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Ribose-5-phosphate isomerase and ribulose-5-phosphate kinase show apparent specificity for a specific ribulose 5-phosphate species

Louise E. Anderson

Department of Biological Sciences, University of Illinois at Chicago, Box 4348, Chicago, IL 60680, USA

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Ribose-5-phosphate isomerase and ribulose-5-phosphate kinase appear to show specificity for a particular ribulose 5-phosphate species. The effect of this specificity will be channeling of ribulose 5-phosphate from the isomerase to the kinase during photosynthesis.

Metabolite channeling; Ribulose-5-phosphate kinase; Ribose-5-phosphate isomerase; Ribulose 5-phosphate; (Chloroplast)

1. INTRODUCTION

In a two enzyme sequence the rate of reaction 2 is limited by the equilibrium constant for reaction 1, because the highest possible concentration of substrate 2 (equals product 1) is the equilibrium concentration of product 1. I now report that the rate of the reaction catalyzed by the enzyme ribulose-5-P kinase (phosphoribulokinase, EC 2.7.1.19) is 3-fold greater than predicted from K_{eq} (ribulose 5-P/ribose 5-P) when the substrate for the two enzyme ribose-5-P isomerase (EC 5.3.1.6) ribulose-5-P kinase reaction is ribose 5-P and kinase activity is limiting.

2. MATERIALS AND METHODS

2.1. *Enzymes*

Ribulose-5-P kinase was purified from 10- to 15-day-old pea (*Pisum sativum* L. var. Little Marvel) plant shoots by a modification of the method of Surek et al. [1]. The enzyme was precipitated from the 35% saturation $(NH_4)_2SO_4$ supernate by dropwise addition of 3 M acetic acid until the pH reached 5. The precipitate was collected by centrifugation, suspended in a minimal amount of 100 mM KCl, 10 mM potassium phosphate, 5 mM mercaptoethanol, pH 7 buffer, and dialyzed overnight against the same buffer. The dialyzed fraction was centrifuged to remove insoluble denatured protein and the supernate was slowly run through a Blue HERD-Sepharose column at room temperature. After the column was washed with approx. 10 column vols of the same buffer, the kinase was eluted by addition of 10 mM ATP in the buffer. A more detailed description of this purification procedure will be published elsewhere. In the experiments described here the active fractions were used without further purification. The specific activity was 284 units · mg protein^{-1} without DTT activation. The isomerase activity of this preparation was less than 3% of the kinase activity.

Ribose-5-P isomerase from *Torula* yeast was purchased from Sigma.

2.2. *Enzyme assays*

Ribulose-5-P kinase activity was assayed as

Correspondence address: L.E. Anderson, Dept of Biological Sciences, University of Illinois at Chicago, Box 4348, Chicago, IL 60680, USA

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described [2]. The coupling enzymes were freed of $(NH_4)_2SO_4$ by gel filtration through Sephadex G-25. The reaction was followed on Varian Cary 210 or 219 recording spectrophotometers. Assay temperature was 23°C. Ribose and ribulose 5-P concentrations were estimated using the same assay and excess ribulose-5-P kinase or ribulose-5-P isomerase and ribulose-5-P kinase.

3. RESULTS AND DISCUSSION

The equilibrium constant for the conversion of ribose 5-P to ribulose 5-P is 0.264 at 25°C [3]. But when ribose-5-P isomerase activity exceeds ribulose-5-P kinase activity the rate of formation of ribulose 1,5-P_2 is the same with either ribose 5-P or ribulose 5-P as the pentose-P substrate (fig.1). At the substrate levels used in the experiment in fig.1 the rate with ribose 5-P as substrate exceeds the predicted maximal rate by a factor of 3. Similar results, but a lesser deviation from the predicted rate, were obtained when crude stromal extracts were used instead of the purified enzymes (not shown). In the experiments reported here ribose 5-P did not serve as a substrate for the kinase reaction unless the isomerase was added. Then contamination of the ribose 5-P with ribulose 5-P cannot account for these results. When the equilibrium mixture of the pentose phosphates (incubated 10 min with isomerase) was used as substrate, the rate was the same whether the initial sugar phosphate incubated with the isomerase was ribose 5-P or ribulose 5-P, and equal to the rate predicted from K_{eq}. Then contamination of the ribulose 5-P with an inhibitor or of the ribose 5-P with an activator cannot account for these results.

