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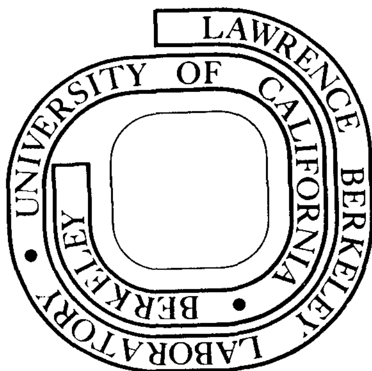

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CARBON METABOLISM OF CHLOROPLASTS IN THE DARK: OXIDATIVE PENTOSE PHOSPHATE CYCLE VERSUS GLYCOLYTIC PATHWAY

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ABSTRACT

The conversion of U-labelled ^{14}C-glucose-6-phosphate into other products by a soluble fraction of lysed chloroplasts has been studied. It was found that both an oxidative pentose phosphate cycle and a glycolytic reaction sequence occur in this fraction.

The formation of bisphosphates and of triose phosphates was ATP-dependent and occurred mainly via a glycolytic reaction sequence including a phosphofructokinase step.

The conversion of glucose-6-phosphate via the oxidative pentose phosphate cycle stopped with the formation of pentose monophosphates. This was found not to be due to a lack in transaldolase (or transketolase) activity, but rather to the high concentration ratios of hexose monophosphate/pentose monophosphate used in our experiments for simulating the conditions in whole chloroplasts in the dark.

Some regulatory properties of both the oxidative pentose phosphate cycle and of the glycolytic pathway were studied.

Key words: Carbon metabolism - chloroplasts - darkness.

ABBREVIATIONS

DHAP = dihydroxyacetone phosphate; GAP = 3-phosphoglyceraldehyde; PGA = 3-phosphoglycerate; HMP = hexose monophosphates, including F6P = fructose-6-phosphate G6P = glucose-6-phosphate GIP = glucose-1-phosphate; 6-PGL = 6-phosphogluconate; PMP = pentose monophosphates, including R5P = ribose-5-phosphate, Ru5P = ribulose-5-phosphate, X5P = xylulose-5-phosphate; E4P = erythrose-4-phosphate; S7P = sedoheptulose-7-phosphate; FBP = fructose-1,6-bisphosphate; SBP = sedoheptulose-1,7-bisphosphate; RuBP = ribulose-1,5-bisphosphate

Footnote (p. 11): [1] If Mg^{++} was completely chelated with EDTA, formation of bisphosphates and DHAP was strongly inhibited (data not shown).

INTRODUCTION

Chloroplastic starch can be degraded by a hydrolytic pathway into maltose and glucose or, via phosphorylase, into G1P (Heldt et al., 1977; Peavey et al., 1977; Steupp et al., 1976). Since fixed carbon can pass through the chloroplast envelope only in the form of triose phosphate or PGA (for a review see Heldt, 1976), the primary products of starch degradation have to be converted into these compounds in order to be exported into the cytoplasm. Theoretically, the conversion of G1P into triose phosphates could occur either via an oxidative pentose phosphate cycle or via a glycolytic pathway mediated by phosphofructokinase.

The occurance of some reactions of oxidative pentose phosphate cycle in chloroplasts is well established (Krause and Bassham, 1969; Lendzian and Ziegler, 1970; Lendzian and Bassham, 1975). The recent purification and characterization of a chloroplastic phosphofructokinase (E.C. 2.7.1.11) gives strong evidence for a glycolytic pathway in chloroplasts (Kelly and Latzko, 1977, a,b). It is still an open question to what extent each process really contributes to a conversion of G1P into triose phosphate and other compounds in the dark metabolism of chloroplasts.

Some recent data on starch degradation in chloroplasts have been obtained with intact spinach chloroplasts which were preloaded with ^{14}C-labelled starch (Heldt et al., 1977; Peavey et al., 1977; Steupp et al., 1976). There are some disadvantages in this method: the specific activity of labelled starch is not exactly known; due to the necessary preincubation and washing procedures, the chloroplasts have already been isolated for a long time when the actual measurement of dark metabolism begins; starch degradation has to be measured at Pi-levels which are far above any Pi-level optimal for

photosynthesis; finally, and most important for regulatory studies, significant features of the stroma composition, such as pH, ionic strength and metabolite levels can be manipulated only to a very limited extent due to the impermeability of the chloroplast envelope.

For the studies reported here we therefore used the soluble fraction of lysed chloroplasts, in which the catabolism of U-labelled ^{14}C-G6P was followed under conditions allowing a separation of the reactions of oxidative pentose phosphate cycle and of a glycolytic pathway.

MATERIALS AND METHODS

Chemicals and Enzymes. U-labelled ^{14}C-G6P was prepared from ^{14}C-glucose with lyophilized hexokinase and stoichiometric amounts of ATP, lyophilized transaldolase (E.C. 2.2.1.1) and transketolase (E.C. 2.2.1.1.) were obtained from Sigma, Stock # 6008 and T-6133, resp.

Plant Material. Spinach was grown in vermiculite fertilized with Hoaglands solution, under artificial light (3000 ft-c) with an 8 h light period and a 16 h dark period at a temperature of 13°C. Chloroplasts were isolated from young leaves according to Jensen and Bassham (1966).

Preparation of stroma enzymes. A suspension of freshly isolated intact chloroplasts containing about 2 mg chlorophyll was centrifuged for 1 min at 1000 g (4°C), and the pellet was resuspended in 1.2 ml of a lysing solution containing 0.025 M HEPES-NaOH pH 7.6, 2 mM $MgCl_2$ and 1 mM EDTA, if not mentioned otherwise. After 10 min at 0°C, the suspension was centrifuged for 15 min at 27,000 g (4°C). The resulting clear supernatant contained the soluble components from the chloroplasts and had an average protein content of about 5 mg protein in 1.2 ml. This solution was usually made CO_2-free by intermittently flushing with N_2 on a Vortex mixer for about 3 min (0°C). The pellet was

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resuspended in 1.2 ml and used for chlorophyll determinations following the procedure of Arnon (1949).

Incubation Conditions. 300 μl of solution of stroma enzymes were added to a solution containing 2 to 6 mM ^{14}C-G6P and different metabolites in a final volume of 500 μl. The solution was assayed under N_2 in serum stoppered flasks in a water bath at 21°C in the dark. At timed intervals, 50 μl samples were removed with syringes and injected into 200 μl of methanol to stop the reactions.

Analysis of Products. The conversion of ^{14}C-G6P into other compounds was followed by descending paper chromatography and radioautography (Jensen and Bassham 1966). 150 μl samples of the methanol extract were spotted on Whatmann #1 paper and the chromatograms were developed in two dimensions as described earlier (Pedersen et al., 1966).

Measurement of CO_2-Release. For measuring release of $^{14}CO_2$ the reaction flasks were slowly flushed with N_2 during the incubation procedure. The gas outlet of the reaction flask was connected to 25 ml scintillation vials containing 20 ml of a quaternary ammonium hydroxide solution (Protosol, New England Nuclear). The reaction was stopped by adding 400 μl methanol containing 0.1 M acetic acid to each flask. Gasing continued for 5 more min, in order to get a quantitative trapping of evolved CO_2. If the time course of CO_2 release was measured, one reaction flask was used for each time point. 0.5 ml of the trapping solution were counted by liquid scintillation.

