Volume 280, number 2, 259–261 FEBS 09506 March 1991

ADONIS 0014579391002143

Chloroplast phosphoribulokinase associates with yeast phosphoriboisomerase in the presence of substrate

Cynthia L. Skrukrud and Louise E. Anderson

Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680, USA

Received 18 January 1991

Pea chloroplastic phosphoribulokinase and yeast phosphoriboisomerase partition independently of one another in a two-phase polyethyleneglycol, dextran system, but apparent interaction is seen when ribose-5-phosphate is added to the two-phase system. It appears that the pea leaf kinase recognizes yeast isomerase when it is carrying metabolite.

Metabolite channeling; Phase partitioning; Calvin cycle; Phosphoriboisomerase; Phosphoribulokinase; Pentose phosphate pathway

1. INTRODUCTION

Phosphoriboisomerase, EC 5.3.1.6, (PRI) and phosphoribulokinase, EC 2.7.1.19, (PRK) catalyze consecutive reactions in the reductive pentose phosphate pathway (Calvin cycle). Together they convert ribose-5-P into ribulose 1,5-bisphosphate, the CO_2-acceptor molecule of photosynthetic carbon fixation. Complexes containing both enzyme activities have been described [1–3], yet the two enzymes can be purified away from one another. This suggests that chloroplastic PRK and PRI interact but that the interaction is relatively weak. At low ribose-5-P levels, PRI and PRK produce ribulose-1,5-bisphosphate at a rate greater than predicted from the equilibrium between ribose-5-P and the enzyme pair's intermediate ribulose 5-P [4]. These kinetic data, likewise, could indicate that PRI and PRK interact.

If proteins interact, they may affect one another's partitioning in an aqueous two-phase system. Enhanced resolution of differences in a protein's partition behavior can be achieved by carrying out repeated partitioning by the countercurrent distribution (CCD) technique [5]. Here we report the results of multiple partition experiments with purified pea chloroplastic PRK and yeast PRI.

Correspondence address: L.E. Anderson, BioSci m/c 066, University of Illinois at Chicago, Box 4348, Chicago, IL 60680, USA

Abbreviations: CCD, countercurrent distribution; DTT, dithiothreitol; PRI, phosphoriboisomerase, EC 5.3.1.6; PRK, phosphoribulokinase, EC 2.7.1.19.

2. EXPERIMENTAL

All procedures were carried out at 4–6°C unless otherwise indicated.

2.1. *Materials*

Polyethylene glycol (M_r 8000), biochemicals, yeast (*Candida utilis*) PRI, and auxillary enzymes were obtained from Sigma. Dextran T-500 and Sephacryl S-200 were produced from Pharmacia. Blue H-ERD Sephacryl was prepared by coupling the procion dye (gift of ICI Sweden) to Sephacryl by the method of Ashton and Polya [6]. All other chemicals were of analytical reagent grade.

The procedure for PRK isolation is a variation based on the method reported for the wheat enzyme [7]. Either a crude whole shoot extract or a chloroplastic extract from 10 to 12 day-old pea plants in 10 mM Hepes-KOH (pH 7), 5 mM 2-mercaptoethanol, and 2.5 mM dithiothreitol (DTT) (extraction buffer) is taken to 30% saturation with ammonium sulfate, and precipitated protein is removed. PRK is then precipitated from the ammonium sulfate supernate by lowering the pH to 5 by dropwise addition of 3 N acetic acid. After centrifugation, the pellet containing PRK is resuspended in the extraction buffer; the pH of the solution is raised to 7 by the addition of 2 N NH_4OH; the solution is dialyzed against buffer containing 10 mM Hepes-KOH (pH 7), 100 mM KCl, 5 mM 2-mercaptoethanol and 2.5 mM DTT; and the dialyzed sample is loaded onto a 40 ml Blue H-ERD Sephacryl column, equilibrated at room temperature with the same buffer. The column is washed at room temperature with the dialysis buffer and with 10 mM potassium phosphate (pH 7) buffer containing 100 mM KCl, 5 mM 2-mercaptoethanol and 2.5 mM DTT; the PRK is eluted using a 0–5 mM ATP, 2.5 mM $MgCl_2$ gradient in 100 ml of the phosphate buffer at room temperature. The specific activities of PRK preparations used in the experiments reported here ranged from 70 to 115 U/mg protein. Note that this specific activity is DTT-activation dependent.

2.2. *CCD experiments*

Prior to being added to the phase partition system, the enzymes were preincubated for 1–2 h in phase partition buffer containing 50 mM potassium phosphate (pH 7) and 2.5 mM DTT. When the two enzymes were partitioned together, they were preincubated together. In some experiments 0.4 mM ribose-5-P or 0.4 mM ribulose-5-P was included in the preincubation buffer and in the phase partition system.

