Synthesis of Spirobenzopyrans Crowned Ether (SP-CE) and Data Collection of its Lithium Ion Uptake

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INTRODUCTION

- Lithium is a naturally occurring alkali metal present in the human body.
- Spirobenzopyrans Crowned Ether (SP-CE) is a sensor that can measure lithium ion fluorescence in living cells.[2]
- As some sodium dependent membrane transporters are also able to accept lithium, SP-CE could be a potential route in testing their activity in cell-based studies.

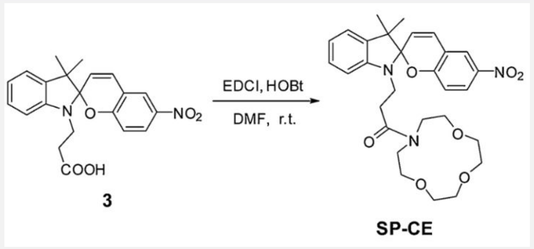

Figure 1. Synthesis Route of SP-CE

HYPOTHESES

1. Since SP-CE is a light sensitive, its optimal *in vitro* fluorescence can be achieved in opaque tubes rather than clear ones.
2. SP-CE added to solutions with lithium ion will have increased fluorescence compared to the negative control.

METHODS

Synthesis of SP-CE[2]

1. Dissolve 0.1 g of the spiropyran, 0.16 g of EDCl, and 0.14 g of HoBt in anhydrous DMF. Reactants are stirred under nitrogen atmosphere for 30 minutes.
2. Add 0.04 g of monoaza-12-crown-4 along with 0.17 mL triethylamine. Stir in dark and room temperature for 24 hours.
3. Pour mixture into 200 mL water. Filter the precipitation and wash with water.
4. The product is dried under vacuum to obtain grey, solid product.
5. Conduct Thin Layer Chromatography (TLC) to test the product's purification.
6. Perform column chromatography in order to isolate SP-CE.
7. Rotary evaporate the remain solvent.
8. Analyze the product's structure using Nuclear Magnetic Resonance (NMR).

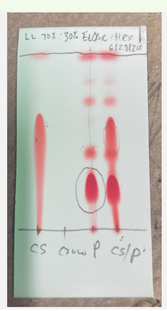

Figure 2. Thin layer chromatography of spiropyran, crown ether, SP-CE, and spiropyran mixed with SP-CE

Figure 3. Column chromatography reaction

RESULTS

Clear vs. Opaque Tubes Fluorescence Data Collection

- 2 negative controls were used by adding 10 μM SP-CE to a buffer and DI water solution with no LiCl into clear and opaque microcentrifuge tubes.
- 2 positive controls were made by adding 10 μM SP-CE to a buffer and DI water solution with 100 μM LiCl into clear and opaque microcentrifuge tubes.
- The excitation fluorescence was measured from 580 to 700 nm, with the excitation wavelength set at 550 nm.

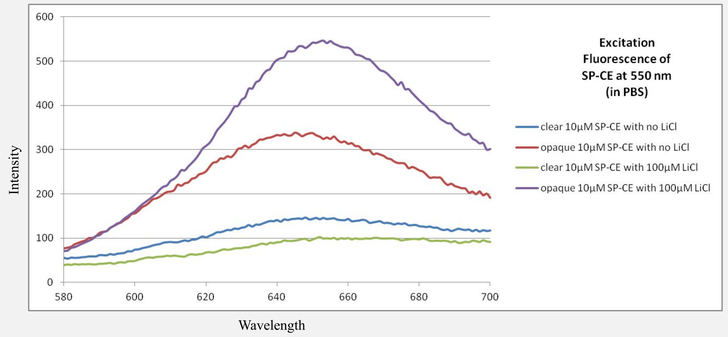

Figure 4. Fluorescent wavelengths of SP-CE samples in PBS and their lithium ion uptake

Data Collection of SP-CE Fluorescence in HEPES buffer

- The 10 μM and 100μM SP-CE solutions are the negative controls.
- Two different concentrations of LiCl, 1mM and 10 mM, were tested as the positive controls (in opaque tubes).
- HEPES buffer was used because it is more compatible with living cells compared to PBS.
- The excitation fluorescence was measured from 580 to 700 nm, with the excitation wavelength set at 550 nm.

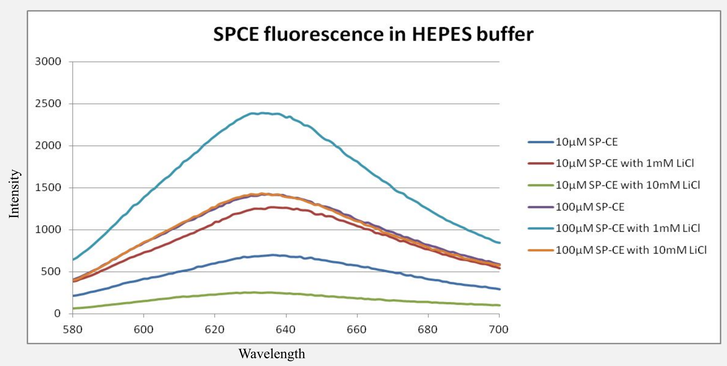

Figure 5. Fluorescent wavelengths of 10 μM and 100μM SP-CE with 0, 1, and 10 mm of LiCl

DISCUSSION

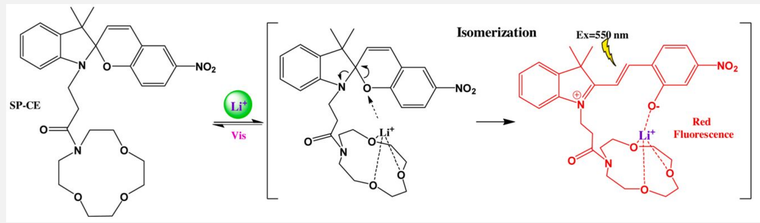

Figure 6. Mechanism of SP-CE responding to Li^+

The SP-CE sensor appeared yellow in frozen temperature and turned purple in room temperature.[3] When lithium is added to the solution, the equilibrium shifts to the colored merocyanine (MC) form.[1] This creates an increase in color, binding affinity, and fluorescent absorption. As shown in Figure 4, SP-CE exhibits greater lithium ion binding when placed in opaque tubes compared to clear ones. In Figure 5, the absorption of 10 μM SP-CE increases upon the addition of 1 mM LiCl. Likewise, 100 μM SP-CE shows a 2000-fold increase in absorbance with 1 mM LiCl. However, when 10 mM LiCl is added, the absorbance of SP-CE decreases, suggesting that the sensor may have exceeded its lithium ion binding capacity

These preliminary experiments indicate a potential increase in fluorescence upon lithium binding to the sensor, though the results are not yet definitive. To ensure data consistency, future experiments will replicate SP-CE fluorescence measurements in both PBS and HEPES buffers. Additionally, varying time, temperature, and concentration conditions will help further evaluate lithium ion uptake by SP-CE.

REFERENCES

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