EXPRESSION AND PURIFICATION OF POTATO TUBER ADP-GLUCOSE PYROPHOSPHORYLASE MUTANTS

Brian Jacobson, Seon-Kap Hwang, Thomas W. Okita

Institute of Biological Chemistry, Washington State University
Pullman, WA

Abstract

Two different AGPase heterotetramer mutants were expressed: L441R and T51K both containing solubility mutation, S302N. The K41R and T51K mutations are responsible for increasing the catalytic activity of the large subunit. Both were expressed in E. coli gplC null mutant and purified. Proteins were extracted from cells using sonication and centrifugation. Purification of AGPase was performed using DEAE-sepharose FF ion exchange, TALON affinity, and POROS 20HQ chromatography. The purity of AGPase was verified by SDS-PAGE gel analysis. The AGPase activity was assayed in the reverse (pyrophosphorylase) direction.

Introduction

ADP-glucose pyrophosphorylase plays a crucial role in glycolgen and starch production in bacteria and plants. AGPase catalyzes the rate-limiting step of the α-glucan biosynthesis pathway by converting ATP and glucose 1-phosphate into ADP-glucose and pyrophosphate. Plant AGPase is a heterotetramer formed by two small subunits and two large subunits. The crystal structure has been determined for the plant small subunit homotetramer (Jin et al. 2005) and the bacterial homotetramer (Cupp-Vickery et al. 2008) but attempts to determine the plant heterotetramer have been unsuccessful thus far. Determination of the crystal structure will allow us to understand the molecular mechanism of the enzyme’s reaction and then make mutations that can enhance the function of AGPase.

Materials and Methods

*All purification steps were performed at 4°C

- Transformation of pSH404 (Swt) into competent E. coli cells (SM345 line) containing pSH431 (Lko) or pSH435 (Lk) both of which have histidine tags located on the large subunit to facilitate purification.

Discussion and Conclusion

AGPase was successfully expressed and purified. Two bands corresponding to large and small subunits can be conspicuously observed. Approximately 50 mg of AGPase has been purified for L441R and T51K and 25 mg for K41R. Minor contamination can be seen after the HQ purification, which could be removed by heat treatment. Unexpectedly, more small subunit proteins compared to large subunit proteins exist in the final protein preparation.

Future Work

Express and purify more AGPase mutant proteins to investigate optimal conditions for protein crystallization. Address unequal ratio of large to small subunits after purification.

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References

