyrene teraphthalate (PET) film as a substrate at the time of cast molding.

BRIEF DESCRIPTION OF DRAWINGS

[0012] FIG. 1 is a graph showing tensile test measurement of a film in one example of the present invention.

[0013] FIG. 2 is a graph showing light transmittance measurement of an unstretched film in one example of the present invention.

[0014] FIG. 3 is a graph showing light transmittance measurement of a stretched film in one example of the present invention.

[0015] FIG. 4 is a graph showing thermogravimetric measurement of a film in one example of the present invention.

[0016] FIG. 5 is a graph showing thermogravimetric measurement of a film in one example of the present invention.

DESCRIPTION OF THE INVENTION

[0017] The film of the present invention has a decomposition temperature of 240 to 260° C., and has high heat resistance. The heat resistance can be measured based on the reduction in weight using a thermo-gravimetric/differential thermal analyzer (TG-DTA). Further, according to transmittance measurement, the film absorbs ultraviolet light having a wavelength of 200 to 300 nm, and has a light transmittance of 85% or more at a wavelength of 400 to 780 nm. The film absorbs ultraviolet light (UV) harmful to the human body, but has a favorable light transmittance at a wavelength of 400 to 780 nm. Preferably, the film has a light transmittance of 90% or more at a wavelength of 400 to 780 nm. This film is transparent and colorless in a visible light region. This property is useful for an optical waveguide, an optical film containing a transparent conductive film, and the like.

[0018] The spider silk protein film can be stretched. Thermal fixation of the stretched film is preferred because the film can be provided with dimensional stability at ambient temperature (0 to 30° C.). The temperature of the thermal fixation after stretching is preferably 50 to 200° C., and further preferably 80 to 200° C. The time for the thermal fixation is preferably 5 to 600 seconds, and further preferably 20 to 300 seconds.

[0019] The refractive index of the spider silk protein film at a wavelength of 590 nm preferably ranges from 1.1 to 1.6, and
further preferably ranges from 1.2 to 1.6. The film having a refractive index in this range is useful for an optical waveguide, an optical film containing a transparent conductive film, and the like. The unstretched spider silk protein film has a haze value of preferably 0.5 to 3.0%, and further preferably 1.0 to 2.0%. Within this range, the film can have favorable transparency.

[0020] It is preferred that the spider silk protein film has moisture absorbency and has a mass reduction of 4 to 8 weight % in the vicinity of 67 to 94° C. in the thermogravimetric/differential thermal analyzer (TG-DTA). This indicates an equilibrium moisture content of the unstretched or stretched film. Further, in the thermogravimetric/differential thermal analyzer (TG-DTA), mass reduction is observed in the unstretched film in the vicinity of 175° C., which probably is remaining solvent—DMSO. Since the unstretched film obtained using the DMSO solvent can be stretched easily, it is considered that the remaining DMSO serves as a plasticizer at the time of stretching.

[0021] The unstretched film of the spider silk protein film has a maximum stress of 6 to 20 MPa, and further preferably 7 to 18 MPa. The unstretched film has a displacement at rupture point (strain) of 20 to 150%, and further preferably 23 to 95%. Moreover, preferably, the film that has been subjected to thermal fixation after stretching has a maximum stress of 40 MPa or more, preferably 40 to 100 MPa, and further preferably 45 to 75 MPa. The film has a displacement at rupture point (strain) of preferably 10 to 50%, and further preferably 15 to 40%. The maximum stress and the displacement at rupture point (strain) in the above ranges are practical as mechanical characteristics.

[0022] In the method of the present invention, a polypeptide derived from spider silk proteins is dissolved in a dimethyl sulfoxide solvent to prepare a dope, and the dope is cast-molded on a surface of a base, followed by drying and/or desolvation. Preferably, the dope has a viscosity of 15 to 80 cP (centipoises) in terms of film formability.

[0023] Preferably, the base to be used at the time of the cast molding is a polyethylene terephthalate (PET) film or a release film in which a silicone compound is fixed on a surface of a PET film. These substrates are advantageous in that they are stable with respect to the DMSO solvent, thereby allowing stable cast molding of the dope and easy separation of the resultant cast films. Although the cast film production is possible also using a glass substrate and a metal substrate, their affinity for the dope is so high that the resultant films are difficult to be detached from the substrates. Meanwhile, a fluorescein substrate (e.g., polytetrafluoroethylene) and a polypropylene (PP) film substrate repel the dope, thereby causing separation of liquid from the substrate and making it difficult to produce cast films.

[0024] It is preferred that the drying and/or desolvation are performed by at least one means selected from vacuum drying, hot-air drying, air drying, and immersion. The immersion for desolvation of cast film may be performed in water using an alcohol solution such as a lower alcohol with a carbon number of 1 to 5 such as methanol, ethanol, and 2-propanol, or in a mixed solution of water and alcohol. The temperature of the desolvation liquid (coagulation liquid) is preferably 0 to 90° C. Preferably, the solvent is removed as much as possible. In the case of stretching in liquid, desolvation can be performed simultaneously with stretching. Note that the desolvation may be performed after stretching.

