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KINETIC PROPERTIES OF PHOSPHOGLYCOLATE PHOSPHATASE FROM *PISUM SATIVUM* AND *PHASEOLUS VULGARIS*

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Key Word Index—*Pisum sativum*; *Phaseolus vulgaris*; Leguminosae; pea; bean; phosphoglycolate phosphatase; 2-phosphoglycolate.

Abstract—High activities of phosphoglycolate phosphatase were observed in *Pisum sativum* and *Phaseolus vulgaris* leaves. The low values for K_m phosphoglycolate and the K_a values for the activation by Mg^{2+} and Cl^- are consistent with the efficient hydrolysis of phosphoglycolate in these plants. This is confirmed by the absence of detectable phosphoglycolate in photosynthetically active leaves of *Pisum sativum*.

INTRODUCTION

In photosynthetic organisms, 2-phosphoglycolate is a product of the ribulose-1,5-bisphosphate oxygenase reaction within the chloroplast. Phosphoglycolate is hydrolysed to glycolate and inorganic phosphate by a specific 2-phosphoglycolate phosphatase [EC, 3.1.3.18] in the chloroplast. This reaction is the first unique step of the oxidative photosynthetic carbon cycle (i.e. C_2 cycle or photorespiratory pathway) [1–3]. Glycolate is then transported out of the chloroplast and is subsequently metabolized in the peroxisomes and mitochondria.

The efficient hydrolysis of phosphoglycolate in plants may be of critical importance in the maintenance of photosynthetic competence because phosphoglycolate is a transition state analogue and potent inhibitor of triosephosphate isomerase [4, 5], an enzyme of the reductive photosynthetic carbon cycle necessary for the regeneration of ribulose-1,5-bisphosphate within the chloroplast. The physiological importance of phosphoglycolate phosphatase is substantiated by the phenotypic characteristics of mutants of plants which do not contain the enzyme [6, 7]. These mutants are unable to survive in air, but can grow in atmospheres containing high concentrations of carbon dioxide, a condition in which the ribulose-1,5-bisphosphate oxygenase reaction forming phosphoglycolate is suppressed by the competing ribulose-1,5-bisphosphate carboxylase reaction.

In a recent review of the properties of phosphoglycolate phosphatase from a variety of photosynthetic organisms [1], we noted a significant variation in the kinetic properties of the enzyme in the leaves of various species of plants. Although K_m values for the hydrolysis of phosphoglycolate are in the range of 10–30 μM for the enzymes from tobacco [8] or spinach leaves [9, 10], much higher values of 2 mM have been reported in *Pisum sativum* [11] and *Phaseolus vulgaris* L. [12] leaves. Such high K_m values could result in the inefficient hydrolysis of phosphoglycolate *in vivo*, and thus might allow phosphoglycolate to play a role in the regulation of photosynthetic rates if significant phosphoglycolate pools accumulate in the chloroplast. In this study we have reinvestigated some of the kinetic properties of phosphoglycolate phosphatase from *Pisum sativum* and *Phaseolus vulgaris* under the experimental conditions previously utilized for our investigation of the properties of the enzyme from *Spinacia oleracea*. In addition, we have measured intracellular phosphoglycolate levels in leaves of *Pisum sativum* to determine whether sufficient phosphoglycolate accumulation occurs to regulate the regeneration of ribulose-1,5-bisphosphate through the inhibition of triosephosphate isomerase.

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RESULTS AND DISCUSSION

The levels and some kinetic properties of phosphoglycolate phosphatase from the leaves of *Pisum sativum* and *Phaseolus vulgaris* have been summarized in Table 1. In addition, values we have obtained previously for *Spinacia oleracea* under identical conditions [9] are provided for comparison, and are similar to those also reported by Rose *et al.* [10]. High levels of the enzyme are observed in all of these species. The enzyme from all three species has a broad pH optimum from 6 to near 9 and low K_m values for the hydrolysis of phosphoglycolate. In addition, low values for the K_a for the activation by Mg^{2+} and Cl^- are observed, indicating that the enzyme is probably saturated with these activators under physiological conditions. However, the degree of activation by these ions differs somewhat between species. The K_m and K_a (Mg^{2+}) values reported here are considerably less than those previously reported for the enzymes from *Pisum sativum* [11] and *Phaseolus vulgaris* [12] under different experimental conditions, and it is not clear if a saturating level of an activating anion was present in the previous investigations. K_a (Cl^-) values have not previously been determined for the enzyme from these species.

