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May 27, 2004

Dr. Grant A. Krafft, Ph.D.
Chairman and Chief Scientific Officer
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Re: Japanese Patent Application No.: 10-533262
National Stage of International Patent App. No.: PCT/US98/02426
MBHB Ref. No.: 97-002-H

Grant:

We are pleased to report that the Japanese Patent Office has granted the patent application identified above and accorded it Japanese Patent Registration No. 3512815.

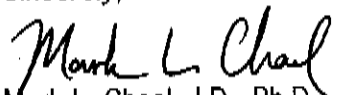
Enclosed please find an original Certificate of Patent as well as a copy of the issued patent. This patent will expire on February 5, 2018, provided that all annuities are timely paid. The next annuity for this patent will be due on January 16, 2007, and yearly thereafter. We will keep you apprised of these dates as they approach.

Also enclosed please find an English translation of the claims that were allowed. You may recall that in order to expedite prosecution of the present patent application, we cancelled the rejected claims that were directed to "compounds that block the formation of ADDLs" (in view of the disclosure of gossypol and tryptic peptides) as well as claims directed to the "treatment of Alzheimer's disease, etc." (in view of Japanese patent policy regarding medical treatment claims).

Please note, however, that we re-filed these rejected claims in a divisional patent application. We are considering alternative claim language for these claims in the divisional application; language that will be acceptable to the Japanese Patent Office. We will forward the particulars for the divisional application as they become available.

Please let me know if you need additional information or have any questions regarding this matter.

Sincerely,

A handwritten signature in black ink that reads "Mark L. Chael". The signature is written in a cursive style with a large, stylized initial "M".

Mark L. Chael, J.D., Ph.D.

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Enc.

Cc: Ms. Barbara Spiegel
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TRANSLATION OF AMENDED CLAIMS

Filed: October 1, 2003

1. An isolated, soluble, non-fibrillar amyloid β protein assembly comprising 3 to 12 amyloid β proteins, wherein the amyloid β protein is the β 1-42 protein and wherein the assembly is neurotoxic.
2. The protein assembly according to claim 1 wherein the assembly comprises trimer, tetramer, pentamer, and hexamer aggregates of amyloid β protein.
3. The protein assembly according to claim 1 or 2 wherein the assembly has a molecular weight of about 26 kD to about 28 kD as measured by non-denaturing gel electrophoresis.
4. The protein assembly according to any of claims 1 to 3 wherein the assembly has a molecular weight of about 22 kD to about 24 kD or about 18 kD to about 19 kD as determined by electrophoresis on a 15% SDS-polyacrylamide gel.
5. The protein assembly according to any of claims 1 to 4 wherein the assembly has comprises globules with dimensions of about 4.7 nm to about 6.2 nm as measured by atomic force microscopy.
6. The protein assembly according to any of claims 1 to 5 wherein the assembly comprises globules with dimensions of about 4.9 nm to about 5.4 nm as measured by atomic force microscopy.

7. The protein assembly according to any of claims 1 to 5 wherein the assembly comprises globules with dimensions of about 5.7 nm to about 6.2 nm as measured by atomic force microscopy.

8. The protein assembly according to any of claims 1 to 5 wherein about 40% to about 75% of the assembly comprises globules with dimensions of about 4.9 nm to about 5.4 nm and dimensions of about 5.7 nm to about 6.2 nm, as measured by atomic force microscopy.

9. A method for assaying the effects of the protein assembly according to any of claims 1 to 8, the method comprising:

(a) administering the protein assembly to the hippocampus of an animal excluding human;

(b) applying an electrical stimulus; and

(c) measuring the cell body spike amplitude over time to determine the long-term potentiation response,

with the proviso that administration of the protein assembly is not done for therapy.

10. The method of claim 9, wherein the long-term potentiation response of the animal is compared to the long-term potentiation response of another animal treated in the same fashion except having saline administered instead of the protein assembly prior to application of the electrical stimulus.

11. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with β amyloid specific antibody; and

(b) detecting binding of the antibody to the protein assembly.

12. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with serum-starved neuroblastoma cells; and

(b) measuring morphological changes in the cells by comparing the morphology of the cells against neuroblastoma cells that have not been contacted with the test material.

13. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with brain slice cultures; and

(b) measuring brain cell death as compared against brain slice cultures that have not been contacted with the test material.

14. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with neuroblastoma cells; and

(b) measuring increases in Fyn kinase activity by comparing Fyn kinase activity in the cells against Fyn kinase activity in neuroblastoma cells that have not been contacted with the test material.

15. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with cultures of primary astrocytes; and

(b) determining activation of the astrocytes as compared to cultures of primary astrocytes that have not been contacted with the test material.

16. A method for detecting in a test material the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting the test material with cultures of primary astrocytes; and

(b) measuring in the astrocytes increases in the mRNA for proteins selected from the group consisting of interleukin-1, inducible nitric oxide synthase, Apo E, Apo J, and α 1-antichymotrypsin by comparing the mRNA levels in the

astrocytes against the corresponding mRNA levels in cultures of primary astrocytes that have not been contacted with the test material.

