

STABILITY OF BOTULINUM NEUROTOXIN COMPLEX IN LIQUID FORMULATION

14TH BOTULINUM RESEARCH CENTER
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BACKGROUND

- Commercially available pharmaceutical compositions of botulinum toxin are typically sold as vacuum-dried form that must be reconstituted with a diluent prior to actual usage.
- One major drawback of using this type of formulation is the limited shelf life of reconstituted botulinum toxin due to surface denaturation, heat, and alkaline conditions.
- The aim of this project is to test the possibility of establishing a stable liquid composition to preserve botulinum neurotoxin.

MATERIALS AND METHODS

- **Materials:**

Lidocaine powder -- lidocaine hydrochloride monohydrate (sigma-aldrich I5647). Make solution with citrate buffer at pH 5.5.

Lidocaine commercial solution -- lidocaine HCl solution: 2%, 20mg/ml in a 50 ml volume. Each ml contains lidocaine hydrochloride anhydrous 20 mg; sodium chloride 6 mg; methylparaben 1 mg added as preservative. may contain HCl and/or NaOH for pH adjustment. pH 6.5 (5.0 to 7.0).

BoNT/A Complex: 1 mg/ml in PBS pH 7.4

pH 5.5 citrate buffer as incubation buffer

pH 7.4 PBST buffer as cleavage buffer

MATERIALS AND METHODS

- **Incubation conditions:** at 4 °c

Complex alone (complex): 50 µl (0.2 mg/ml) (1:4 with citrate buffer)

Complex +0.5% lidocaine prepared from powder (50 µl)

(3:1 Complex + Citrate to LD)

Complex +1% lidocaine prepared from powder (25 µl)

} [Complex + LD (p)]

Complex +0.5% lidocaine from liquid (50 µl)

Complex +1% lidocaine from commercial liquid (25 µl)

} [Complex + LD (l)]

- **Cleavage conditions:** at 37 °c

4 µm of t-snagr was incubated with 50 nm BoNT/A Complex after the incubation with different lidocaine solutions. The cleavage was carried out in 37 °c for 3 hrs.

- Cleavage analysis on day 0, 1, 5, 8, 12, 19, and on going

CLEAVAGE ANALYSIS

- The cleavage of t-SNAGR shown as the decrease in substrate and increase in product bands.
- Relative density calculation: five bands for each cleavage sample;
Normalization: ratio with inert band.

Substrate: sum of substrate bands 1+2,

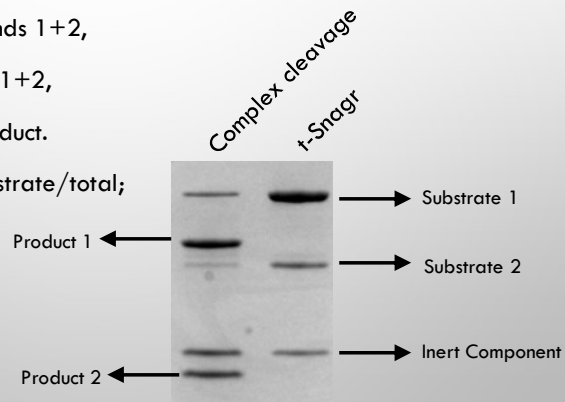
Product: sum of product bands 1+2,

Total: sum of substrate and product.

% quantity of sub: ratio of substrate/total;