RESULTS AND DISCUSSION

Oxidative Pentose Phosphate Cycle or Glycolytic Pathway? When ^{14}C-G6P (2 mM), $NADP^+$ (1 mM) and ATP (1 mM) are added to the soluble fraction of lysed chloroplasts in the dark, several labelled metabolites of the Calvin cycle are formed with rates of 1-3 μg atoms carbon/mg chlorophyll (fig. 1). At the same time the formation of 6-Pgluconate and the release of CO_2 at comparable

rates indicate flow of carbon via G6P-dehydrogenase (E.C. 1.1.1.49) and 6-Pgluconate dehydrogenase (E.C. 1.1.1.44) which catalyze the first steps of the oxidative pentose phosphate cycle (fig. 2). These rates are in the range of reported rates of starch degradation in isolated intact chloroplasts (Heldt et al., 1977; Peavey et al., 1977; Steupp et al, 1976). The question arises whether the bisphosphates and triose phosphates are formed via oxidative pentose phosphate cycle or whether a phosphofructokinase reaction is responsible for the formation of these products in the dark reactions of chloroplasts.

It is known that the oxidative pentose phosphate cycle occurs mainly at high $NADP^+$/NADPH ratios (Lendzian and Bassham 1975). In fact, by replacing $NADP^+$ (1 mM) with NADPH (1 mM), both the formation of 6-Pgluconate and the release of CO_2 are nearly completely inhibited (fig. 2). PMP formation is reduced to about 50%. The formation of DHAP and FBP + SBP is not inhibited at all. Under these conditions, therefore, the conversion of G6P into bisphosphates and triose phosphates occurs independently from operation of the oxidative pentose phosphate cycle. Also, at least part of the pentose monophosphate seems to be formed by reactions different from those of the oxidative pentose phosphate cycle.

Conversion of G6P into triose phosphates via a glycolytic pathway requires ATP. Thus, in the complete absence of ATP, the glycolytic pathway should be prevented, whereas the oxidative pentose phosphate cycle should still occur. As demonstrated in fig. 2, in the presence of an ATP-trapping system (glucose + hexokinase), the formation of bisphosphates and of DHAP is nearly completely inhibited, whereas the formation of 6-Pgluconate and release of CO_2 as well as pentose monophosphate formation are hardly influenced.

These experiments indicate clearly that under our conditions the formation of bisphosphates and of DHAP occurs in a strictly ATP-dependent reaction, presumably via conversion of F6P to FBP with phosphofructokinase. It also appears from fig. 2 that the reaction sequence of oxidative pentose phosphate cycle stops at the level of pentose monophosphates. This might be due to a lack of transaldolase or transketolase activity in our preparation. However, by addition of commercially available transaldolase (and also of transketolase, not shown here) the formation of DHAP and of bisphosphates was not influenced at all and remained very low in the absence of ATP (fig. 3).

On the other hand it was already shown in fig. 1 that a certain amount of pentose monophosphate is formed from G6P even if the oxidative pentose phosphate cycle is completely inhibited, indicating a flow of carbon from triose phosphates and F6P into pentose monophosphates, presumably via transketolase and transaldolase. This shows again that transketolase and transaldolase are not the limiting factors for the conversion of pentose monophosphates into triose phosphates.

For a consideration of the thermodynamics of this conversion, the participating reactions have been listed below, together with the standard free energy changes (data from Bassham and Krause, 1969).

(1)	X5P + E4P	F6P + GAP	$\Delta G^{\circ\prime} = -1.47$ Kcal
(2)	X5P + R5P	S7P + GAP	$\Delta G^{\circ\prime} = -0.10$ Kcal
(3)	S7P + GAP	F6P + E4P	$\Delta G^{\circ\prime} = +0.38$ Kcal
(4)	2 X5P + R5P	2 F6P + GAP	$\Delta G^{\circ\prime} = -1.19$ Kcal

From the equilibrium constants of the reactions catalyzed by triose phosphate isomerase, pentose phosphate isomerase and hexosephosphate isomerase (Bassham and Krause, 1969), the steady state concentrations of the compounds

participating in reactions (1) to (4) have been calculated from measured amounts of total hexose- and pentose monophosphates and of DHAP in whole Chlorella cells (Bassham and Krause, 1969), from whole chloroplasts in the light (Lilley et al, 1977) and whole chloroplasts in the dark (Kaiser and Bassham, in press). From these data, the physiological free energy change for the overall reaction (4) has been calculated to be +2.43 for Chlorella cells, +2.48 for whole chloroplasts in the light and +1.17 for whole chloroplasts in the dark. These positive values indicate that reaction (4) is proceeding in the direction of pentose monophosphate synthesis even in the dark. A significant flow of carbon from pentose monophosphates into triose phosphates via transketolase and transaldolase could be expected only if the pool size of triose phosphate (and also of hexosemonophosphate) were kept very low. This might occur *in vivo* under conditions of high cytoplasmic Pi concentrations, when triose phosphates are effectively exported. Such high external Pi concentrations have been shown to be essential for starch degradation in whole chloroplasts in the dark (Heldt et al., 1977; Peavey et al., 1977; Steupp et al., 1976), and the high level of hexosemonophosphates in whole chloroplasts in the dark was also strongly decreased under conditions of high external [Pi] (Kaiser and Bassham, in press). The possibility that under certain conditions *in vivo* the reactions of oxidative pentose phosphate cycle might also contribute to the conversion of G1P into triosephosphates cannot be excluded, in spite of the fact that under our experimental conditions, the flow of carbon from G6P into triose phosphate occurred mainly via a glycolytic pathway (including phosphofructokinase).

Regulatory Aspects of Oxidative Pentose Phosphate Cycle. It is evident that in order to avoid a waste of energy in futile cycles, the oxidative pentose phosphate cycle as well as the glycolytic pathway have to be precisely

regulated. It is well known (and is also obvious from fig. 1) that the activity of chloroplastic G6P-dehydrogenase depends on NADPH/$NADP^+$ ratios as they exist in whole chloroplasts (Lendzian and Bassham, 1975, 1976). Recently it was found that there are complex interactions between NADPH/ $NADP^+$-ratio, pH, Mg^{++} and substrate concentration which all influence the activity of G6P dehydrogenase (Lendzian, 1978). Furthermore, it has been shown that G6P-dehydrogenase is inhibited by dithiol compounds (Anderson et al., 1974, compare fig. 7). Recently we observed that during light-dark-light transitions, the levels of PGA and of the hexose monophosphates undergo dramatic changes, which might be also important for modulating the activity of certain enzymes (Kaiser and Bassham, in press). Therefore, we studied the effect of different concentrations of G6P and PGA (as they were found in whole chloroplasts in light and dark) on the reactions of the oxidative pentose phosphate cycle (i.e. in the complete absence of an ATP-dependent phosphofructokinase reaction). The release of CO_2 and the formation of pentose monophosphates increase linearly with increasing concentrations of G6P, reaching V_{max} at about 6 mM G6P (fig. 4). This is the concentration of hexose monophosphate found in intact chloroplasts in the dark (Kaiser and Bassham, in press). The net rate of 6-Pgluconate accumulation increases only at the higher hexose monophosphate concentration, when the pentose monophosphate concentration is already maximal (fig. 4). The total reaction rate of the oxidative pentose phosphate cycle is more than doubled by a change in total hexosemonophosphate from 2 mM to 6 mM (fig. 4).

