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Abstract

Glycolysis is an essential metabolic pathway that occurs in virtually all organisms. In this pathway, glucose, $C_6H_{12}O_6$, is converted into pyruvate, $C_3H_3O_3^-$, releasing free energy that is responsible for the formation of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH). The enzyme, phosphoglycerate kinase (PGKase), catalyzes the reaction that results in the reversible transfer of a phosphate group from 1,3 bisphosphoglycerate to ADP. The cytoplasmic PGK exists as two molecular forms in wildtype rice and a RNAi rice line expressing low levels of the RNA binding protein Tudor-SN (Fig. 1). To determine the molecular basis for these two PGK forms, we attempted to purify the enzyme from developing rice seeds. Isolation of the enzyme was obtained by obtaining a crude extract of developing seeds of the rice cultivar Kitaake and then purification through successive column chromatography steps using DEAE, heparin (Fig. 2) and ATP affinity resins. Analysis of the various fractions obtained by column chromatography by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) indicated that the enzyme was significantly purified. While only one band was expected, the gel exhibited two protein bands, the first of PGK and the second degraded form (Fig. 3 and 4). The final protein concentration, specific activity, and percent yield were calculated to be 0.11 mg/mL, 579.6 $\mu\text{mol}/\text{min}/\text{mg}$, and 9%, respectively.

Introduction

Phosphoglycerate kinase is an enzyme that plays a crucial role in glycolysis and the formation of ATP.



Previous studies have found that there is a difference of 0.1 between the isoelectric points of the PGK enzyme in the wild-type, Kitaake rice strain, vs. the mutant, which expresses low amounts of the RNA binding protein Tudor-SN. There is a high degree of structural homology between the 2 isoenzymes, however, the noted variation in isoelectric points enable separations to be possible on basis of difference in overall charge of protein.

