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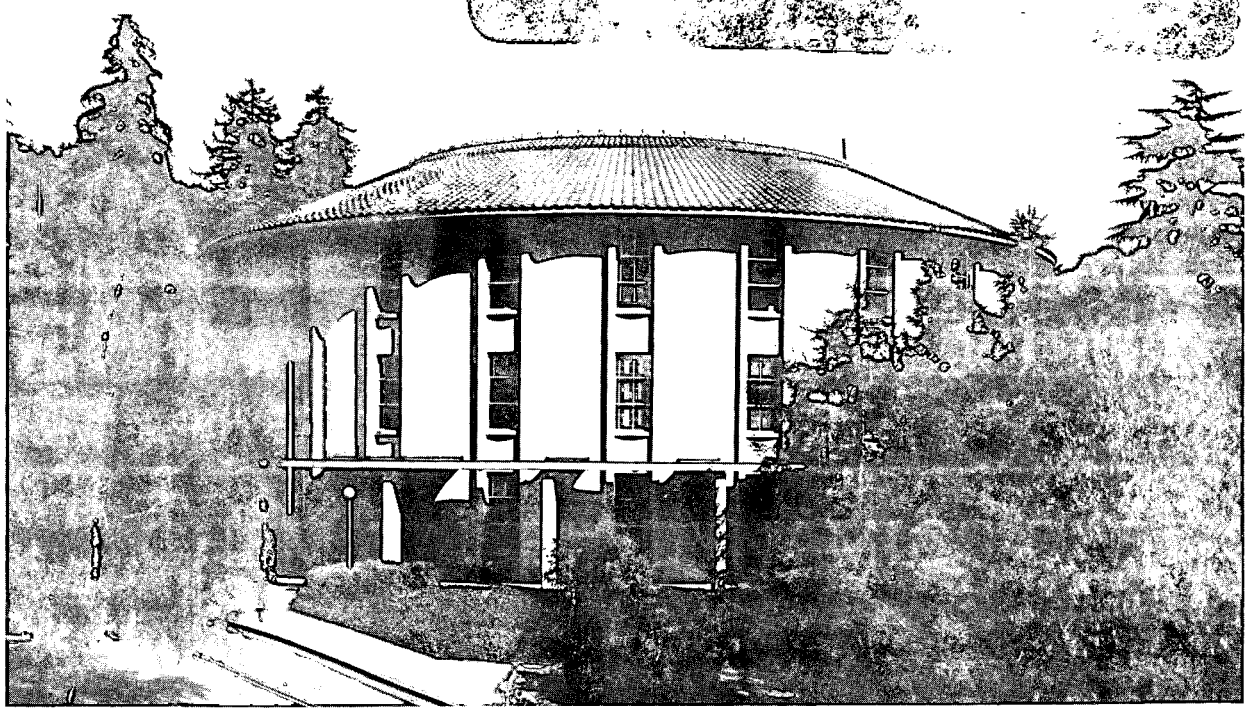

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DISCLAIMER

Control of Carbon Metabolism at Phosphoenolpyruvate in Photosynthetic Cells*

James A. Bassham

To Be Presented at the Meeting of the Japanese Society of Plant Physiologists
Kanazawa, Japan, April, 1984

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Control of carbon metabolism at phosphoenolpyruvate in photosynthetic cells.

Productivity of agricultural crops depends on many factors of which one is the primary photosynthetic productivity. When all environmental conditions are optimal, photosynthetic efficiency may be limiting. This is indicated by a comparison of record yields of C_4 and C_3 cereal grain crops (Table 1).

Table 1. Record Yield of Some Cereal Grain Crops (Key, 1981)

Species	Grain Yield kg/ha	Author
C_4-Plant species		
Maize	23,900	Wittwer 1975
Sorghum	21,500	Wittwer 1975
C_3-Plant species		
Wheat	14,500	Anonomous 1966
Barley	11,300	Wittwer 1975
Oats	10,500	Wittwer 1975
Rice	9,900	Murata & Matsushima 1975

The maximum grain yields are 50% to 100% more for the C_4 crops. The nearly ideal conditions which must be approached to obtain such yields probably included high solar incidence and hot climate, environmental factors which permit photorespiration and hence reduced primary efficiency in C_3 plants but do not so adversly affect efficiency in C_4 plants. Quality of product, such as nitrogen content of the grain also might be affected by primary photosynthetic cell metabolism.

There is much interest in the possibility of improving photosynthetic productivity and consequently agricultural yield and quality through genetic modification. This might be accomplished either through conventional breeding

methods or by techniques of genetic engineering. Possibilities for molecular genetic engineering include the introduction of genes derived from one species into another species. A more immediately promising approach may be to alter the amount of expression of genes already present. Such changes could be used to control the amounts and activities of enzymes which mediate key rate-limiting steps in carbon and nitrogen metabolism. Important controlled steps of this type are to be found within each of the several functions of green cells.

The primary functions of such cells are: 1. to capture light energy and use this energy to transfer electrons from water to ferredoxin, and to convert ADP and P_i to ATP; 2. to use reduced ferredoxin and ATP in the reduction of carbon dioxide to triose phosphates, nitrate to ammonia, sulfate to sulfhydryl groups, and glutamine and oxoglutarate to glutamate (the GOGAT reaction) as part of the nitrogen cycle; and 3. to convert triose phosphates to sucrose and other carbohydrates, and also to convert triose phosphates and ammonia to primary amino acids, such as alanine, asparate, glutamate, glutamine, and asparagine.

In higher plants, the principal exports from the photosynthetic cell are sucrose and varying amounts of primary amino acids, or in some species, other nitrogenous carbon compounds. Light capture and conversion, and reductive steps occur mostly in the chloroplasts, whereas transformation of triose phosphates and ammonia to secondary products takes place mostly in the cytoplasm, peroxisomes, and mitochondria, although glutamate synthesis also requires the GOGAT reaction in the chloroplasts (Figure 1.)