CCD experiments were performed in a cold room. The aqueous two-phase system contained 9% (wt/wt) dextran T-500, 7.2% (wt/wt)

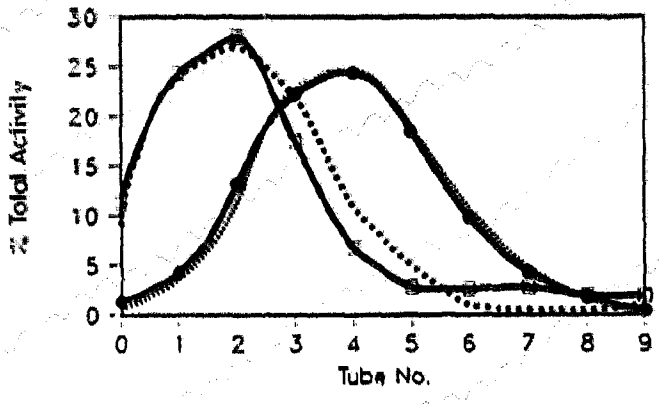

Fig. 1. Countercurrent distribution of pea chloroplastic phosphoribulokinase and yeast phosphoriboisomerase. Phosphoribulokinase (8 U) (●) and phosphoriboisomerase (0.04 U) (□) were partitioned together. Phase system (pH 7) containing the enzymes was placed in tube 0; 9 transfers were performed. The dashed lines show how each enzyme was distributed when it was partitioned individually under the same conditions: (.....) phosphoribulokinase; (-----) phosphoriboisomerase. Similar results were obtained in a total of 5 experiments.

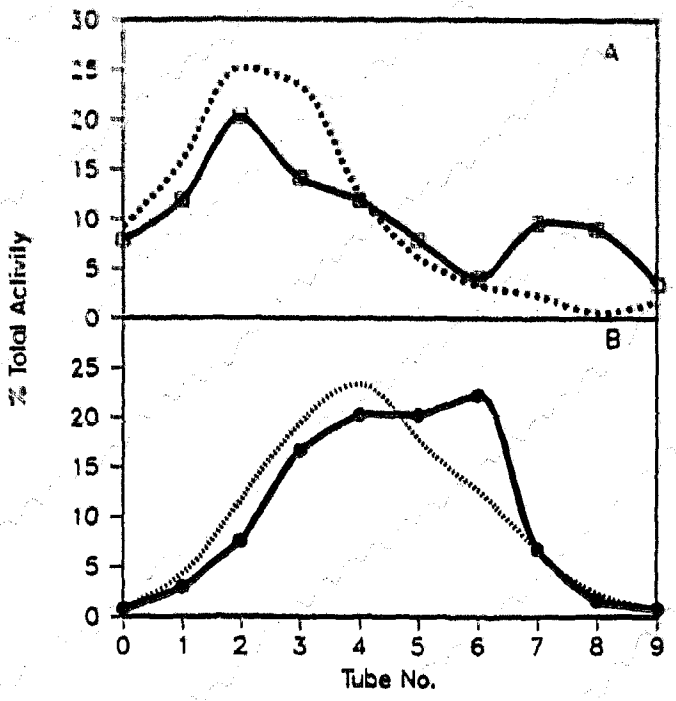

Fig. 2. Countercurrent distribution of pea chloroplastic phosphoribulokinase with yeast phosphoriboisomerase in the presence of ribose-5-phosphate. Phosphoribulokinase (●) and phosphoriboisomerase (□) were partitioned together in phase system (pH 7) containing 0.4 mM ribose-5-phosphate. The activity ratio was 1:140 phosphoriboisomerase (0.035 U)/phosphoribulokinase (4.9 U). The experiment was performed twice with similar results. The dashed lines show how each enzyme was distributed when it was partitioned individually in the presence of its substrate: (.....) phosphoribulokinase with 0.4 mM ribulose-5-phosphate; (-----) phosphoriboisomerase in the presence of 0.4 mM ribose-5-phosphate. Similar results were obtained when the kinase was partitioned individually in the presence of 0.4 mM ribose-5-phosphate.

polyethylene glycol 8000, 50 mM potassium phosphate (pH 7), and 2.5 mM DTT. Tubes contained 2.1 g of the system; one gram of top phase was transfered manually to the next tube in the series with the fresh upper phase from the final tube being transfered to tube 0. Phases were mixed by 30 inversions and were separated by centrifuging for 2 min at 2 333 × *g*. At the end of the run (1 h), 2.9 ml 50 mM potassium phosphate (pH 7), 2.5 mM DTT was added to each tube to obtain a homogeneous solution. The fractions were collected and analyzed for enzymatic activity.