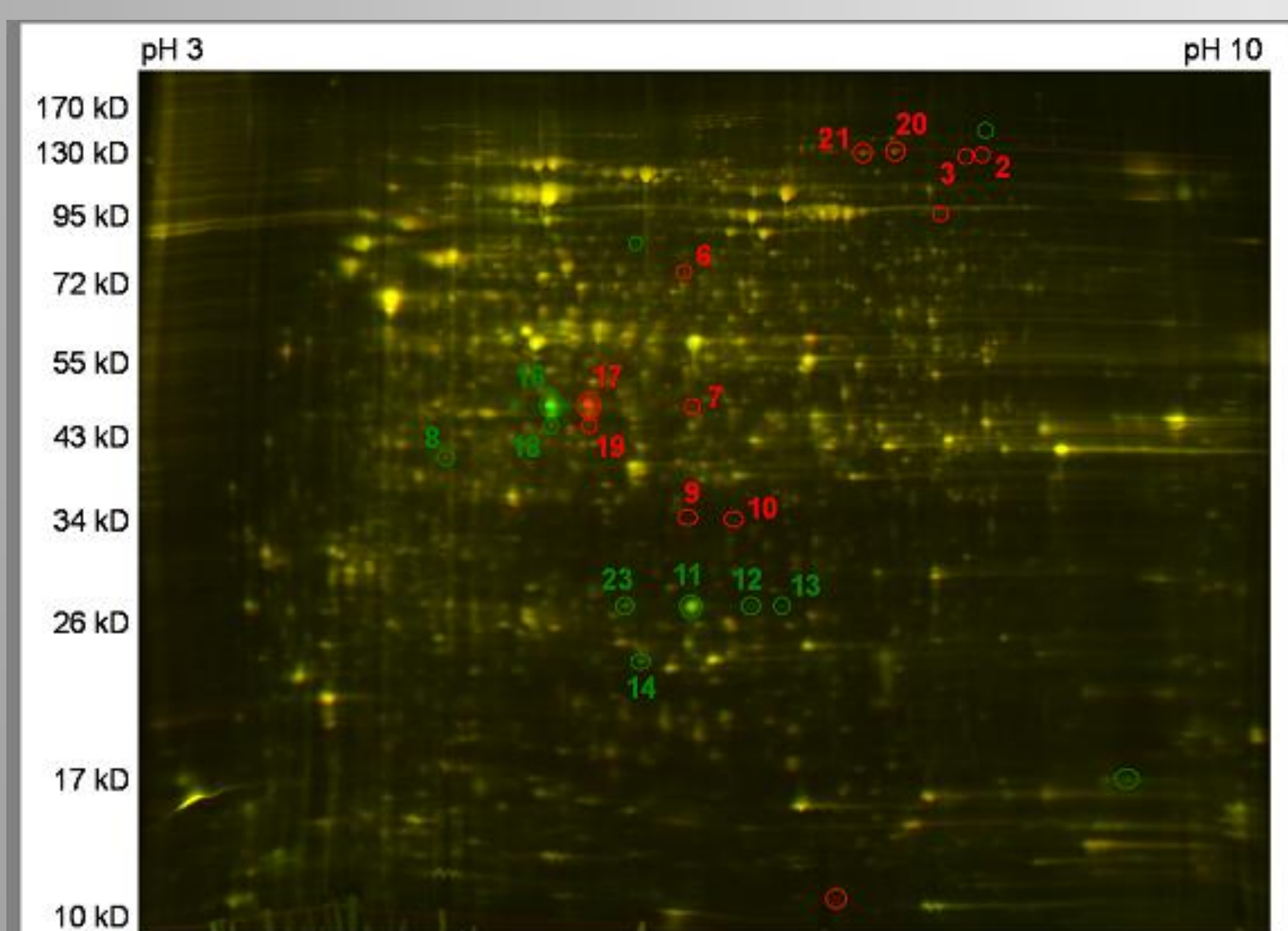


Figure 1. 2D PAGE of 12 day old developing rice seeds from wildtype Kitaake (red) and OsTudor-SN RNAi plant (green). PGK in wildtype (polypeptide band 17 in red) is more basic than the PGK found in the RNAi plant (polypeptide band 16 in green) that is expressing low amounts of the RNA binding protein Tudor-SN.

The present isolation and purification experiment was conducted to enable a deeper comprehensive study and understanding of the differences in properties and structure of the two distinct PGK forms that occur in wildtype rice plants and in the RNAi plant expressing low levels of the RNA binding protein Tudor-SN.