Fig. 4

If the hexose monophosphate concentration is kept constant at 4 mM and the concentration of PGA is varied in the range which exists in whole chloroplasts during light-dark transition (Kaiser and Bassham, in press), the formation of pentose monophosphates and the release of CO_2 are both

inhibited by increasing PGA concentrations (fig. 5). At the same time, the pool size of 6-Pgluconate is increased at higher PGA. Therefore, we assume that PGA inhibits the 6-Pgluconate dehydrogenase, leading to an accumulation of 6-Pgluconate. At this time, the physiological significance of this observation remains unclear, but it is noteworthy that 6-Pgluconate can activate or inhibit the RuBP-carboxylase, depending on conditions (Chu and Bassham, 1972).

Fig 5
6

Regulatory Aspects of the Glycolytic Pathway. The regulatory properties of an isolated and purified chloroplast phosphofructokinase have recently been investigated (Kelly and Latzko, 1977a,b). It was found that the enzyme was inhibited by phosphoglycolate, PGA, or non-Mg^{++}-bound ATP. However, the physiological significance of some of these findings might be questioned, as the level of phosphoglycolate in whole chloroplasts is usually extremely low, and the existence of non-Mg^{++}-bound ATP in whole chloroplasts has yet to be proved. Therefore, an investigation of some regulatory properties of the glycolytic pathway in the soluble fraction of lysed chloroplasts seemed warranted.

The overall rate of the formation of DHAP and bisphosphates depends on the concentration of the added G6P in a way similar to that of the reactions of the oxidative pentose phosphate cycle, increasing linearly up to a concentration of about 6 mM (fig. 6). A change in the hexose monophosphate concentration (here of G6P) similar to that occurring in intact chloroplasts during light-dark-light transition (Kaiser and Bassham, in press), increases the rate of DHAP formation by about 150% (fig. 6).

Fig. 6

We also studied the effects of varying PGA-concentrations and of phosphoglycolate on the above reactions. A change in [PGA] from 1.4 mM to 4 mM (as in whole chloroplasts during dark-light transition, Kaiser and Bassham, in press) usually caused only a slight inhibition of the formation of bisphosphates

and of DHAP (not shown). Phosphoglycolate at a concentration of 1 mM remained without any effect (also not shown here). These findings are somewhat contradictory to the results of Kelly and Latzko (1977, a,b), but it seems possible that in a complex enzyme system such as the stroma extract used in our experiments, certain side reactions (like a hydrolysis of phosphoglycolate) might alter the effect of the added metabolites. On the other hand it might as well be that in a stroma extract (where all components of the stroma are still present and only diluted, enzymes are kept in a more "natural" state and therefore react differently from a purified enzyme preparation.

Changing the Mg^{++} concentration in the reaction medium from 0.2 mM to 5 mM at constant ATP had no significant effect on the formation of bisphosphates and of DHAP[1)] (Table I). However, an increase in ATP from 0.2 mM to 2 mM nearly doubled the rate of bisphosphate and DHAP formation partly at the expense of PGA formation which decreased to about 50% (Table I). It is important to realize that in the experiment shown in Table I, ATP never inhibited the conversion of G6P into bisphosphates and DHAP, even if the concentration of added ATP exceeded the concentration of Mg^{++} 10-fold. Assuming a very high activity of Mg^{++} in intact chloroplasts of about 50 mM, and a chloroplast volume of 25 μl/mg chlorophyll in a reaction volume of 1 ml (as in the experiment shown in Table I), would give an endogenous Mg^{++} concentration of only 0.75 mM. If 2 mM ATP and 0.2 mM Mg^{++} are added (Table I), the concentration of non-Mg^{++} bound ATP should still be about 1 mM, a concentration which was found inhibitory for the purified phosphofructokinase (Kelly and Latzko, 1977, a,b). Nevertheless we found no inhibition of phosphofructokinase-dependent DHAP and bisphosphate formation.

Besides the Mg^{++} concentration, a change in stroma pH and the formation of reduced sulfhydryl groups have been found to be most important modulators

1) see footnote

of chloroplast enzymes (for a review see Kelly and Latzko, 1976). A change in pH from 7.3 to 8.0 has no significant effect on the conversion of G6P into the different products of both the oxidative pentose phosphate cycle and of the glycolytic pathway (fig. 7).

In contrast, addition of DTT (10mM) produced a dramatic effect. The formation of 6-Pgluconate, of bisphosphates, DHAP and pentose monophosphates are strongly inhibited, whereas the formation of RuBP (and in some experiments also of PGA) are strongly stimulated (fig. 7). This is indicative of the complete change in the direction of carbon flow which occurs from dark metabolism to light-metabolism. It is presumably caused by the well known stimulation of the bisphosphatases, of phosphoribulokinase and perhaps also of GAP-dehydrogenase by reduced dithiols (for a review see Kelly and Latzko, 1976). It cannot be decided from our experiment (fig. 7) whether there is in addition a direct inhibition of phosphofructokinase by DTT. However, no evidence for such a direct inhibition was found by other authors (Kelly and Latzko, 1977, a,b). Therefore, it might be that in the presence of DTT, the activity of phosphofructokinase is overshadowed by the much higher activity of fructose bisphosphatase. Under these conditions, a futile cycle would occur.

CONCLUSIONS

Our experiments demonstrate the occurence of both an oxidative pentose phosphate cycle and a glycolytic pathway in the soluble fraction of lysed chloroplasts (fig. 8). This is consistent with similar results obtained by a comparison of the conversion of C_1-labelled and C_6-labelled G6P into other products in a reconstituted chloroplast system (Schaedle and Bassham, in preparation). However, when the steady state concentrations of hexose monophosphates (6 mM) and pentose monophosphates (0.4 mM or less) in whole

chloroplasts in the dark (Kaiser and Bassham, in press) are simulated, the flow of carbon from pentose monophosphates into triose phosphates via transaldolase and transketolase is extremely low. A consideration of the thermodynamics of the participating reactions shows that the flow of carbon is in fact slightly favored in the direction of pentose monophosphate synthesis. Since the oxidative pentose phosphate cycle stops therefore at the level of pentose monophosphate, its main physiological function might be to provide the chloroplast with NADPH and pentose monophosphate in the dark. Some of the formed pentose monophosphates might diffuse into the cytoplasm, and part of the NADPH could be exported via a malate/oxaloacetate shuttle (for a review, see Heldt, 1976), thereby providing e.g. NADPH for fatty acid synthesis, dependent on acetyl CoA formed outside the chloroplast.