[0025] The unstretched film after the drying and/or desolvation can be stretched uniaxially or biaxially in water. The biaxial stretching may be either sequential stretching or simultaneous biaxial stretching. Multistage stretching composed of two or more stages may be performed. The stretch ratio is preferably 1.01 to 6 times, and further preferably 1.05 to 4 times in both of the horizontal and vertical directions. Within this range, a balance between the stress and strain can be adjusted easily. The thickness of the unstretched or stretched film is preferably 1 to 1000μm. The condition of the stretching in water is preferably at a water temperature of 20 to 90° C. The film after the stretching is preferably subjected to thermal fixation by dry heat at 50 to 200° C for 5 to 600 seconds. The thermal fixation provides the film with dimensional stability at ambient temperature. Incidentally, the film stretched uniaxially will be a uniaxially-oriented film, and the film stretched biaxially will be a biaxially-oriented film.

[0026] In the present invention, DMSO, a polar solvent, is used as a dope of the polypeptide derived from natural spider silk proteins. DMSO has a melting point of 18.4° C. and a boiling point of 189° C. DMSO has a much higher boiling point than hexafluoroisopropanol (HFIP) and hexafluoroacetone (HFAc) having boiling points of 59° C. and -26.5° C., respectively, which have been used in conventional methods. Further, in view of the fact that DMSO has been used also in general industrial fields for acrylic fiber polymerization and acrylic fiber spinning solutions, and as solvents for polymide polymerization, they are low cost substances with proven safety.

[0027] The protein of the present invention is a polypeptide derived from spider silk proteins. The polypeptide derived from spider silk proteins is not limited particularly as long as it is derived from natural spider silk proteins or an analog of natural spider silk proteins. In terms of excellent tenacity, the polypeptide derived from spider silk proteins is preferably derived from major dragline silk proteins produced in major ampullate glands of spiders. Examples of the major dragline silk proteins include major ampullate spiderins MaSp1 and MaSp2 derived from Nephila clavipes, and ADF3 and ADF4 derived from Araneus diadematus, etc.

[0028] The recombinant spider silk proteins may be derived from minor dragline silk produced in minor ampullate glands of spiders. Examples of the minor dragline silk proteins include minor ampullate spiderins MiSp1 and MiSp2 derived from Nephila clavipes.

[0029] Other than these, the recombinant spider silk proteins may be derived from flagelliform silk proteins produced in flagelliform glands of spiders. Examples of the flagelliform silk proteins include flagelliform silk proteins derived from Nephila clavipes, etc.

[0030] Examples of the polypeptide derived from major dragline silk proteins include a polypeptide containing two or more units of an amino acid sequence represented by the formula 1: REPI-REP2 (1), preferably a polypeptide containing four or more units thereof, and more preferably a polypeptide containing six or more units thereof. In the polypeptide derived from major dragline silk proteins, units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) may be the same or different from each other.

[0031] In the formula (1), the REP1 represents polyalanine. In the REP1, the number of alanine residues arranged in succession is preferably 2 or more, more preferably 3 or more, further preferably 4 or more, and particularly preferably 5 or more. Further, in the REP1, the number of alanine residues
arranged in succession is preferably 20 or less, more preferably 16 or less, further preferably 12 or less, and particularly preferably 10 or less. In the formula (1), the REP2 is an amino acid sequence composed of 10 to 200 amino acid residues. The total number of glycine, serine, glutamine, proline and alanine residues contained in the amino acid sequence is 40% or more, preferably 50% or more, and more preferably 60% or more with respect to the total number of amino acid residues contained therein.

[0032] In the major dragline silk, the REP1 corresponds to a crystal region in a fiber where a crystal β sheet is formed, and the REP2 corresponds to an amorphous region in a fiber where most of the parts lack regular configurations and that has more flexibility. Further, the [REP1-REP2] corresponds to a repetitive region (repetitive sequence) composed of the crystal region and the amorphous region, which is a characteristic sequence of dragline silk proteins.

[0033] An example of the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) is a recombinant spider silk protein derived from ADF3 having an amino acid sequence represented by any of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3. The amino acid sequence represented by SEQ ID NO: 1 is an amino acid sequence obtained by the following mutation: in an amino acid sequence of ADF3 to the N-terminal of which has been added an amino acid sequence (SEQ ID NO: 4) composed of a start codon, His 10-tag and HRV3C Protease (Human rhinovirus 3C Protease) recognition site, 1st to 13th repetitive regions are about doubled and the translation ends at the 1154th amino acid residue. The amino acid sequence represented by SEQ ID NO: 2 is an amino acid sequence obtained by adding the amino acid sequence (SEQ ID NO: 4) composed of a start codon, His 10-tag and HRV3C Protease (Human rhinovirus 3C Protease) recognition site, to the N-terminal of a partial amino acid sequence of ADF3 (NCBI Genebank Accession No.: AAC47010.1: 1263287) obtained from the NCBI database. The amino acid sequence represented by SEQ ID NO: 3 is an amino acid sequence obtained by the following mutation: in an amino acid sequence of ADF3 to the N-terminal of which has been added the amino acid sequence (SEQ ID NO: 4) composed of a start codon, His 10-tag and HRV3C Protease (Human rhinovirus 3C Protease) recognition site, 1st to 13th repetitive regions are about doubled. Further, the polypeptide containing two or more units of the amino acid sequence represented by the formula 1: REP1-REP2 (1) may be a polypeptide that is composed of an amino acid sequence represented by any of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3 in which one or a plurality of amino acids have been substituted, deleted, inserted and/or added and that has repetitive regions composed of crystal regions and amorphous regions.

