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PHOTOSYNTHESIS OF ISOLATED CHLOROPLASTS. V. EFFECT ON FIXATION RATE AND METABOLITE TRANSPORT FROM THE CHLOROPLASTS CAUSED BY ADDED FRUCTOSE-1,6-DIPHOSPHATASE

J. A. Bassham, A. M. El-Badry, M. R. Kirk, H.C.J. Ottenheym*
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Summary

Addition of fructose-1,6-diphosphatase (FDPase) plus Mg^{++} ions in increasing concentrations causes stimulation and then inhibition of photosynthesis in isolated spinach chloroplasts. The effects are similar to those previously found with leaf extract additions, including modifications of the effects caused by added inorganic pyrophosphate (PP_i). Under the conditions of pH, Mg^{++} ion concentration, and PP_i concentration in which these effects are seen, the added enzyme has only negligible activity as FDPase. Thus the enzyme appears to be exerting its effect through some function other than as an FDPase. It is tentatively suggested that this other function is in the transport of metabolites, especially fructose-1,6-diphosphate, out through the chloroplast outer membrane. This hypothesis is supported by the following observations:

1. Addition of FDPase plus Mg^{++} to chloroplasts photosynthesizing with $^{14}CO_2$ causes an increase in the total labeled fructose-1,6-diphosphate and dihydroxyacetone phosphate (which appear mostly in the suspending medium), and a <u>decrease</u> in the total labeled fructose-

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6-phosphate and of all compounds following fructose-1,6-diphosphate in the carbon reduction cycle, including the carboxylation substrate, ribulose-1,5-diphosphate. Furthermore, the amounts of all metabolites retained in the chloroplasts decrease.

2. Addition of Mg^{++} ions alone, in concentrations slightly higher than PP_i concentration, causes some loss of all compounds from the chloroplasts to the medium.

3. Addition of 5 mM PP_i decreases the loss of hexose and heptose monophosphates and of ribulose-1,5-diphosphate, but does not decrease the loss of FDP and of dihydroxyacetone phosphate from the chloroplasts to the medium.

Introduction

The rate of photosynthetic fixation of carbon dioxide is strongly affected by additions of small amounts of clarified spinach juice[1]. The degree of inhibition or stimulation is a function of the ratio of amount of added spinach juice to the concentration of added inorganic pyrophosphate (PP_i). Fractionation of the juice by gel filtration gave two components which act synergistically to produce the stimulation or inhibition. The component of high molecular weight was identified as fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, E.C.3.1.3.11)[2], hereafter referred to as FDPase. The component of low molecular weight, called "Factor B" in the previous reports[1,2], has now been found to

be an impure mixture of inorganic salts, of which the active constituent is Mg^{++}. The synergistic stimulation and inhibition, and their dependence on PP_i, has been duplicated by additions to photosynthesizing chloroplasts of selected ratios of FDPase and Mg^{++}.

Addition of fructose-1,6-diphosphate (Fru-1,6-P_2) to relatively inactive isolated spinach chloroplasts stimulates their fixation rate[3], while its addition to more active chloroplasts maintains a higher rate after 15 minutes of photosynthesis, when the rate begins to decline[4]. Other tested metabolites were generally inhibitory or without effect[4]. Thus the level of Fru-1,6-P_2 in chloroplasts might play some role in the regulation of the carboxylation of ribulose-1,5-diphosphate to give 3-phosphoglyceric acid.

The carboxylation reaction and the conversion of Fru-1,6-P_2 to Fru-6-P, as well as the conversion of sedoheptulose-1,6-diphosphate, appear to be metabolically-regulated steps in the photosynthetic carbon reduction cycle[5-9]. Both the carboxylase and the diphosphatases, when isolated and purified, exhibit a dependence of activity on pH and Mg^{++} concentration[10,11] which could account at least in part for their regulation <u>in vivo</u>[12]. Among the labelled metabolites formed in isolated spinach chloroplasts photosynthesizing with ^{14}C-labelled bicarbonate, there are pronounced differences in the rates of movement out of the chloroplasts into the suspending medium[13].

Those compounds which just precede the reactions mediated by the diphosphatases (Fru-1,6-P_2, dihydroxyacetone phosphate and glyceraldehyde phosphate) are found to a much greater extent outside the chloroplasts after a few minutes photosynthesis, whereas the hexose and heptose monophosphates, including Fru-6-P, and ribulose-1,5-diphosphate (Ribul-1,5-P_2), are very well retained. If Fru-1,6-P_2 moves out of the chloroplasts by means of facilitated transport, a part of the apparent regulation of FDPase *in vivo* could be in fact a regulation of this transport, provided FDPase in the cytoplasm is less active than that inside the chloroplasts.

We have now investigated the effects of FDPase, Mg^{++} and PP_i, added together and separately, on the levels of labelled metabolites formed in photosynthesizing chloroplasts, and the effects of these additions on the movement of metabolites out of the chloroplasts. Most of the effects reported were under conditions of pH, Mg^{++} concentration and PP_i level under which the added FDPase could have little or no activity for the conversion of Fru-1,6-P_2 to Fru-6-P. The effects in the medium of the added FDPase seem therefore to be indicative of another activity of this protein.

Experimental

Chloroplasts were prepared from fresh spinach as described previously[14]. Kinetic studies of the formation of labelled compounds and their movement from the chloroplasts to the medium during photosynthesis by the isolated chloroplasts with ^{14}C-labelled bicarbonate were as described earlier[13].

In some experiments, an attempt was made to remove enzymes external to the chloroplasts by "washing." The chloroplast pellet from the initial centrifugation[14] was resuspended in 10 ml Solution B or Solution C and again centrifuged at 2000 x g for about 50 sec, after which the chloroplasts were stored for less than 10 min at 0°C until the beginning of the illumination at 20°.

In those experiments in which the distribution of metabolites between chloroplasts and medium was to be determined, a 100 µl sample of the suspension of photosynthesizing chloroplasts was removed at the indicated times, and this sample was subjected to 10 sec centrifugation with a Spinco microfuge. The pellet and the supernatant solution were separated and each was killed in 80% methanol. The elapsed time from sampling to killing was about 25 sec. Some samples were killed immediately, without separation of chloroplast from medium, to permit subsequent evaluation of metabolite conversion during centrifugation.

The supernatant solution could not be completely separated from the pellet, and a contamination of the pellet by 10% of

the supernatant solution is estimated. In those cases where a very large portion of a given compound is found in the supernatant solution, a correction has been made in the results. This correction, equal to 0.10 of the ^{14}C found in the compound in the supernatant solution, is subtracted from the value found for the pellet and added to the value found for the supernatant solution.

For determination of the rates of photosynthetic incorporation of $^{14}CO_2$, aliquot samples of the killed chloroplasts-methanol-water mixtures were dried on filter paper, free CO_2 was driven off by addition of acetic acid, and the ^{14}C content was measured with a Geiger-Müller tube and scaler. Chlorophyll content was determined in a sample of the chloroplast suspension, and rate of photosynthesis was calculated from known specific radioactivity of bicarbonate used, ^{14}C content and chlorophyll content[14]. Products of photosynthesis were analyzed by two-dimensional paper chromatography and autoradiography, as previously described[5,6].