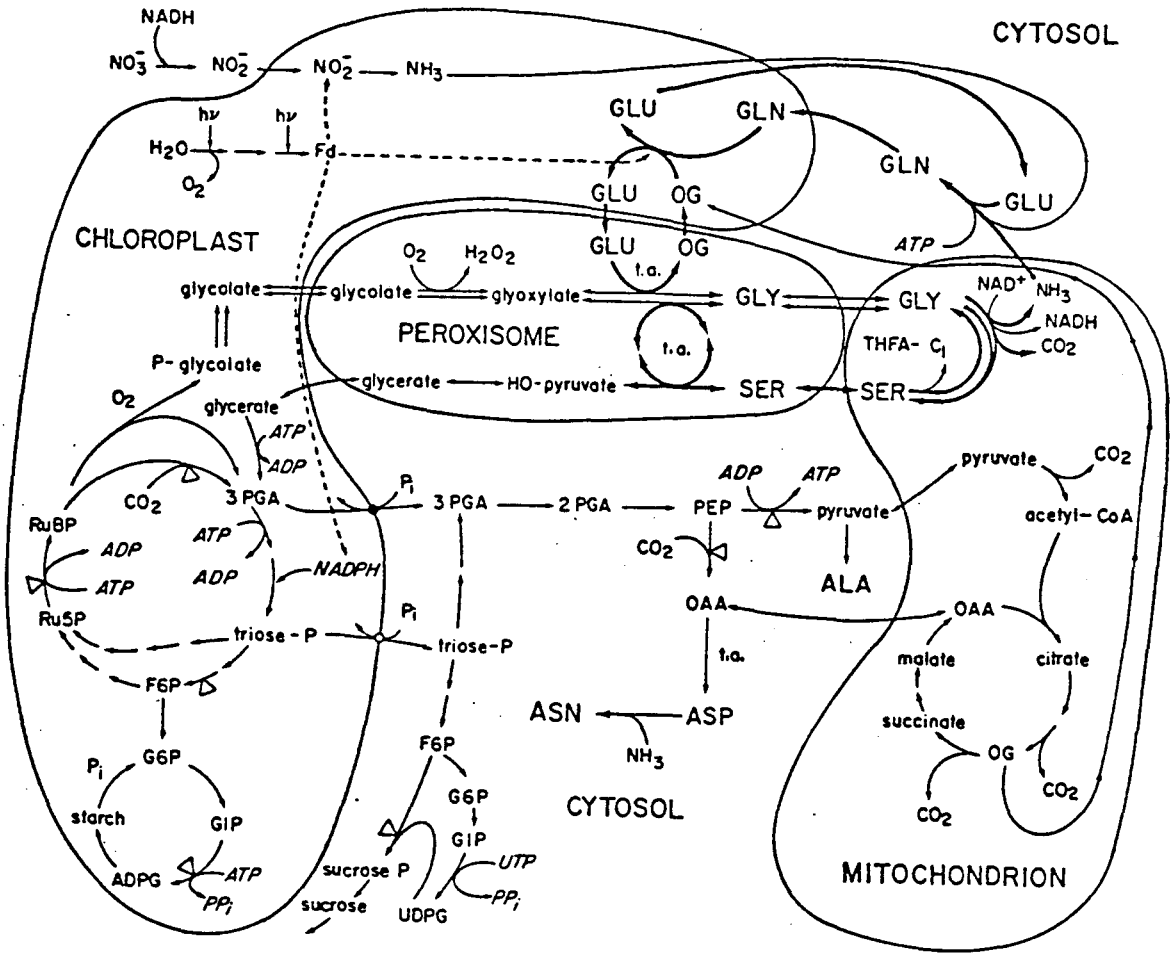

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Figure 1. Primary carbon-nitrogen pathways in photosynthetic leaf cells

This scheme shows some of the primary pathways of carbon and nitrogen metabolism in photosynthetic cells with emphasis on effects of cytoplasmic ammonia. Abbreviations: 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; F6P, fructose-6-phosphate; Ru5P, ribulose-5-phosphate; RuBP, ribulose-1,5-bisphosphate; PEP, phosphoenolpyruvate; OAA, oxaloacetate; OG, oxoglutarate; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; ADPG, adenosine diphosphoglucose; UDPG, uridine diphosphoglucose; GLU, glutamate, GLN, glutamine; ASP, aspartate; ALA, alanine.

Δ indicate some sites of metabolic regulation

Some potentially important possibilities for altering primary productivity and/or quality are to be found in carbon metabolism associated with reactions using and forming the key metabolite, phosphoenolpyruvate (PEP). This metabolite is found in both chloroplasts and cytoplasm, is involved in C_4 metabolism and respiratory metabolism, and occupies a central position from which several biosynthetic pathways begin.

Utilization of PEP in metabolism is strongly controlled by effects of cytoplasmic ammonia turnover on activation of the enzyme phosphoenolpyruvate carboxylase (PEPC) and of pyruvate kinase (PK). These enzymes mediate respectively the carboxylation of PEP (1) and its conversion to pyruvate (2). These reactions are of critical importance to the interactions of carbon and nitrogen metabolism in photosynthetic cells. Also, PEP carboxylation (1) is the initial step in the C_4 photosynthetic carbon cycle.

1. PEP + HCO_3^- ---> oxaloacetate + P_i

2. PEP + ADP -----> pyruvate + ATP

A reaction forming PEP from pyruvate which is of great importance to both C_4 photosynthesis and to interactions between carbon and nitrogen metabolism in C_4 and C_3 plant seeds is mediated by the enzyme pyruvate orthophosphate dikinase (PPDK) (3):

3. pyruvate + P_i + ATP ---> PEP + AMP + PP_i

One place this reaction occurs is in the chloroplasts of C_4 leaf mesophyll cells where it is an essential step in C_4 photosynthetic carbon metabolism (Hatch and Slack, 1968). PPDK is now known to have a much wider occurrence in both C_3 and C_4 plants in leaves and in seeds, where it may have a role in amino acid transformations. Conceievably, an increased expression of this enzyme and of PEPC in leaves of C_3 plants could have important effects on net primary photosynthesis. These possibilities will be considered later.

Effects of ammonia turnover on PEPC and PK activities.

Addition of ammonia salts to the medium of the unicellular alga, Chlorella pyrenoidosa or other Chlorella species in the dark causes increased uptake of CO_2 (Holm-Hansen, et al. 1959), increased metabolism of sugar, and increased flow of carbon into acids of the TCA cycle and amino acids, increased rate of respiration, and decreased the ATP/ADP ratio (Kanazawa, et al. 1972, Miyachi, et al. 1977, Miyachi and Miyachi 1980). During photosynthesis, ammonia addition to Chlorella causes similar shifts in metabolism such as decreased sucrose formation (though sometimes increased) and increased formation of certain amino acids and TCA acids (Hiller 1970, Kanazawa, et al. 1970c, 1972, Miyachi and Miyachi 1980). With synchronously-growing algae, similar shifts in metabolic carbon flow correlated with changes in the ability of the cells to reduce nitrate, suggesting a physiogical role of regulation by ammonia turnover (Kanazawa et al. 1970a, b).