[0034] In the present invention, one or a plurality of it refers to 1 to 40, 1 to 35, 1 to 30, 1 to 25, 1 to 20, 1 to 15, 1 to 10, or 1 or a few, for example. Further, in the present invention, “one or a few” refers to 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 1.

[0035] An example of the recombinant spider silk protein derived from minor dragline silk proteins is a polypeptide containing an amino acid sequence represented by the formula 2: REP3 (2). In the formula 2, the REP 3 indicates an amino acid sequence composed of (Gly-Gly-Z)n(Gly-Ala)n (A)n, where Z indicates any one of amino acids, particularly, it is preferably an amino acid selected from the group consisting of Ala, Tyr and Gin. Further, n is preferably 1 to 4, l is preferably 0 to 4, and r is preferably 1 to 6.

[0036] Among spider silks, the minor dragline silk is wound spirally from the center of a spider net, and used as a reinforcement of the net and a yarn to wrap a captured prey. The minor dragline silk is inferior to the major dragline silk in tensile strength, but is known to have high stretchability. The reason for this is considered to be as follows: in the minor dragline silk, since many crystal regions are composed of regions where glycine and alanine are arranged alternately in succession, hydrogen bonds of the crystal regions weaken easily as compared with the major dragline silk whose crystal regions are composed only of alanine.

[0037] Examples of the recombinant spider silk protein derived from flagelliform silk proteins include a polypeptide containing an amino acid sequence represented by the formula 3: REP4 (3). In the formula 3, the REP 4 indicates an amino acid sequence composed of (Gly-Pro-Gly-Gly-X)n, where X indicates any one of amino acids, particularly, it is preferably an amino acid selected from the group consisting of Ala, Ser, Tyr and Val. Further, n indicates a number of 4 or larger, preferably 10 or larger, and more preferably 20 or larger.

[0038] Among spider silks, the flagelliform silk does not have crystal regions but has repetitive regions composed of amorphous regions, which is a major characteristic of the flagelliform silk. It is considered that since the major dragline silk and the like have repetitive regions composed of crystal regions and amorphous regions, they have both high stress and stretchability. Meanwhile, regarding the flagelliform silk, the stress is inferior to that of the major dragline silk but the stretchability is high. The reason for this is considered to be that the flagelliform silk is composed mostly of amorphous regions.

[0039] The polypeptide can be produced using a host that has been transformed by an expression vector containing a gene encoding a polypeptide. A method for producing a gene is not limited particularly, and it may be produced by amplifying a gene encoding a natural spider silk protein from a cell derived from spiders by a polymerase chain reaction (PCR) or the like, and cloning it, or may be synthesized chemically. A method for chemically synthesizing a gene also is not limited particularly, and it can be synthesized as follows, for example: based on information of amino acid sequences of natural spider silk proteins obtained from the NCBI web database or the like, oligonucleotides that have been synthesized automatically with AKTA oligopilot plus 100/100 (GE Healthcare Japan Corporation) are linked by PCR or the like. At this time, in order to facilitate purification and observation of protein, a gene may be synthesized that encodes a protein having the above-described amino acid sequence to the N-terminal of which has been added an amino acid sequence composed of a start codon and His 10-tag. Examples of the expression vector include a plasmid, a phage, a virus and the like that can express protein based on a DNA sequence. The plasmid-type expression vector is not limited particularly as long as it allows a target gene to be expressed in a host cell and it can amplify itself. For example, in the case of using Escherichia coli Rosetta (DE3) as a host, a pET22B(+) plasmid vector, a pCold plasmid vector and the like can be used. Among these, in terms of productivity of protein, it is preferable to use the pET22B(+) plasmid vector. Examples of the host include animal cells, plant cells, microbes, etc.
When the dope of the present invention is 100 mass %, the concentration of a solute (the polypeptide derived from natural spider silk proteins) is preferably 3 to 50 mass %, more preferably 3.5 to 35 mass %, and particularly preferably 4.2 to 15.8 mass %.

After removing dusts and bubbles, the viscosity of the dope is preferably 15 to 80 cP (centipoises), and further preferably 20 to 70 cP. The film cast molding is performed using the dope of this viscosity. Specifically, preferably on a PET film substrate on which a release layer is formed, the dope is cast so as to produce a wet film having a constant thickness using a film thickness control means such as an applicator, a knife coater, and a bar coater. In the case of a dry system, the solvent is dried, and the obtained film is subjected to drying and/or desolvation by vacuum drying, hot-air drying, air drying, etc. In the case of a wet system, the cast film is immersed into a desolvation bath (also referred to as a coagulation bath) to remove the solvent. Then, the film may be stretched as described above.

