## Enzymatic synthesis of c-di-GMP by recombinant protein

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Cellulose biosynthesis is one of the important biological activities, which has been maintained by many living organisms for more than hundred millions years on the earth. Cellulose synthase is known to be membrane protein complex, which is clearly difficult to study. A cellulose-producing bacterium *Gluconacetobacter xylinus* is chosen as an experimental model in this study because it is a prokaryotic single-cell organism, which makes experimental design simpler. In this bacterium, c-diGMP (cyclic-diguanyl monophosphate) plays an important role in cellulose biosyntheis as an allosteric effector. This study aims to establish the enzymatic synthesis of c-diGMP.

### Experiments

Enzyme that synthesizes c-diGMP is known as DGC (diguanyl cyclase) or "GGDEF" protein. First, VCA0956 from *Vibrio cholerae*, which was previously shown to have DGC activity *in vitro* [1], was selected and its genomic DNA was inquired to ATCC (American Type Culture Colllection). However, the DNA of this pathogenic bacterium was not available. Then a homologue was found by blastp search: DGC from *Shewanella oneidensis* MR-1 (hereafter called as SoDGC). The *sodgc* gene was amplified by PCR and inserted into pBAD vector (Invitrogene Inc., US) for expression by *E. coli*. Recombinant DGC expressed with hexahistidine-tag fused was purified by IMAC (Immobilized Metal Affinity Chromatography), and mixed with GTP to convert it to c-diGMP. The resultant c-diGMP was finally purified by anion-exchange chromatography with mobile phase of the gradient of ammonium carbonate, as previously described [2].

### Results

Recombinant DGC protein expressed by *E. coli* was successfully purified as confirmed by SDS-PAGE (Figure 1). Processing of the c-di-GMP synthesis reaction by anion-exchange chromatography gives two peaks of elution at 1.6% and 3.2% ammonium carbonate (Figure 2). By MALDI-ToF MS, it was indicated that the former and the latter contain GTP and c-diGMP, respectively. Successful synthesis of c-diGMP was further confirmed by LCMS-IT-TOF analysis: fragments obtained by MS/MS was consistent with the molecular formula

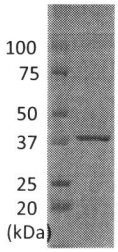


Figure 1 SDS-PAGE stained by Coomasie Brilliant Blue. Lane 1: marker, Lane 2: recombinant DGC purified by IMAC.

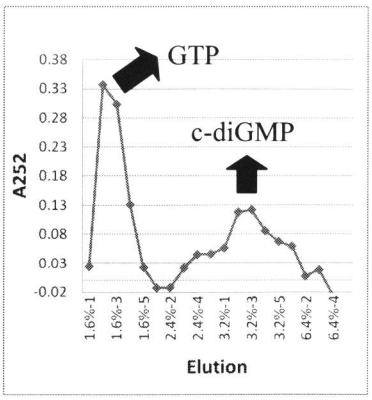


Figure 2. Elution profile of the resultant c-diGMP synthesis reaction on anion-exchange chromatography with ammonium carbonate gradient.

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### References

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[2] Ross, P., Aloni, Y., Weinhouse, C. "An unusual guanyl oligonucleotide regulates cellulose synthesis in *Acetobacter xylinum*", *FEBS letters*, vol. 186, no. 2, pp 191-196, 1985.