Ammonia also affects the transformation of carbon compounds in photosynthetic cells isolated from leaves of higher plants (Hammell et al. 1979, Lawyer et al. 1981, Larsen et al. 1981, Woo and Canvin 1980) in ways similar in many respects to those observed with Chlorella. Although there were some differences among the various studies and species, the general pattern of increased rate of carbon flow through the pyruvate kinase step and of PEP carboxylation was seen.

In higher plant cells it appears that one physiological role of this effect of increased ammonia in the cell on carbon metabolism is related to the increased ammonia turnover occuring during conditions of photorespiration (Lawyer, et al., 1981). Ammonia released during the glycolate pathway by the conversion of two molecules of glycine to CO_2 and one molecule of serine must be refixed via glutamine synthesis from glutamate. Glutamine and oxoglutarate are converted to glutamate in the photorespiratory nitrogen cycle (Keys, et al., 1978). Glutamate is used partly for glutamine synthesis and partly for the conversion of glyoxylate to glycine with aminotransferase.

Apparently, the rapid operation of this cycle necessitates an increased rate of synthesis of the carbon skeletons of the C_5 amino acids involved, to prevent the pool size of glutamate from dropping too low. Even with the increased rate of flow of carbon into such synthesis there is a decrease in the glutamate pool size when leaf cells are exposed to ammonia (Table 2), along with an immediate large increase in level of glutamine and of glyoxylate (Figs. 2, 3). Ammonia level and turnover can also be increased internally by

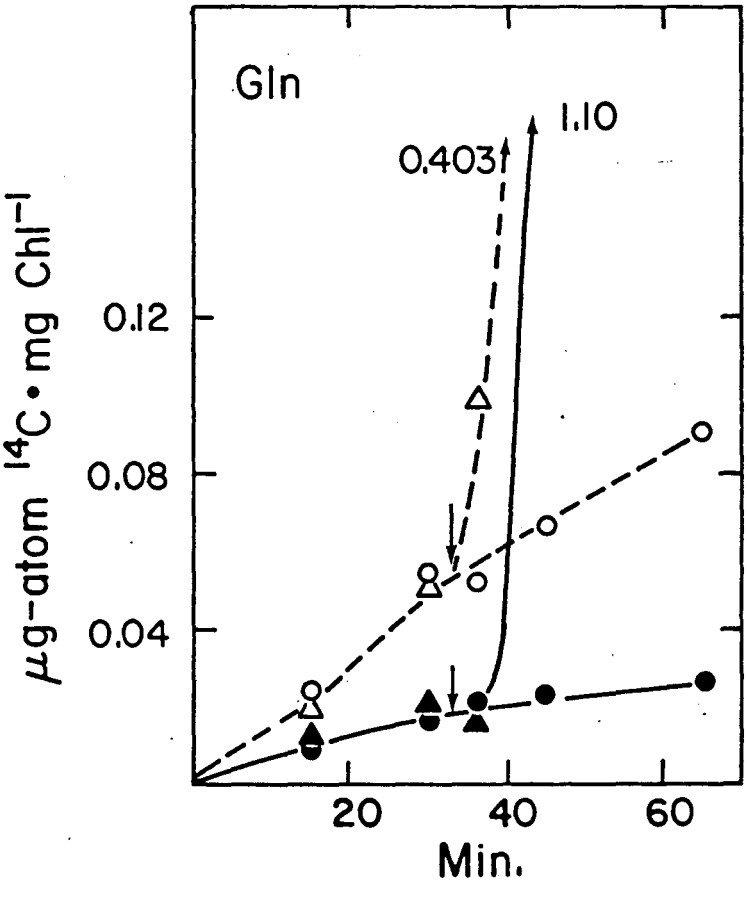

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Figure 2. Effects of addition of ammonia on labelling of glutamine in isolated spinach cells.

Open symbols: photorespiration conditions (20% O_2, 0.03% CO_2)
Closed symbols: nonphotorespiratory conditions (2% O_2, 0.2% CO_2)
Circles: Controls
Triangles: Ammonia addition

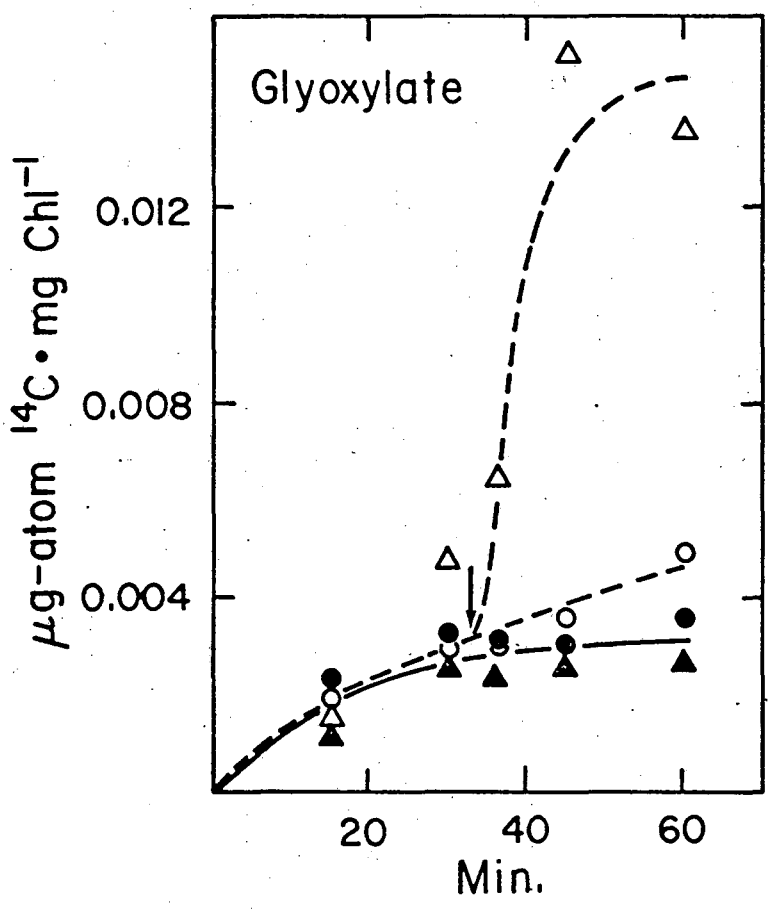

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Figure 3. Effects of addition of ammonia on labelling of glyoxylates in isolated spinach cells.
Symbols as in Figure 2.

an increased rate of nitrate reduction in photosynthetic cells, as occurs at certain stages of the cell cycle of uncellular algae, or at certain stages of leaf development in leaves of higher plants.

