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Abstract

Two different AGPase heterotetramer mutants were expressed: L^{K41R,T51K,S302N} and L^{S302N} 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 glgC* 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 glycogen 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.

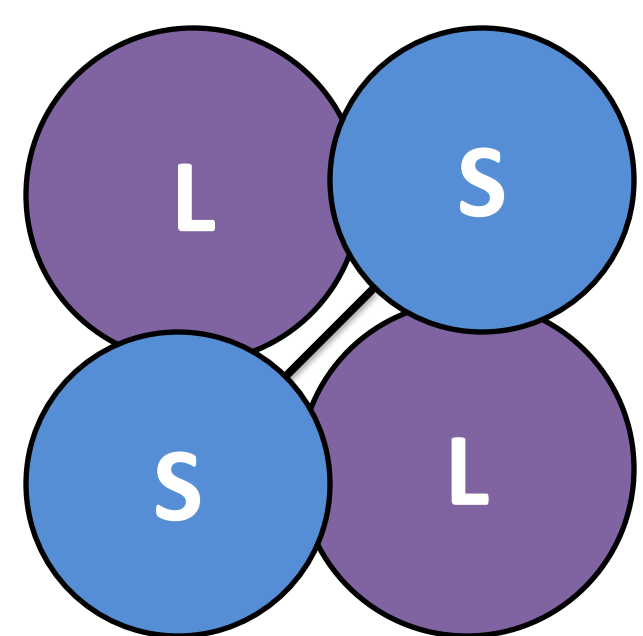


Figure 1 A. Heterotetramer of Plant AGPase

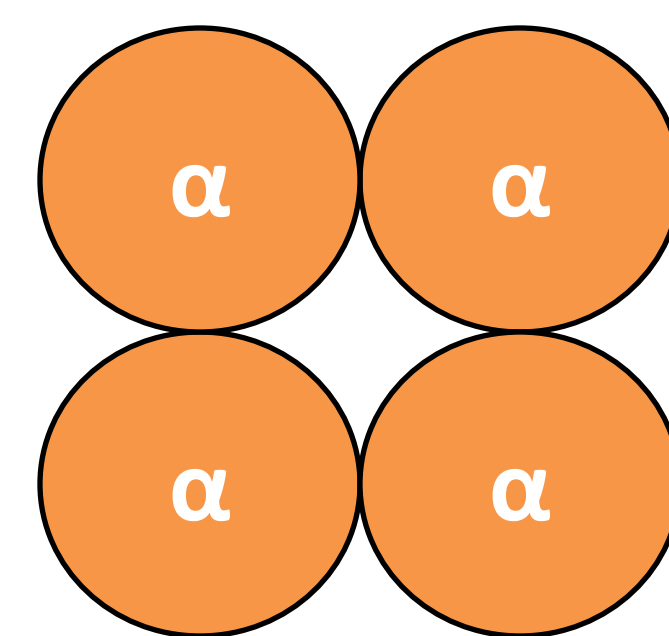


Figure 1 B. Homotetramer of Bacterial AGPase

A. Starch Biosynthesis Pathway

B. Regulation of AGPases

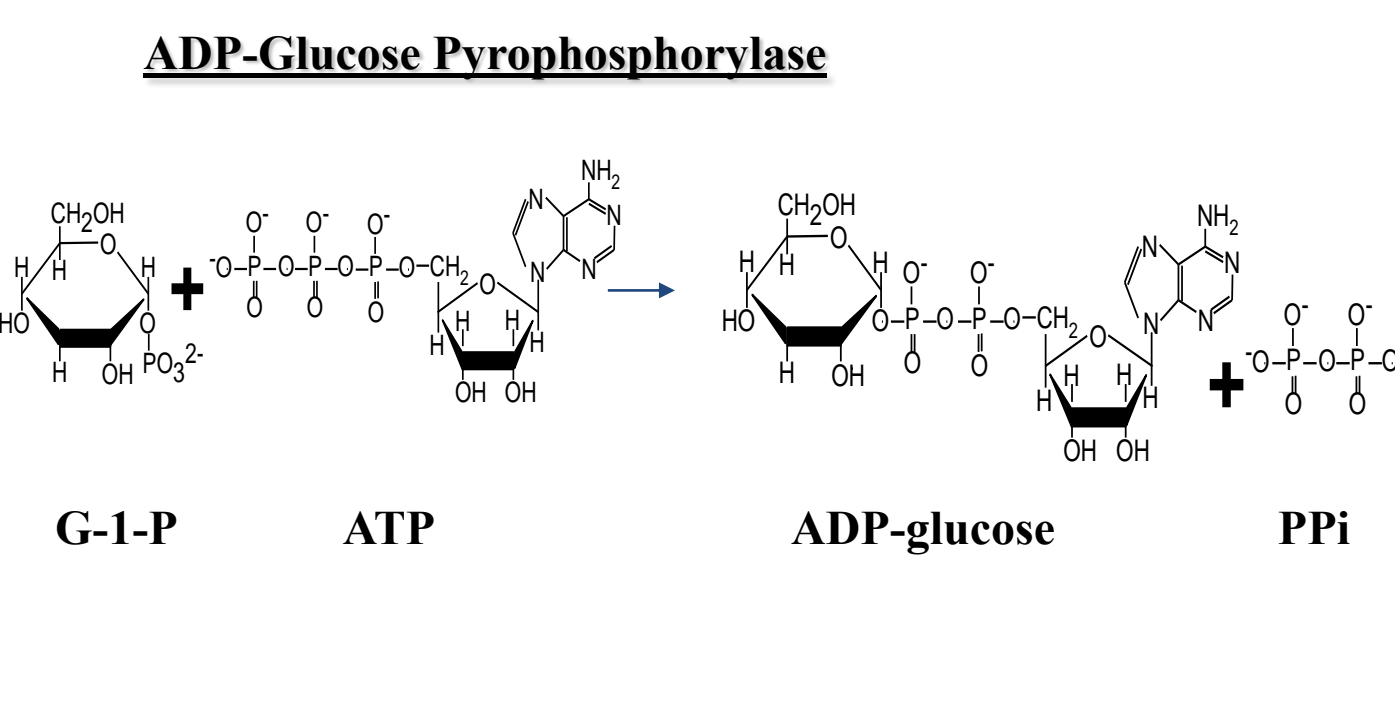


Figure 2 A. AGPase catalyzes the rate limiting step for the glucan biosynthesis pathway. 2 B. The allosteric regulation of AGPases from different organisms with each organism representing a different class of AGPase.

Materials and Methods

*All purification steps were performed at 4°C

• Transformation of pSH404 (S^{WT}) into competent *E. coli* cells (SM345 line) containing pSH431 (L^{RKN}) or pSH435 (L^N) both of which have histidine tags located on the large subunit to facilitate purification.

- Plate cells on NZCYM media containing penicillin and kanamycin
- 4-6 single colonies selected from iodine staining
- Transfer colonies to 25 mL NZCYM media
- Incubate overnight
- Transfer the seed culture 1 L culture and incubate until optical density is 0.8-1.2
- Induce expression of AGPase using IPTG (0.2 mM) for 18 h at room temperature
- Harvest cells
- Disrupt cells in buffer A containing proteinase inhibitors
- Sonication for 1 min twice and centrifuge
- Purify crude extract using DEAE-sepharose FF ion exchange chromatography with a linear gradient of 0-0.5 M NaCl in buffer A
- Analyze the DEAE fractions in the reverse direction for AGPase activity
- Pool active fractions for further purification
- Perform TALON affinity chromatography for His-tagged AGPase purification
- Precipitate AGPase in 67% (w/v) ammonium sulfate
- Perform POROS 20HQ ion chromatography to remove AGPase large subunit homotetramer using buffer A containing 0.15 M NaCl
- Concentrate AGPase using a ultrafiltration membrane (cut-off MW, 10 kDa) apparatus and nitrogen gas
- Freeze AGPase and store at -80 °C

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 L^{K41R,T51K,S302N} and 25 mg for L^{S302N}. 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.