It has been further demonstrated that the oxidative pentose phosphate cycle is very well regulated by $NADP^+$/NADPH ratios (fig. 1, cf Lendzian and Bassham, 1975, 1976; Lendzian, 1978), by reducing sulfhydryl groups (fig. 7, cf Anderson et al., 1974), and by changes in the substrate level (fig. 4), compare Lendzian (1978).

The conversion of G6P into triose phosphates, which is a necessary prerequisite for an effective export of carbon into the cytoplasm, has been shown to be strictly ATP-dependent under our conditions. This conversion thus occurs almost exclusively via a glycolytic pathway (fig. 8). We found only two effective modulations of this process: a change in substrate concentration and the presence or absence of sulfhydryl groups. In addition, a less effective modulation might be caused by changes in [PGA].

The existence of a futile cycle in the light cannot be ruled out. The hexose monophosphate concentration which exists in whole chloroplasts in the

light (about 2 mM) could permit a rate for the phosphofructokinase mediated reaction of about 0.285 µmoles FBP formed (and ATP consumed)/mg chlorophyll x h (data from fig. 6). Comparing this rate of ATP consumption with the ATP formation during photosynthesis at saturating light conditions (about 100 µmoles CO_2 fixed/mg chlorophyll x h, equivalent to 300 µmoles ATP formed/mg chlorophyll x h), a futile cycle would consume only about 0.095% of the energy produced by the light reactions. Even at maximal activity, phosphofructokinase (2.5 µmoles FBP formed/mg chlorophyll x h, see Kelly and Latzko, 1977, a,b) would still consume less than 1% of the total ATP formed in the light. The retention of this much phosphofructokinase activity in the light would thus be too low to cause a significant waste of energy at least under saturating light conditions.

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Table I; Effect of Mg^{++} and ATP at different concentration ratios on the formation of 3-phosphoglycerate (PGA), dihydroxyacetone phosphate (DHAP), sedoheptulose-1,7-bisphosphate (SBP) + fructose-1,6-bisphosphate (FBP) and pentose monophosphates (PMP) from U-labelled ^{14}C-G6P in the absence of oxidative pentose phosphate cycle (i.e. with 1 mM NADPH). Rates were calculated from a 12 min time course. In this experiment the lysing solution contained only 0.2 mM Mg^{++}, and no EDTA. For further details see Materials and Methods.

Conditions	µg-atoms carbon/mg chlorophyll x h			
	PGA	DHAP	SBP + FBP	PMP
$MgCl_2$ 0.2 mM				
ATP 0.2 mM	0.375	0.600	0.785	0.465
ATP 2.0 mM	0.185	1.130	1.395	0.520
$MgCl_2$ 5.0 mM				
ATP 0.2 mM	0.340	0.640	0.735	0.440
ATP 2.0 mM	0.250	0.885	1.145	0.145

LEGENDS

Figure 1: Conversion of U-^{14}C-G-6-P into the different metabolites (indicated in the fig.) by the soluble fraction of lysed chloroplasts in the dark. The reaction was started by adding 20 µl of U-^{14}C-G-6-P (50 µCi) to the reaction mixture to make a final concentration of 2 mM. The reaction mixture was as described under Materials and Methods, but contained in addition: ATP (1 mM), and $NADP^+$ (1 mM) or NADPH (1 mM) as indicated in the Fig. All reactions were carried out under N_2. $^{14}CO_2$ release was measured with the same flask from which the samples for chromatography were taken. However, only one time point was taken for the CO_2-release in this experiment, since the samples had to be acidified in order to get quantitative release of CO_2. For further details see Materials and Methods.

Figure 2: Conversion of U-^{14}C-G-6-P into other metabolites by the soluble fraction of lysed chloroplasts in the dark, in the presence of ATP (1 mM), or in the absence of ATP (i.e. with an ATP-trapping system of glucose 4 mM, and hexokinase 3.6 U). In addition, all flasks contained $NADP^+$ (1 mM). Other conditions as in Fig. 1 or as described under Materials and Methods.

Figure 3: Effect of transaldolase (2 U/flask) on the conversion of U-^{14}C-G-6-P (2 mM) in the absence of ATP (i.e. with glucose and hexokinase), and with $NADP^+$ (2 mM). Other conditions as in Fig. 1 or as described in Materials and Methods.

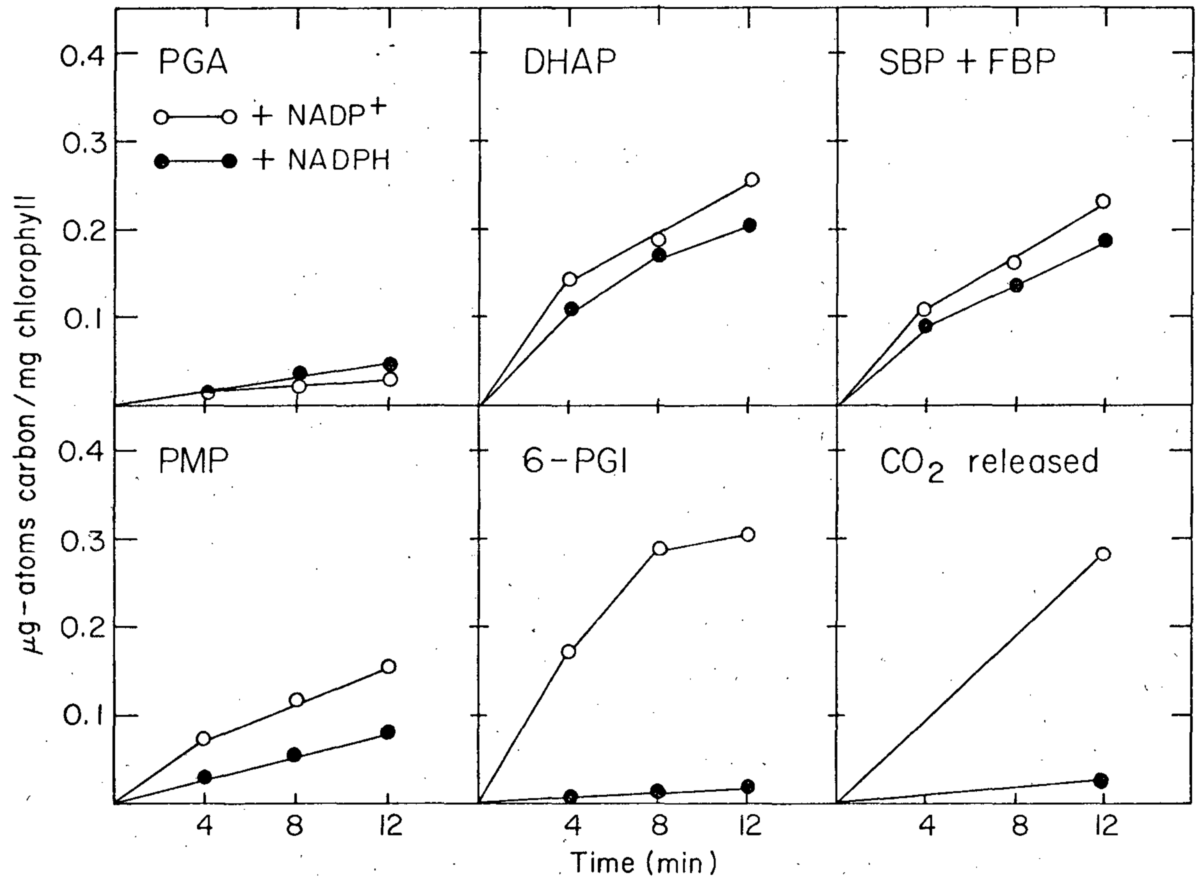
Figure 4: Formation of products of the oxidative pentose phosphate cycle from U-^{14}C-G-6-P in the absence of ATP (i.e. with glucose and hexokinase), at different substrate concentrations. The reaction medium contained in addition $NADP^+$ 1 mM, glucose 4 mM and hexokinase 3.6 U). After 10 min, the reaction was stopped by adding a methanol/acetic acid mixture and flushed with N_2 for 5 min more. 100 µl samples of the acidified mixture were used for paper chromatography. For further details see Materials and Methods.