Table 2

POOL SIZES OF AMINO ACIDS (from Larsen, et al., 1981)

Light, Spinach Cells, with NH_4^+ or NO_3^- as N source, 15 min after start of Exp.

Amino Acid	nmol/mg Chl NO_3^-	nmol/mg Chl NH_4^+	%difference
glutamate	750	480	- 36
glutamine	180	450	+150
aspartate	300	450	+ 50
alanine	570	610	+ 7

From many such studies it has become clear that any ammonia-induced drop in glutamate concentration caused by its accelerated conversion to glutamine is accompanied by an accelerated conversion of triose phosphates to glutamate via the PK- and PEPC-mediated steps. The next problem was to discover the mechanisms of these regulations of carbon flow.

Regulation of PEPC activity by ammonia turnover

The stimulation of PEPC activity with increased ammonia turnover in the cytoplasm might be explained as a direct consequence of pH increase (Hammel, et al., 1979). PEPC has a pH maximum in activity at a pH more alkaline than

the expected usual cytoplasmic pH (7.0-7.5). It has been suggested (Davies, 1973) that PEPC could provide a cytoplasmic pH stat, since PEP carboxylation produces an additional acid equivalent. Recent studies with the ammonia analog, methylamine and Chlorella in both light and dark (Kanazawa, et al., 1983) supported this hypothesis. Many of the effects seen with ammonia were also seen with the non-biological analog. In particular, changes in rates of formation of some products derived from PEP carboxylation (citrate, malate, fumarate) increased even more with methylamine addition than with ammonia. (Figs. 4 -5). In contrast, formation of a product derived from the PK reaction such as alanine (Fig. 6) was much less stimulated with methylamine than with ammonia. The formation of glutamine itself was only slightly stimulated since of course methylamine cannot be used in its formation (Fig.7). Accelerated glutamine synthesis per se is thus not the cause of increased PEP carboxylation.

Regulation of PK activity by ammonia turnover

While a small cytoplasmic pH increase thus may explain the increased activity of PEPC when internal ammonia turnover increases, it cannot explain the change in PK activity. Plant PK has a very broad pH optimum. The kinetic properties of spinach leaf PK very recently were investigated (Baysdorfer and Bassham, 1984). Two isoforms of PK were separated and partially purified (Fig. 8). The isoforms are similar in many respects including inhibition by ATP and oxalate and stimulation by AMP. Where they differ is in response to citrate, aspartate, and glutamate (Table 3). Isoform I is inhibited by high citrate levels (K_i= 1.2 mM), but is not affected by physiological levels of aspartate or glutamate We think it is regulated for respiratory metabolism. Isoform II