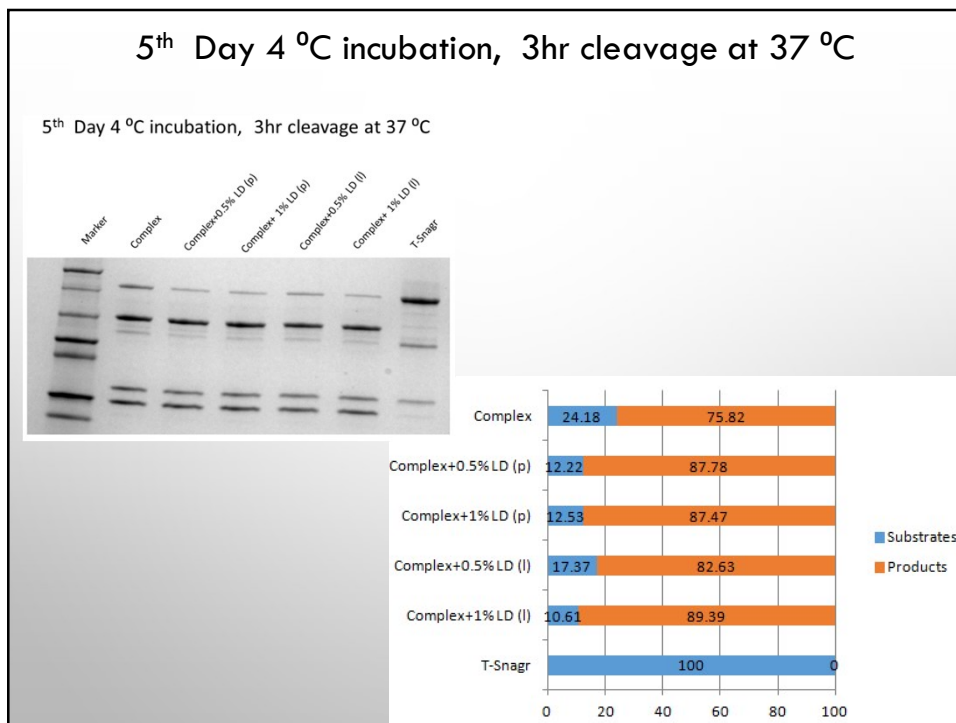
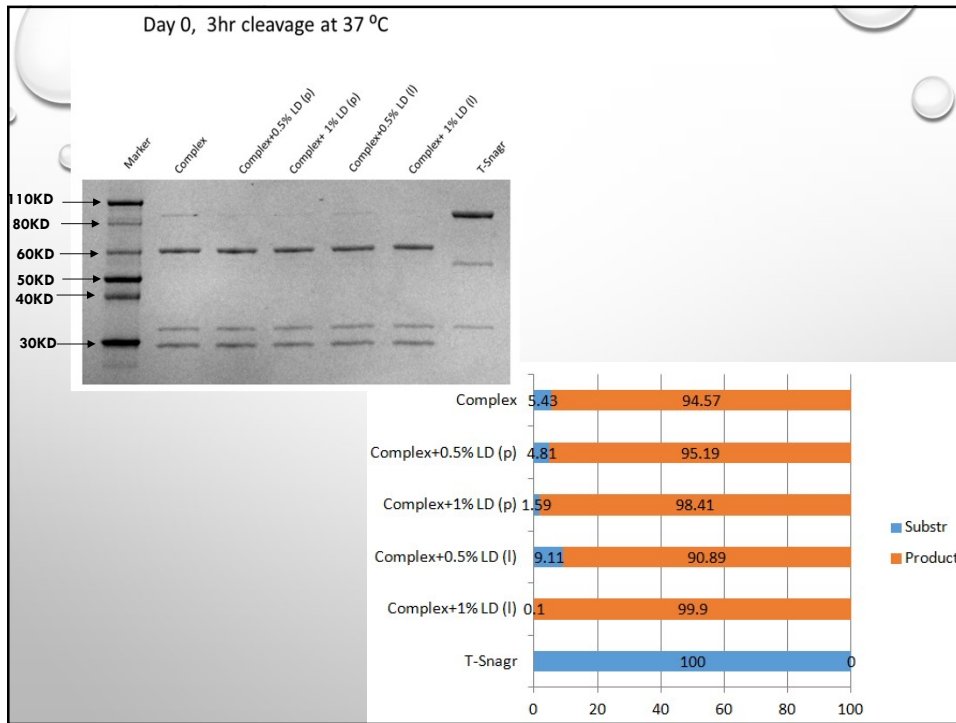
% quantity of prod:

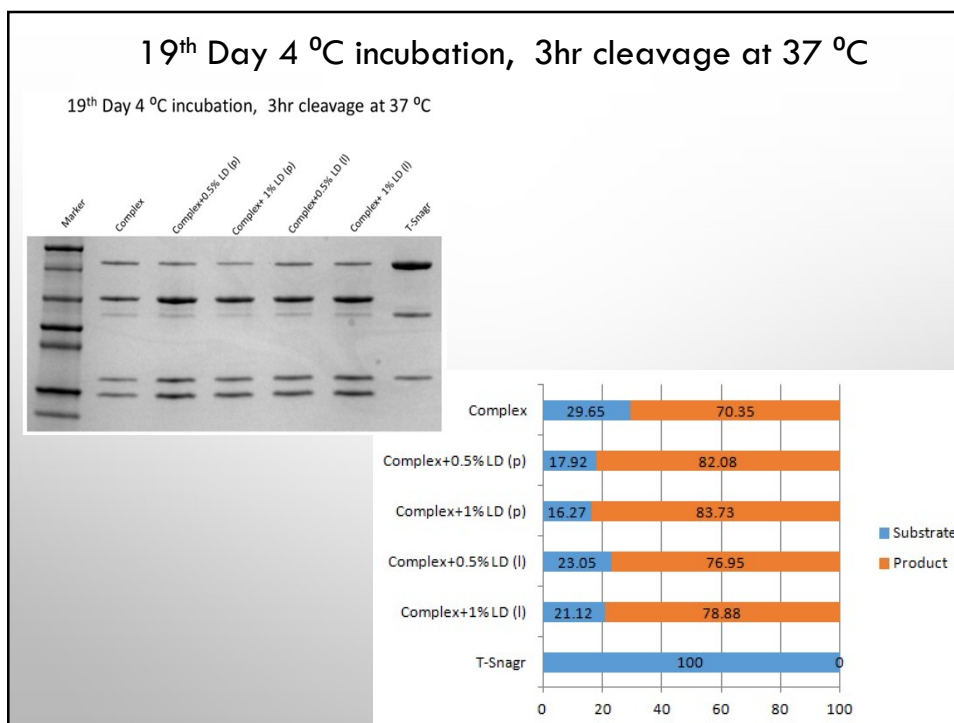
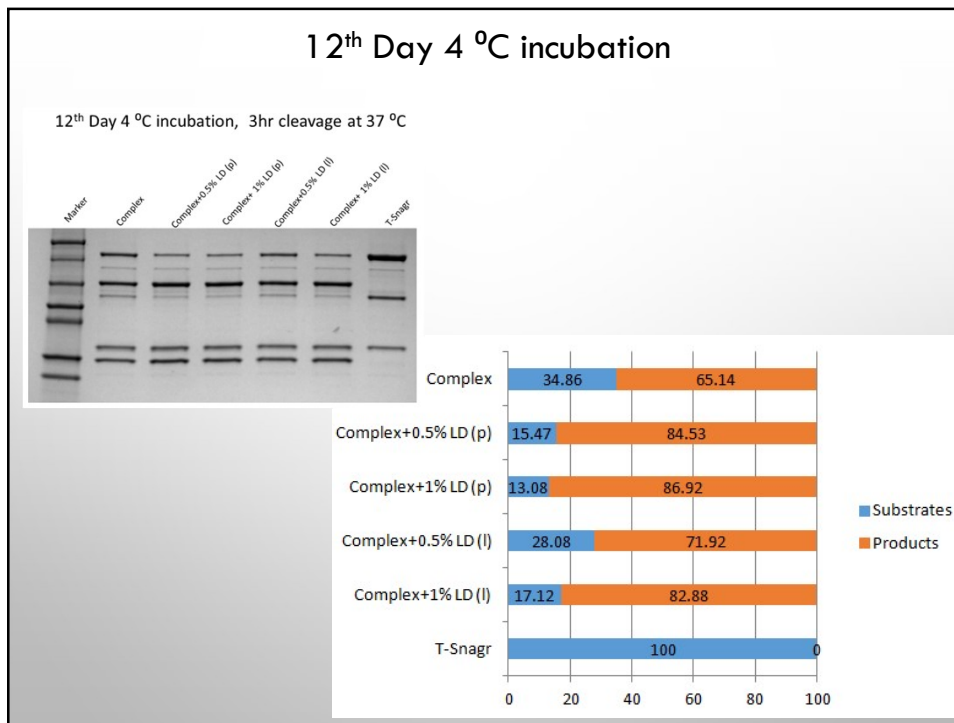
ratio of product/total