Figure 5: Time course of the formation of 6-Pgluconate, PMP and $^{14}CO_2$-release from U-^{14}C-G-6-P by the soluble fraction of lysed chloroplasts, at different concentrations of PGA. The reaction medium contained ^{14}C-G-6-P (2 mM), $NADP^+$ (1 mM), glucose and hexokinase as in Fig. 4, and PGA as indicated. Other conditions as in Fig. 4.

Figure 6: Conversion of U-^{14}C-G-6-P into products of the glycolytic pathway by the soluble fraction of lysed chloroplasts, at different substrate concentrations. The reaction mixture contained in addition $NADP^+$ 1 mM, ATP 1 mM and U-^{14}C-G-6-P as indicated in the Fig. For further details see Materials and Methods.

Figure 7: Time course of the conversion of G-6-P (2 mM) via glycolytic pathway and oxidative pentose phosphate cycle at pH 7.3 and pH 8.0, respectively in the presence and absence of DTT (10 mM). reaction mixture contained in addition $NADP^+$ 1 mM, ATP 1 mM, and DTT as indicated. Chloroplasts were lysed at pH 7.3 or pH 8.0 (for details see Materials and Methods). Other conditions as in Fig. 1.

Figure 8: Two possible pathways for the conversion of G6P (e.g. from phosphorolytic starch degradation) into triose phosphate in chloroplasts in the dark: oxidative pentose phosphate cycle (left) and glycolytic pathway (right).