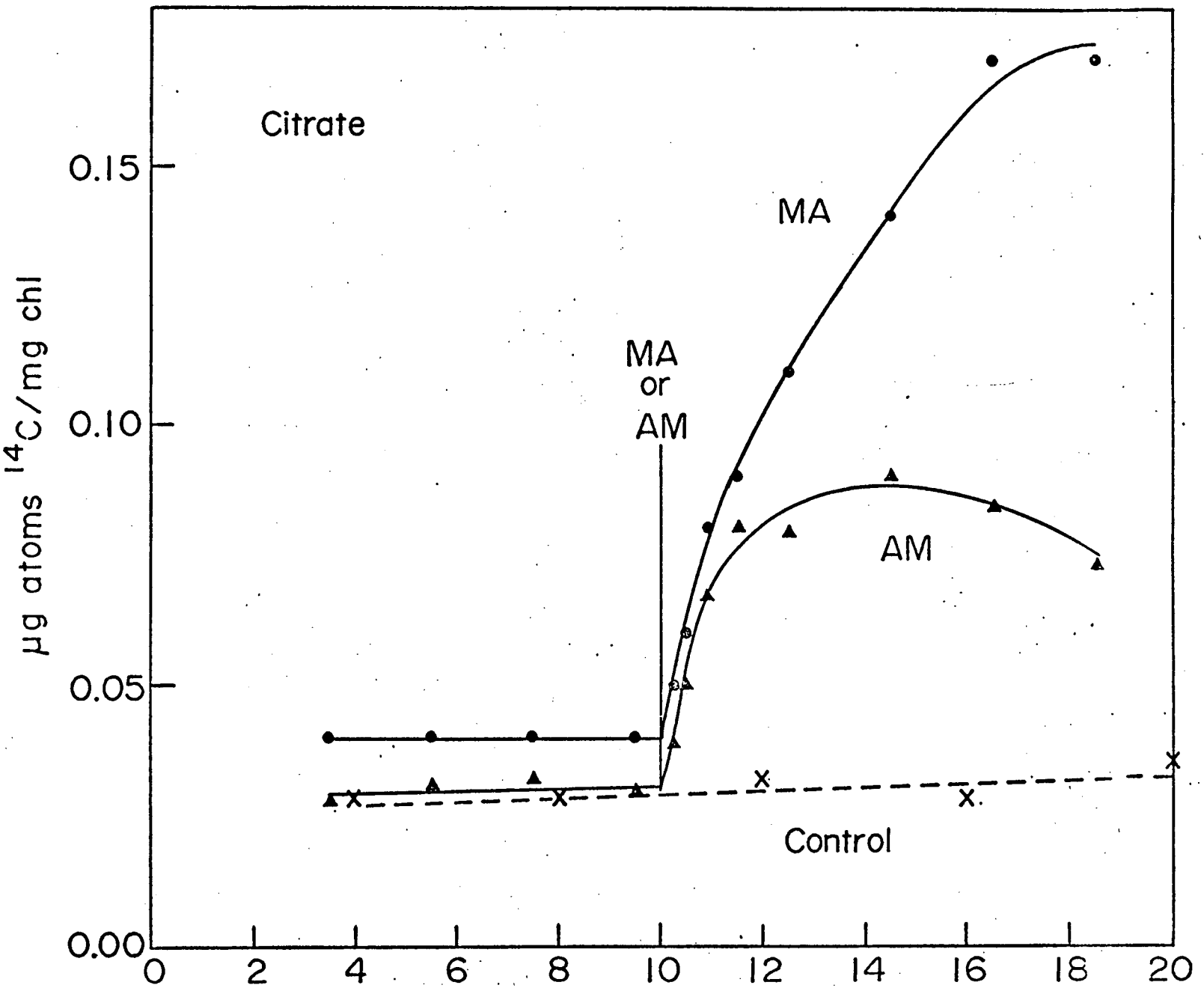

Figure 4. Effects of addition of ammonia and of methylamine on labelling of citrate in Chlorella pyrenoidosa (dark, following photosynthesis)

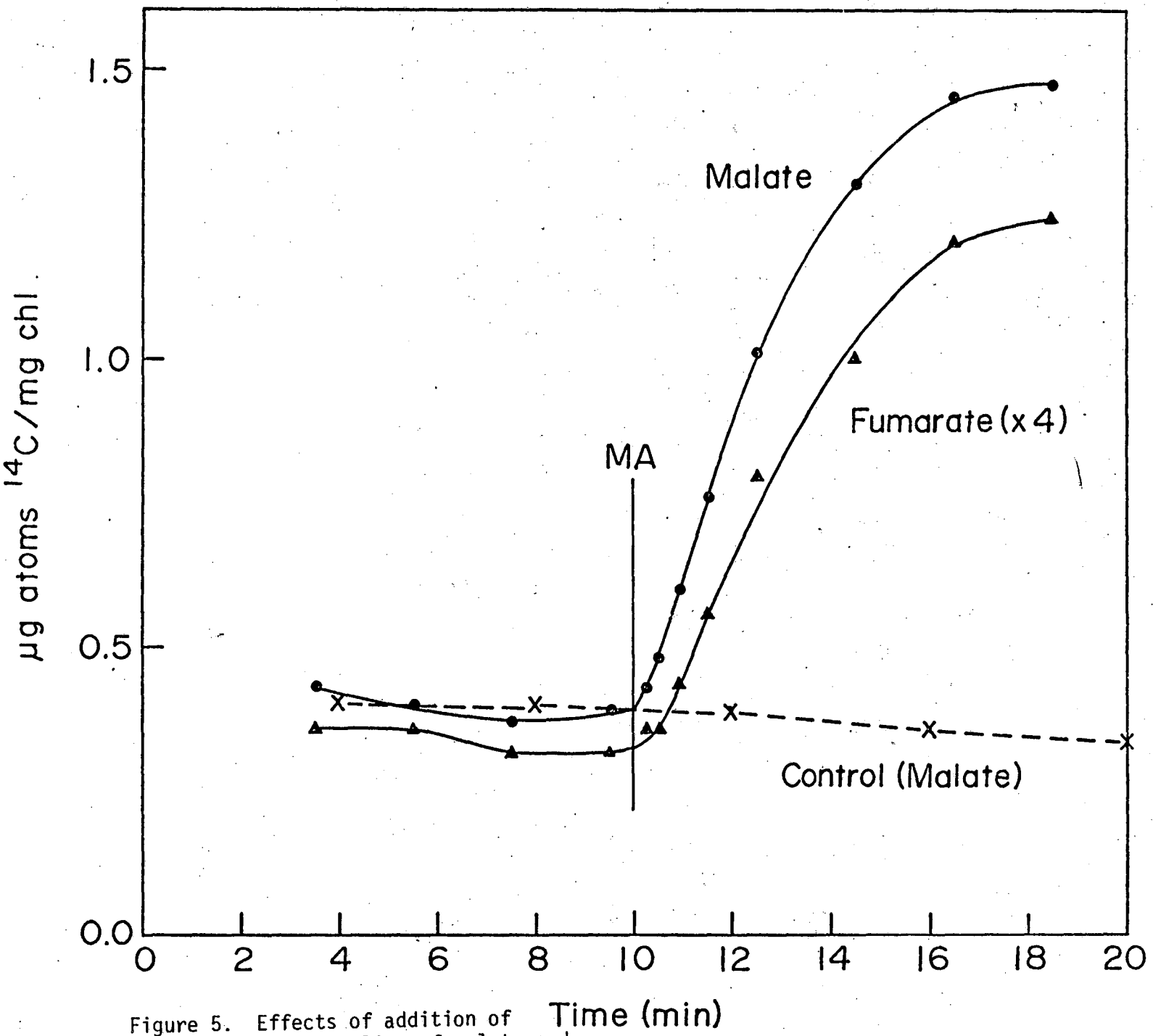

Figure 5. Effects of addition of methylamine on labelling of malate and fumarate in Chlorella pyrenoidosa (light)