A solution of purified fructose diphosphatase was prepared from isolated spinach chloroplasts as reported earlier[2]. The eluate from the gel filtration through Sephadex G-200 was dialyzed against "Solution C"[14] minus PP_i and ascorbate, and the dialyzed enzyme solution was diluted 25 times in Solution C minus ascorbate and PP_i. Amounts of this diluted solution, ranging up to 30μg of protein, were added to the chloroplasts in the illumination flasks, and the suspensions were made up to 0.5 ml for rate experiments or 1.0 ml for kine-

tic experiments, as indicated in the Results. Other additions of Mg^{++}, PP_i, etc. are given under Results.

Results

The rate of photosynthesis by isolated spinach chloroplasts is stimulated 25% by the addition of small amounts of magnesium ion and purified FDPase and is strongly inhibited by the addition of greater amounts of Mg^{++} and FDPase (Fig. 1). The shape of this stimulation-inhibition curve and its dependence on the presence of 5 mM PP_i are exactly analogous to the effects earlier observed upon the addition of various amounts of spinach juice to the isolated photosynthesizing chloroplasts[1].

The inhibition of photosynthetic rate by Mg^{++} and FDPase is reversed by higher levels of PP_i (Fig. 2). As was the case with spinach juice[1], or with FDPase plus "Factor B"[2], the concentration of added PP_i required to reverse inhibition is proportional to the amount of inhibiting substances added. Thus, it is clear that the effects formerly observed upon adding small amounts of spinach juice to the isolated chloroplasts, including dependence on PP_i concentration, can be reproduced by the addition of purified FDPase and Mg^{++}.

Analysis of purified "Factor B"[2], by atomic absorption spectroscopy showed that it contained approximately 10% Mg^{++}. In the presence of the usual suspending medium ($-Mg^{++}$) which contained 5 mM PP_i, addition of 21 μg of purified FDPase caused 25% stimulation in the rate of photosynthesis (Table I), whereas addition of

enough Mg^{++} to make its concentration 2 mM, caused a 10% stimulation in the absence of added FDPase but an 85% inhibition in the presence of the FDPase. Since the total volume was 0.5 ml, the added Mg^{++} was 1 mM above the usual reaction mixture concentration and corresponded to 0.5 μmole, or 12 μg, of Mg^{++}. This may be compared with the synergistic effect found previously[2] upon the addition of 24 μg of fructose diphosphatase and 150 μg of "Factor B", corresponding to 15 μg of Mg^{++}.

At 1 mM PP_i (Table I) the inhibition by fructose diphosphatase plus Mg^{++} is even more striking. It should be particularly noted that whereas the stimulation with fructose diphosphatase alone was 90%, and the stimulation by 1.5 mM Mg^{++} was 12%, the addition of both factors caused an inhibition of 96%.

When FDPase and Mg^{++} were added to photosynthesizing chloroplasts after 6 min of photosynthesis with ^{14}C-labelled bicarbonate, rather surprising effects on the levels of labelled metabolites occurred (Figs. 3 and 4). As compared with the levels of metabolites in the control chloroplasts, the level of Fru-1,6-P_2 in chloroplasts with added FDPase increased, while the level of Fru-6-P decreased. This behaviour has proved to be reproducible, as have other differences observed (Table II). These include an increased level of dihydroxyacetone phosphate, which is rapidly equilbrated with fructose-1,6-diphosphate in all of our experiments, due to high aldolase activity both in the chloroplasts and in the medium. The kinetic curves for

dihydroxyacetone phosphate are, in all cases, similar to the ones for fructose-1,6-diphosphate. Compounds with levels which decreased upon FDPase and Mg^{++} addition included glucose-6-phosphate, sedoheptulose-7-phosphate, and sedoheptulose-1,6-diphosphate. The level of sedoheptulose-1,7-diphosphate declined very rapidly upon FDPase and Mg^{++} addition (Fig. 5). The carboxylation substrate, ribulose-1,5-diphosphate (Ribul-1,5-P_2) also decreased considerably, and this could be partly responsible for the decreased rate of CO_2 fixation. However, it should be noted that the levels of pentose monophosphate, which is the sum of ribulose-5-phosphate, xylulose-5-phosphate and ribose-5-phosphate, were not changed by the addition of Mg^{++} and FDPase.

The effects of addition of FDPase and Mg^{++} on the distribution of metabolites between the chloroplasts and the suspending medium are very large (Table III). In the chloroplasts with these additions the level of fructose-1,6-diphosphate in the medium increased 11-fold, and the level of dihydroxyacetone phosphate increased 4-fold. At the same time, the level of fructose-6-phosphate in the pellet declined 75%, its level in the medium increased 2-fold, and its total level declined about 40%. Thus, the addition of FDPase and Mg^{++} ions has increased greatly the appearance of metabolites in the suspending medium. However, the added FDPase has not increased conversion of fructose-1,6-diphosphate to fructose-6-phosphate in the total, although some increased conversion in the medium may have occurred.

When enough FDPase and Mg^{++} are present from the start of photosynthesis to cause inhibition, the relative effects on the distribution of metabolites are similar to the effects when Mg^{++} and FDPase are added after several minutes of photosynthesis (Table IV). The ^{14}C levels in all compounds are, of course, decreased, but relatively greater decreases are seen in hexose and heptose monophosphate compared with dihyroxyacetone phosphate which is proportional to Fru-1,6-P_2.

A comparison of $^{14}CO_2$ fixation rates in the presence of added FDPase and bovine serum albumin (Table V), each added with Mg^{++}, shows that inhibition is not a general effect of protein addition.

The effects of addition of FDPase alone, without enough Mg^{++} ion to cause a synergistic effect in the presence of 5 mM PP_i, were minor (Table VI). There was a slight stimulation of $^{14}CO_2$ fixation rate, but levels of individual compounds in chloroplasts and medium were virtually unchanged.

The effects of addition of 5 mM Mg^{++} alone, after 7 min photosynthesis in the normal medium (Solution C), were both qualitatively very similar to the effects of adding both FDPase and Mg^{++} in one experiment (Table VII). However, the photosynthetic rate in this experiment with Mg^{++} addition only was only 30 μmoles CO_2 fixed (mg chlorophyll hr)$^{-1}$, lower than usual, suggesting a greater than usual breakage of chloroplasts during the course of the experiment and, consequently,

the presence of considerable FDPase in the medium, coming from broken chloroplasts. This sort of difficulty can never be entirely overcome under the conditions which we use, and it is probable that there is always present in the medium some FDPase, as well as all of the other enzymes from the chloroplasts.

In another experiment (Table IV) Mg^{++} concentration was such that no inhibition was seen without added FDPase. In that case, no increased transport of metabolites was seen with Mg^{++} without added FDPase.

