BBA 66296

CHLOROPLAST AND CYTOPLASMIC ENZYMES

III. PEA LEAF RIBOSE 5-PHOSPHATE ISOMERASES

LOUISE E. ANDERSON

With the technical assistance of ROBERT GLASSMAN

Department of Biological Sciences, University of Illinois at Chicago Circle, Chicago, Ill. 60680 (U.S.A.)

(Received November 6th, 1970)

SUMMARY

1. The apparent isoelectric points of pea *(Pisum sativum)* leaf chloroplast and cytoplasmic ribose-5-*P* isomerases (D-ribose 5-phosphate ketol-isomerase, EC 5.3.1.6) differ by 0.2 pH unit.

2. The two isoenzymes have the same optimum pH, 7.5. The K_m for ribose-5-*P* is 2.3 mM for the chloroplast, and 2.1 mM for the cytoplasmic, enzyme.

3. Ribulose-1,5-P_2 is a positive modulator (increases v_{max}) of both isoenzymes; AMP is a competitive inhibitor. These data suggest that ribose-5-*P* isomerase is involved in regulating the reductive pentose phosphate cycle in the pea leaf chloroplast.

INTRODUCTION

Ribose-5-*P* isomerase (D-ribose 5-phosphate ketol-isomerase, EC 5.3.1.6) catalyzes the interconversion of ribose-5-*P* and ribulose-5-*P*. In the green plant this enzyme is essential for the operation of the reductive pentose phosphate cycle within the chloroplast, and, in the cytoplasm, in heterotrophic pentose metabolism and purine nucleotide biosynthesis. Although ribose-5-*P* isomerases from a number of different sources including two higher plants and a photosynthetic bacterium have been purified and characterized[1-6] the chloroplast and cytoplasmic forms of the plant enzyme have not been separated and studied previously. The purposes of the present study were to determine whether there are two forms of ribose-5-*P* isomerase in the green leaf and to characterize the isoenzymes.

The isoelectric points of the pea leaf chloroplast and cytoplasmic ribose-5-*P* isomerases as determined by isoelectric focusing differ by 0.2 pH unit, one isoenzyme occurring in the chloroplast and the other in the cytoplasm. The two isomerases have similar or identical pH optima, K_m's for ribose-5-*P*, and K_i's (competitive) for AMP.

Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine.

Both are positively modulated (v_{max} is increased) by ribulose-1,5-P_2, a key metabolite in the reductive pentose phosphate cycle. The type of modulation and the nature of the two compounds affecting the activity of the isoenzymes suggest that ribose-5-P isomerase is involved in regulating pentose phosphate metabolism, and in particular the reductive pentose phosphate cycle, in the pea plant. No differences, other than in isoelectric point, were found between the two enzymes.

MATERIALS AND METHODS

Preparation of cytoplasmic and chloroplast fractions

Chloroplast and cytoplasmic extracts for isoelectric focusing were prepared and the protein content estimated as described previously[7]. Partially purified isomerase fractions were used in the other experiments described here. Crude extracts were prepared as for isoelectric focusing, except that the preparation was scaled up for 100 g tissue and chloroplasts were suspended in 10 mM potassium phosphate, 5 mM mercaptoethanol buffer, pH 7, prior to disruption. The fraction precipitating between 35–65% $(NH_4)_2SO_4$ saturation was collected, dissolved in minimal amounts of buffer and passed through Sephadex G-150 columns (2.5 cm × 30 cm, 40–120 μm) in 10 mM pH 7.5 HEPES (potassium salt), 5 mM mercaptoethanol buffer. Active fractions were stored at −20°. This procedure results in about 50-fold purification of the enzyme.

Enzyme assays

Sodium ribose-5-P was used as substrate in all cases. The assay of AXELROD AND JANG[1] was used to estimate enzyme activity in isoelectric focusing experiments. The color that develops in the presence of ribulose-5-P was measured at 540 nm with a Bausch and Lomb Spectronic 20 colorimeter.

Initial velocity measurements were made at 25° by a modification of the procedure of AXELROD AND JANG[1]. Reaction mixtures contained ribose-5-P, 50 μmoles HEPES (potassium salt), pH 7.5, and enzyme, in a total volume of 1.0 ml. Reaction was initiated by the addition of enzyme. Samples (200 μl) were removed as rapidly as possible into 1.0 ml of an ice-cold mixture of H_2SO_4–water (225:75, by vol). 50 μl 0.12% carbazole in absolute ethanol and 50 μl 1.5% cysteine·HCl were added with mixing after each addition. The solution was incubated for exactly 30 min at 37° and the absorbance measured at 540 nm with a Gilford 2400 spectrophotometer. Initial velocity was determined by extrapolating to zero time.