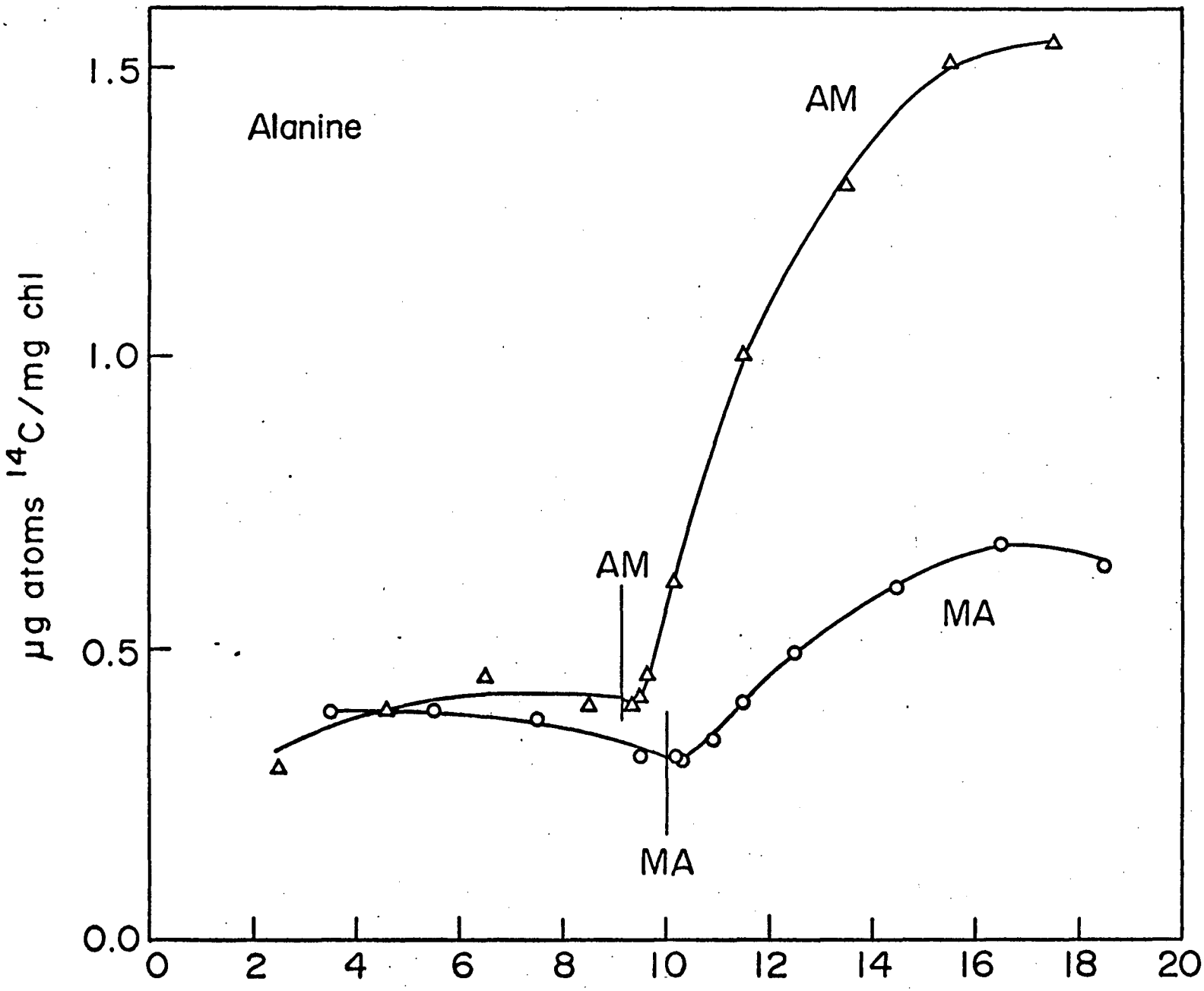

Figure 6. Effects of addition of ammonia and of methylamine on labelling of alanine in Chlorella pyrenoidosa (during photosynthesis)

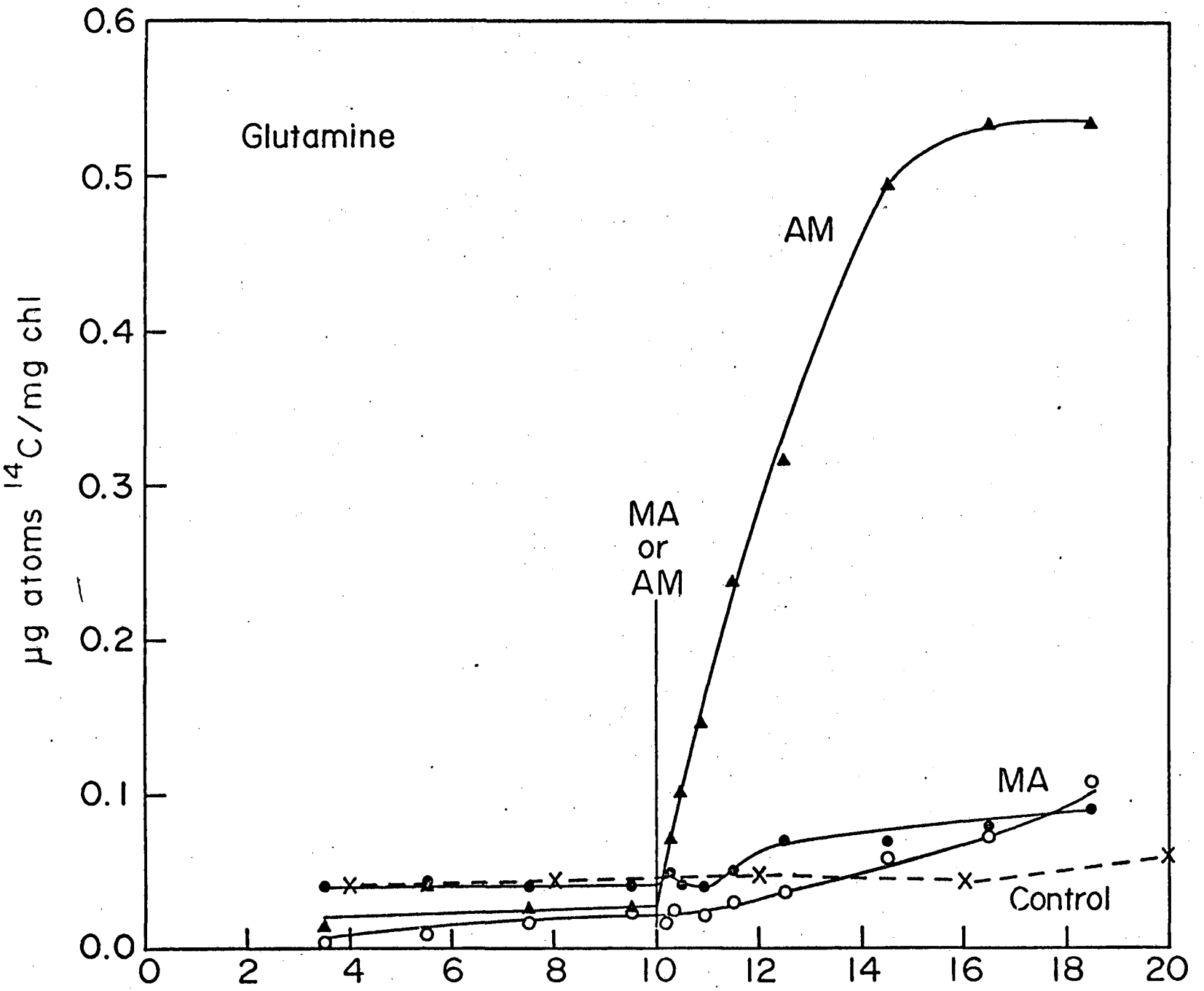

Figure 7. Effects of ammonia and methylamine on labelling of glutamine in Chlorella pyrenoidosa

triangles, dark, ammonia; crosses, control; closed circles, methylamine in dark; open circles, methylamine in light