XBL 786-3986

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Fig. 1

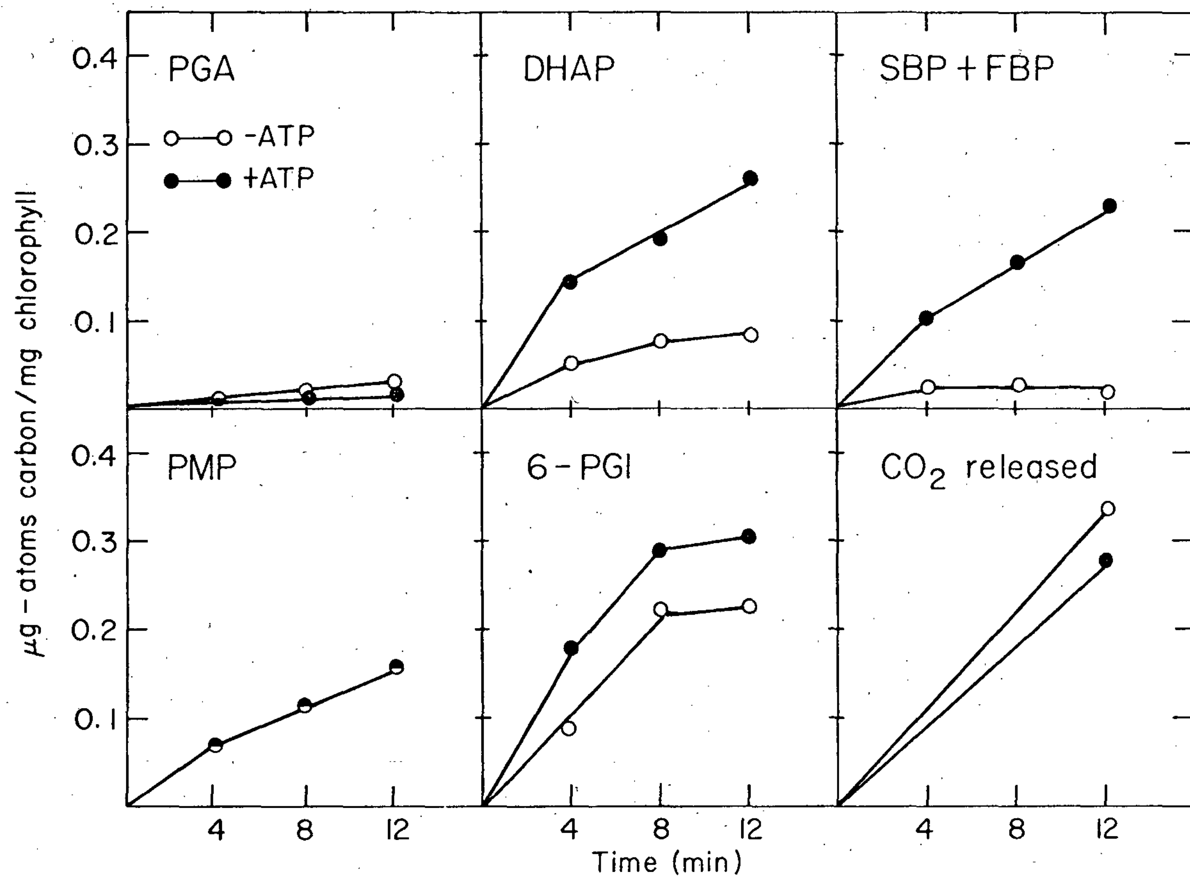

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Fig. 2

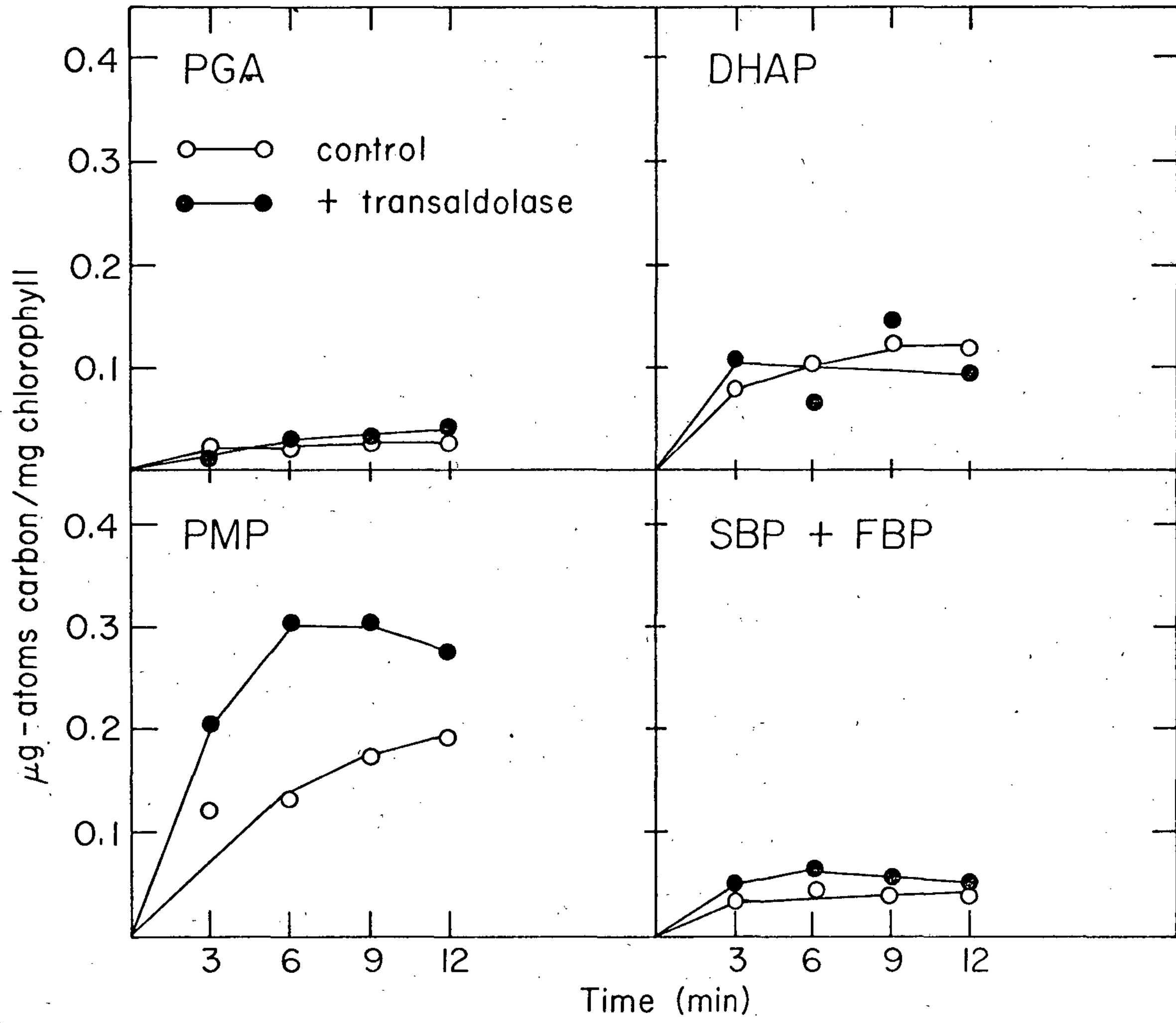

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Fig. 3