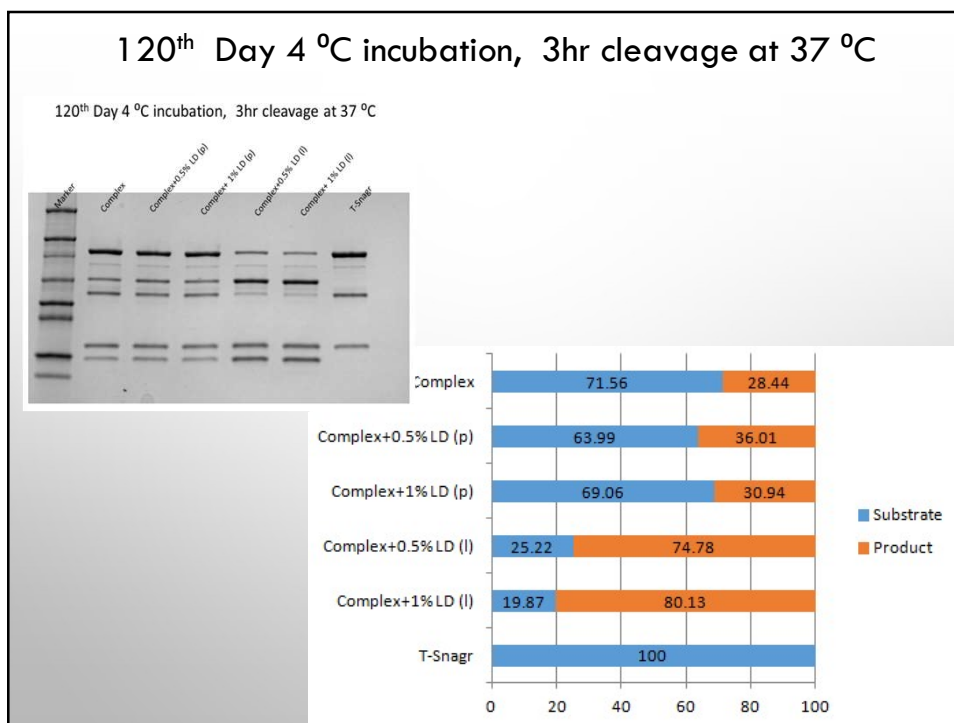
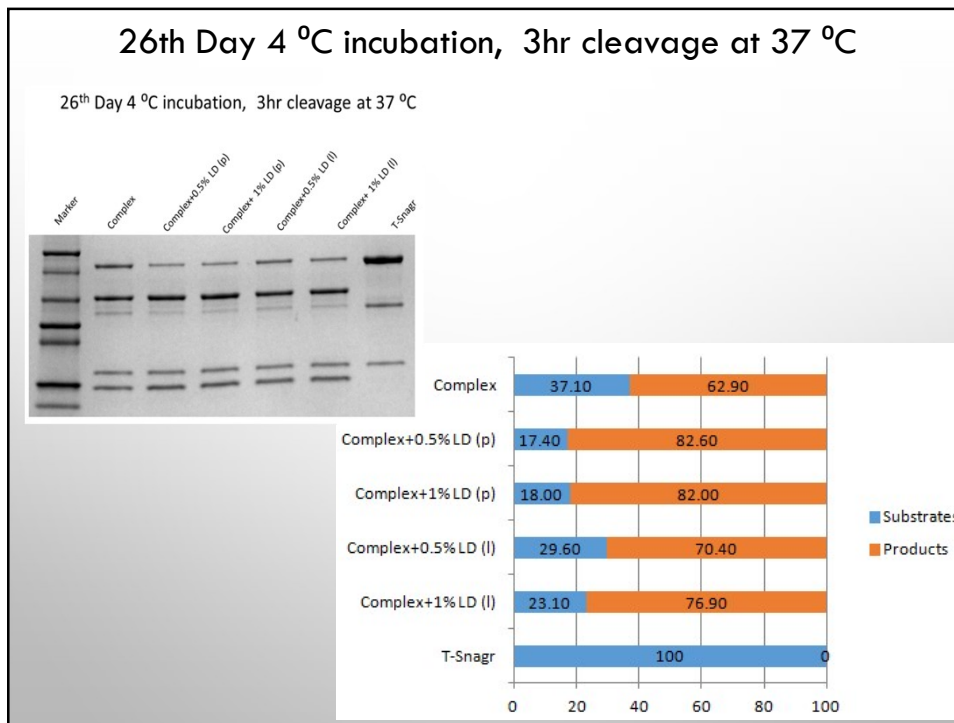