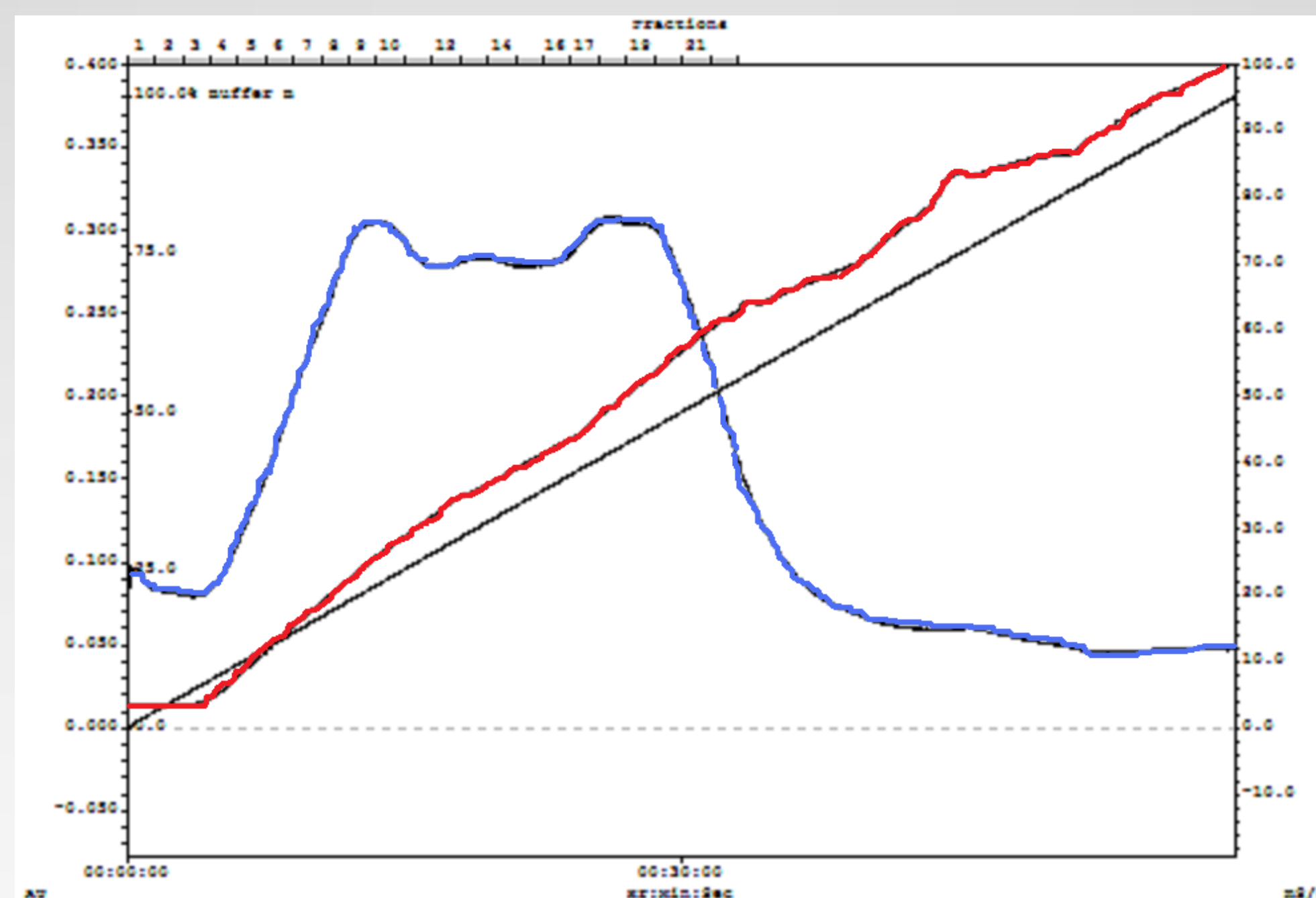