Although most of these enzymes retain some activity in the medium, we have found that there is no appreciable activity of either FDPase or ribulose diphosphate carboxylase (E.C.4.1.1.39) in the medium. In other words, although the FDPase protein is present in the medium, as a consequence of chloroplast breakage, its activity as FDPase is negligible. When chloroplasts were placed in the light in Solution C for 5 min and centrifuged from the medium, assay of the medium for FDPase activity showed an activity of less than 0.1 nmoles Fru-1,6-P_2 per min which may be compared with the CO_2 fixation rate of the chloroplasts of 20 nmoles per min, based on a rate of 60 μmoles (hr·mg chlorophyll)$^{-1}$ and 50 μg chlorophyll in the chloroplasts in the flask. Also, it may be shown that the 10 μg of FDPase added gave an activity of only 0.2 nmoles Fru-1,6-P_2 converted per min in Solution C, but would have an activity of 100 nmoles/min at pH 8.7 and 5 mM Mg^{++}.

When photosynthesis and metabolite transport of chloroplasts with 5 mM PP_i are compared with that of chloroplasts without PP_i (Solution C minus PP_i), the principal effects are seen to be the improved retention (with PP_i) in the chloroplasts of those metabolites which, under the usual conditions, tend to be retained, namely, hexose and heptose monophosphates and ribulose-1,5-diphosphate (Table VIII).

Additions of low concentrations of Fru-1,6-P_2 chloroplast suspensions (Table IX) before the start of photosynthesis result in some stimulation. Larger concentrations of added Fru-1,6-P_2 produce inhibition of CO_2 uptake. These effects also depend somewhat on the concentration of added PP_i, the stimulation being somewhat more pronounced with 1 mM PP_i than with 5 mM PP_i whereas the inhibition is greater in the presence of 5 mM PP_i.

Addition of 1 mM Fru-1,6-P_2 to chloroplasts after 5 min of photosynthesis with $^{14}CO_2$ (Table X) causes inhibition (1.9 μmoles $^{14}CO_2$ fixed per mg chlorophyll during the next 6 min compared with 6.7 μmoles fixed in the control and 7.7 μmoles fixed in the presence of added Fru-6-P). The effects of added Fru-6-P are rather minor, suggesting little penetration of this compound into the chloroplasts. The effects that are seen with added Fru-1,6-P_2 (note the "flushing of the ^{14}C from the Fru-6-P and other subsequent pools) suggest that the Fru-1,6-P_2 does enter the chloroplasts readily.

Discussion

The principal regulatory effects on the rate of photosynthesis by isolated chloroplasts previously ascribed to a substance of high molecular weight plus a substance of low molecular weight in the spinach juice[1] can be completely duplicated by the addition of appropriate amounts of fructose diphosphatase and of Mg^{++}. Fru-1,6-P_2 comes out rapidly from the isolated photosynthesizing spinach chloroplasts, while Fru-6-P is well retained[13]. It might have been postulated that added FDPase converts Fru-1,6-P_2 to Fru-6-P which cannot re-enter the chloroplast. This would remove carbon from the photosynthetic carbon reduction cycle, leading to a shortage of carboxylation substrate, ribulose-1,5-diphosphate. The fact that PP_i is an inhibitor of fructose diphosphatase[16] while Mg^{++} is an activator of this enzyme, would be consistent with this assumption.

However, the data presented force the conclusion that the primary effect of added FDPase on photosynthesis by isolated chloroplasts is not produced by means of its function in converting Fru-1,6-P_2 to Fru-6-P. 1) FDPase is nearly inactive under the conditions that exist in the suspending medium of the chloroplasts. 2) The level of labelled Fru-1,6-P_2 increases and the level of labelled Fru-6-P decreases on addition of the fructose diphosphatase. 3) Addition of FDPase leads to lower levels of hexose and heptose monophosphates and of Ribul-1,5-P_2 within the pellet and to an increased level of

Fru-1,6-P_2 in the supernatant solution. Similar effects are seen with added Mg^{++} and the effects of Mg^{++} and FDPase are synergistic.

The ratio of the volume of the supernatant solution to the chloroplast volume is of the order of 50:1. Thus, even for those metabolites which rapidly appear in the suspending medium, active transport is not required. Nevertheless, given the very considerable difference in rates of movement of metabolites from chloroplast to medium (e.g., Fru-1,6-P_2 vs. Fru-6-P), it seems clear that some kind of facilitated transport is required. We conclude that the facilitated transport of Fru-1,6-P_2 from the chloroplast to the suspending medium is enhanced by the addition of Fru-1,6-P_2 to the medium, and by the addition of Mg^{++} to the medium. We tentatively conclude that the protein which can act as a fructose diphosphatase under suitable conditions is capable of being absorbed somewhere in the outer chloroplast double membrane and there functions as a transport enzyme. Such activity apparently would be stimulated by Mg^{++} and inhibited by pyrophosphate, just as is its activity as a diphosphatase. Studies of metabolite transport and other physical and chemical properties of the chloroplast membrane probably will be required before such a postulate can be proved or disproved.

The effect of added pyrophosphate is to increase the levels of hexose and heptose monophosphates and of Ribul-1,5-P_2

within the chloroplasts and to diminish their appearance in the supernatant solution. However, the levels of Fru-1,6-P_2 and of dihydroxyacetone phosphate appearing and remaining in the supernatant solution are greater in the presence of pyrophosphate than in its absence. This may be due to the higher rate of photosynthesis and larger amount of reduced carbon when pyrophosphate is present.

It now appears that only a few steps in the photosynthetic carbon reduction cycle are necessarily restricted to the inside of the chloroplasts in the suspension of isolated chloroplasts which unavoidably contains broken chloroplasts and soluble enzymes in the medium. The steps which apparently occur exclusively inside the chloroplasts are, as might be expected, the rate-limiting steps or regulated steps, that is, the diphosphatase and the carboxylation reactions. Reactions mediated by aldolase, transketolase and isomerases appear to occur to some extent outside the chloroplasts. Probably the reduction of phosphoglyceric acid to triose phosphate and the phosphorylation of ribulose-5-phosphate to ribulose diphosphate also occur almost entirely in the chloroplasts.

It was found in earlier studies[13] and again in the present investigation (see Table VIII) that levels of Fru-1,6-P_2 and dihydroxyacetone in the supernatant solution rise very high by about 6 to 10 min, and then decline between 10 and 20 min of photosynthesis in isolated chloroplasts. Apparently, the longer the concentration of these metabolites stays above a low level, the longer the rate of CO_2 uptake is maintained at

a high level. It was found earlier that addition of Fru-1,6-P_2 to the chloroplasts tends to maintain a high rate at a time when the rate would decline. The finding that small amounts of Fru-1,6-P_2 stimulates fixation of CO_2 while larger amounts inhibits provides further evidence for the regulatory effects of Fru-1,6-P_2.

