



US007227010B2

(12) **United States Patent**
Smith

(10) **Patent No.:** **US 7,227,010 B2**

(45) **Date of Patent:** **Jun. 5, 2007**

(54) **RECOMBINANT LIGHT CHAINS OF BOTULINUM NEUROTOXINS AND LIGHT CHAIN FUSION PROTEINS FOR USE IN RESEARCH AND CLINICAL THERAPY**

(75) Inventor: **Leonard A. Smith**, Clarksburg, MD (US)

(73) Assignee: **United States of America as Represented by the Secretary of the Army**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/012,269**

(22) Filed: **Nov. 6, 2001**

(65) **Prior Publication Data**

US 2007/0104737 A1 May 10, 2007

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/910,186, filed on Jul. 20, 2001, now Pat. No. 7,081,529, which is a continuation of application No. 09/611,419, filed on Jul. 6, 2000, which is a continuation of application No. 08/123,975, filed on Sep. 21, 1993, now abandoned.

(60) Provisional application No. 60/133,865, filed on May 12, 1999, provisional application No. 60/133,866, filed on May 12, 1999, provisional application No. 60/133,867, filed on May 12, 1999, provisional application No. 60/133,868, filed on May 12, 1999, provisional application No. 60/133,869, filed on May 12, 1999, provisional application No. 60/133,873, filed on May 12, 1999, provisional application No. 60/246,774, filed on Nov. 6, 2000, provisional application No. 60/311,966, filed on Aug. 9, 2001.

(51) **Int. Cl.**

C07H 21/02 (2006.01)
C07H 21/04 (2006.01)
C12P 21/06 (2006.01)
C12P 21/04 (2006.01)
C12N 15/09 (2006.01)
C12N 7/00 (2006.01)
C12N 1/12 (2006.01)

(52) **U.S. Cl.** **536/23.7**; 536/23.1; 536/23.4; 435/69.3; 435/69.7; 435/69.1; 435/252.3; 435/252.1; 435/235.1; 435/252.35; 435/252.33; 435/252.8; 435/71.1; 435/71.2

(58) **Field of Classification Search** 536/23.1, 536/23.6, 23.4, 23.7; 435/69.1, 320.1, 252.3, 435/70.1, 71.1, 69.7, 69.3, 71.2, 252.1, 235.1, 435/252.35, 252.33, 252.8
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,196,193 A 3/1993 Carroll

5,601,823 A * 2/1997 Williams et al. 424/167.1
5,919,665 A 7/1999 Williams
5,939,070 A * 8/1999 Johnson et al. 424/194.1
6,365,158 B1 * 4/2002 Williams et al. 424/190.1
6,444,209 B1 * 9/2002 Johnson et al. 424/194.1
6,461,617 B1 * 10/2002 Shone et al. 424/236.1
6,495,143 B2 * 12/2002 Lee et al. 424/199.1
6,500,436 B2 * 12/2002 Donovan 424/239.1
6,573,003 B2 * 6/2003 Williams et al. 424/190.1
6,613,329 B1 * 9/2003 Kink et al. 424/164.1
6,641,820 B1 * 11/2003 Donovan 424/239.1

(Continued)

FOREIGN PATENT DOCUMENTS

WO WO 95/327380 12/1995

(Continued)

OTHER PUBLICATIONS

Poulain et al, *J. Biological Chemistry*, 1991, 266/15:9580-9585.*
Hutson et al, *Current Microbiology*, 1994, 28:101-110.*
Santos-buelga et al, *Current Microbiology*, 1998, 37:312-318.*
Campbell et al, *J. Clinical Microbiology*, 1993, 31/9:2255-2262.*
Dasgupts et al, *Biochimie*, 1988, 70:811-817.*
Ahmed SA et al., 2001, "Enzymatic autocatalysis of botulinum A neurotoxin light chain" *J. Protein Chem.* 20(3):221-231.
Schmidt JJ et al., 2001, "High-throughput assays for botulinum neurotoxin proteolytic activity: serotypes A, B, D, and F" *Analytical Biochemistry* 296:130-137.

(Continued)

Primary Examiner—Nita Minnifield
(74) *Attorney, Agent, or Firm*—Elizabeth Arwine

(57) **ABSTRACT**

Botulinum neurotoxins, the most potent of all toxins, induce lethal neuromuscular paralysis by inhibiting exocytosis at the neuromuscular junction. The light chains (LC) of these dichain neurotoxins are a new class of zinc-endopeptidases that specifically cleave the synaptosomal proteins, SNAP-25, VAMP, or syntaxin at discrete sites. The present invention relates to the construction, expression, purification, and use of synthetic or recombinant botulinum neurotoxin genes. For example, a synthetic gene for the LC of the botulinum neurotoxin serotype A (BoNT/A) was constructed and over-expressed in *Escherichia coli*. The gene product was purified from inclusion bodies. The methods of the invention can provide 1.1 g of the LC per liter of culture. The LC product was stable in solution at 4° C. for at least 6 months. This rBoNT/A LC was proteolytically active, specifically cleaving the Glu-Arg bond in a 17-residue synthetic peptide of SNAP-25, the reported cleavage site of BoNT/A. Its calculated catalytic efficiency k_{cat}/K_m was higher than that reported for the native BoNT/A dichain. Treating the rBoNT/A LC with mercuric compounds completely abolished its activity, most probably by modifying the cysteine-164 residue located in the vicinity of the active site. About 70% activity of the LC was restored by adding Zn^{2+} to a Zn^{2+} -free, apo-LC preparation. The LC was nontoxic to mice and failed to elicit neutralizing epitope(s) when the animals were vaccinated with this protein. In addition, injecting rBoNT/A LC into sea urchin eggs inhibited exocytosis-dependent plasma membrane resealing.