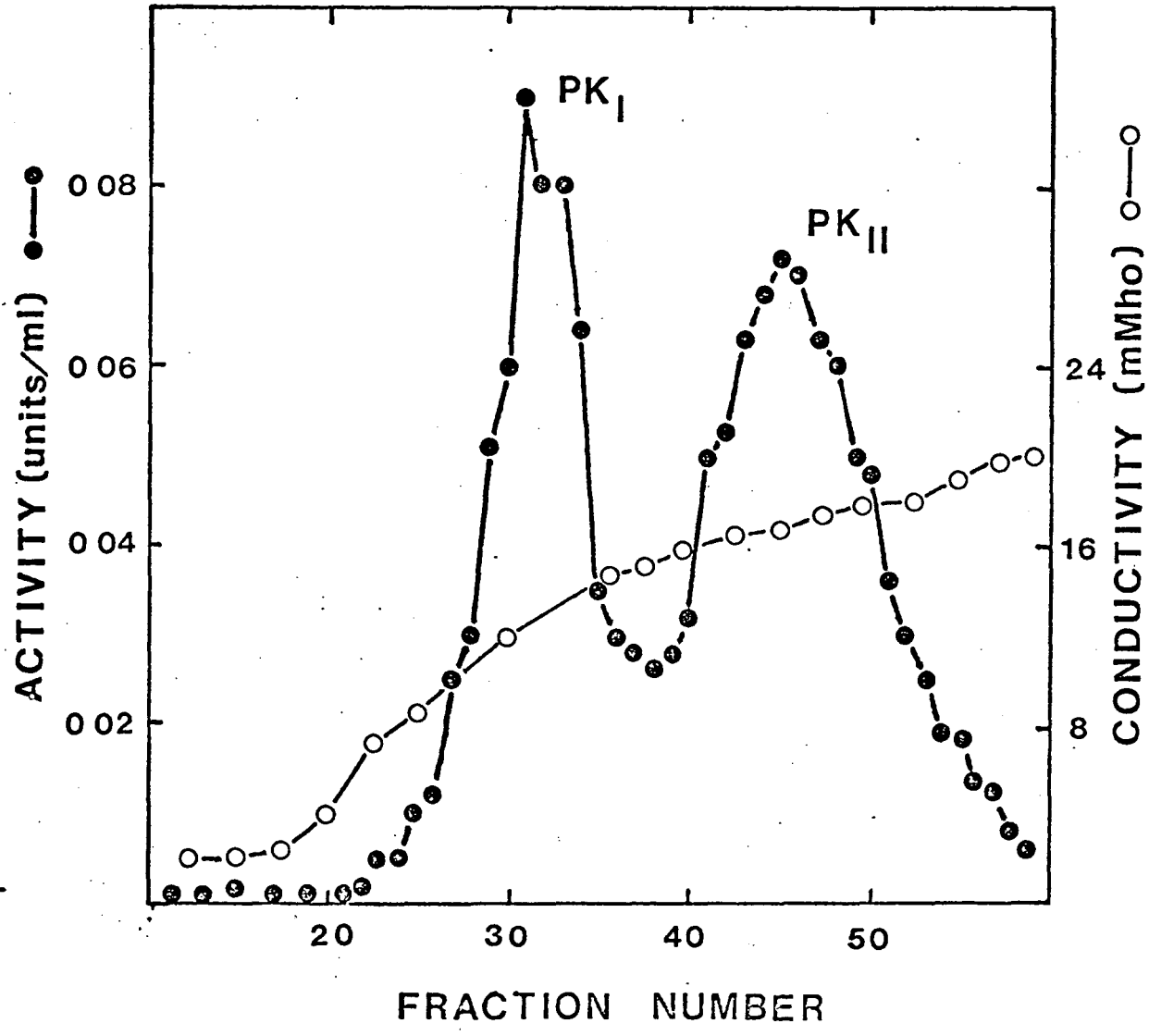

Figure 8. Separation of two isoforms of pyruvate kinase from spinach.

is inhibited by glutamate (K_i = 0.68 mM), but is stimulated by rather low levels of aspartate (K_a = 0.05 mM). These properties seem to relate it to the observed effects of increased ammonia turnover.

Table 3

Some Properties of Spinach PK Isoforms
(Baysdorfer & Bassham, Plant Physiol. 74, 374-379, 1984)

	PK_I	PK_{II}
K_mPEP	0.038	0.045
K_mADP	0.101	0.114
$K_i$citrate	1.20	n.e.
$K_i$glutmate	n.e.	0.680
$K_a$aspartate	n.e.	0.050
$K_i$oxalate	0.30	0.27

n.e. = negligible effect

It should be noted that while many studies with $^{14}CO_2$ show an increased rate of labelling of both glutamate and aspartate with ammonia addition, determination of intracellular pool sizes in isolated spinach cells showed a 50 % increase in aspartate pool size and 35% decrease in glutamate pool size 15 min after ammonia addition, compared to the control. Moreover, an estimated 80% of cellular aspartate and 50% of glutamate is in a slowly metabolized pool (possibly vacuolar) so that the active metabolic pool in the cytoplasm may change much more when ammonia is added. These considerations lead us to the hypothesis that much of the increase in rate of the PK reaction seen with addition of ammonia may be due to increases in aspartate and decreases in

glutamate in the cytoplasm. These changes would be expected to increase the activity of PK isoform II. Additional acceleration of PK might be due to small increases in the level of substrate ADP, since the K_ms for both isoforms for ADP were about 0.1 mM, probably within the range of physiological levels in the cytoplasm.

The similarities in certain properties (especially pH profiles) of the two PK isoforms strongly suggest that they are both cytoplasmic. So far in our laboratory we have not been able to find a spinach chloroplastic PK as has been reported for other species (Ireland, et al., 1979). Preliminary studies in our laboratory (Baysdorfer) indicated that while the amount of PK I remained rather constant during leaf development, the amount of PK II was low in the very young leaf, rose to a maximum during leaf development and then declined in the mature leaf. Whether this precise pattern will be substantiated upon further investigation is not known, but it does suggest that the expression of these isoforms is programmed in accord with physiological need.