The simplest explanation of the regulatory effects on CO_2 fixation rate of FDPase, Mg^{++} and PP_i is based on the respective effects of these factors on the movement of metabolites out from the chloroplasts into the suspending medium. The retention of hexose and heptose monophosphates, and especially of ribulose-1,5-diphosphate in the chloroplasts, probably helps to maintain CO_2 fixation. In addition, it appears that there may be a direct regulatory effect of Fru-1,6-P_2 concentration on the rate of the carboxylation reaction in whole chloroplasts. The mechanism of such a regulation might operate either on the carboxylation enzyme or on the CO_2 transport into the chloroplasts.

It seems possible that FDPase in the outer chloroplast membrane might play an important role in regulation of photosynthetic metabolism <u>in vivo</u>. The postulated role would be in the movement of carbon compounds from the carbon reduction cycle in the chloroplast to other types of metabolism in the cytoplasm. The control of this "transport" activity could be largely mediated by Mg^{++} ion and PP_i concentration inside and outside the chloroplast outer membrane.

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TABLE I

Effects of Mg^{++} and of FDPase on Rates of $^{14}CO_2$ Uptake by Isolated Spinach Chloroplasts

PPi (mM)	Enzyme (μg)	Mg^{++} (mM)	Rate $\frac{\mu moles\ ^{14}C}{mg.Chl.hr.}$	% Stimulation (+) or Inhibition (-)
5	0	0	101	0
5	21	0	126	+25
5	0	1.0	115	+15
5	21	1.0	138	+38
5	0	2.0	110	+10
5	21	2.0	17	-83
5	0	5.0	48	-52
5	21	5.0	2.3	-98
1	0	0	83	--
1	8.4	0	158	+90
1	0	1.0	115	+38
1	8.4	1.0	108	+30
1	0	1.5	93	+12
1	8.4	1.5	3.5	-96

TABLE II

Effect of FDPase and Mg^{++} on ^{14}C Incorporation into Metabolites

Compound	μmoles ^{14}C/mg. chlorophyll		
	After 5 min. PS	After 10 min. PS	
		W/O Mg^{++} & FDPase (control)	4 min. after addn. of Mg^{++} & FDPase
Total ^{14}C uptake	3.65	9.00	6.68
3-P-glycerate	1.38	3.18	2.39
dihydroxyacetone	0.28	0.42	1.15
Fru-1,6-P_2	0.07	0.265	0.37
Fru-6-P	0.166	0.25	0.134
Glc-6-P	0.115	0.375	0.150
Sed-7-P	0.20	0.325	0.060
Sed-1,7-P_2	0.073	0.08	0.009
pentose-P	0.070	0.22	0.22
Ribul-1,5-P	0.019	0.028	0.006
Origin	0.40	1.26	0.80
Misc. spots	0.363	0.528	0.39
Total on chromatograms	3.14	6.93	5.68
% accounted for	85.9	77.0	85.0
PS rate	43.8 μm/mg/hr	54.0 μm/mg/hr	40.1 μm/mg/hr

TABLE III

Effect of Added Mg^{++} and FDPase on Distribution of Metabolites between Chloroplasts and Suspending Medium

Compound	μmoles ^{14}C/mg chlorophyll								
	after 5 min. PS av. of A & B			after 12 min. PS & 6.5 min. after Mg^{++} and FDPase in B					
	Pellet	Supernatant	Total	Pellet		Supernatant		Total	
				A	B	A	B	A	B
Total ^{14}C incorp.			6.50					13.72	15.61
3-P-glycerate	0.55	2.32	3.53	0.75	0.40	3.08	3.75	7.92	5.70
dihydroxyacetone-P	0.04	0.73	0.95	0.04	0.03	0.60	2.45	0.74	3.01
Fru-1,6-P_2	0.02	0.19	0.27	0.01	0.01	0.11	1.32	0.19	1.49
Fru-6-P	0.094	0.024	0.186	0.202	0.054	0.054	0.110	0.303	0.184
Glc-6-P	0.240	0.040	0.275	0.529	0.095	0.080	0.136	0.588	0.280
Sed-7-P	0.36	0.03	0.034	0.715	0.185	0.090	0.075	0.675	0.190
Sed-1,7-P_2	0.015	0.012	0.135	0.014	0.004	0.005	0.029	0.115	0.066
pentose-P	0.03	0.084	0.13	0.043	0.025	0.227	0.146	0.320	0.180
Ribul-1,5-P_2	0.016	0.005	0.046	0.038	0.013	0.008	0.109	0.078	0.166
origin			0.80					1.73	1.55
disaccharide	0.01	0.018	0.018	0.020	0.012	0.130	0.082	0.112	0.08
glyceraldehyde-3-P	0.004	0.062	0.075	0.006	--	0.048	0.279	0.050	0.431
miscellaneous								0.095	0.931
Total from chromatograms			6.76					12.92	14.26
% accounted for			104.0					94.1	91.3
PS rate (μm ^{14}C/mg chl/hr)			78.0						

Flask A: control {1 mM Mg^{++}, 5 mM PPi}

Flask B: {Same as Flask A for 5.5 min. of PS time; Mg^{++} to make 6 mM and 10.2 μg. FDPase added after 5.5 min. "washed chloroplasts"}

TABLE IV

Effect of Mg^{++} and FDPase Added from the Beginning of Photosynthesis on the Level of Metabolites Seen after 5 min with $^{14}CO_2$

1 mM Mg^{++} in control; 5 mM PP_i

Compound	% of total fixed					
	control	2 mM Mg^{++}	3 mM Mg^{++}	2 mM Mg^{++} 21 µg FDPase	3 mM Mg^{++} 21 µg FDPase	21 µg FDPase
3-P-glycerate	15	17	16	15	9.6	26
dihydroxyacetone-P	28	27	29	35	39	26
Frc-6-P	2.3	2.0	1.9	1.6	1.4	1.6
Sed + Glc-P	5.3	4.8	4.3	3.8	3.8	4.0
Pentose-P	1.2	1.2	1.3	1.2	1.3	1.3
	µmoles ^{14}C/mg chlorophyll					
Total ^{14}C fixed	5.43	5.68	5.39	3.83	0.770	4.32

TABLE V

Comparison of Effects of Addition of FDPase and of Bovine Serum Albumin on Photosynthesis Rate in Isolated Chloroplasts

5 min. photosynthesis

1 mM PP_i

1.5 mM Mg^{++}

µg protein/ flask	Relative rate of photosynthesis	
	FDPase	Bovine Serum Albumin
Control: "0"	1.0	1.0
8.4 µg	0.17	1.26

PS rate of control: 44.3 µm ^{14}C/mg.chl/hr

TABLE VI

Effect of Added FDPase on Distribution of Metabolites between Chloroplasts and Suspending Medium

1 mM Mg^{++}
5 mM Ppi

"washed chloroplasts"