Determination of kinetic constants

Seven substrate levels varied at even reciprocal intervals between 0.6 and 10 mM were used. Values and standard error for v_{max}, K_m, K_i and K_A (calculated as is non-competitive inhibition) were estimated as described previously[8].

Reagents

The sodium salts of ribose-5-P and of ribulose-1,5-P_2, AMP, HEPES, 2-(N-morpholino)ethanesulfonic acid (MES), and N,N-bis(2-hydroxyethyl)glycine (Bicine) were obtained from Sigma, ampholine solution from LKB, Sephadex from Pharmacia,

and ultra-pure $(NH_4)_2SO_4$ from Mann. All other chemicals were analytical reagent grade.

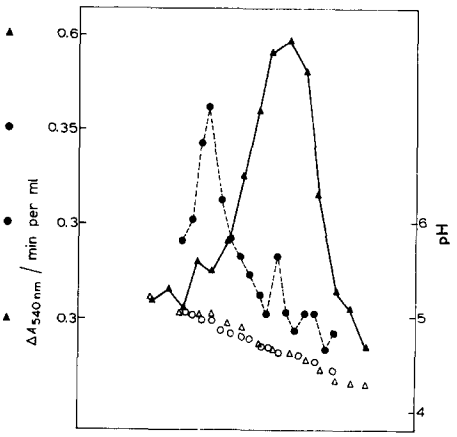
RESULTS

The apparent isoelectric point of the chloroplast enzyme is 5.0, and of the cytoplasmic enzyme, 4.8 (Fig. 1). The experiment was repeated twice with chloroplast, and 3 times with cytoplasmic extracts with consistent results. There was no evidence for additional isoenzymic forms when whole leaf extracts were focused on 3–10 ampholyte.

The two isomerases have broad, apparently identical pH activity curves and the same optimum pH, 7.5 (Fig. 2). The spinach[3] and alfalfa[1] enzymes also have broad pH activity curves.

The isoenzymes have K_m's for ribose-5-*P* which are similar or identical (Table I). These values are close to the value reported by KNOWLES *et al.*[3] for the spinach enzyme, but 5-fold higher than the value reported by RUTNER[4]. They are 10-fold higher than the Michaelis constant of the *Rhodospirillum rubrum* isomerase[6].

Both of the pea leaf isomerases are inhibited competitively by AMP. Ribose-5-*P* isomerases of alfalfa[1], *Escherichia coli*[9] and *R. rubrum*[6] are also strongly inhibited by AMP; the spinach enzyme is not[4]. There is no significant inhibition of either pea



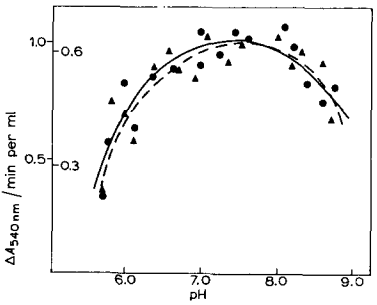


Fig. 1. Isoelectric focusing patterns of pea leaf ribose-5-*P* isomerases. Chloroplast (circle) and cytoplasmic (triangle) extracts containing 10 mg protein were subjected to electrophoresis for 1.5 days at 500 V in 4–6 ampholyte in a 110-ml LKB column as previously described[7] except that sorbitol was substituted for sucrose. The cathode was at the bottom of the column. 9-drop (0.7 ml) fractions were collected and analyzed for enzyme activity. Ribose-5-*P* isomerase activity is given by the filled symbols, pH of the fractions by open symbols. Data were plotted for best fit to pH values. No units are given on the abscissa. The units used (drops) were consistent within each run but not between the 2 runs. Some of the color which develops is due to the reaction of sorbitol with the reagents used to detect ribulose-5-*P*.

Fig. 2. Dependence of pea leaf chloroplast and cytoplasmic ribose-5-*P* isomerases on pH. Purified chloroplast (●, outside ordinate) or cytoplasmic (▲, inside ordinate) isomerase was incubated with 5 μmoles ribose-5-*P* and 100 μmoles buffer (potassium salt), in a total volume of 2 ml. The pH of the assay mixture was determined with a Radiometer 26 pH meter after removal of aliquots for ribulose-5-*P* determinations.

TABLE I

PROPERTIES OF PEA LEAF RIBOSE-5-*P* ISOMERASE

For details see text. Number of determinations in parentheses.