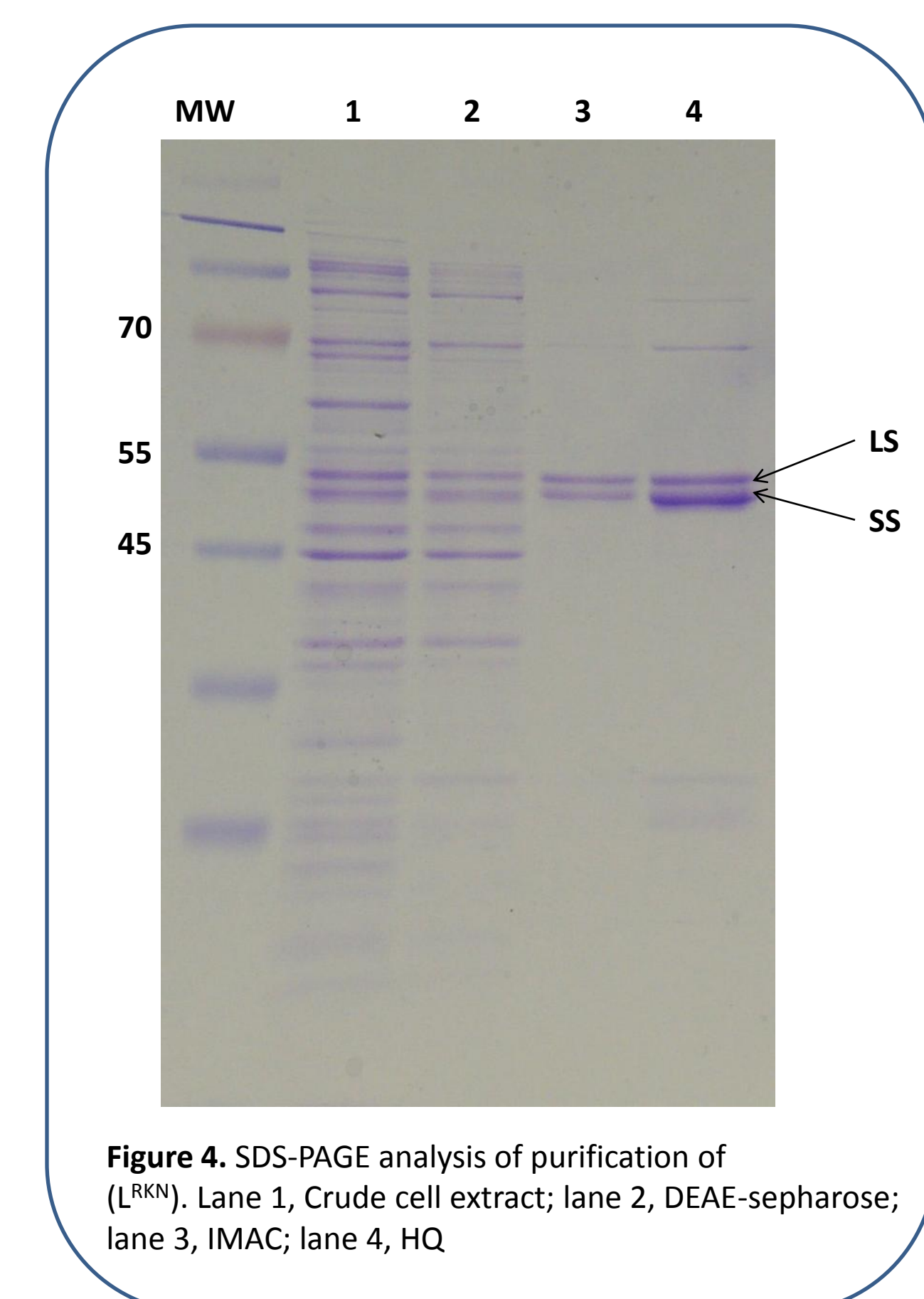


Figure 4. SDS-PAGE analysis of purification of (L^{RKN}). Lane 1, Crude cell extract; lane 2, DEAE-sepharose; lane 3, IMAC; lane 4, HQ

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.