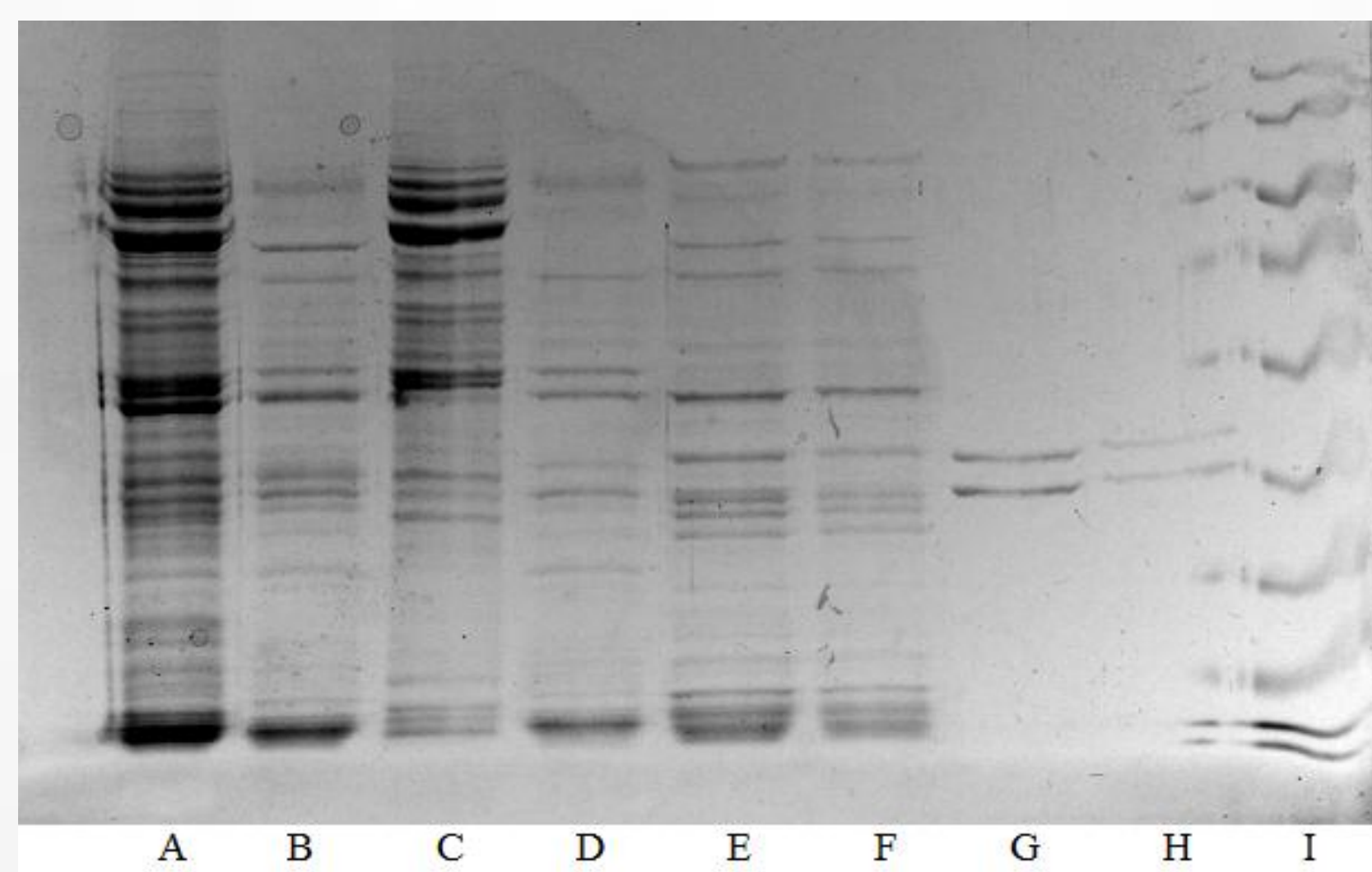