17. A method for identifying compounds that modulate the effects of the protein assembly according to any of claims 1 to 8, the method comprising:

(a) administering either saline or a test compound to the hippocampus of an animal;

(b) applying an electrical stimulus;

(c) measuring the cell body spike amplitude over time to determine the long-term potentiation response; and

(d) comparing the long-term potentiation response of animals having saline administered to the long-term potentiation response of animals having test compound administered,

with the proviso that administration of the test compound is not done for therapy.

18. The method of claim 17 which further comprises administering the protein assembly to the hippocampus either before, along with, or after administering the saline or test compound, with the proviso that administration of the protein assembly is not done for therapy.

19. A method for identifying compounds that block the neurotoxicity of the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting separate cultures of neuronal cells with the protein assembly either in the presence or absence of the compound;

(b) measuring the proportion of viable cells in each culture; and

(c) comparing the proportion of viable cells in each culture, with compounds that block the neurotoxicity of the oligomeric structure being identified as resulting in an increased proportion of viable cells in the culture as compared to the corresponding culture contacted with the oligomeric structure in the absence of the compound.

20. A method for identifying compounds that block binding to a cell surface protein of the protein assembly of any of claims 1 to 8, the method comprising:

(a) contacting separate cultures of neuronal cells with the protein assembly either in the presence or absence of the compound;

(b) adding a reagent that binds to the protein assembly, the reagent being fluorescent;

(c) analyzing the separate cell cultures by fluorescence-activated cell sorting; and

(d) comparing the fluorescence of the cultures, with compounds that block binding to a cell surface protein of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound.

21. A method for identifying compounds that block binding to a cell surface protein of the protein assembly of any of claims 1 to 8, the method comprising:

(a) forming the protein assembly from amyloid β protein such that it becomes a labeled protein assembly comprising a binding moiety capable of binding a fluorescent reagent;

(b) contacting separate cultures of neuronal cells with the labeled oligomeric structure either in the presence or absence of the compound;

(c) adding a fluorescent reagent that binds to the protein assembly;

(d) analyzing the separate cell cultures by fluorescence-activated cell sorting; and

(e) comparing the fluorescence of the cultures, with compounds that block binding to a cell surface protein of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound.

22. A method for identifying compounds that block formation or binding to a cell surface protein of the protein assembly oligomeric structure of any of claims 1 to 8, the method comprising:

(a) preparing separate samples of amyloid β

protein that either have or have not been mixed with the compound;

(b) forming the protein assembly in the separate samples;

(c) contacting separate cultures of neuronal cells with the separate samples;

(d) adding a reagent that binds to the protein assembly, the reagent being fluorescent;

(e) analyzing the separate cell cultures by fluorescence-activated cell sorting; and

(f) comparing the fluorescence of the cultures, with compounds that block formation or binding to a cell surface protein of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound.

23. A method for identifying compounds that block formation or binding to a cell surface protein of the protein assembly oligomeric structure of any of claims 1 to 8, the method comprising:

(a) preparing separate samples of amyloid β protein that either have or have not been mixed with the compound;

(b) forming the protein assembly in the separate samples such that it becomes a labeled protein assembly comprising a binding moiety capable of binding a fluorescent

reagent in each of the separate samples;

(c) contacting separate cultures of neuronal cells with the separate samples;

(d) adding a fluorescent reagent that binds to the protein assembly;

(e) analyzing the separate cell cultures by fluorescence-activated cell sorting; and

(f) comparing the fluorescence of the cultures, with compounds that block formation or binding to a cell surface protein of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound.

24. The method of claim 22 or 23, wherein the fluorescence of the cultures further is compared with the fluorescence of cultures that have been treated in the same fashion except that instead of adding or not adding the compound prior to formation of the protein assembly, the compound either is or is not added after formation of the protein assembly,

with compounds that block formation of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound, only when the compound is added prior to the protein assembly, and

compounds that block binding to a cell surface

protein of the protein assembly being identified as resulting in a reduced fluorescence of the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the, when the compound is added either prior to or after the protein assembly.

25. A method of detecting binding to a cell surface protein of the protein assembly of any of claims 1 to 8, the method comprising:

(a) forming the protein assembly from amyloid β protein;

(b) contacting a culture of neuronal cells with the protein assembly;

(c) adding an antibody that binds the protein assembly, the antibody including a conjugating moiety;

(d) washing away unbound antibody;

(e) linking an enzyme to the antibody bound to the protein assembly by means of the conjugating moiety;

(f) adding a colorless substrate that is cleaved by the enzyme to yield a color change; and

(g) determining the color change as a measure of binding to a cell surface protein of the protein assembly.