A: control B: 30 μg FDPase added after 5.5 min. PS

Compound	μmoles ^{14}C/mg chlorophyll								
	after 5 min. PS av. of A & B			after 12 min. PS-FDPase in B for 6.5 min.					
	Pellet	Supernatant	Total	Pellet		Supernatant		Total	
				A	B	A	B	A	B
Total ^{14}C incorp.			3.27					6.82	7.55
3-P-glycerate	0.35	1.09	1.52	0.460	0.460	2.28	2.64	2.49	3.16
dihydroxyacetone-P	0.060	0.390	0.60	0.115	0.030	0.615	0.560	0.953	0.816
Fru-1,6-P_2	0.007	0.053	0.105	0.016	0.009	0.121	0.076	0.196	0.178
Fru-6-P	0.095	0.014	0.175	0.157	0.109	0.033	0.028	0.277	0.187
Glc-6-P	0.225	0.033	0.210	0.380	0.210	0.055	0.063	0.425	0.042
Sed-7-P	0.270	0.010	0.183	0.500	0.370	0.082	0.065	0.460	0.363
Sed-1,7-P_2	0.017	0.003	0.085	0.020	0.007	0.005	0.003	0.116	0.022
pentose-P	0.025	0.085	0.125	0.058	0.041	0.245	0.320	0.380	0.392
Ribul-1,5-P_2	0.016	0.002	0.027	0.017	0.007	0.003	0.003	0.038	0.050
misc. spots, inc. origin			0.344					1.235	1.339
Total from chromatograms			3.37					6.57	6.93
% accounted for			103.0					96.3	91.8
PS rate at 5 min (μm ^{14}C/mg.chl/hr)			39.2						

TABLE VII

Effect of Addition of Mg^{++} (5 mM) on ^{14}C Incorporation into Metabolites in Chloroplasts and Suspending Medium

5 mM PPi "washed chloroplasts"

Compound	μmoles ^{14}C/mg chlorophyll								
	After 6 min. PS		no Mg++ av. of 2 flasks	after 15 min. PS		8 min. after addn. of 5 mM Mg^{++} fo flask B			
	Pellet	Supernatant	Total	Pellet		Supernatant		Total	
				A	B	A	B	A	B
Total ^{14}C incorp.			3.32					8.6	7.8
3-P-glycerate	0.127	1.22	1.62	0.25	0.10	3.75	3.00	5.0	3.2
dihydroxyacetone-P	0.033	0.48	0.58	0.03	0.01	0.27	0.59	0.41	0.90
Fru-1,6-P_2	0.01	0.18	0.18	0.012	0.005	0.09	0.70	0.15	0.85
Fru-6-P	0.055	0.009	0.078	0.076	0.022	0.028	0.12	0.11	0.14
Glc-6-P	0.132	0.008	0.111	0.154	0.05	0.05	0.076	0.227	0.171
Sed-7-P	0.158	0.006	0.095	0.228	0.05	0.043	0.065	0.244	0.14
Sed-1,7-P_2	0.015	0.008	0.087	0.021	0.003	0.005	0.019	0.100	0.03
pentose-P	0.021	0.048	0.073	0.025	0.008	0.13	0.165	0.17	0.19
Ribul-1,5-P_2	0.007	0.001	0.017	0.02	0.006	0.003	0.002	0.04	0.02
origin			0.46					1.41	1.21
miscellaneous			0.83					0.556	0.56
Total from chromatograms			3.38					8.42	7.45
% accounted for			102					97.9	95.4
PS rate (μm ^{14}C/mg chl/hr)			33.2						

TABLE VIII

Effect of PPi on ^{14}C Incorporation into Metabolites in Chloroplasts and Suspending Medium

Compound	µmoles ^{14}C/mg chlorophyll											
	after 6 min. PS						after 15 min. PS					
	Pellet		Supernatant		Total		Pellet		Supernatant		Total	
	+PPi	-PPi	+PPi	-PPi	+PPi	-PPi	+PPi	-PPi	+PPi	-PPi	+PPi	-PPi
Total ^{14}C incorp.					6.1	3.92					9.1	6.1
3-P-glycerate	0.45	0.27	1.19	1.15	1.41	1.22	0.84	0.33	1.63	1.29	1.77	1.60
dihydroxyacetone-P	0.013	0.013	0.50	0.35	0.32	0.19	0.01	0.001	0.150	0.045	0.10	0.013
Fru-1,6-P_2	0.010	0.008	0.215	0.086	0.275	0.162	0.003	0.001	0.020	0.001	0.05	0.005
Fru-6-P	0.102	0.043	0.018	0.035	0.165	0.090	0.145	0.020	0.028	0.035	0.198	0.090
Glc-6-P	0.282	0.10	0.030	0.020	0.290	0.133	0.40	0.050	0.067	0.073	0.494	0.193
Sed-7-P	0.390	0.145	0.022	0.010	0.261	0.125	0.34	0.090	0.085	0.022	0.44	0.135
Sed-1,7-P_2	0.006	0.001	0.007	0.012	0.033	0.024	0.003	--	0.002	0.001	0.015	0.003
pentose-P	0.017	0.007	0.080	0.050	0.129	0.064	0.030	0.008	0.123	0.024	0.181	0.053
Ribul-1,5-P_2	0.016	0.010	0.002	0.002	0.029	0.018	0.037	0.010	0.004	0.002	0.054	0.020
P-enolpyruvate	--	--	0.002	0.004	0.002	0.005	--	0.0014	0.0024	.013	0.003	0.023
origin					0.717	0.672					1.08	0.95
disaccharide	0.014	0.010	0.028	0.023	0.036	0.028	0.037	0.022	0.180	0.127	0.201	0.206
miscellaneous					0.267	0.193					1.012	0.922
total on chromatograms					3.934	2.924					5.598	4.213
% accounted for					64.5	74.6					61.5	69.1
PS rate (µm ^{14}C/mg chl/hr)					61.0	39.2						

TABLE IX

Effect of Concentration of Added Fru-1,6-P_2 on $^{14}CO_2$ Uptake During 5 min Photosynthesis by Isolated Chloroplasts

Conc. Fru-1,6-P_2 added, mM	Fixation of ^{14}C µmoles (mg.chlorophyll)$^{-1}$	
	1 mM PP_i	5 mM PP_i
0	3.6	4.5
0.1	5.5	5.3
0.2	6.4	5.6
0.5	5.3	3.8
1.0	3.7	1.9

Rates (no Fru-1,6-P_2 added):

43.4 µm/mg/hr with 1.0 mM pp_i;

53.8 µm/mg/hr with 5.0 mM PP_i

TABLE X

Effect of Added Fru-1,6-P_2 and of Fru-6-P on Photosynthesis Rate and Incorporation of ^{14}C into Metabolites

Compound	μmoles of ^{14}C/mg chlorophyll			
	after 5 min PS no addns	control	6 min after addn 1 mM	6 min after addn 1 mM Fru-1,6-P_2
Total ^{14}C incorp.	8.1	14.8	15.8	10.0
3-P-glycerate	2.65	4.75	4.60	2.70
dihydroxyacetone-P	0.93	1.19	1.27	1.38
Fru-1,6-P_2	0.23	0.74	0.50	1.15
Fru-6-P	0.205	0.26	0.37	0.05
Glc-6-P	0.225	0.435	0.425	0.10
Sed-7-P	0.235	0.25	0.35	0.045
Sed-1,7-P_2	0.115	0.145	0.460	0.075
pentose-P	0.120	0.180	0.66	0.11
Ribul-1,5-P_2	0.035	0.064	0.062	0.01
origin	0.70	1.92	2.00	0.85
miscellaneous	0.425	0.896	1.028	0.81
Total on chromatogram	5.87	10.83	11.73	7.28
& accounted for	72.5	73.2	76.6	72.8
PS rate (μm ^{14}C/mg.chl/hr)$^{-1}$	97.2			

