Comparison of Activity of Botulinum Neurotoxin Type A Holotoxin and Light Chain Using SNAPtide® FRET Substrates

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Abstract

The holotoxin neurotoxins consist of two subunits, a 50 kDa heavy chain and a 130 kDa light chain. The light chain contains the catalytic site of the enzyme, while the heavy chain is responsible for entry into the target cell and for maintenance of the holotoxin activity. The light chain is a zinc-dependent metalloprotease. The eukaryotic substrate for botulinum neurotoxin type A is SNAP-25, a holotoxin and light chain. One of the substrates contains an oAbz/DNP FRET pair and the other a FITC/DABCYL FRET pair.

In this study, we determined the specificity of the holotoxin and light chain for each of the substrates. The specific activity was also determined for the holotoxin and light chain. To measure the enzymatic activity, we used the SNAPtide® fluorogenic substrate assay. The substrate was incubated with the enzyme in buffer at 37°C for 15 minutes. The enzymatic activity was measured by determining the decrease in the number of nmoles cleaved.

Results

Figure 1: Specificity of LcA and LcB using SNAPtide® assay.

A) The top graphs are representative calibration curves generated by measuring the initial rate of hydrolysis of SNAPtide® #520 at 200 nM LcA and SNAPtide® #521 at 20 nM LcB. B) The limit of detection in this assay for LcA is around 25 pM. This value is true for both fluorogenic substrates and when using excess substrate. When compared to BTA, the LOD of LcA is about 2 fold lower on a molar scale.

Materials

SNAPtide® 520@8 (8 µM SNAPtide® substrate #521 and calibration peptide #529) corresponds to SNAPtide® substrate #520. The calibration peptides were diluted to 10 nM LcB and 10 nM LcA in 10 mM Tris/HCl buffer, pH 7.4. The concentration of LcB was determined to contain 12.3 +/- 0.4 mg/mL LcA.

Conclusions

Specific Activity

The specific activity of the holotoxin was measured in 20 mM HEPES buffer containing 0.008 M ZnCl2, 0.063 M ZnCl2, 0.125 M ZnCl2, and 0.5 M ZnCl2. The concentration of LcA was determined to contain 12.3 +/- 0.4 mg/mL LcA. The specific activity was also determined for the holotoxin and light chain. The specific activity was also determined for the holotoxin and light chain.

Figure 2: Specificity of LcA and LcB using SNAPtide® assay.

A) The top graphs are representative calibration curves generated by measuring the initial rate of hydrolysis of SNAPtide® #520 at 200 nM LcA and SNAPtide® #521 at 20 nM LcB. B) The limit of detection in this assay for LcA is around 25 pM. This value is true for both fluorogenic substrates and when using excess substrate. When compared to BTA, the LOD of LcA is about 2 fold lower on a molar scale.