

Example 7

[0089] Structural modifications will be made to the substrate(s) described herein in order to enable intracellular localization of the substrate. Intracellular localization of the substrates will allow assay of botulinum protease activity inside cells. Examples of such modifications include, but are not limited to, addition of the HIV TAT transmembrane sequence to a substrate, or addition of the peptide called “penetratin” to a substrate. Any method of allowing the substrate be located inside the cell wall may be used.

[0090] Intracellular localization of the substrate will also allow the substrates to be used as competitive inhibitors to compete with endogenous SNAP-25 for the BoNT-A Lc active site. The binding of substrates to BoNT-A Lc will protect endogenous SNAP-25 from cleavage to an extent. These competitive inhibitors may be given to a subject in need of treatment of a condition due to botulinum toxin. In an embodiment, multiple doses of the substrates may be administered to the subject. In an embodiment, the dose or doses may be administered enterally, parenterally, or other suitable method. The dose or doses may be in the form of a liquid, tablet, capsules, or other suitable form. The dosage given is that necessary to provide a partial or full inhibition of BoNT A.

Example 8

[0091] A kit will be used to detect the presence of serotype A botulinum neurotoxin in human or animal samples (including, but not limited to, blood, stool, organs and tissues), in environmental samples (including, but not limited to, soil, food, and water), and in other samples. In addition to detection, the kit may also be used to quantify the amount of serotype A botulinum neurotoxin in the sample.

[0092] In one embodiment, the kit will comprise the following:

[0093] (a) A vial containing a pH-buffering compound in dry form. The composition of this buffer is such that, upon dissolving in water, the pH is 7.5 ± 0.5 . Suitable examples include sodium hydroxyethylpiperazine sulfonate, commonly abbreviated as “HEPES”, and sodium phosphate. The vial will also contain one or more agent(s) known to stabilize BoNT A, such as bovine serum albumin. Before use, the buffer is dissolved in a suitable volume of purified water (g). Polysorbate 20 (i) is added to a final concentration of 0.05-0.10% (v/v).

[0094] (b) A vial containing a pH-buffering compound in dry form. The composition of this buffer is such that, upon dissolving in water, the pH is 7.5 ± 0.5 . Suitable examples include sodium hydroxyethylpiperazine sulfonate, commonly abbreviated as “HEPES”, and sodium phosphate. The vial will also contain, in dry form, a reducing agent (for example, dithiothreitol or tris-(carboxyethyl)-phosphine), and a zinc salt (for example, zinc chloride or zinc acetate). The vial will also contain one or more agent(s) known to stabilize BoNT A, such as bovine serum albumin. Before use, the buffer is dissolved in a suitable volume of purified water (g). Polysorbate 20 (i) is added to a final concentration of 0.05-0.10% (v/v).

[0095] (c) A vial containing one of the FRET substrates described herein, in dry form. Before use, the substrate is dissolved in an appropriate volume of dimethylsulfoxide (h).

[0096] (d) Polymeric beads (or other suitable solid material), coated with antibodies specific for BoNT A.

[0097] (e) The same solid material as in (d), coated with the same type of immunoglobulins as in (d), but with NO antibodies to BoNT A.

[0098] (f) A vial containing a known quantity of lyophilized BoNT A, with suitable stabilizing excipients (if needed), for preparation of standard curves. Before use, lyophilized BoNT A is dissolved in buffer (a).

[0099] (g) A bottle containing highly purified water, free of contaminants that could compromise the assay.

[0100] (h) A bottle containing highly purified dimethylsulfoxide, free of contaminants that could compromise the assay.

[0101] (i) A bottle containing a suitable non-ionic detergent (for example, Polysorbate 20), free of contaminants that could compromise the assay.

[0102] In one embodiment, the kit would be used as follows:

[0103] (1) The sample in question is dissolved in or extracted with buffer (a), then mixed with the antibody-coated beads (d). Any BoNT A in the sample will be captured and immobilized on the beads by the antibodies. This is called the test assay.

[0104] (2) After an appropriate incubation time, the beads are then washed with buffer (a) to remove unwanted material in the sample.

[0105] (3) Simultaneously, a second assay is prepared in the same way, but using beads (e). This is called the control assay.

[0106] (4) After washing, the beads from the test assay and from the control assay are suspended, in separate tubes, in a solution containing buffer (b) and the FRET substrate (c).

[0107] (5) After a suitable incubation period, the solutions are separated from the beads and the fluorescence intensities of the solutions are measured. If the fluorescence of the assay that contained beads (d) (the test assay) is higher than the assay that contained beads (e) (the control assay), the presence of BoNT A in the test sample is indicated.

[0108] (6) The concentration of BoNT A in the test sample may be determined by comparison of the fluorescence intensity of the test assay with a standard curve using known concentrations of BoNT A, prepared using vial (f).

[0109] In sum, the HPLC-based and fluorogenic BoNT A substrates reported here represent significant improvements, compared to current widely-used BoNT A substrates, in terms of hydrolysis rates, binding constants, assay sensitivity, and ease of synthesis. These improvements and characteristics result from non-conservative amino acid substitutions and truncations to a peptide which, in its original form, corresponded to residues 187-203 of the neuronal protein, SNAP-25. With regard to FRET substrates, these advantages are particularly apparent when comparing the kinetic properties of fIP6 ([DabcylK] (SEQ ID NO: 2) [SFC]) with those of a previously-described BoNT A FRET substrate from another laboratory (27). The substrates disclosed herein are useful in BoNT A mechanistic studies, high-throughput inhibitor searches, and in therapy for botulism. The substrates could also be employed to further enhance the sensitivity of a recently-reported attomolar-level BoNT A detection system (29).