It is apparent that the levels and kinetic properties of the enzyme in *Pisum sativum* are sufficient to hydrolyse all of the phosphoglycolate phosphatase formed by the

Table 1. Properties of phosphoglycolate phosphatase

Species	Levels in Leaves (units/mg Chl)	pH optimum	K_m (μM)	$K_a(Mg^{2+})$ (μM)	Fold activation by Mg^{2+}	$K_a(Cl^-)$ (mM)	Fold activation by Cl^-
*Spinacea oleracea**	15	6–8.5	26	21 ± 3	9.3	0.45	5.6
Pisum sativum	8 ± 3	6–9	40 ± 5	72 ± 15	5.5 ± 1.6	0.37 ± 0.04	2.0 ± 0.2
Phaseolus vulgaris	18 ± 4	6–9	30 ± 2	115 ± 15	3.8 ± 0.8	0.56 ± 0.20	1.6 ± 0.3

*Values from ref. [9].

ribulose-1,5-bisphosphate oxygenase reaction. When leaves from plants carrying out photosynthesis in air were subjected to the enzymatic analysis of phosphoglycolate levels measured as described in the Experimental, no phosphoglycolate was detected using an assay of sufficient sensitivity to measure a level of *ca* 5 μM within the chloroplast of the plant. These results indicate that a specific phosphoglycolate phosphatase with high activity and affinity for phosphoglycolate efficiently hydrolyses phosphoglycolate as it is formed in the plant, and thus phosphoglycolate accumulation is an unlikely regulator of photosynthetic activity in the leaves of C_3 plants. However, since the K_i for inhibition of pea chloroplast triosephosphate isomerase is 15 μM [5], the possibility of some regulation of the enzyme by levels of phosphoglycolate below that of our detection limits can not be eliminated.

In summary, in contrast to previous studies, the kinetic properties of phosphoglycolate phosphatase from *Pisum sativum* and *Phaseolus vulgaris* are similar in many respects to those we have measured for the enzyme from *Spinacia oleracea*. Furthermore, the absence of detectable levels of phosphoglycolate within the leaves of *Pisum sativum* is consistent with the efficient hydrolysis of phosphoglycolate by this enzyme and discounts the possibility of a significant physiological regulation of ribulose-1,5-bisphosphate regeneration via the inhibition of triosephosphate isomerase by phosphoglycolate.

EXPERIMENTAL

Enzyme assays. Phosphoglycolate phosphatase activity was determined by measuring the liberation of inorganic phosphate from the substrate 2-phosphoglycolate and kinetic parameters were determined as described in ref. [9].

Enzyme enrichment. Phosphoglycolate phosphatase was partially purified from *Pisum sativum* cv Burpeana and *Phaseolus vulgaris* cv Tendercrop by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography as described previously for the spinach enzyme [9]. The resultant enzyme fractions were specific for the hydrolysis of 2-phosphoglycolate; the rate of hydrolysis of a variety of other phosphorylated compounds including *p*-nitrophenyl phosphate, 3-phosphoglycerate and ribose-5-phosphate were all less than 5% the rate of 2-phosphoglycolate hydrolysis.

Measurement of 2-phosphoglycolate levels in leaves. Pea leaves were removed from plants growing in the field under bright sunlight at 1 p.m., and 5 g was immediately added to 10 ml 1 M $HClO_4$ and homogenized. A tracer level of ^{14}C-phosphoglycolate (0.01 μCi), prepared by the pyruvate kinase catalysed phosphorylation of ^{14}C-glycolate [13], was added to serve as an internal standard for the recovery of phosphoglycolate through subsequent steps (the recovery was normally 40–50%). The extract was neutralized with 4 M KOH and the solid $KClO_4$ was removed by centrifugation. The supernatant was applied to a 5 ml Dowex-1 (formate) column, washed with 20 ml 4 M HCO_2H, and eluted with a 20 ml gradient of 4–8 M HCO_2H. The fractions (2 ml) were concd by lyophilization, and resuspended in 0.1 ml 20 mM Mes, 5 mM $MgCl_2$, pH 6.3. To half of the sample was added 5 units of the phosphoglycolate specific phosphoglycolate phosphatase purified from spinach leaves as described in ref. [9]. The amount of inorganic phosphate liberated by the enzyme relative to that present in the absence of the enzyme was determined by the malachite green assay [14], and was used to calculate the phosphoglycolate levels within the leaf. The sensitivity of the procedure is such that 2 nmol of phosphoglycolate could be detected as determined by adding a known amount of 2-phosphoglycolate (Sigma) to a leaf extract prior to the processing of the sample and corresponds to a concentration of phosphoglycolate of *ca* 5 μM within the chloroplast stroma, assuming a chloroplast volume of 25μl/mg Chl [15].

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