

CLEAVAGE SENSITIVE ANTIBODIES AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/234,813, filed Aug. 18, 2009, which is hereby incorporated by reference in its entirety.

RIGHT IN THE INVENTION

[0002] This invention was made with support from the United States Government and, specifically, the United States Army Medical Research Institute of Infectious Diseases, and, accordingly, the United States government has certain rights in this invention.

BACKGROUND

[0003] 1. Technical Field

[0004] This invention is directed generally to a method and system of assaying biologically relevant activity and, in particular, the assay of protease activity using cleavage sensitive antibodies.

[0005] 2. Background

[0006] Botulism is a potentially fatal disease caused by botulinum neurotoxins (BoNTs) secreted by anaerobic spore-forming bacteria *Clostridium botulinum* (1, 2). Historically, botulism has been associated with food poisoning. The first account of the disease was recorded in 1735 when an outbreak of botulism in Europe was linked to tainted sausage (botulus is Latin for sausage) (3). Today, sporadic outbreaks of food-borne botulism generally result from contaminations occurring at commercial canneries, with the most recent incident in the United States occurring in 2007 (4). Also, infant botulism leads to the hospitalization of nearly 100 children annually in the U.S. alone (5, 6).

[0007] Though BoNTs are the most potent of biological poisons, purified BoNTs have found widespread use in medical clinics, and are used to treat a wide array of ailments including the cosmetic appearance of facial wrinkles, cervical dystonia, migraine headaches, and anal fissure (7-10). However, the growing use of these toxins as therapeutic agents makes unintentional overdosing increasingly likely. In addition to accidental or unintentional environmental exposure, the current political climate makes the malicious misuse of BoNTs, through acts of terrorism, a serious possibility (11). Hence, the health threat posed by BoNTs continues to grow. Yet at this time, only limited therapeutic options are available to treat botulism (12). The most common treatment consists of long-term supportive care involving mechanical respiration. Additionally, treatment with BABYBIG™ (anti-BoNT immunoglobulins derived from human serum, California Department of Health Services, Berkeley, Calif.) decreases the length of hospitalization in cases of infant botulism, adding credence to the potential benefits of anti-toxin intervention against this disease (13). Currently, BABYBIG and bivalent (BoNTs A/B) equine antitoxin (approved for use in adults) are the only FDA-approved treatments available. Clearly, there is a need to develop novel therapeutics to aid in the recovery from botulism.

Pathophysiology

[0008] There are seven biochemically distinct BoNT serotypes (designated A-G). BoNT holotoxins are composed of

two subunits: a heavy chain (HC) and light chain (LC), which are connected by a disulfide bridge (2, 14). For all BoNT serotypes, the mechanism of toxicity involves two basic steps: toxin entry into neurons followed by soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein cleavage (15). The toxin entry step is mediated by the HC, as domains located within this subunit interact with neuronal surface receptors that trigger endocytosis (2, 14). Within the endosome, the toxin dissociates into subunits, where the HC may serve an additional function by acting as a chaperone that assists refolding of the LC into its catalytically active conformation(s) (16). Once activated, neuronal function is disrupted by the proteolytic activity of the LC. Specifically, the LC (also referred to as the catalytic domain) is a zinc (Zn) metalloprotease that cleaves SNARE proteins, which mediate the exocytosis of neurotransmitter contained within synaptic vesicles (2). Different BoNT serotypes cleave different SNARE protein components, and/or different sites within the same component. For BoNT serotype A (BoNT/A), the cleavage of SNARE component SNAP-25 (synaptosomal-associated protein of 25 kDa) between glutamine 197 and arginine 198 results in the inhibition of acetylcholine release into neuromuscular synapses, and the flaccid paralysis ensues (17, 18).

High-Throughput Assay

[0009] In order to rapidly identify and characterize BoNT inhibitors in cellular models, high-throughput assays must be established. Primary chick neurons are a sensitive cellular model system for studying BoNT intoxication, and successfully measure toxin activity in neurons by quantitating the cleavage of the BoNT/A substrate, SNAP-25, using commercially available antibodies in conjunction with immunoblotting (19). While this analytical method has been reliable, the assay is not amenable to high-throughput screening. To eliminate this research bottle neck the present invention discloses BoNT/A cleavage sensitive (BACS) antibodies which are capable of measuring the BoNT/A catalyzed proteolysis of SNAP-25 in a variety of assay formats. All assays can be conducted in multi-well plates and are amenable for high-throughput analysis. Thus when coupled with cellular models these assays can be used for a broad range of applications such as drug development (the evaluation of toxin antagonists, the evaluation of toxin activators, compound library screening) and BoNT biopharmaceutical manufacturing assays (quality control, product formulation requirements).

[0010] Botulinum types A and E toxins cleave protein SNAP-25. Botulinum types B, D, F and G and tetanus toxins cleave vesicle-associated membrane protein (VAMP-also called synaptobrevin). Botulinum type C toxin cleaves the protein syntaxin.

[0011] While protease assays are known in the art, they are based on synthetic substrates that are only viable in vitro. The novelty and utility of the present invention is that the disclosed assays can detect the cleavage of proteins such as endogenous SNAP-25 making them effective in vivo as well as in vitro which has far reaching implications for the advancement of the art.

[0012] U.S. Pat. No. 5,965,699 to Schmidt et al. discloses a label-based assay for the determination of type A botulinum toxin enzymatic (proteolytic) activity. However, the assay relies on labeling SNAP-25 residues with fluorescamine in vitro and is specifically designed to avoid using animals and cell cultures.