REGULATION OF SPINACH RIBULOSE 5-PHOSPHATE KINASE BY 3-PHOSPHOGLYCERATE

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1. Introduction

Ru5P kinase is a key regulatory enzyme of the Calvin cycle. A light-dependent interconversion of this enzyme by reduction of thiol groups via thioredoxin and ferredoxin–thioredoxin reductase has been described [1]. In intact chloroplasts, the half-times of activation and inactivation were 30 s and 100 s [2], respectively. When photosynthesizing chloroplasts are darkened, CO_2 fixation is found to be inhibited within 30 s, although the stromal levels of ATP are not depleted [3]. These results indicated that during a light–dark transient Ru5P kinase can be rapidly inactivated by other mechanisms apart from a reversal of the light-dependent enzyme interconversion. The observed alterations of pH and Mg^{2+} concentration alone were not sufficient [3] to account for this inhibition and it appeared that a regulation by metabolite levels was taking place.

Ru5P kinase can be regulated by the ATP/ADP ratio [2,4]. In [5] no influence by ADP but an inhibition by 3-phosphoglycerate (PGA) was found [5]. These authors speculated that PGA did not act directly on the enzyme, but caused an inhibition by chelating the Mg^{2+} needed for enzyme activity [5]. Here, the influence of PGA on Ru5P kinase activity is reinvestigated.

2. Materials and methods

Spinach (*Spinacia oleracea*) was grown in water culture [6] and chloroplasts were prepared as in [7]. For incubation of chloroplasts see [2]. Assay of Ru5P kinase by $H[^{14}C]O_3$ incorporation [2] was carried out with 50 μM Ru5P, 0.2 mM ATP, 20 mM $MgCl_2$, 10 mM $NaH[^{14}C]O_3$, 100 mM Tris–HCl (pH 7.8) and 0.2% (v/v) Triton X-100, unless otherwise stated (section 3). When PGA was present during the assay, the PGA was adjusted after the acidification step to the same concentration in all samples before the second incubation with added RuBP carboxylase (2 U/ml) was performed. Standards were included to check that this PGA concentration did not impair the quantitative conversion of RuBP to [^{14}C]PGA.

Abbreviations: PGA, 3-phosphoglycerate; Ru5P, ribulose 5-phosphate

3. Results and discussion

3.1. *Nature of the inhibition by PGA*

The activated enzyme assayed immediately after chloroplast rupture appears to have a higher substrate affinity than those enzyme preparations tested by other authors. Thus the K_m-value for Ru5P (25 μM in fig.1, 26–66 μM in other experiments not shown here) is much below the values given for the isolated enzyme (0.2 mM) in [8,9]. Also, the K_m-value for ATP (35–65 μM, not shown) again was well below reports of 280 μM [8] and 650 μM [9]. The inhibition of Ru5P kinase by PGA was competitive to Ru5P (fig.1). With ATP, the inhibition of Ru5P kinase was non-competitive (not shown). To ensure that the inhibition by PGA was not due to chelation of Mg^{2+}, all experiments with PGA were done in the presence of 20 mM Mg^{2+}. Maximal enzyme activity was obtained already with 5–10 mM Mg^{2+}.

In cloroplasts the levels of PGA, ADP and ATP are linked via phosphoglycerate kinase and glyceraldehyde 3-phosphate dehydrogenase, such that a fall in ATP is frequently accompanied by an increase of both ADP and PGA. The interaction between the inhibition by

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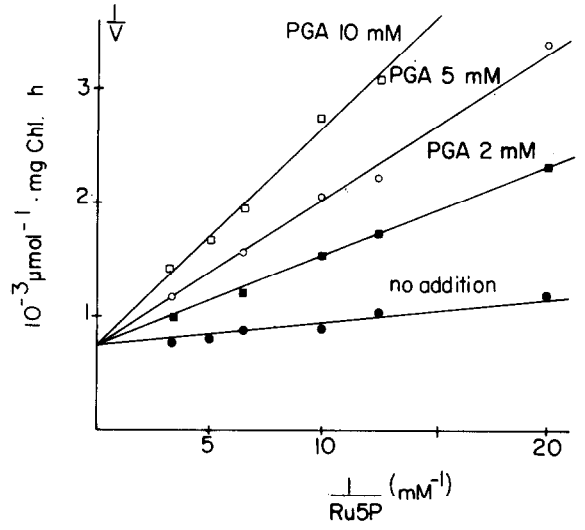

Fig.1. Competitive inhibition of Ru5P kinase by PGA with respect to Ru5P.

