Facile immobilization of recombinant *Clostridium thermocellum* endoglucanase CelA by artificial oil bodies

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Introduction and objective

Lignocellulosic biomass, consisting of cellulose, hemicellulose, and lignin, is recognized as the most abundant renewable bioresource in nature. After enzymatic hydrolysis, lignocellulose provides many fermentable sugars that can be converted to sustainable bio-based products and biofuels with microorganisms. In particular, cellulose can be hydrolyzed to produce D-glucose by the coordinated act of cellulolytic enzymes (e.g. cellulase), including endoglucanase, cellobiohydrolase, and β-glucosidase. Endoglucanases cleave glucosidic linkages in the cellulose molecule at random and are of great importance for their useful applications in many bio-related fields.

Enzymatic degradation of cellulose is conventionally administrated at high temperatures. This makes an industry-scale production of thermostable cellulolytic enzymes highly desirable. Unfortunately, overproduction of these recombinant cellulases in bioprocess-friendly bacteria, such as *Escherichia coli*, is greatly hindered by many inherent problems. Of these, production of misfolded recombinant proteins that tend to aggregate remains the biggest challenge. To address this issue, we have explored artificial oil bodies (AOBs) as an inert matrix for one-step refolding and immobilization of recombinant cellulases.

Results and conclusion

AOBs have a spherical structure of triacylglycerol core surrounded by a monolayer of structural proteins-bound phospholipids (PLs). Technologically, AOBs can be reconstituted *in vitro* by plant oils, PLs, and OBs-associated structural proteins, such as oleosin (Ole). As a first attempt, CelA of *Clostridium thermocellum* (CtCelA) was chosen for illustration. CtCelA is a thermally stable and acidphilic β-1,4 endoglucanase. Accordingly, CtCelA was first fused to the C-terminus of sesame Ole to create a hybrid gene, Ole-CtCelA. By overexpressing in *E. coli*, Ole-CtCelA was produced in an insoluble form with a yield reaching 20% of total protein content. After

plant oils were added, cell debris containing Ole-CtCelA was subject to sonication. Followed by brief centrifugation, self-assembled AOBs floating on the top of solution were recovered and shown to exhibit endoglucanase activity. Moreover, no endoglucanase activity could be detected after AOBs were treated with trypsin (e.g. protease). This result indicates the presence of active CtCelA on the surface of AOBs.

The enzyme immobilization condition was further optimized based on the methodology of Box-Behnken design. Three factors, including the weight ratio of oil to protein (O/P), pH, and temperature, were selected for optimization and 15 trials were performed for analysis of variance (ANOVA) using JMP 5.1. Consequently, it led to an optimal condition for enzyme immobilization as 24^{o}C, pH 8.4, and O/P at 1:2. Moreover, the optimal reaction condition for immobilized CtCelA was also analyzed by the central composite design. By ANOVA, the highest activity of immobilized CtCelA could be obtained at 68 ^{o}C and pH 6.3. At these optimal conditions, the half-life of immobilized CtCelA was 6 h after exposed to 70^{o}C. In particular, immobilized CtCelA could be reused for 8 cycles and retained more than 50% of the maximal activity.

In conclusion, the results indicate the promise of our proposed method for simple preparation of immobilized endoglucanase. This method shall provide a novel method for effective degradation of cellulose.

Reference

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