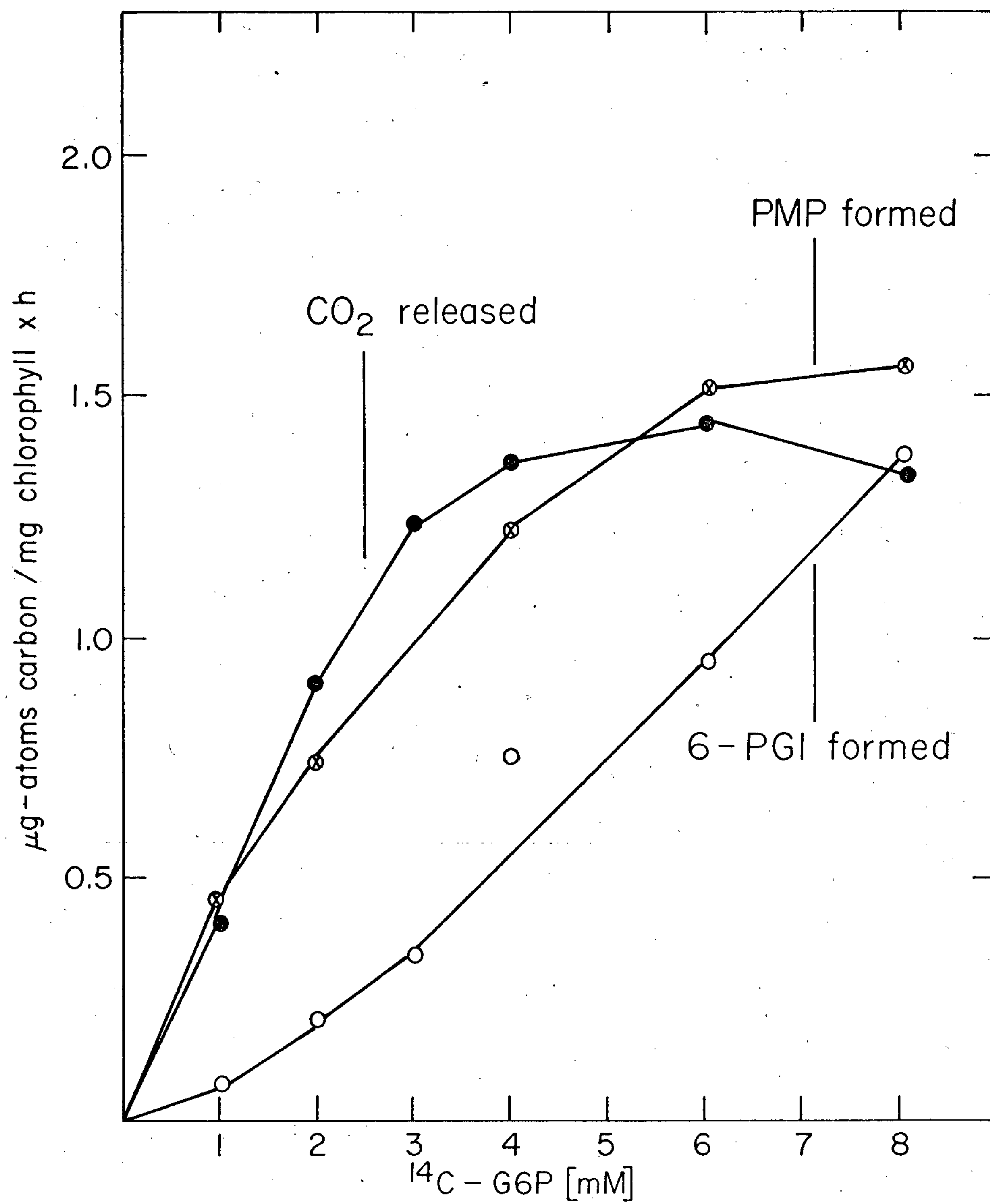

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Fig. 4

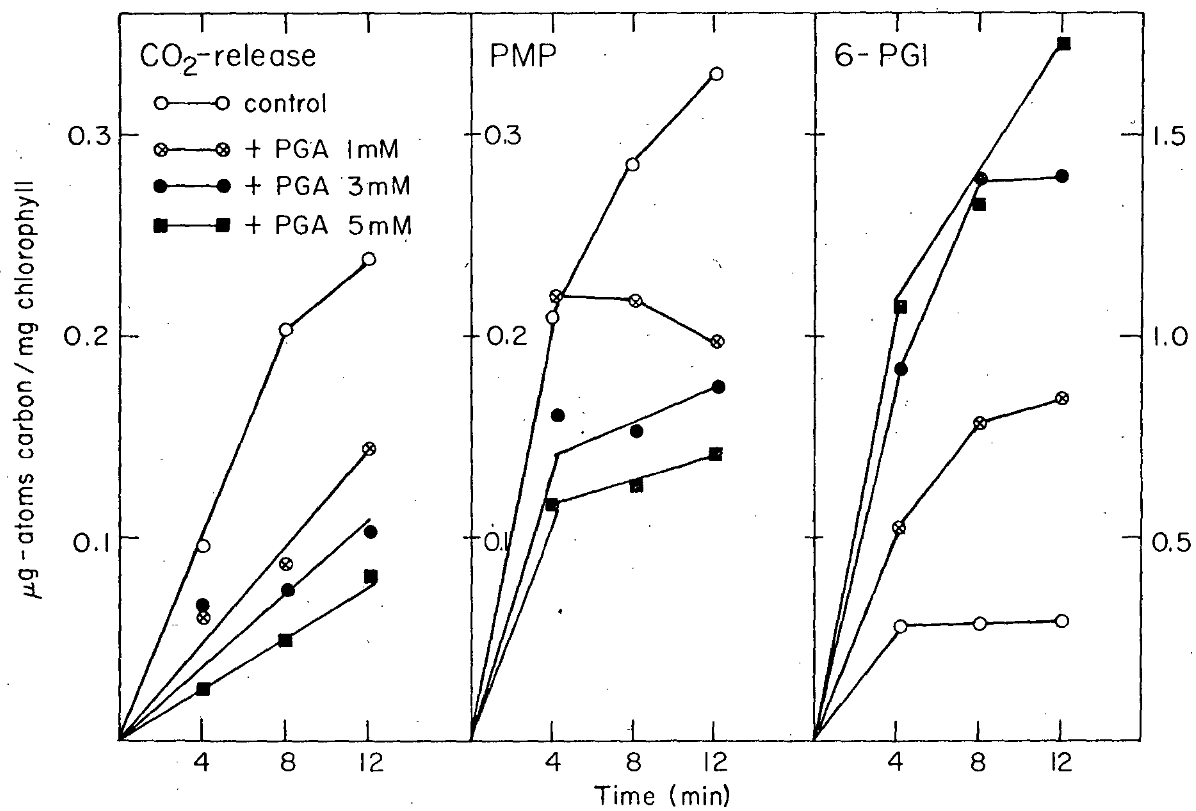

Fig. 5

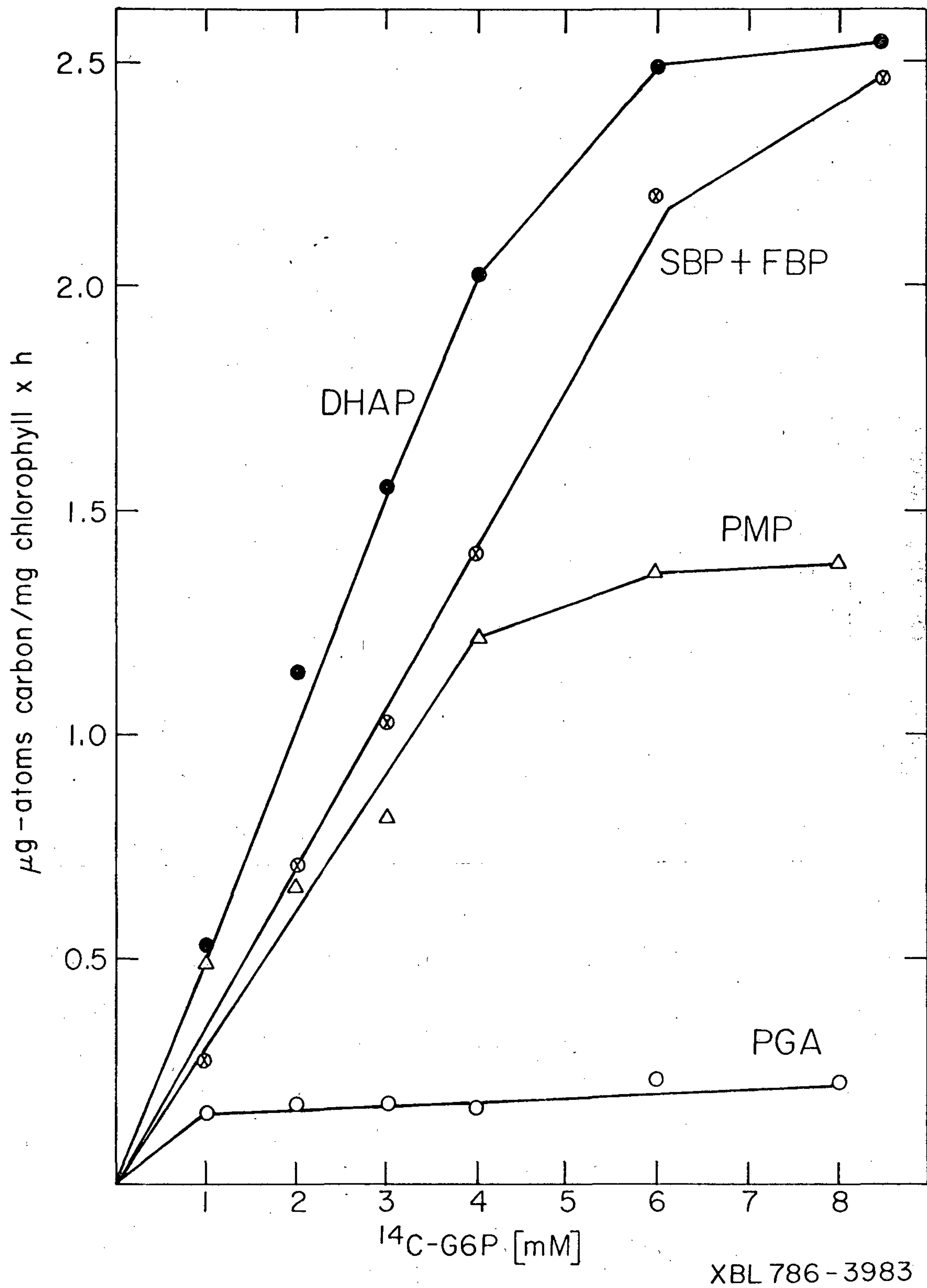

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Fig. 6