Enzymes show specificity for substrate species. One explanation for the results in fig.1 is that the ribulose 5-P species produced by the isomerase and utilized by the kinase is not the predominant species in aqueous solutions of ribulose 5-P. Since the pentulose 5-phosphates are acyclic, anomers are not a possibility. The mechanism of the isomerase reaction probably dictates the formation of the free keto form of ribulose 5-P (since an enediol intermediate is involved, see [4]), but this is apparently the predominant carbonyl form in aqueous solution (see NMR spectra for ribulose 5-P in [5]). The active form of ribulose 5-P might be in slow equilibrium with some other form of, or derivative of, ribulose 5-P. Knowles et al. [6] have noted spectral changes in solutions of ribulose 5-P.

A second possibility is that the substrate for the kinase is ribulose 5-P bound to the isomerase, by analogy to P-glycerate kinase/glyceraldehyde-3-P dehydrogenase (see [7]). But addition of isomerase to the kinase reaction mixture (substrate ribulose 5-P) does not increase the reaction rate appreciably (fig.2). If ribulose 5-P bound to the isomerase were the true substrate, addition of isomerase should

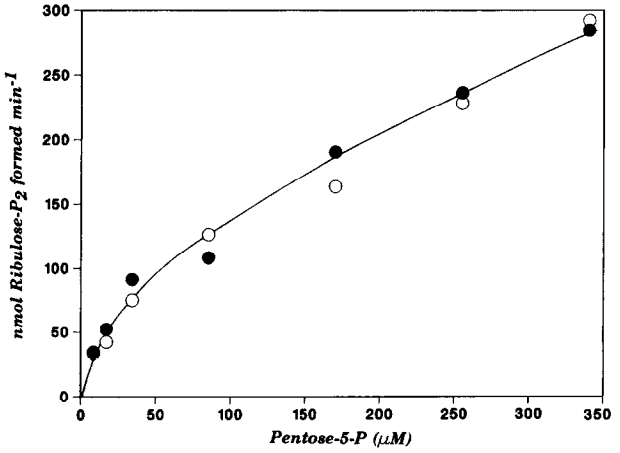

Fig.1. Ribulose-5-P kinase activity as a function of pentose 5-P concentration. Cuvettes contained 0.1 units ribulose-5-P kinase, other assay components as listed in section 2 and ribulose 5-P (●) or 2.9 units ribose-5-P isomerase and ribose 5-P (○). Similar results were obtained in another experiment where ribose-5-P isomerase was added to all of the cuvettes.