A color film also can be produced in the present invention. First, a colorant such as a dye is dissolved or dispersed in DMSO to prepare a DMSO coloring liquid. The colorant dissolves or disperses in DMSO easily. The coloring liquid is added to the dope, or the dope is added to the coloring liquid and mixed together, followed by film cast molding in the same manner as described above. Then, the resultant is subjected to drying and/or desolvation, and may be formed into an unstretched or stretched color film. The obtained color film can be applied to a reflector, a marker, an ultraviolet preventing film, a slit yarn, etc.

Examples

Hereinafter, the present invention will be described in further detail by way of examples. Note that the present invention is not limited to the following examples.

(1) Light Transmittance

A UV-Vis-NIR spectrophotometer manufactured by Shimadzu Corporation was used.

(2) Thermal Analysis

A Thermo-Gravimetric/Differential Thermal Analyzer (TG-DTA) manufactured by Seiko Instruments Inc. was used.

(3) Refractive Index

In accordance with JIS K 7142, an Abbe Refractometer 2T manufactured by ATAGO Co., Ltd. was used to measure refractive index under the following conditions: measurement temperature: 23° C.; light source: Na lamp (D beam/589 nm); the number of measurements: 3; contact liquid: diiodomethane.

(4) Viscosity

An EMS machine manufactured by Kyoto Electronics Manufacturing Co., Ltd. was used.

(5) Tensile Test

A tensile testing machine manufactured by Shimadzu Corporation was used.

(6) Film Thickness Measurement

A digital outside micrometer manufactured by Nippon Seiki Co., Ltd. was used.

(7) Measurement of Remaining Amount of Solvent

As an internal standard, 1,2-dichloroethane-formic acid solution at a concentration of 3,100 ppm (0.00310 mg/ml) was prepared. 500 μl of a protein solution (0.1 g of the protein film was dissolved in 10 ml of formic acid) and 500 μl of an internal standard solution were mixed. Further, an acetonitrile deuterated solvent for H-NMR measurement was added to the mixed solution in an amount approximately equivalent to that of the mixture solution so as to dilute the solution to about two times, and then H-NMR measurement was performed (NMR model: JNM-ECEX 100 manufactured by JOEL Ltd.). The H-NMR integrated intensity of 1,2-dichloroethane (internal standard sample) was compared with the H-NMR integrated intensity of DMSO. A calibration curve was formed by preparing a DMSO-formic acid solution at 3 ppm to 3000 ppm and following the above protocol. By comparison with the calibration curve, the concentration of DMSO in the protein solution was calculated. A nuclear magnetic resonator (NMR) manufactured by JOEL Ltd. was used for the measurement of the concentration of DMSO.

Examples 1-4, Comparative Example 1

1. Preparation of Polypeptide Derived from Spider Silk Proteins

<Gene Synthesis>

(1) Gene Synthesis of ADF3Kai

A partial amino acid sequence of ADF3 (GI: 1263287), which is one of two principal dragline silk proteins of Araneus diadematus, was obtained from the NCBI web database, and synthesis of a gene encoding an amino acid sequence (SEQ ID NO: 2) was outsourced to GenScript, Inc. The amino acid sequence (SEQ ID NO: 2) is an amino acid sequence obtained by adding an amino acid sequence (SEQ ID NO: 4) composed of a start codon, His 10-tag and HRV 3C Protease (Human rhinovirus 3C Protease) recognition site, to the N-terminal of said partial amino acid sequence of ADF3. Consequently, a pUC57 vector to which a gene of ADF3Kai having a base sequence represented by SEQ ID NO: 5 had been introduced was obtained (having an Nde I site immediately upstream of 5' terminal of the gene and an Xba I site immediately downstream of 5' terminal thereof). Thereafter, the gene was subjected to a restriction enzyme treatment with Nde I and EcoR I, and recombined into a pET122b(+)-expression vector.

(2) Gene Synthesis of ADF3Kai-Large

The half of the gene sequence of ADF3Kai on the 5' side (hereinafter, referred to as a sequence A) was amplified by the PCR reaction using ADF3Kai as a template, and a T7 promoter primer (SEQ ID NO: 8) and a Rep Xba I primer (SEQ ID NO: 9). The obtained DNA fragment of the sequence A was recombined into a pUC118 vector that in advance had been subjected to the restriction enzyme treatment with Nde I and Xba I using a Mighty Cloning Kit (manufactured by TAKARA BIO INC.). Similarly, the half of the gene sequence of ADF3Kai on the 3' side (hereinafter, referred to as a sequence B) was amplified by the PCR reaction using ADF3Kai as a template, and an Xba I Rep primer (SEQ ID NO: 10) and a T7 terminator primer (SEQ ID NO: 11). The obtained DNA fragment of the sequence B was recombined into a pUC118 vector that in advance had been subjected to the restriction enzyme treatment with Xba I and EcoR I using the Mighty Cloning Kit (manufactured by TAKARA BIO INC.). The pUC118 vector to which the sequence A had been introduced and the pUC118 vector to which the sequence B had been introduced were subjected to the restriction enzyme treatment with Nde I, Xba I and Xba I, EcoR I, respectively, and target DNA fragments of the sequences A and B were purified by gel cut. The DNA frag-
ments A, B and the pET22b(+) that in advance had been subjected to the restriction enzyme treatment with Nde I and EcoR I were subjected to a ligation reaction and transformed into *Escherichia coli* DH5a. After confirmation of the insertion of the target DNA fragments by a colony PCR using a T7 promoter primer and a T7 terminator primer, plasmid was extracted from a colony where a target band size (3.6 kbp) was obtained, and the entire base sequence was checked by a sequence reaction using a 3130xl Genetic Analyzer (Applied Biosystems). Consequently, the construction of a gene of ADF3Kai-Large represented by SEQ ID NO: 6 was confirmed. The amino acid sequence of ADF3Kai-Large is as represented by SEQ ID NO: 3.