FIGURE CAPTIONS

Figure 1. Stimulation and inhibition of $^{14}CO_2$ fixation in isolated spinach chloroplasts by added fructose-1,6-diphosphatase and Mg^{++} ○ 5 mM PP_i; ● no PP_i. Relative rate of 1.0 obtained with 5 mM PP_i and 1.0 mm Mg^{++} (Solution C)was 74.4 μmoles ^{14}C (mg chlorophyll hr)$^{-1}$. Mg^{++} and FDPase were added to the reaction flasks (total volumes 0.5 ml) at the start of the preillumination period to give the concentrations indicated. After 5 min preillumination, $H^{14}CO_3^-$ was added and the chloroplasts were allowed to photosynthesize for 5 min.

Figure 2. Dependence of inhibition of CO_2 fixation on concentration of added PP_i.

▲ 1 mM Mg^{++}, no FDPase

○ 3 mM Mg^{++}, 4.2 μg FDPase

● 6 mM Mg^{++}, 10.5 μg FDPase

Control rate of 1.0, with 1 mM Mg^{++}, 5 mM PP_i (Solution C) was 120 μmoles $^{14}CO_2$ fixed (mg chlorophyll hr)$^{-1}$. Other conditions as in Fig. 1.

Figure 3. Effect of addition of FDPase and Mg^{++} on level of labelled Fru-1,6-P_2 in photosynthesizing spinach chloroplasts.

○ Control (chloroplasts in Solution C)

● 17 μg FDPase and Mg^{++} to give 4 mM added after 6 min photosynthesis

Figure 4. Effect of addition of FDPase and Mg^{++} on level of labelled Fru-6-P in photosynthesizing spinach chloroplasts

○ Control (chloroplasts in Solution C)

● 17 μg FDPase and Mg^{++} to give 4 mM Mg^{++}

Figure 5. Effect of addition of FDPase and Mg^{++} on level of labelled Sedul-1,7-P_2 in photosynthesizing spinach chloroplasts

○ Control (chloroplasts in Solution C)