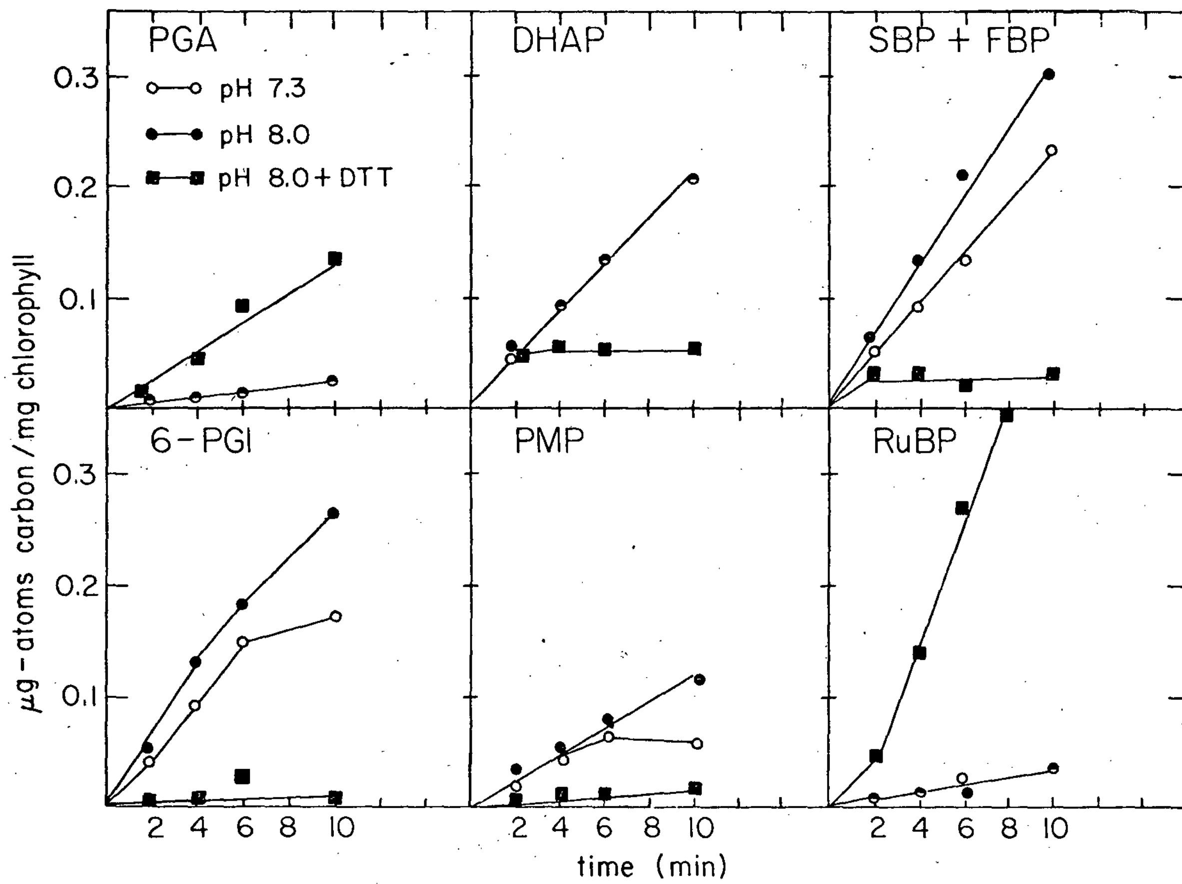

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Fig. 7

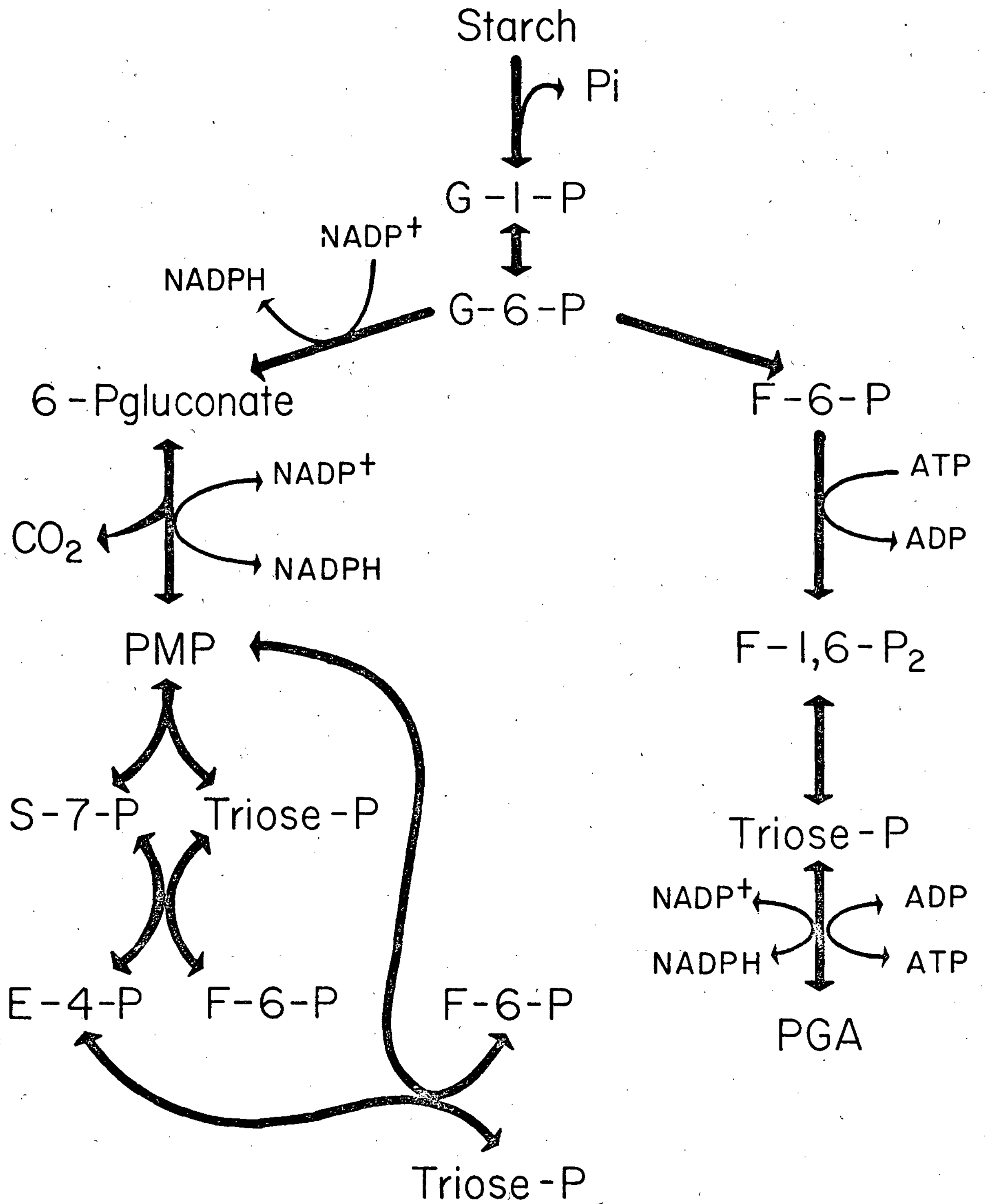

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Fig. 8

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