[0064] (3) Gene Synthesis of ADF3Kai-Large-NRSH1

[0065] With a pET22b(+) vector to which the gene of ADF3Kai-Large obtained above had been introduced used as a template, through site-directed mutagenesis using a PrimeSTAR Mutagenesis Basal Kit (manufactured by TAKARA BIO INC.), a codon GCC corresponding to the 1155th amino acid residue, i.e., glycine (Gly), in the amino acid sequence of ADF3Kai-Large (SEQ ID NO: 3) was mutated into a stop codon TAA, and a gene of ADF3Kai-Large-NRSH1 represented by SEQ ID NO: 7 was constructed on the pET22b(+). The accuracy of the introduction of the mutation was checked by the sequence reaction using the 3130xl Genetic Analyzer (Applied Biosystems). The amino acid sequence of ADF3Kai-Large-NRSH1 is as represented by SEQ ID NO: 1.

[0066] <Expression of Protein>

[0067] The pET22b(+) expression vector containing the gene sequence of ADF3Kai-Large-NRSH1 was transformed into *Escherichia coli* Rosetta (DE3). The obtained single colony was incubated for 15 hours in 2 mL of an LB culture medium containing ampicillin. Thereafter, 1.4 mL of said culture solution was added to 140 mL of an LB culture medium containing ampicillin, and incubated to an OD600 of 3.5 under the conditions of 37° C. and 200 rpm. Next, the culture solution with the OD600 of 3.5 was added to 7 L of a 2xYT culture medium containing ampicillin, together with 140 mL of 50% glucose, and incubated further to the OD600 of 4.0. Thereafter, isopropyl-[β]-thiogalactopyranoside (IPTG) was added to the obtained culture solution with the OD600 of 4.0 so that the final concentration would be 0.5 mM, thereby inducing the expression of protein. After a lapse of two hours from the addition of IPTG, the culture solution was centrifuged and bacterial cells were collected. Protein solutions prepared from the bacterial cells before the addition of IPTG and after the addition of IPTG were each electrophoresed in a polyacrylamide gel. Consequently, a target band size (about 101.1 kDa) was observed with the addition of IPTG, and the expression of the target protein was confirmed.

[0068] Purification

[0069] (1) About 50 g of bacteria cells of the *Escherichia coli* expressing the ADF3Kai-Large-NRSH1 protein and 300 ml of a buffer solution M (20 mM Tris-HCl, pH 7.4) were placed in a centrifuge tube (1000 ml). After dispersing the bacteria cells with a mixer (“T18 basic ULTRA TURRAX” manufactured by IKA, level 2), the dispersion was centrifuged (11,000 g, 10 minutes, room temperature) with a centrifuge (“Model 7000” manufactured by Kubota Corporation), and a supernatant was discarded.

[0070] (2) To a precipitate (bacteria cells) obtained by the centrifugation, 300 mL of the buffer solution M and 3 mL of 0.1 M PMSF (dissolved by isopropanol) were added. After dispersing the precipitate for 3 minutes with the above mixer (level 2) manufactured by IKA, the bacteria cells were disrupted repeatedly for three times using a high-pressure homogenizer (“Panda Plus 2000” manufactured by GEA Niro Soave).

[0071] (3) To the disrupted bacterial cells, 300 mL of a buffer solution B (50 mM Tris-HCl, 100 mM NaCl, pH 7.0) containing 3 w/v % of SDS was added. After dispersing well the bacterial cells with the above mixer (level 2) manufactured by IKA, the dispersion was stirred for 60 minutes with a shaker (manufactured by TAIITEC CORPORATION, 200 rpm, 37° C). Thereafter, the stirred dispersion was centrifuged (11,000 g, 30 minutes, room temperature) with the above centrifuge manufactured by Kubota Corporation, and a supernatant was discarded, whereby SDS washing granules (precipitate) were obtained.

[0072] (4) The SDS washing granules were suspended in a DMSO solution containing 1M lithium chloride so that the concentration would be 100 mg/mL, and heat-treated for 1 hour at 80° C. Thereafter, the heated suspension was centrifuged (11,000 g, 30 minutes, room temperature) with the above centrifuge manufactured by Kubota Corporation, and the supernatant was collected.

