Using Cyanophycin Production as a Screen for Cyanophycin Synthetase Activity

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Introduction

Biopolymers offer biodegradable substitutes to many petroleum-derived plastics used today. Cyanophycin synthetase (CphA) is an attractive enzyme to target for novel polymer production due to its intriguing synthesis mechanism and the polymer's unique chemical structure[1]. Cyanophycin granular protein (CGP) is a biopolymer composed of a poly-L-aspartic acid backbone and multi-L-arginine side chains that are polymerized by CphA (Fig 1)[2]. This project outlines an assay that can be used in directed evolution to find mutant CphA variants which produce useful cyanophycin analogues that contain novel side chains.

CGP has already been extracted and purified on a large scale, but this assay examines CGP purification on a smaller scale. New challenges arise when purifying CGP at reduced volumes due to its solubility in both acidic and basic conditions. This project focuses on optimizing parameters to maximize CGP yield. Monitoring CphA activity through CGP production on a small scale is useful for screening large quantities of colonies efficiently, which is critical for directed evolution.

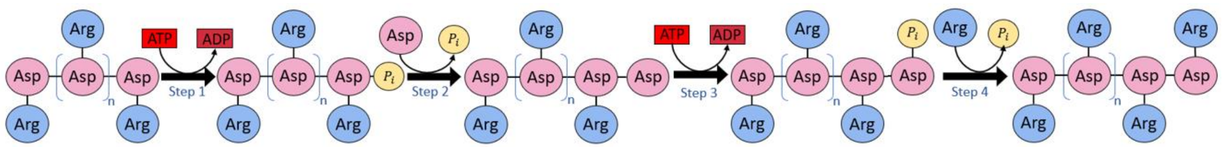

Fig 1: Proposed mechanism for the elongation of the cyanophycin polymer. ATP (red) is hydrolyzed by CphA to attach a phosphate moiety (yellow) onto the C-terminus side of the aspartic acid (pink) backbone (step 1). A free aspartic acid then replaces the phosphate moiety (step 2). ATP is again hydrolyzed to attach another phosphate moiety onto the sidechain of the newly attached aspartic acid (step 3). A free arginine (blue) then replaces the phosphate moiety (step 4).

Findings – CGP extraction optimization

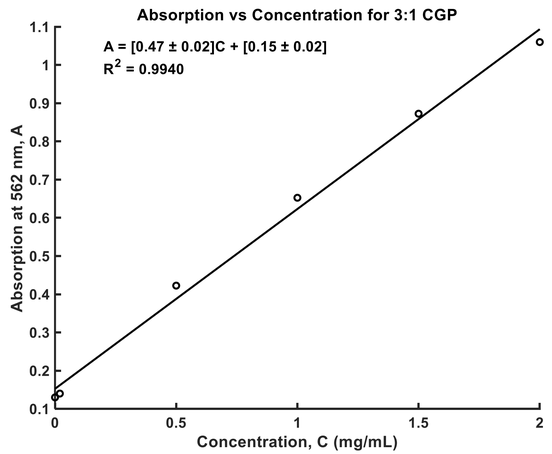

Fig 4: Absorption-concentration relationship for large scale purified CGP with a 3:1 ratio of soluble to insoluble polymer[3]. A 95% confidence interval test was performed to determine a linear relationship between concentration and absorption.

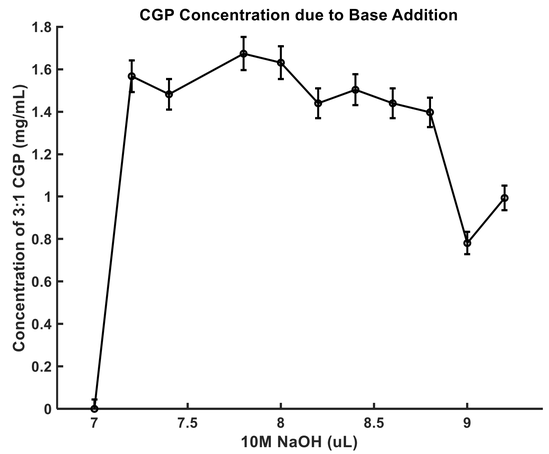

Fig 5: CGP concentration after different volumes of 10M NaOH were added. The concentrations were calculated using the absorption-concentration relationship in figure 4.

Methods

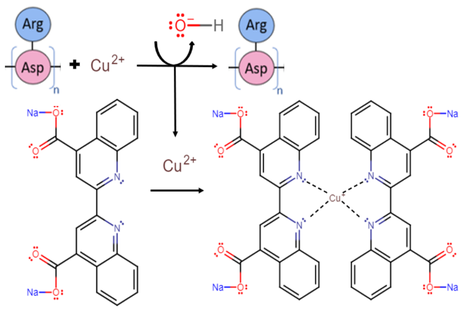

Fig 2: BCA reaction pathway. CGP polymer oxidizes copper from its +2 state to +1, which allows bicinchoninic acid to form a complex with it that fluoresces at 562 nm.

BL21-DE3 *E. coli* strains were used for growing CphA cultures that were incubated with LB at 37ºC for 12 hours. They were then expressed with IPTG at 30ºC for 48 hours. The cultures were pelleted in 1 mL increments by centrifugation. After, the cell pellets were soaked in 3% HCl to lyse cells and allow CGP to solubilize. The solution was centrifuged, and the supernatant was taken and neutralized with tris buffer and different volumes of 10 M NaOH. Two parts of ice-cold ethanol were then added so that CGP precipitated out of solution. CGP was pelleted and the Bicinchoninic Acid assay[4] (BCA) procedure was followed. The solution's absorbance was measured at 562 nm.

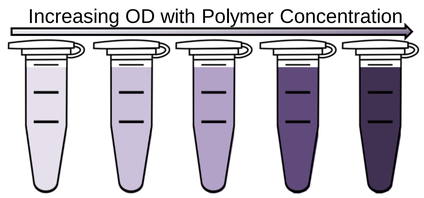

Fig 3: Various concentrations of CGP in 1.5 mL centrifuge tubes after BCA reagent was added.

Discussion

The absorption-concentration relationship shown in figure 4 is what allows the concentrations of CGP extracted at a small scale to be determined. Figure 5 suggests the greatest yield of CGP results from the addition of 7.8 µL 10M NaOH. Additionally, the range in which CGP can be extracted efficiently is approximately 0.8 µL NaOH.

Using the optimal volume of NaOH to neutralize the CGP solution will allow for the maximum yield of CGP. This assay can be adapted to 96 deep well plates so that large quantities of 1 mL cultures can be tested at once. This will allow for the determination of a dynamic range for how much CGP native CphA can produce, which is critical in identifying CphA mutants during directed evolution.

Acknowledgements

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