Figure 2. Heparin Column Chromatography

This figure shows the elution pattern of protein (blue line) from the heparin chromatography column using a 0-0.7M NaCl gradient. The black line represents the theoretical salt gradient while the red line indicates the measured conductivity. PGK enzyme activity was measured and found to be eluted from the heparin column in fractions 6-12.

A.



B.

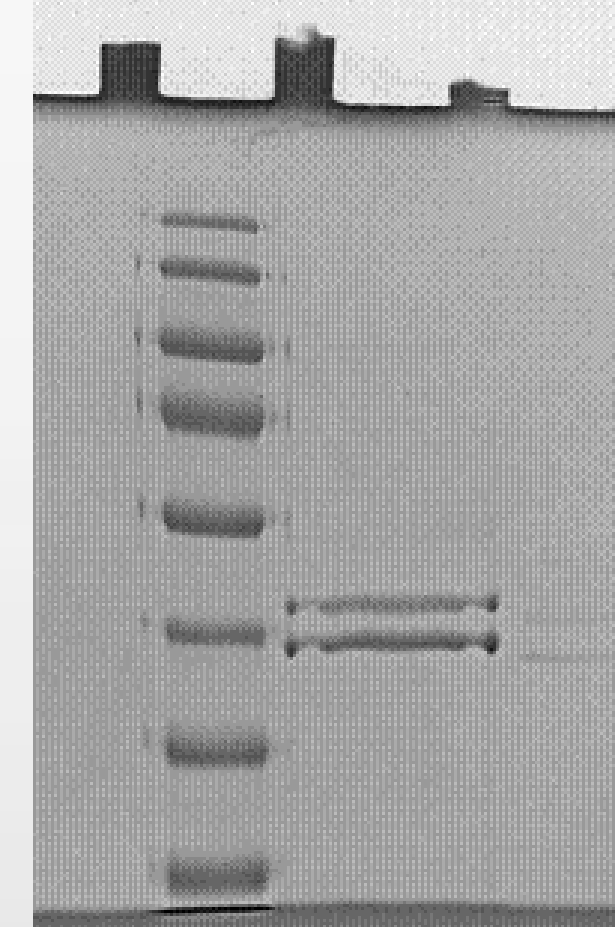


Figure 3. Coomassie Blue-stained SDS-Polyacrylamide gel

A) Developing rice seeds were grounded and extracted in 20mM Tris HCl PH 8.0, 5mM $MgSO_4$ buffer containing 1 $\mu\text{g}/\text{mL}$ leupeptin, 1 $\mu\text{g}/\text{mL}$ pepstatin, and 0.2 mM PMSF. The homogenate was then filtered and centrifuged at 12,000 RPM for 15 min. The supernatant (crude extract, lane A) was collected and passed through a DEAE ion exchange column. The unbound flow through fractions (lane B) contained about 70% of the total PGK enzyme activity with the remainder binding to the DEAE column and eluting in fractions 7-13 when subjected to a 0.02 to 0.5M NaCl salt gradient. The DEAE flow through fraction was then subjected to heparin chromatography (see Fig. 2) followed by ATP affinity chromatography. (D) Flow through from the Heparin column; (E) Fractions collected off the heparin column; (F) Flow through from the ATP column; (G) and (H) hold the most purified form of the PGK enzyme—fractions from .7 NaCl and ATP elutions from the ATP column

(I) Protein standard

B) SDS-PAGE analysis of concentrated PGK fraction obtained from ATP affinity column (right lane). The enzyme, phosphoglycerate kinase, has a molecular weight of 42 kDa. Analysis of gel bands determined the upper band to be at 42.8kDa and the lower band at 39.8 kDa. Left lane contains proteins standards of known molecular size.

