



US007037680B2

(12) **United States Patent**
Smith et al.

(10) **Patent No.:** **US 7,037,680 B2**
(45) **Date of Patent:** **May 2, 2006**

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

(75) Inventors: **Leonard A. Smith**, Clarksburg, MD
(US); **Melody Jensen**, Frederick, MD
(US)

(73) Assignee: **The 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 444 days.

(21) Appl. No.: **10/011,588**

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

(65) **Prior Publication Data**

US 2002/0168727 A1 Nov. 14, 2002

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/910,186,
filed on Jul. 20, 2001, 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, appli-
cation No. 09/611,419.

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

(51) **Int. Cl.**

C12P 21/06 (2006.01)
C12P 21/04 (2006.01)
C12N 15/09 (2006.01)
C12N 1/12 (2006.01)
C12N 1/20 (2006.01)

(52) **U.S. Cl.** **435/69.1**; 435/69.7; 435/69.3;
435/70.1; 435/71.1; 435/71.2; 435/842; 435/252.3;
435/252.33; 435/254.23; 435/252.1; 435/320.1;
536/23.1; 536/23.4; 536/23.7; 536/24.1

(58) **Field of Classification Search** 435/69.1,
435/69.3, 320.1, 235.1, 325, 172.3, 69.7,
435/70.1, 71.1, 71.2, 842, 252.1, 252.3, 252.33,
435/254.23; 536/23.1, 23.6, 23.4, 23.7, 24.1
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 B1 * 12/2002 Lee et al. 424/199.1
6,573,003 B1 * 6/2003 Williams et al. 424/190.1
6,613,329 B1 * 9/2003 Kink et al. 424/164.1
6,670,148 B1 * 12/2003 Mundschenk et al. 435/69.1
6,737,251 B1 * 5/2004 Marchetti et al. 435/69.1
6,822,075 B1 * 11/2004 Bjorck et al. 530/350
2003/0219457 A1 * 11/2003 Williams 424/199.1
2005/0143289 A1 * 6/2005 Hunt 514/2
2005/0169442 A1 * 8/2005 Nakano 379/88.16

FOREIGN PATENT DOCUMENTS

WO WO 98/07864 A1 * 2/1998
WO 27 9715394 5/1998
WO 12 0012890 11/2000
WO WO 00/67700 A2 * 11/2000

(Continued)

OTHER PUBLICATIONS

Hutson et al, Current Microbiology, 1994, 28:101-110.*

(Continued)

Primary Examiner—N. M. 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.

16 Claims, 20 Drawing Sheets