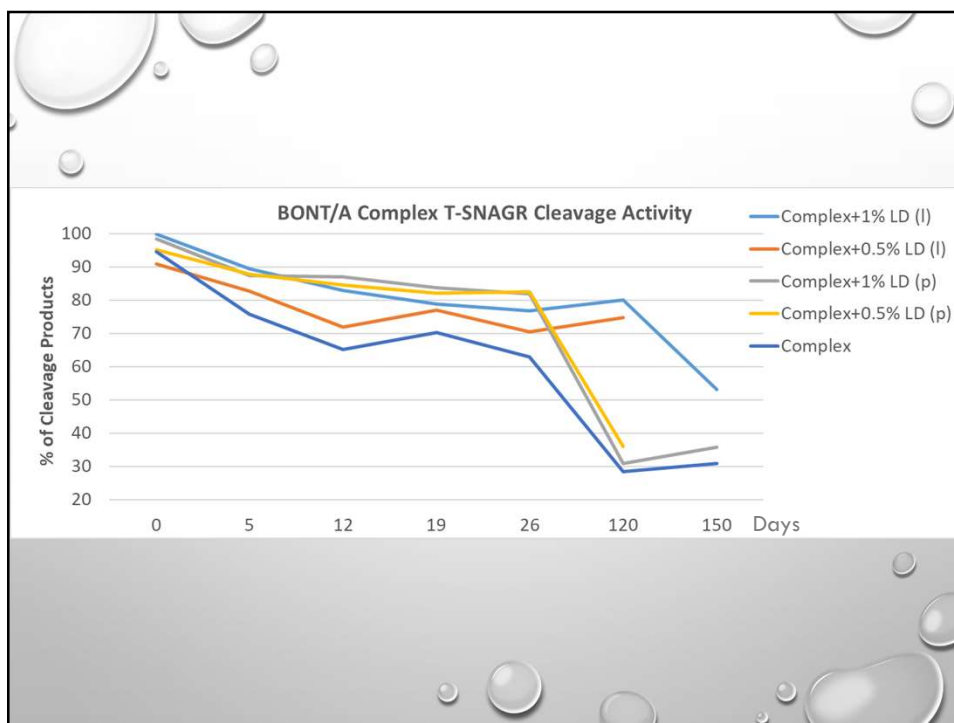
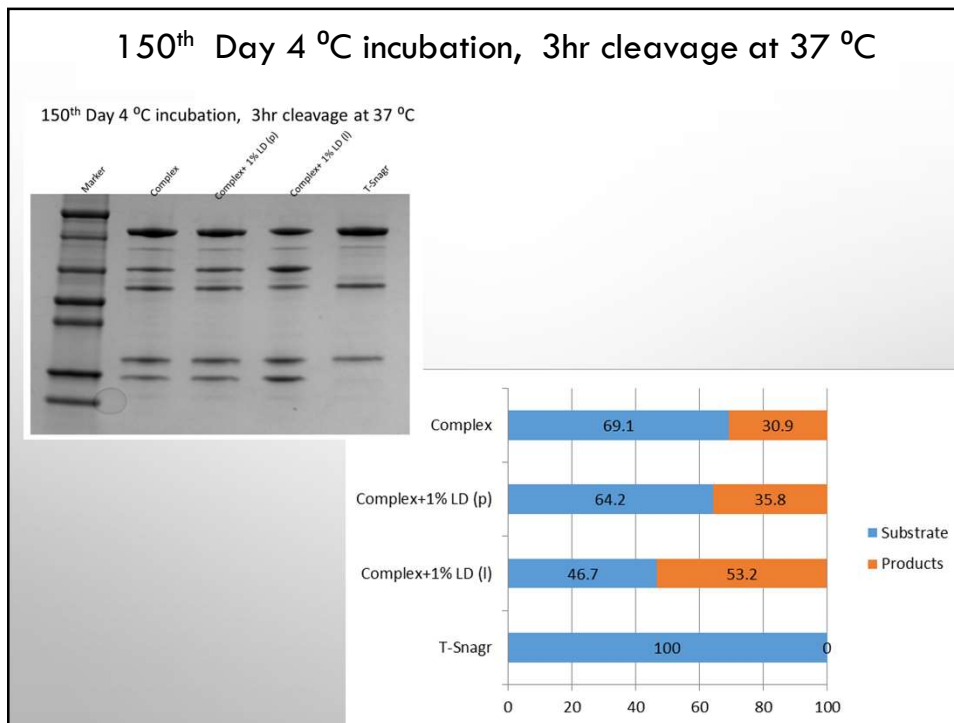
RESULTS

- The SDS PAGE gel for each time point.
- The relative quantity of substrates and cleavage products for each time point.
- Changes of the relative quantity of substrates and products over time.









CONCLUSION

- In the presence of lidocaine (both powder formula and liquid formula), the cleavage activity of BoNT/A complex was enhanced compared to complex alone.
- 1% lidocaine has more enhanced effect on the cleavage activity of BoNT/A complex than 0.5% .
- Overall, liquid lidocaine has higher enhance effects than powdered lidocaine.