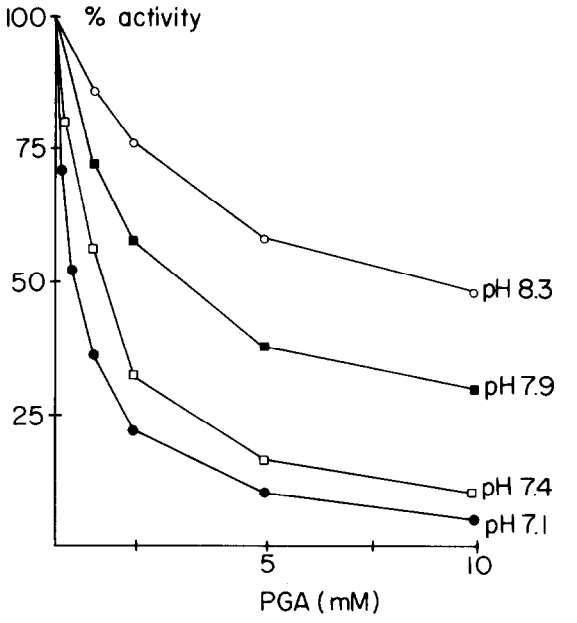

Fig.2. pH-dependence of the inhibition by PGA.

ADP and PGA was therefore investigated. As shown in table 1, the inhibition through ADP and PGA is always additive. Similar additivity was found for the form of the Ru5P kinase obtained from darkened chloroplasts (not shown).

3.2. *pH-Dependence of the PGA inhibition*

During light–dark transition, the pH in the stroma changes from ~7.9 to ~7.1 [3]. The pH dependence of the inhibition by PGA was investigated. The inhibition of Ru5P kinase by PGA is strongly increased by lowering the pH (fig.2). Over this range, PGA occurs in 2 forms (PGA^{2-}, PGA^{3-}, pK 7.1). Only one of these forms may inhibit Ru5P kinase. Indeed, when the data in fig.2 are recalculated to give the corresponding [PGA^{2-}], over the whole pH range, a clear correlation between [PGA^{2-}] and the relative inhibition of the enzyme is found (fig.3). Obviously, PGA^{2-} is the inhibitory form. A K_i for PGA^{2-} of ~130 µM is calculated. The pH dependence of PGA inhibition could alternatively have been caused by an increased K_m for Ru5P in acid conditions. This alternative, however, can be dismissed since with decreased pH even a slight decrease of the K_m for Ru5P was found (not shown). Previously, Ru5P kinase has been found to show only a weak pH-dependence [2], in contrast to fructose- and sedoheptulose bisphosphatase which have a strong pH dependence [2,10]. However, because of the pH-dependence of the inhibition by PGA, Ru5P kinase will show a marked sensitivity to changes of pH between 7–8 in the presence of physiological PGA concentrations. For example, lowering the pH from 7.9–7.1 leads to only a 25% decrease in activity in the absence of PGA, but to a 75% and 84% inhibition with 2 and 5 mM PGA, respectively (calculated from fig.2).