The partition ratio *G*, the ratio between the amount of substance in the mobile and stationary part of the system, was estimated for each enzyme using the relationship $G = i/(n-i)$ where i is the number of the tube where the peak of enzyme activity appears and n is the number of transfers [5].

2.3. *Enzyme assays*

All enzymes were assayed at ambient temperature (22°C) spectrophotometrically (ΔA_{340}). The PRK assay mixture contained 100 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM ATP, 0.5 mM phosphoenolpyruvate, 0.4 mM ribulose-5-P, 0.2 mM NADH, 0.9 U/ml lactate dehydrogenase, and 0.2 U/ml pyruvate kinase. PRI activity was measured using the same method and same basic assay mixture, but 0.4 mM ribose-5-P was the substrate, and excess PRK was included as a coupling enzyme.

3. RESULTS AND DISCUSSION

Yeast PRI and chloroplastic PRK showed unique distribution patterns in the two-phase system (Fig. 1). PRK partitioned with a *G* value of 0.8. Yeast PRI had a greater affinity for the lower dextran-rich phase, partitioning with a *G* value of 0.3. No shift in the partition pattern was seen when PRI and PRK were preincubated and partitioned together (Fig. 1) which indicates a lack of physical interaction.

In a second set of experiments, PRK paired with yeast PRI was preincubated and partitioned in the presence of 0.4 mM ribose-5-P. Different ratios of the enzymes were tested since the limiting component of a complex should show the greatest alteration in its distribution pattern [8]. When the PRK/PRI activity ratio was high (140 U PRK/1 U PRI), a new peak of yeast PRI activity with a *G* value of 3.5 appeared (Fig. 2A), and PRK activity was shifted towards this new PRI peak (Fig. 2B). If the new activity peaks represent the partitioning of weakly complexed PRI and PRK undergoing repeated association and dissociation, the final positions of the new peaks will probably not be coincident, as is seen here. When PRI activity was greater than PRK activity, only the shift in PRK activity distribution was seen (data not shown). Inclusion of either ribulose-5-P or ribose-5-P did not alter the distribution pattern of either enzyme partitioned individually. This result indicates that PRK is only capable of recognizing yeast PRI when it has ribose-5-P, or more likely ribulose-5-P, bound.

To our knowledge, this is the first time a physical interaction between two enzymes has been demonstrated to be dependent on the presence of their common metabolite. This substrate requirement for interaction

between pea chloroplastic PRK and yeast PRI suggests that the recognition of PRI-bound ribulose-5-P by PRK is the source of the physical interaction seen for the two enzymes. This physical interaction could facilitate direct transfer of ribulose-5-P between the two enzymes. Interaction followed by transfer of ribulose-5-P from PRI to PRK could explain the unexpectedly high rate of ribulose-1,5-bisphosphate formation at low ribose-5-P levels by this enzyme pair [4].

Acknowledgements: We thank Göte Johansson, Lunds Universitet, for hosting one of us (C.L.S.) in his laboratory during which time initial experiments leading to those reported here were carried out, and for much helpful discussion and advice. Lawrence Sykora and staff of the Greenhouse of the University of Illinois at Chicago cultivated the pea plants, and Binh Duy Trân provided excellent technical assistance with the CCD experiments. C.L.S. was a postdoctoral fellow of the Laboratory of Cellular, Molecular and Developmental Biology, University of Illinois at Chicago and was a recipient of a Wood/Whelan Research Fellowship from the International Union of Biochemistry for travel to Sweden. This work was supported in part by U.S. Department of Energy Grant FG02-85ER60367.

REFERENCES

[1] Gontero, B., Cárdenas, M.L. and Ricard, J. (1988) Eur. J. Biochem. 173, 437–443.
[2] Joint, I.R., Morris, I. and Fuller, R.C. (1972) J. Biol. Chem. 247, 4833–4838.
[3] Salnis, J.K. and Harris, G.C. (1986) Biochem. Biophys. Res. Commun. 139, 947–954.
[4] Anderson, L.E. (1987) FEBS Lett. 212, 45–48.
[5] Albertsson, P.-Å. (1986) Partition of Cell Particles and Macromolecules, 3rd edn, pp. 121–132, Wiley-Interscience, New York.
[6] Ashton, A.R. and Polya, G.M. (1978) Biochem. J. 175, 501–506.
[7] Surek, B., Heilbronn, A., Austen, A. and Latzko, E. (1985) Planta 165, 507–512.
[8] Backman, L. and Shanbhag, V.P. (1979) J. Chromatogr. 171, 1–13.