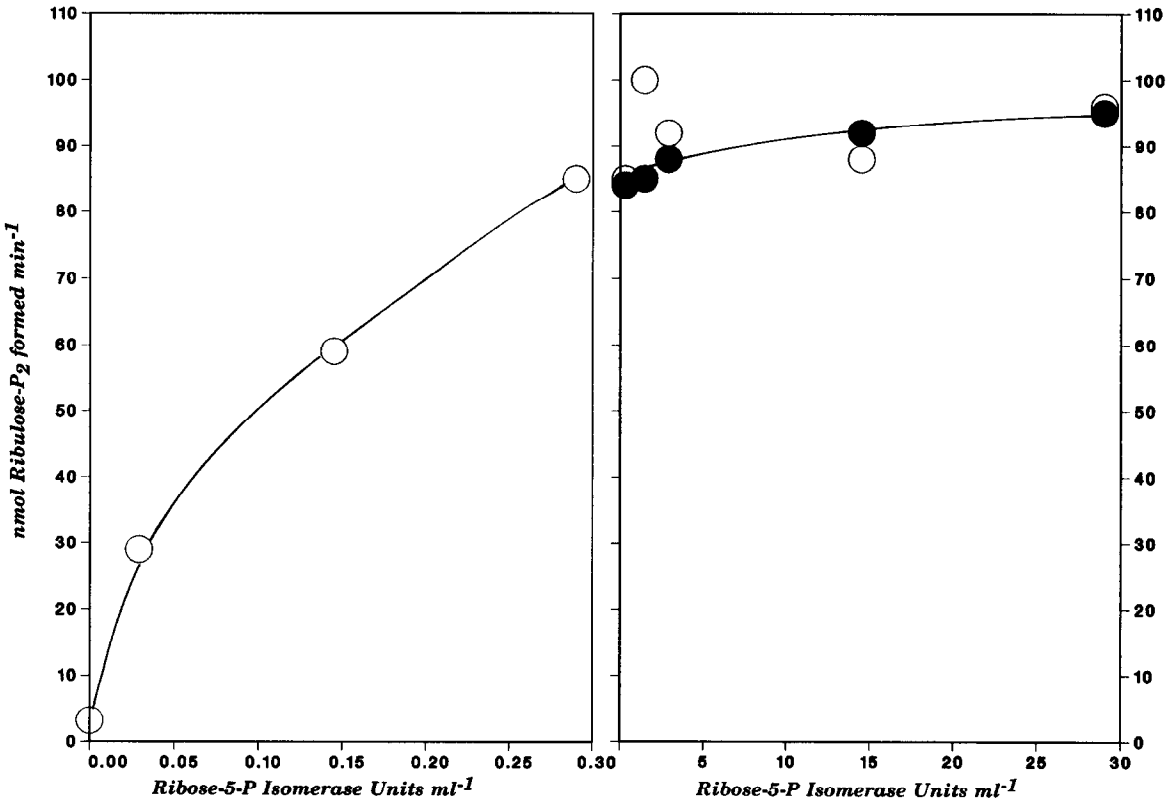

Fig.2. (Left) Dependence of ribulose-5-P kinase catalyzed ribulose-P_2 formation on ribose-5-P isomerase when the substrate is ribose 5-P. (Right) Effect of high levels of ribose-5-P isomerase on ribulose-P_2 formation when the substrate is ribulose 5-P (●) or ribose 5-P (○).

have increased the rate. Therefore in these experiments the substrate for the kinase does not appear to be isomerase bound ribulose 5-P.

Channeling of substrate from the isomerase to the kinase within an enzyme complex could also account for the observed results. Sainis and Harris [8] have presented some new evidence for complexing between ribulose-5-P isomerase, ribulose-5-P kinase and ribulose-P_2 carboxylase. If complexing is responsible for the results observed here, then this might be seen in measurements of the time required to reach steady-state rates in the two enzyme system. Plots of the reciprocal of transient time (τ) versus ribulose-5-P kinase concentration (isomerase rate limiting) were linear, however (not shown). It should be noted that I was forced to use a coupled enzyme assay for kinase activity, which adds two additional transient steps to the measurement of τ and therefore τ estimated may not have been only τ for the build up of steady-state levels of ribulose 5-P. Increasing the kinase concentration 4-fold did not increase the rate of ribulose-P_2 formation in this experiment (not shown). Likewise increasing the isomerase concentration in the experiment shown in fig.2 (kinase limiting) did not appreciably increase the rate of ribulose-P_2 formation. If complexing were involved increasing the concentration of either enzyme should have increased the reaction rate. These results, then, do not implicate complexing, but because of the problems involved in using a coupled enzyme assay and in all experiments of this type (see [9]), they cannot conclusively eliminate that possibility. Complexing between enzymes from the same organism is more likely than between enzymes of different origin: it will be of interest to repeat these experiments with ribose-5-P isomerase from pea chloroplasts.

These experiments indicate that ribose-5-P isomerase and ribulose-5-P kinase produce and utilize, respectively, a form of ribulose 5-P which is not the predominant form in aqueous solution. Whatever the active species, this specificity will serve to channel ribulose 5-P from the isomerase to the kinase in the intact chloroplast.

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