Acknowledgements

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Dr. Mikiko Matsui & Okita Lab Members

References

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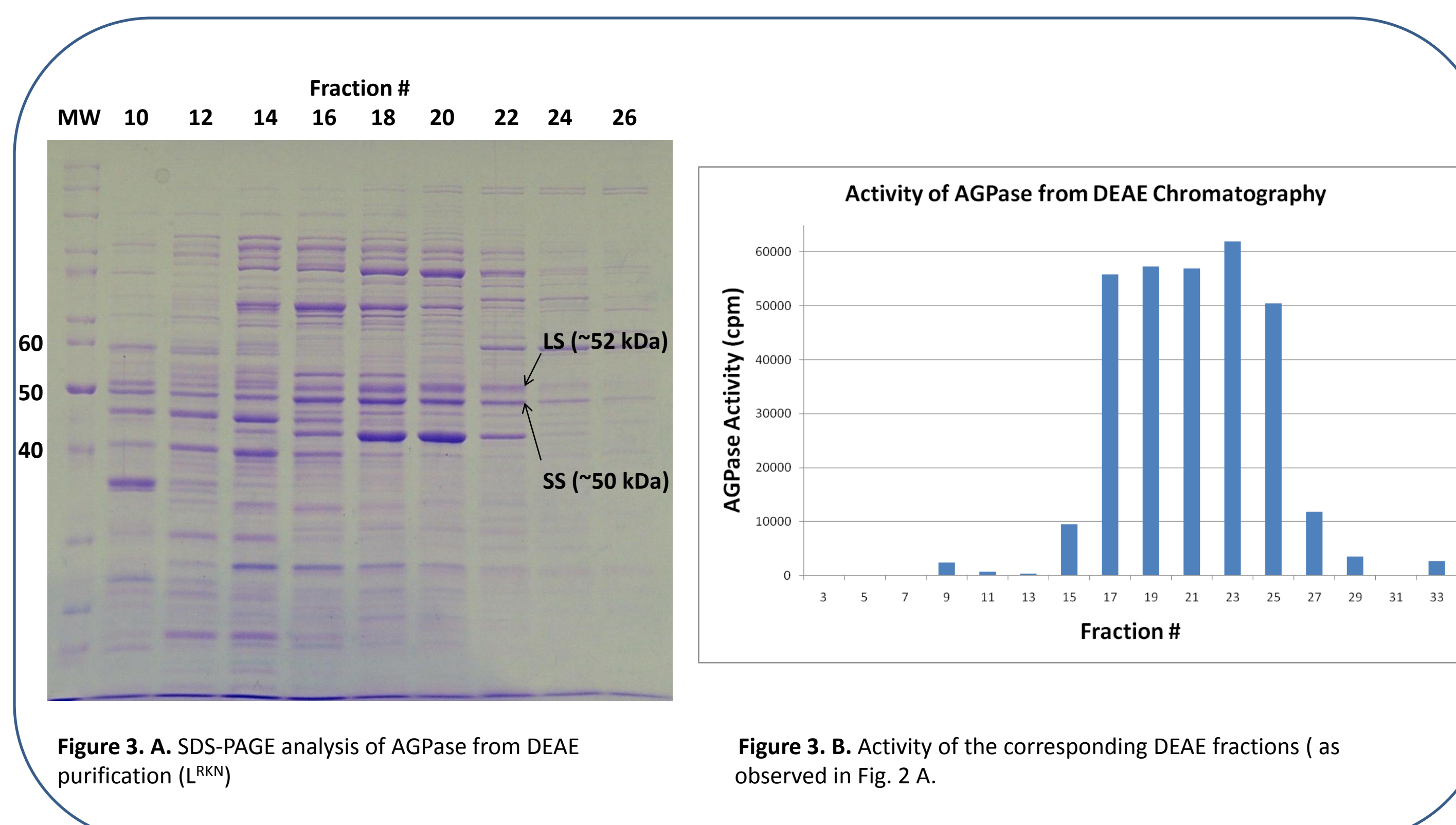


Figure 3. A. SDS-PAGE analysis of AGPase from DEAE purification (L^{RKN})

Figure 3. B. Activity of the corresponding DEAE fractions (as observed in Fig. 2 A.