26. A method for identifying compounds that block binding to a cell surface protein of the protein assembly of any of claims 1 to 8, the method comprising:

(a) preparing separate samples of amyloid β protein that either have or have not been mixed with the compound;

(b) forming the protein assembly in the separate samples;

(c) contacting separate cultures of neuronal cells with the separate samples;

(d) adding an antibody that binds the protein assembly, the antibody including a conjugating moiety;

(e) washing away unbound antibody;

(f) linking an enzyme to the antibody bound to the protein assembly by means of the conjugating moiety;

(g) adding a colorless substrate that is cleaved by the enzyme to yield a color change; and

(h) comparing the color change produced by each of the separate samples, with compounds that block formation or binding to a cell surface protein of the protein assembly being identified as resulting in a reduced color change produced by the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound.

27. The method of claim 26, wherein the color change produced by the cultures further is compared with the color change produced by cultures that have been treated in the same fashion except that instead of adding or not adding the compound prior to formation of the protein assembly, the

compound either is or is not added after formation of the protein assembly,

with compounds that block formation of the protein assembly being identified as resulting in a reduced color change produced by the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound, only when the compound is added prior to the protein assembly, and

compounds that block receptor binding of the protein assembly being identified as resulting in a reduced color change produced by the culture as compared to the corresponding culture contacted with the protein assembly in the absence of the compound, when the compound is added either prior to or after the protein assembly.

28. A method for identifying compounds that block formation of the protein assembly of any of claims 1 to 8, the method comprising:

- (a) preparing separate samples of amyloid β protein that either have or have not been mixed with the compound;
- (b) forming the protein assembly in the separate samples;
- (c) assessing whether any protein assemblies have formed in the separate samples using a method selected from the group consisting of electrophoresis, immunorecognition, and atomic force microscopy; and
- (d) comparing the formation of the protein

assemblies in the separate samples, with compounds that block formation of the protein assembly being identified as resulting in decreased formation of the protein assembly in the samples as compared with a sample in which the protein assembly is formed in the absence of the compound.

29. A method of preparing the protein assembly according to any of claims 1 to 8, wherein the method comprises:

(a) obtaining a solution of monomeric amyloid β protein, the amyloid β protein being capable of forming the protein assembly;

(b) diluting the protein solution into an appropriate media to a final concentration of about 5 nm to about 500 μ m.

(c) incubating the media resulting from step (b) at about 4°C for about 2 hours to about 48 hours.

(d) centrifuging the solution at about 14,000g at about 4°C; and

(e) recovering the supernatant resulting from the centrifugation as containing the amyloid β protein assembly.

30. The method of claim 35, wherein the method comprises incubating the media resulting from step (b) at about 4°C in the presence of clusterin.

31. A protein assembly prepared according to claim 29 or 30.

32. The use of a protein assembly according to any of claims 1 to 8 to alter the long-term potentiation response of a nerve cell, comprising contacting the cell with the protein assembly (excluding contacting in vivo in human).

33. The use of a protein assembly according to any of claims 1 to 8 to alter the learning or memory of an animal excluding human, comprising administering the protein assembly to the animal.

34. The use of a protein assembly according to any of claims 1 to 8 to cause morphological change of a nerve cell, comprising contacting in vitro the cell with the protein assembly.

35. The use according to claim 34, wherein the morphological change includes an effect selected from the group consisting of cell killing, altering Fyn kinase activity, altering fyn kinase subcellular localization, and altering mRNA levels for proteins including interleukin-1, inducible nitric oxide synthase, Apo E, Apo J, and α 1-antichymotrypsin.

36. The use of a protein assembly according to any of claims 1 to 8 to cause astrocyte activation, comprising contacting in vitro the astrocyte with the protein assembly.

37. The use of a protein assembly according to any of claims 1 to 8 to identify test compounds that block the neurotoxicity of the protein assembly, comprising contacting in vitro a nerve cell with the protein assembly and the test compound.

38. The use of a protein assembly according to any of claims 1 to 8 to identify test compounds that block the binding to a cell surface protein of the protein assembly, comprising contacting in vitro a nerve cell with the protein assembly and the test compound.

39. The use of a protein assembly according to any of claims 1 to 8 to identify test compounds that block the formation of the protein assembly, comprising contacting in vitro amyloid β protein with the test compound during incubation to form the protein assembly.

40. A method for protecting a nerve cell against ADDL-induced aberrant neuronal signaling due to the effects of a protein assembly according to any of claims 1 to 8, the method comprising contacting the cell with a compound that blocks the activity of the protein assembly that lead to ADDL-induced aberrant neuronal signaling (excluding treating in vivo human cells).

41. A method for detecting in a test material the protein assembly of any of claims 1 to 8 comprising:

(a) contacting in vitro the test material with a nerve cell; and

(b) determining whether the cell exhibits ADDL-induced aberrant neuronal signaling.

42. The use of a protein assembly according to any of claims 1 to 8 to cause ADDL-induced aberrant neuronal signaling of a nerve cell, comprising contacting in vitro the cell with the protein assembly.

43. Use of a compound that blocks the formation or activity of a protein assembly according to any of claims 1 to 8 for the preparation of a medicament for protecting an animal against decreases in learning or memory due to the effects of the protein assembly.

44. Use of a compound that blocks the formation or activity of a protein assembly according to any of claims 1 to 8 for the preparation of a medicament for reversing in an animal decreases in learning or memory due to the effects of the protein assembly.