# INHIBITION OF $CO_2$ FIXATION IN INTACT SPINACH CHLOROPLASTS BY 3-PHOSPHOGLYCERIC ACID[1]

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Received December 30,1974

SUMMARY: Glycerate-3-P inhibits $CO_2$ fixation of isolated spinach chloroplats at concentrations higher than 1 mM but does not inhibit $O_2$ evolution. Glycerate-3-P inhibition of photosynthesis is not overcome by higher bicarbonate concentrations.

## INTRODUCTION.

Intermediates of the photosynthetic carbon reduction cycle such as ribose-5-P, fructose-1,6-diP, glyceraldehyde-3-P, dihydroxyacetone-P and 3-phosophoglyceric acid (3-PGA) can stimulate photosynthesis and overcome the initial lag phase of photosynthesis when isolated chloroplasts are assimilating $CO_2$ in the light (1). It has been proposed that the stimulation of $CO_2$ fixation and also the overcoming of the lag phase for $CO_2$ fixation in isolated chloroplasts by the addition of intermediates to the reaction media were due to a filling of depleted substrate pools. (2). However, 3-PGA appears to have a unique two-phase effect on chloroplast activities because we have observed that 1 to 2 mM concentrations of 3-PGA can also inhibit $CO_2$ fixation.

In this paper we report on the effects of exogenously added 3-PGA on the rates of $CO_2$ fixation and $O_2$ evolution of isolated chloroplast suspensions fixing $CO_2$ in the light in order to elucidate the site of 3-PGA regulation.

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[1]Supported by National Institutes of Health Special Fellowship GM 54213-01 and in part by National Institutes of Health Grant GM 17868-03 and a grant from the National Science Foundation.

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## MATERIALS AND METHODS.

Chloroplasts were isolated from spinach leaves (Spinacia oleracea L.) by the following procedure: 10 g of diced leaves from the growth chamber were homogenized for 2 sec in 40 ml of a solution of pH 6.8, containing 0.33 M sorbitol, 50 mM Hepes-KOH buffer, 2 mM EDTA, 1 mM $Na_4P_2O_7$, 1 mM $MgCl_2$ and 1 mM $MnCl_2$. The homogenate was filtered through Miracloth, centrifuged at 755 x g for 90 sec., and the pellet of chloroplasts was suspended in 2 ml of the above solution containing 5 mM ascorbate at pH 7.8. The chlorophyll concentration was usually between 60-100 ug chlorophyll $ml^{-1}$.

## RESULTS.

The data in Table 1 compare the rates of $CO_2$ fixation in isolated chloroplasts treated with 0 and 2 mM 3-PGA. The reactions were in photosynthetic reaction systems containing 1, 5, and 10 mM bicarbonate. Strong inhibition of $CO_2$ fixation was always observed with 2 mM 3-PGA at all bicarbonate concentrations.

Data for the effects of 3-PGA on $O_2$ evolution from isolated chloroplasts fixing $CO_2$ in the reaction mixtures are also listed in Table 1. The treatments consisted of 0 and 2 mM 3-PGA at 1, 5, 10 mM bicarbonate levels. Clearly, 3-PGA did not inhibit $O_2$ evolution in chloroplasts fixing $CO_2$.

## DISCUSSION.

The experimental evidences presented in Table 1 show that 3-PGA inhibited $CO_2$ fixation but not $O_2$ evolution when intact chloroplasts were assimilating $CO_2$. We assume therefore that the site for 3-PGA stimulated inhibition of $CO_2$ fixation was not associated with the photosystem, the electron transport system nor with the reductive phase of carbon metabolism. If the reductive step of the Calvin cycle or any of the pathways involved in the transfer of

Table 1

THE EFFECTS OF 3-PHOSPHOGLYCERATE ON $CO_2$ FIXATION AND $O_2$ EVOLUTION OF INTACT SPINACH CHLOROPLASTS*

| Concentration of 3-PGA | Concentration of $HCO_3^-$ in Each Reaction | | |
|---|---|---|---|
| | 1 mM | 5 mM | 10 mM |
| | μmoles $CO_2$ fixed/mg chlorophyll$^{-1}$ hour$^{-1}$ | | |
| Control | 14.89 | 22.67 | 23.49 |
| 2 mM | 5.89 | 7.76 | 9.35 |
| | μmoles of $O_2$ evolved/mg chlorophyll$^{-1}$ hour$^{-1}$ | | |
| Control | 9.85 | 16.10 | 22.17 |
| 2 mM | 14.09 | 17.11 | 26.01 |

*The rate of $CO_2$ fixation was determined by $^{14}CO_2$ (25 uc umole$^{-1}$) uptake in test tubes at 25° C in a water bath. Illumination was by four 150 w flood lamps at 120 V. Oxygen evolution was measured polarigraphically with a modified Clark-type electrode inserted into a thermostated, 1 ml, lucite chamber at 25° C. Light was provided by a 150 w flood lamp with a 5 cm water filter. Light intensities at the reaction vessels were comparable in the $CO_2$ and $O_2$ experiments. The reaction mixtures contained 0.33 M sorbitol, 50 mM Hepes KOH, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 1 mM $Na_4P_2O_7$, 5 mM ascorbate. In addition the mixtures contained 3-phosphoglycerate and $NaHCO_3$ in the concentrations listed above.

reducing power from light to the Calvin cycle were inhibited by the presence of 3-PGA this should be reflected as a reduction of the rate of $O_2$ evolution from intact chloroplasts in the process of photosynthesis. It appears therefore that the site of the 3-PGA-stimulated inhibition of $CO_2$ fixation is associated with processes which regenerate the phosphorylated carbohydrate acceptor for $CO_2$. We consider that in our preparations the carboxylation step is not involved as the site for the 3-PGA inhibition although Paulsen and Lane (4) showed that 3-PGA inhibited purified ribulose-1,5-diP carboxylase. They

observed that 3-PGA performed as a competitive inhibitor for bicarbonate. Our studies clearly show that the 3-PGA inhibition of $CO_2$ fixation of isolated plastids was not affected by higher amounts of bicarbonate.

On the other hand, Heldt (3) has demonstrated in spinach chloroplasts that 3-PGA can be exchanged for glyceraldehyde-3-P. This exchange would result in an inhibition of $CO_2$ fixation but not in $O_2$ evolution. We believe that this mechanism would explain our observations. Finally, it is possible that in the presence of excess 3-PGA, glycerate-1,3-diP accumulates which may inhibit a reaction of the photosynthetic carbon reduction cycle.

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