● 17 μg FDPase and Mg^{++} to give 4 mM Mg^{++}

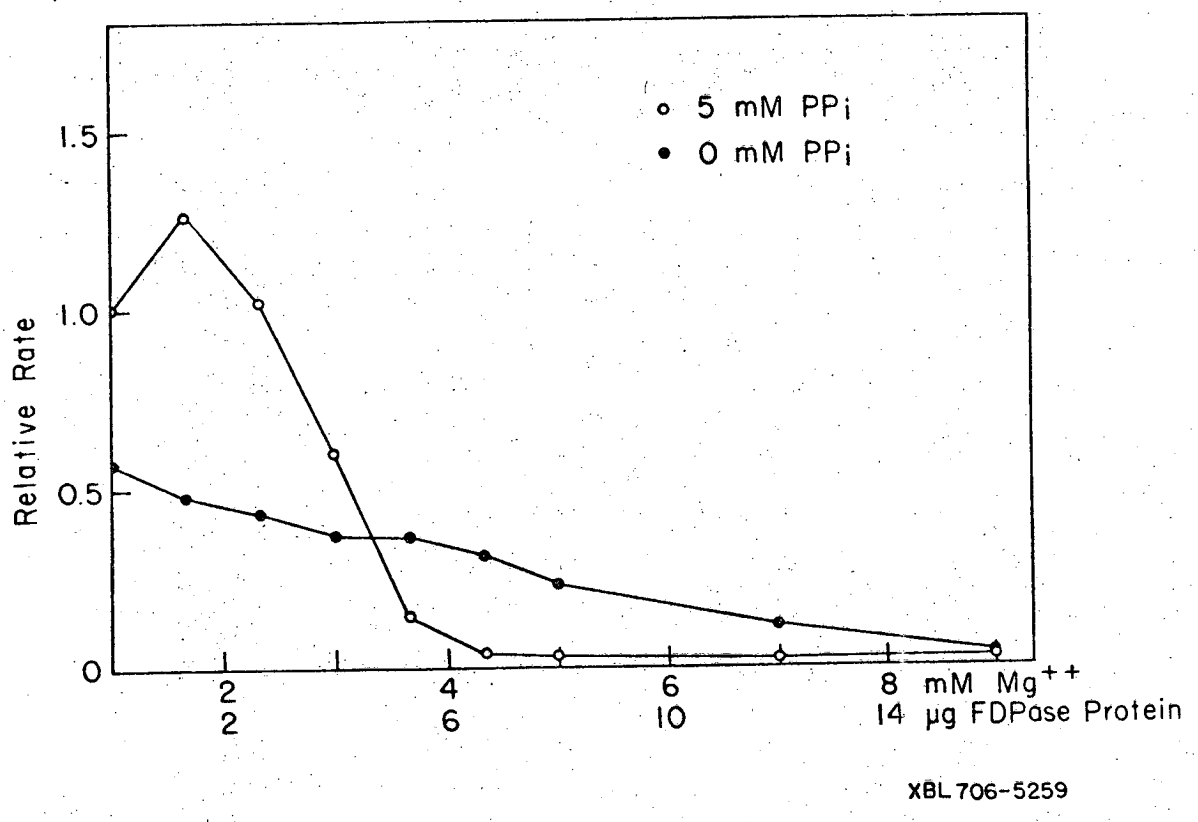
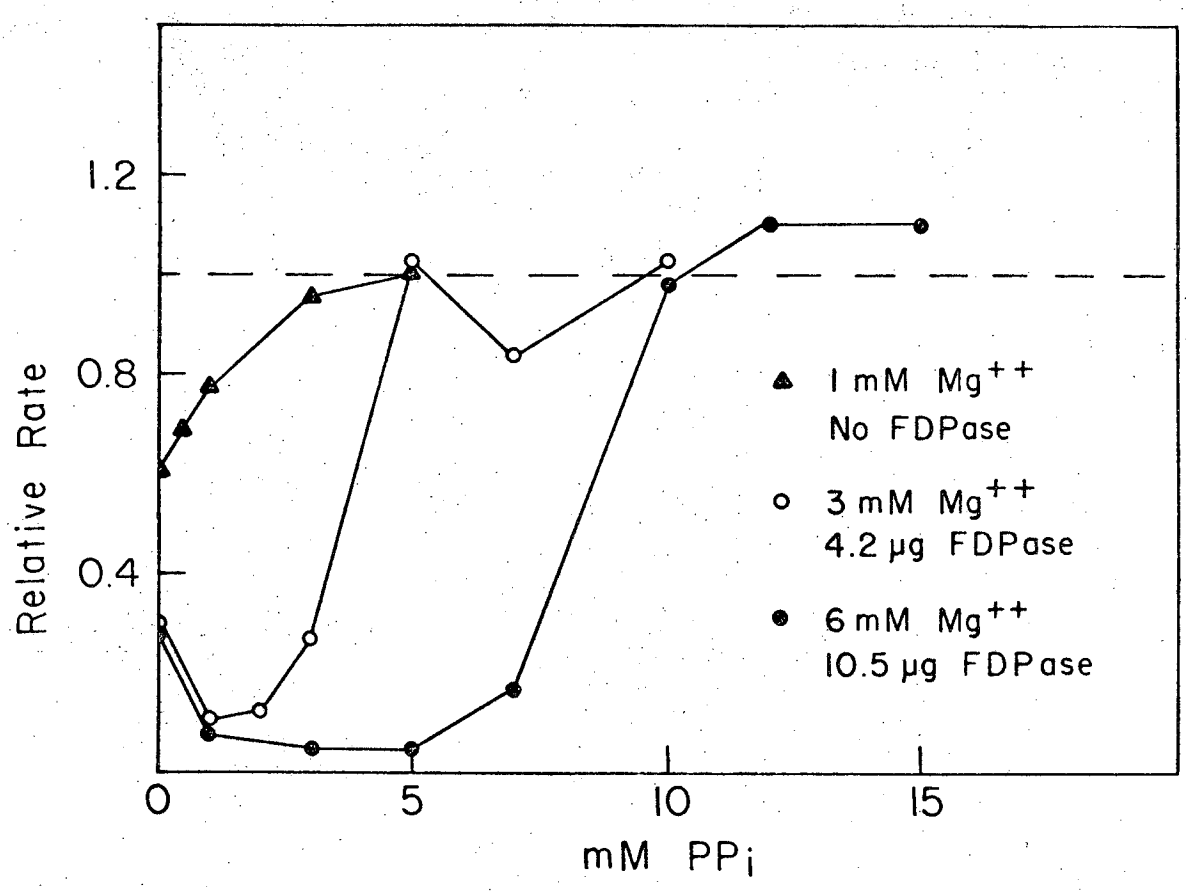
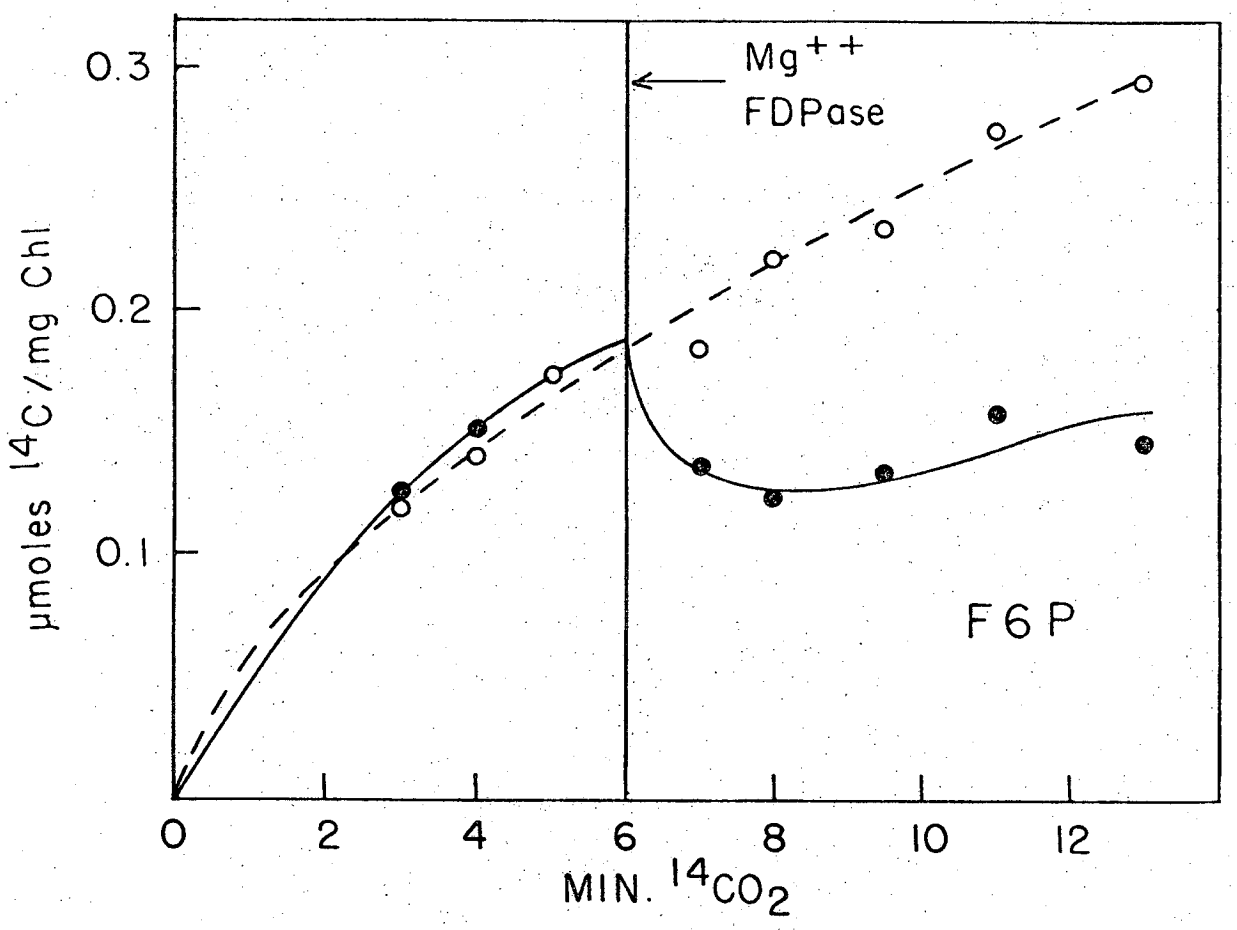

5 mM PPi
0 mM PPi
1.5
1.0
0.5
0
Relative Rate
2
2
4
6
6
10
8 mM Mg++
14 µg FDPase Protein
XBL 706-5259