Table 1
Additive inhibition by ADP and PGA

Assay conditions		Activity
3-PGA (mM)	ADP (mM)	µmol/mg chl . h
0	0	806
3	0	438
0	0.6	598
3	0.6	300 (322)

Chloroplasts were incubated 6–12 min in light and then injected into radioactive assay mix (pH 7.9) containing 0.2 mM ATP, 50 µM Ru5P, 20 mM $MgCl_2$ and other additions as specified below. The figures in parentheses represent the activity calculated from the separate inhibition by ADP and PGA assuming that the two inhibitors are strictly additive

3.3. *Regulation of Ru5P kinase by PGA*

In [5], the inhibition by PGA was weak and seemed

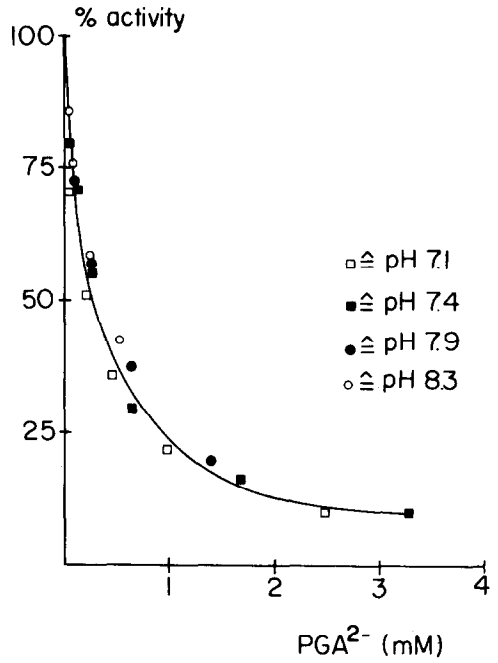

Fig.3. Inhibition of Ru5P kinase by PGA^{2-}. The values are calculated from the experiment of fig.3 using the formula $[PGA^{2-}]/[PGA^{2-}] + [PGA^{3-}] = [H^+]/[H^+] + 10^{-pK}$ (pK = 7.1). Data obtained at pH 7.1 (□), 7.4 (■), 7.9 (●) and 8.3 (○) are given.

of little physiological importance, but the assays had been carried out at alkaline pH, when the PGA was predominantly in the ineffective form (PGA^{3-}). Also, saturating Ru5P levels were used which were far above the physiological concentration [3], so that the competitive inhibition by PGA was masked. When physiological Ru5P levels are used, the concentrations of PGA needed for strong inhibition of Ru5P kinase are in the range of those concentrations measured in the stroma of isolated and in situ chloroplasts [11]. This effect of PGA could be of significance, both for the inactivation of Ru5P kinase during light–dark transients, and for the fine regulation of the Calvin cycle in the light.

During a light–dark transient with chloroplasts a fall of pH from 7.9–7.1, and a fall of the ATP/ADP ratio from 2–0.33 was observed [3]. In isolated chloroplasts the PGA level was found to remain constant at 3 mM [3] while in the stroma of intact protoplasts it rose from 3–6 mM (M. S., H. W. H., in preparation). In experiments where these conditions are simulated (table 2), a 78% inhibition of the Ru5P kinase is found when the PGA is held constant, and a 92% inhibition

Table 2
Inhibition of Ru5P kinase in a simulated light–dark transition

Assay conditions			Ru5P kinase act.
pH	3-PGA (mM)	ADP (mM)	(μmol/mg chl . h)
7.9	3	0.1	470
7.1	3	0.1	129
7.1	3	0.6	103
7.1	6	0.6	36

Chloroplasts were incubated for 6–12 min in light and then injected into radioactive assay mix containing 0.2 mM ATP, 50 μM Ru5P, 20 mM $MgCl_2$ and other additions and pH as shown below

when the PGA rises from 3–6 mM. Thus, independent of enzyme interconversion, a rapid inhibition of Ru5P kinase is achieved as soon as pH, ATP, ADP and PGA levels change. This pH-dependent inhibition by PGA, and the control through adenylates together with the reported enzyme interconversion allows an exceptionally effective regulation of Ru5P kinase.

During photosynthesis the additive inhibition of Ru5P kinase by increases in the ADP and PGA levels, provides an effective mechanism to balance the activity of Ru5P kinase and PGA kinase. If the Ru5P kinase is too active, the ATP/ADP ratio will fall and the PGA concentration rise, leading to a feedback inhibition of Ru5P kinase. The effectiveness of this inhibition will be increased since the accompanying fall in triose phosphate will ultimately result in lowered Ru5P levels [12].

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