[0073] (5) Ethanol in an amount three times greater than that of the collected supernatant was prepared. The collected supernatant was added to the ethanol, and left to stand still for 1 hour at room temperature. Thereafter, the resultant was centrifuged (11,000 g, 30 minutes, room temperature) with the above centrifuge manufactured by Kubota Corporation to collect aggregated protein. Next, a process of washing aggregated protein using pure water and a process of collecting aggregated protein by centrifugation were repeated three times, and thereafter water was removed by a freeze dryer to collect freeze-dried powder. The purification degree of the target protein ADF3Kai-Large-NRSH1 (about 56.1 kDa) in the obtained freeze-dried powder was checked by analyzing images of the results of polyacrylamide gel electrophoresis (CB staining) of said protein powder using Totallab (non-linear dynamics Ltd.). As a result, the purification degree of ADF3Kai-Large-NRSH1 was about 85%.

2. Adjustment of Dope

[0074] A dope was prepared by dissolving the spider silk protein in a DMSO solvent so that the concentration of the protein would be 5.98 mass %. After 3 hours of dissolution using a shaker, dusts and bubbles were removed from the dope. The solution viscosity of the dope was 23.5 cP (centipoises).

3. Film Cast Molding

[0075] In Example 1, a release film (manufactured by Mitsui Chemicals Tobelco, Inc., product number “SP-PET-01-75-BU”), in which a silicone compound is fixed on a surface of a 75 μm-thick polyethylene terephthalate film (PET), was used as a substrate. The dope was cast-molded on the surface of the substrate using a film applicator (150 mm, with a micrometer, manufactured by MTI Corporation). Thereby, a wet film was produced. In the other Examples and Comparative Examples, wet films were produced in the same manner as in Example 1 using substrates shown in Table 1.

4. Drying

[0076] After 16 hours of standing at 60° C, each film was left to stand still for another 16 hours in a vacuum dryer at 60°
C. to be dried. The unstretched spider silk protein film thus obtained was detached from the substrate. If the film could not be detached in air, it was immersed in water to be detached.

Table 1 summarizes the results of the unstretched films obtained by the above-described manner. The evaluations in Table 1 are based on the following.

**Table 1**

<table>
<thead>
<tr>
<th>Type of substrate</th>
<th>Casting property</th>
<th>Detachability Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example 1</td>
<td>PET film (75 µm) + silicone releasing treatment</td>
<td>A</td>
</tr>
<tr>
<td>Example 2</td>
<td>PET (100 µm) film</td>
<td>A</td>
</tr>
<tr>
<td>Example 3</td>
<td>Glass plate</td>
<td>A</td>
</tr>
<tr>
<td>Example 4</td>
<td>Acrylic plate</td>
<td>B</td>
</tr>
<tr>
<td>Comparative</td>
<td>Copper plate</td>
<td>A</td>
</tr>
<tr>
<td>Example 1</td>
<td></td>
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</table>

As described above, in Examples 1 and 2 of the present invention, favorable unstretched films were obtained because the PET film or the film in which a silicone releasing thin film was formed on a PET film was used as the substrate. Although there were some problems in detachability in Example 3 and in the casting property and detachability in Example 4, films could be formed. On the other hand, in Comparative Example 1, a film could be formed but the detachability was poor.

**Table 2**

<table>
<thead>
<tr>
<th>Temperature of water (°C.)</th>
<th>Length of film before stretching</th>
<th>Length at the time of rupture (mm)</th>
<th>Maximum stretch ratio (times)</th>
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</thead>
<tbody>
<tr>
<td>Example 5</td>
<td>5</td>
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<tr>
<td>Example 6</td>
<td>25</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Example 7</td>
<td>48</td>
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</tr>
<tr>
<td>Example 8</td>
<td>79</td>
<td>30</td>
<td>50</td>
</tr>
</tbody>
</table>

**Remarks**

As can be seen from Table 2, in the range of 5 to 79°C, the film could be stretched in water. In the range of 25 to 48°C, the stretch ratio of two times or more was achieved.

**Example 9**

It was found that the film obtained by uniaxially stretching the unstretched film of Example 1 in water shrank at room temperature of 25°C. In order to stop such shrinkage, the film was subjected to thermal fixation. Table 3 shows the various conditions and results.

**Table 3**

<table>
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<th>Conditions of stretching</th>
<th>Shrinkage</th>
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<td>Temperature of water (°C.)</td>
<td>Stretch ratio (times)</td>
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<td>Example 9</td>
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</table>
As can be seen from Table 3, the shrinkage could be stopped by thermal fixation after stretching.

Examples 10-11

An unstretched film was produced in the same manner as in Example 1. The film had a thickness of 23.4 μm (Example 10). This unstretched film was stretched uniaxially to 1.5 times in water at 50°C. The film had a thickness of 19.8 μm (Example 11). Table 4 below shows the results of tensile test on the respective films obtained. The tensile test was performed under environments of 25°C and 60% RH (humidity). The values of the uniaxially-stretched film of Example 11 shown in Table 4 are values in the stretching direction. FIG. 1 summarizes the data.