Fig. 1



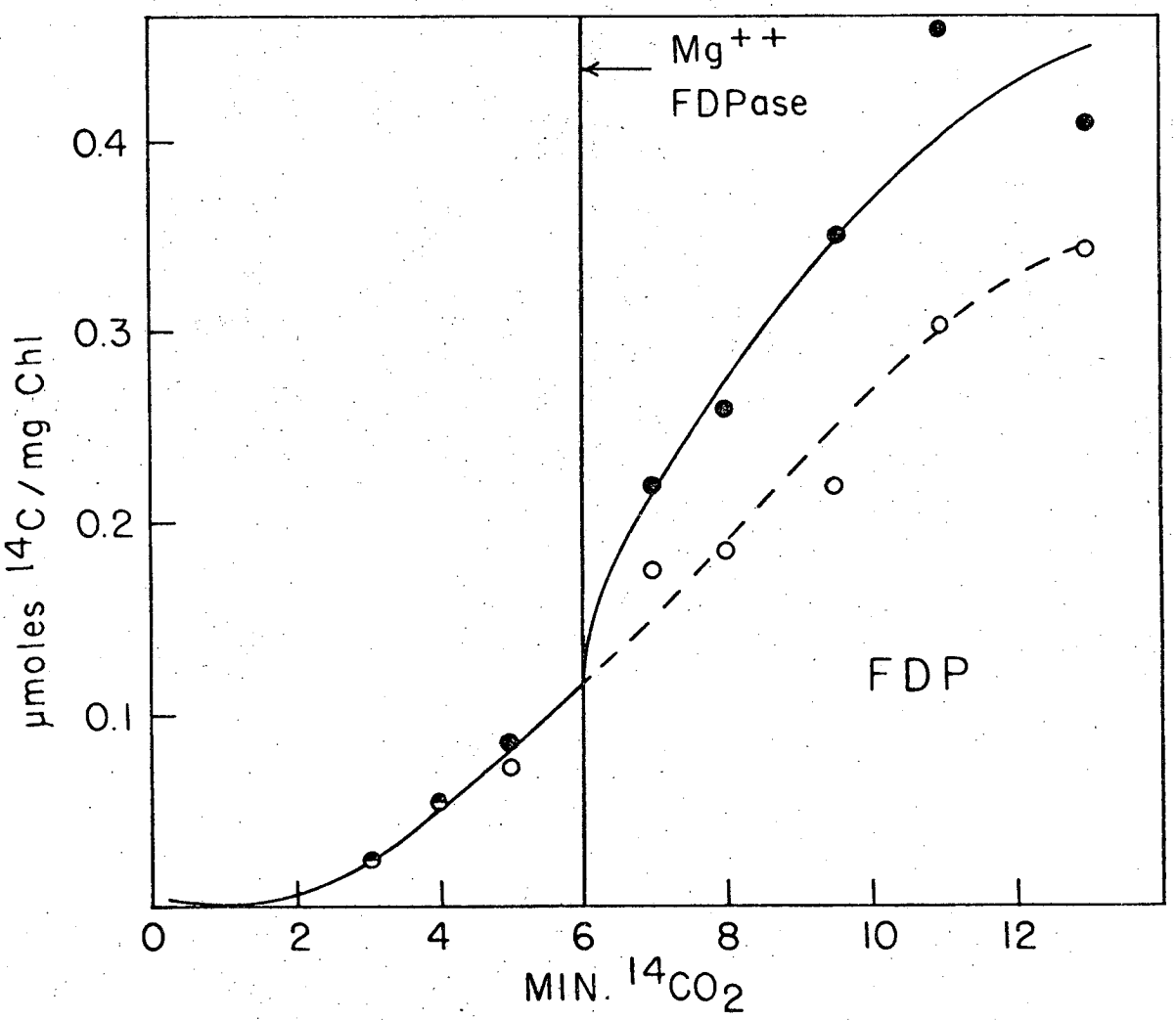
XBL 705-5201

Fig. 2



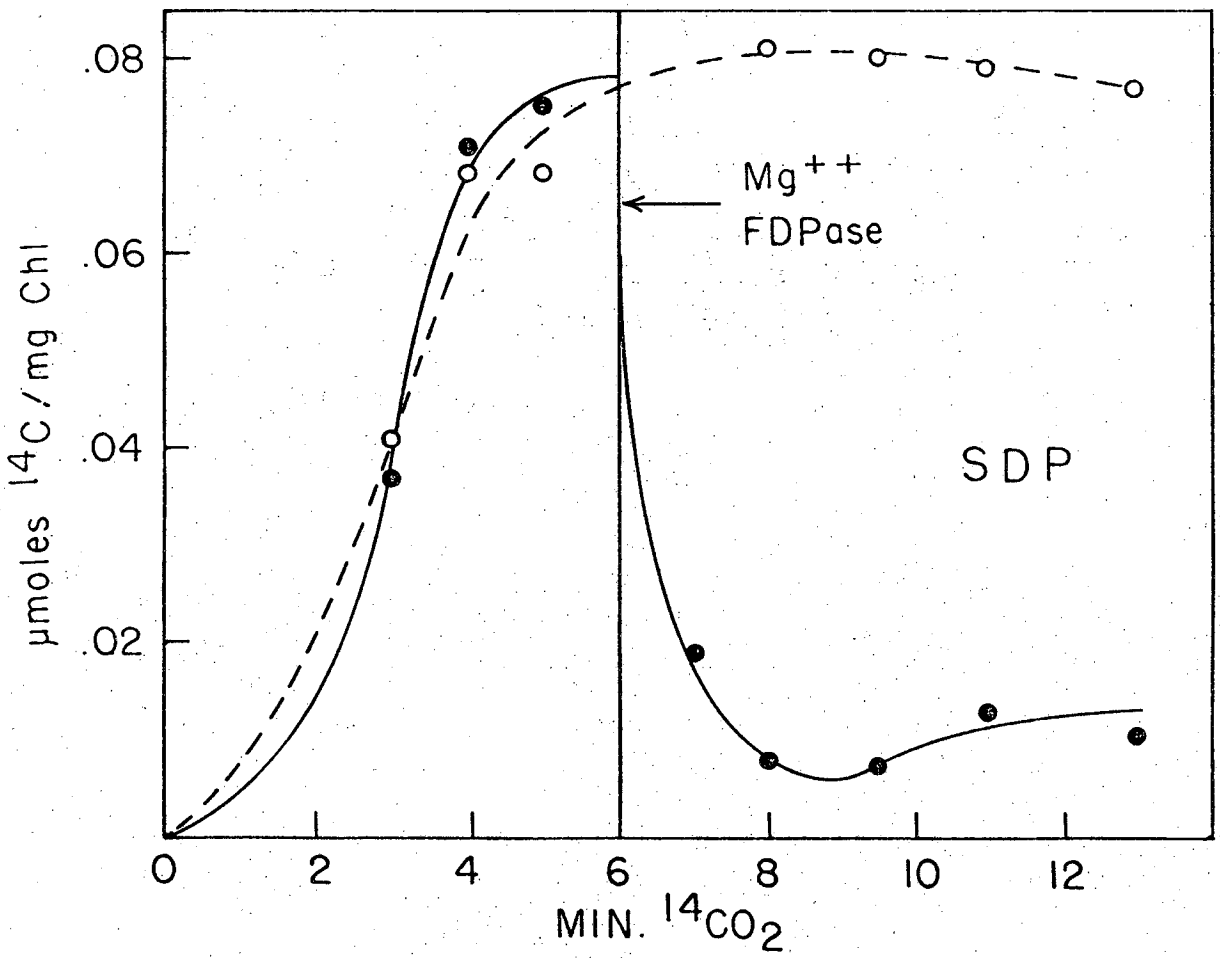
XBL 704-5181

Fig. 3



XBL 704-5179

Fig. 4



XBL 704-5182

Fig. 5

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