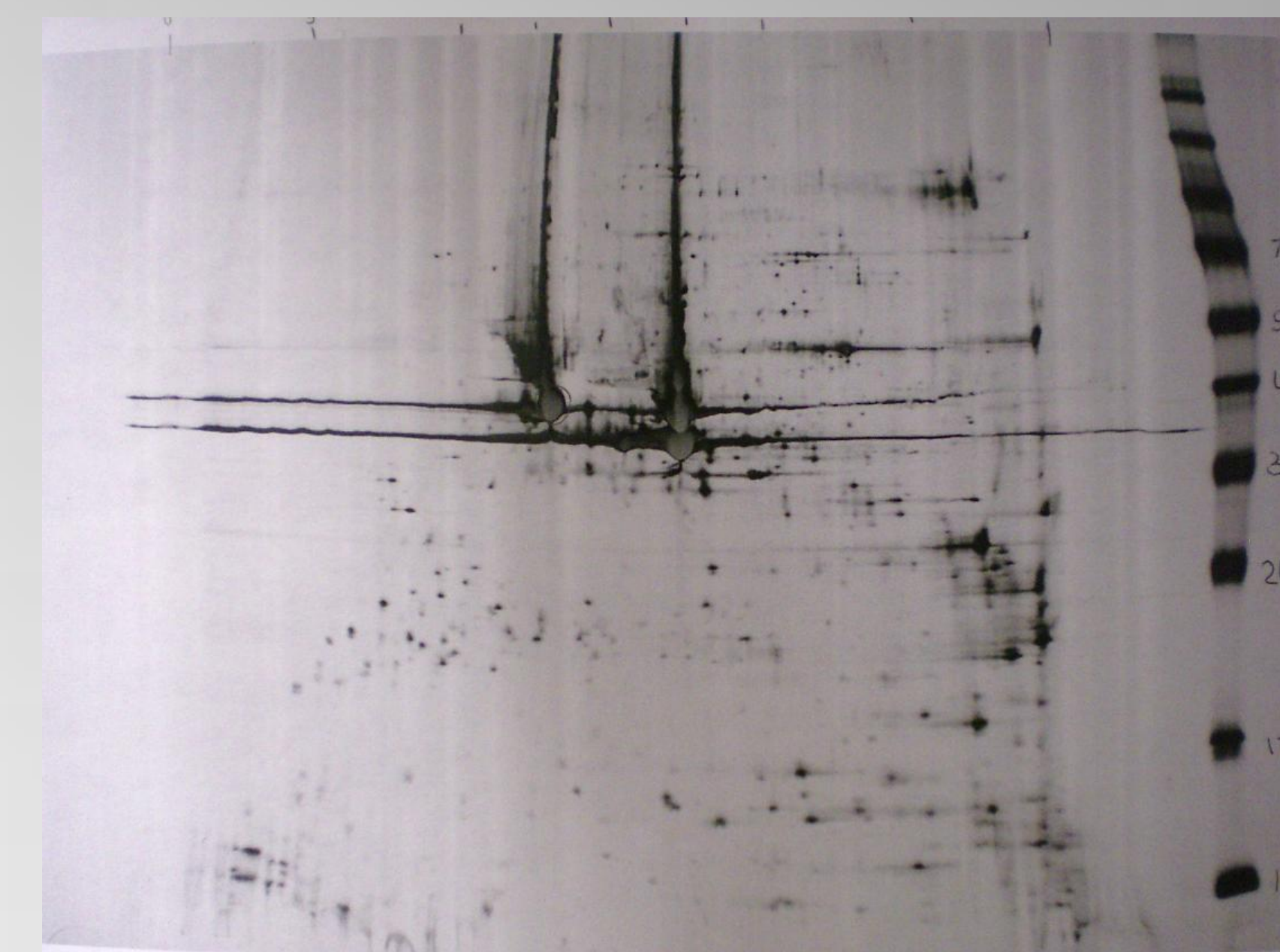


Figure 4. 2D DIGE on the Two Purified PGK Gel Bands

A 2D DIGE gel and mass spectrometry data confirmed that the upper band from the SDS gel (left spot) was that of the phosphoglycerate kinase enzyme, at approximately 42 kDa. The lower band (right spot) was determined to also be the PGK enzyme, but in its degraded form—enabling it to travel further down the SDS-gel (<42kDa) with a more basic net charge.

Conclusion

Sample	Volume (mL)	Protein Concentration (mg/mL)	Specific Activity (units/mg)	Total Activity (units)	Purification Fold	Percent Yield
Crude	140	4.40	1.74	1071.8	1	100%
DEAE Flow Through	119	0.75	3.61	327.6	2	31%
Heparin Flow Through	127	1.11	0.16	22.5	0.1	2%
Heparin Fractions	17	0.80	11.77	160.1	7	15%
Concentrated ATP Fraction	1.5	0.11	579.6	95.63	333	9%

1. The PGK enzyme was able to be extracted and isolated from other non-specific proteins
2. Purification was more than 333x fold
3. About 0.16 mg of purified PGK, or a yield of 9%, was obtained from ~60 g of developing rice seeds. The specific activity of the purified PGK enzyme was determined to be 579.6 $\mu\text{mol}/\text{min}/\text{mg}$ of protein.

Future Work

Future experiments will include the complete purification of the, wild-type, kitaake PGK protein, in order to obtain a single protein band on the SDS gel, and the extraction and purification of the RNAi plant expressing low levels of the RNA binding protein, Tudor-SN.