<table>
<thead>
<tr>
<th>Example 10</th>
<th>Example 11</th>
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<tr>
<td>(unstretched film)</td>
<td>(uniaxially-stretched film)</td>
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<tr>
<td>Maximum stress (calculated in all areas) (MPa)</td>
<td>9.08</td>
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<tr>
<td>Displacement at rupture point (strain), last 100% (ε%)</td>
<td>90.62</td>
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<tr>
<td>Elastic modulus, maximum inclination, 2 points (MPa)</td>
<td>214.53</td>
</tr>
<tr>
<td>Maximum test force (calculated in all areas) (N)</td>
<td>2.83</td>
</tr>
<tr>
<td>Distance between grippers (mm)</td>
<td>20.00</td>
</tr>
<tr>
<td>Thickness (μm)</td>
<td>31.2</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>10.00</td>
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</tbody>
</table>

Both of the unstretched films obtained in Examples 1 and 10 had a refractive index nD of 1.556. FIGS. 2 and 3 show graphs of light transmission measurement. FIG. 2 is a graph showing the light transmission measurement of the film of Example 10, and FIG. 3 is a graph showing the light transmission measurement of the film of Example 11. The films absorbed ultraviolet light having a wavelength of 200 to 300 nm, and exhibited a light transmission of 85% or more at a wavelength of 400 to 780 nm.

FIG. 4 is a graph showing thermal analysis measurement of the film of Example 10, and FIG. 5 is a graph showing thermal analysis measurement of the film of Example 11. Both of the films had a mass reduction of 4 to 8 mass % in the vicinity of 67 to 94°C, which probably is the amount of equilibrium moisture absorbed. It also was found that the decomposition temperature of the film was present in the vicinity of 240 to 260°C. Further, the unstretched film of Example 10 had a mass reduction also in the vicinity of 175°C, which probably is the remaining solvent—DMSO.

Example 12

The unstretched film obtained in Example 1 (50 mm in length, 50 mm in width and 32 μm in thickness) was stretched biaxially to 1.26 times in an X-axis direction and 1.26 times in a Y-axis direction simultaneously under environments of 78 to 80 RH % (humidity) and 24°C (temperature). The tensile speed was 30 mm/min. A film biaxial stretching machine manufactured by Imoto Machinery Co., Ltd. was used as a stretching machine. Simultaneous biaxial stretching was possible.

Comparative Example 2

Hexafluoropropanol (HFIP) was used as a solvent, and the same spider silk protein as that of Example 1 was used. A dope was prepared by dissolving the spider silk protein in the HFIP solvent so that the concentration of the protein would be 5.98 mass %. The test was performed in the same manner as in Example 1 except that the same substrates as those of Examples 1 and 2 were used as the casting substrates. As a result, the PET substrates were swelled and dissolved by HFIP, and could no longer be used as the substrates.

To cope with this, a glass substrate was used for casting. However, the resultant film was difficult to be detached from the substrate, and needed to be detached gradually in water with great effort to obtain an unstretched film. A sample 20 mm in length and 40 mm in width of this unstretched film was set in a manual uniaxial stretching machine (manufactured by Imoto Machinery Co., Ltd.) (the distance between grippers: 20 mm) and pulled in hot water at 50°C. As a result, the film dissolved partially and tore, and hence stretching was impossible.

Example 13

In this example, methods of removing the solvent DMSO were tested. The desolvation shown in Table 5 was performed using the unstretched film (thickness: 55.2 μm) obtained in Example 1. The remaining amount of DMSO was quantified using NMR. Table 5 shows the results.

<table>
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<tr>
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<th>Remaining amount of DMSO in film (mass %)</th>
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</thead>
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<td>1</td>
<td>Unstretched film, untreated</td>
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<tr>
<td>2</td>
<td>Vacuum drying at 60°C, 24 hours</td>
<td>11.1</td>
</tr>
<tr>
<td>3</td>
<td>Vacuum drying at 80°C, 48 hours</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>100% methanol, 25°C, 24 hours</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>100% methanol, 25°C, 48 hours</td>
<td>Not detected</td>
</tr>
<tr>
<td>6</td>
<td>50% methanol/aqueous solution, 25°C, 24 hours</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>50% methanol/aqueous solution, 25°C, 48 hours</td>
<td>Not detected</td>
</tr>
<tr>
<td>8</td>
<td>Water at 50°C, 24 hours</td>
<td>0.02</td>
</tr>
<tr>
<td>9</td>
<td>Water at 50°C, 48 hours</td>
<td>Not detected</td>
</tr>
<tr>
<td>10</td>
<td>Water at 50°C, stretching for 5 seconds</td>
<td>1.2</td>
</tr>
</tbody>
</table>

As shown in Table 5, DMSO was difficult to remove by vacuum drying at 60°C. On the other hand, DMSO was removed efficiently by methanol, methanol/water, and hot water at 50°C. Films containing DMSO at an undetectable level can be used also as films compatible with the human
body. Further, only by stretching the film in hot water, the amount of DMSO was reduced by about one-tenth.

Example 14

[0100] In this example, color film production was tested. First, 0.5 w/v % of each dye shown in Table 6 was added to DMSO, followed by dissolution at 60°C for 2 hours while shaking, and further dissolution at 40°C for 16 hours. The respective coloring solutions were added so that the concentration of the spider silk protein would be 5.98 mass %, Films were produced in the same manner as in Example 1. Table 6 shows the results.