	Chloroplast	*Cytoplasmic*
p*I'*	4.95 (2)	4.75 (3)
Optimum pH	7.5 (3)	7.5 (3)
K_m (mM), ribose-5-*P*	2.3 ± 0.3 (11)	2.1 ± 0.2 (9)
K_a (mM), ribulose-1,5-P_2	1.2 ± 0.1 (5)	1.22 ± 0.02 (4)
K_i (mM), AMP	1.3 ± 0.4 (2)	1 ± 0.2 (5)

leaf enzyme by ADP, ATP, *P*-enolpyruvate, 3-*P*-glyceric acid, or citrate. Phosphate is a competitive inhibitor; K_i's are around 10 mM. The *R. rubrum* isomerase is inhibited by ADP, citrate, and by inorganic phosphate[6].

Ribulose-1,5-P_2, a key intermediate in the reductive pentose phosphate cycle and an analog of ribulose-5-*P*, enhances the activity of both pea leaf isomerases by causing an increase in v_{max}. The *R. rubrum* enzyme is competitively inhibited by ribulose-1,5-P_2 (ref. 6).

DISCUSSION

Pea leaf chloroplast and cytoplasmic ribose-5-*P* isomerases have slightly different isoelectric points as do the chloroplast and cytoplasmic forms of triose-*P* isomerase, fructose-1,6-P_2 aldolase (EC 4.1.2.7) and *P*-glyceric acid kinase (EC 2.7.2.3)[10].

The ribose-5-*P* isomerases are functionally similar if not identical (Table I); the pH activity curves and optima are identical and the apparent affinity for substrate is the same for both enzymes. In contrast chloroplast and cytoplasmic triose-*P* isomerases do not have identical pH optima, and have different inhibitor and Michaelis constants[7]. At the level of enzyme control there seems to be no advantage to the plant in having two essentially identical ribose-5-*P* isomerases.

The competitive inhibition of the ribose-5-*P* isomerase isoenzymes by AMP may be due to the similarity between ribose-5-*P* and the ribosyl-*P* portion of the adenylic acid. Nevertheless this inhibition is probably metabolically significant. In the chloroplast high AMP levels will reflect lack of light energy. Inhibition of ribose-5-*P* isomerase by AMP probably provides one mechanism for the control of the reductive pentose phosphate cycle by light.

Ribulose-1,5-P_2 resembles ribulose-5-*P* and hence might be expected to be a competitive inhibitor of ribose-5-*P* isomerase. Instead this compound is a positive modulator. Stimulation of chloroplast ribose-5-*P* isomerase by ribulose-1,5-P_2 will act in an autocatalytic manner to cause the formation (*via* the ribulose-5-*P* kinase reaction) of yet more ribulose-1,5-P_2, thereby reinforcing the operation not only of the isomerase but of the entire reductive pentose phosphate cycle.

The ribose-5-*P* isomerases of pea leaf chloroplast and cytoplasm are very similar enzymes, with the same pH dependence and Michaelis constants for ribose-5-*P*. Both are affected by a compound important in energy metabolism, AMP, and by ribulose-1,5-P_2, an intermediate in the reductive pentose phosphate cycle. Fine-

level modulation of isomerase activity by these compounds through alteration of the kinetic constants will change, to a greater or lesser degree, the steady-state levels of ribose-5-*P* to ribulose-5-*P* and the flow of carbon *in vivo* will be shifted.

ACKNOWLEDGMENTS

We thank Mark Bresnik for assisting with the determination of the kinetic constants. This research was supported by grants from the University of Illinois, Chicago Circle, Research Board and from the National Science Foundation, GB 8626.

REFERENCES

1 B. AXELROD AND R. JANG, *J. Biol. Chem.*, 209 (1954) 847.
2 J. HURWITZ, A. WEISSBACH, B. L. HORECKER AND P. Z. SMYRNIOTIS, *J. Biol. Chem.*, 218 (1956) 769.
3 F. C. KNOWLES, M. K. PON AND N. G. PON, *Anal. Biochem.*, 29 (1969) 40.
4 A. C. RUTNER, *Biochemistry*, 9 (1969) 178.
5 L. ANDERSON, L. E. WORTHEN AND R. C. FULLER, in K. SHIBATA, A. TAKAMYIA, A. T. JAGENDORF, AND R. C. FULLER, *Comparative Biochemistry and Biophysics of Photosynthesis*, University Park Press, State College, Pa., 1968, p. 379.
6 L. E. ANDERSON AND R. C. FULLER, in H. METZNER, *Progress in Photosynthesis Research*, Vol. 3, Metzner, Tübingen, 1969, p. 1618.
7 L. E. ANDERSON, *Biochim. Biophys. Acta*, 235 (1971) 237.
8 L. E. ANDERSON AND R. C. FULLER, *J. Biol. Chem.*, 244 (1969) 3105.
9 J. DAVID AND H. WIESMEYER, *Biochim. Biophys. Acta*, 208 (1970) 56.
10 L. E. ANDERSON AND V. R. ADVANI, *Plant Physiol.*, 45 (1970) 583.