**TABLE 6**

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<th>No.</th>
<th>Dye</th>
<th>Result</th>
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</thead>
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<td>Acid dye: Acid Yellow RW</td>
<td>A color film colored in bright yellow was obtained.</td>
</tr>
<tr>
<td>2</td>
<td>Acid dye: Acridine Sky Blue FSE</td>
<td>A color film colored in bright blue was obtained.</td>
</tr>
<tr>
<td>3</td>
<td>Acid dye: Polar Red B 125%</td>
<td>A color film colored in bright red was obtained.</td>
</tr>
<tr>
<td>4</td>
<td>Fluorescent dye: NKP-8315 Yellow</td>
<td>A color film colored in yellow fluorescence was obtained. When illuminated with ultraviolet light, the film emitted green fluorescence.</td>
</tr>
</tbody>
</table>

Example 15

[0101] Unstretched films were produced in the same manner as in Example 1. One of the films obtained had a thickness of 69.0 μm and the other had a thickness of 10.1 μm. The unstretched films 30 mm in length and 30 mm in width were set at a Haze Meter (model HZ-2P, manufactured by Suga Test Instruments Co., Ltd.). Haze values of the respective films were measured using C light sources. The haze values were obtained from the following formula.

\[
\text{Haze rate} = \left( \frac{T_0 \text{ diffuse transmittance}}{T_1 \text{ total light transmittance}} \right) \times 100
\]

[0102] The results of the measurement were as follows.

[0103] (1) Thickness: 69.0 μm, Td: 1.39, Tt: 91.65, Haze: 1.5%(%)

[0104] (2) Thickness: 10.1 μm, Td: 0.93, Tt: 92.5, Haze: 1%

From these results, it was confirmed that the unstretched films have high transparency.

**INDUSTRIAL APPLICABILITY**

[0105] The film of the present invention has favorable light transmittance and comparatively high refractive index, and hence is useful for an optical waveguide, an optical film containing a transparent conductive film, and the like. It also is useful as a color film.

[0106] Sequence Listing Free Text

[0107] SEQ ID NOS: 1-4 amino acid sequence

[0108] SEQ ID NOS: 5-7 base sequence

[0109] SEQ ID NOS: 8-11 primer sequence

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690 695 700
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1. A spider silk protein film comprising a polypeptide derived from spider silk proteins, wherein a decomposition temperature of the film is 240 to 260°C, the film absorbs ultraviolet light having a wavelength of 200 to 300 nm and has a light transmittance of 85% or more at a wavelength of 400 to 780 nm, and the film is transparent and colorless in a visible light region.

2. The spider silk protein film according to claim 1, wherein the spider silk protein film is unstrained, and the unstrained film before desolvation contains dimethyl sulfoxide.

3. The spider silk protein film according to claim 1, wherein the spider silk protein film is a uniaxially-stretched film.

4. The spider silk protein film according to claim 1, wherein the spider silk protein film has a refractive index ranging from 1.2 to 1.6 at a wavelength of 590 nm.

5. The spider silk protein film according to claim 1, wherein the spider silk protein film has moisture absorbency and has a mass reduction in the vicinity of 67 to 94°C in a thermo-gravimetric/differential thermal analyzer (TG-DTA).

6. The spider silk protein film according to claim 1, wherein the unstretched film has a maximum stress of 6 to 20 MPa and a displacement at rupture point (strain) of 20 to 150%.

7. The spider silk protein film according to claim 1, wherein the spider silk protein film is colored by addition of a colorant that can dissolve or disperse in dimethyl sulfoxide.

8. The spider silk protein film according to claim 3, wherein the stretched spider silk protein film has a maximum stress of 40 MPa or more and a displacement at rupture point (strain) of 10 to 50%.

9. A method for producing a spider silk protein film containing a polypeptide derived from spider silk proteins, comprising:

dissolving a polypeptide derived from spider silk proteins in a dimethyl sulfoxide solvent to prepare a dope; and cast-molding the dope on a surface of a base.
10. The method for producing a spider silk protein film according to claim 9, wherein the dope has a viscosity of 15 to 80 cP.

11. The method for producing a spider silk protein film according to claim 9, wherein the base to be used at the time of the cast molding is a polyethylene terephthalate (PET) film or a release film in which a silicone compound is fixed on a surface of a PET film.

12. The method for producing a spider silk protein film according to claim 9, wherein the film is subjected to drying and/or desolvation by at least one selected from vacuum drying, hot-air drying, air drying, and immersion.

13. The method for producing a spider silk protein film according to claim 9, wherein the film after the drying and/or desolvation is stretched uniaxially or biaxially in water, or the film is subjected to desolvation simultaneously with stretching.

14. The method for producing a spider silk protein film according to claim 13, wherein the film after the stretching is subjected to thermal fixation by dry heat at 50 to 200°C.

15. The method for producing a spider silk protein film according to claim 9, further comprising:
   - dissolving or dispersing a colorant in dimethyl sulfoxide (DMSO) to prepare a DMSO coloring liquid; and
   - adding the coloring liquid to the dope or adding the dope to the coloring liquid and mixing them to prepare a dope for film casting.