It will be important to learn more about control of genetic expression of these isoforms. For example, are there two different genes and two kinds of mRNA, or is there just one gene and perhaps one mRNA for PK ? In the latter case there could be modification of the protein to convert one isoform to the other. This modification might be controlled by expression of a protein modifier such as a protein kinase. When the mechanism for genetic control is found, it might prove to be coordinately controlled with metabolically related activities, for example, nitrate reductase.

When the mechanisms of such genetic control are sufficiently understood, it may become possible for genetic engineers to manipulate them, altering the relative amounts of primary carbon and nitrogen products from the photosynthetic cells and perhaps influencing protein content of storage organs such as seeds.

PPDK activity in leaves and seeds of C_3 and C_4 plants.

PPDK is an essential enzyme in the pathway of photosynthetic carbon dioxide assimilation in many C_4 plants (Fig. 9) (Hatch and Slack, 1969) and in some CAM plants (Kluge and Osmond, 1971; Sugiyama and Laetsch, 1975). Like PK and PEPC, PPDK exhibits interesting regulatory behavior (Sugiyama, 1973). Though thought for a time to be unique in plants to C_4 and CAM species, PPDK activity was reported to be present in the pericarp tissue of barley seeds (Duffus and Rosie 1973), in immature wheat grains (Meyer, et al., 1982), in young leaves of tobacco (Kisaki, et al., 1973), in epidermal strips of Commelina benghalensis (Das and Raghavendra, 1974), and in Vicia faba L. in both mesophyll and guard cell protoplasts (Schnabl, 1981).

The occurence of PPDK in C_3 leaf tissue remained somewhat in controversy, however. PPDK activity was not detected in epidermal strips from Commelina cyanea R. Br. (Thorpe, et al., 1978) or in protoplast expracts from leaves of Vicia faba (Outlaw, et al. 1981). This apparent discrepancy can be explained by the facts that the amount of PPDK in C_3 leaf tissues

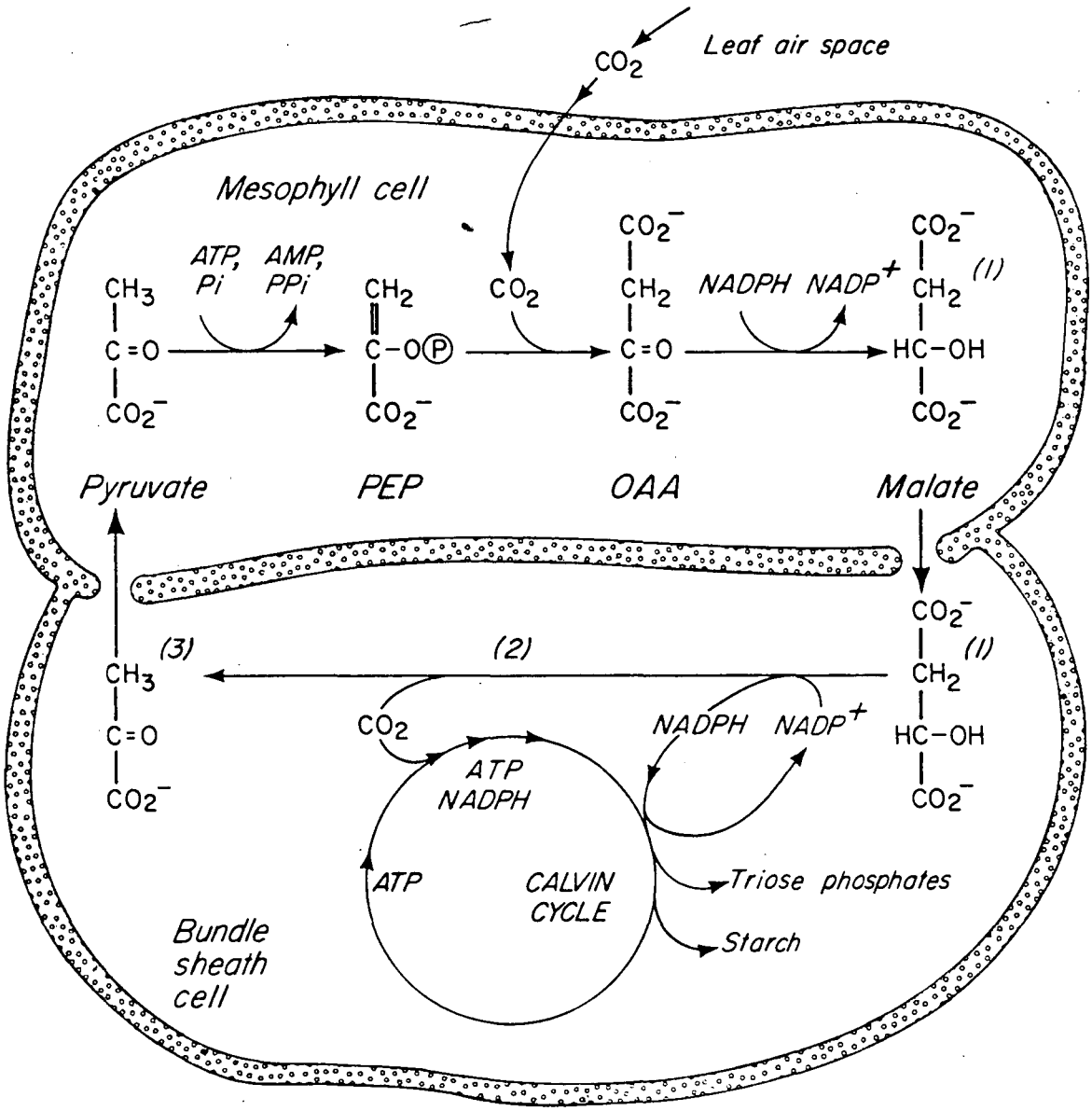

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Figure 9. C_4 photosynthetic CO_2 fixation pathway (maize).

is at best only 1-2% of the amount in C_4 leaves, the enzyme activity is affected by leaf illumination, and is cold labile, and the assay usually employed can give misleading results.

From the standpoint of possible genetic manipulation, some important questions are: is the PPDK gene generally present in C_3 plants, what regulatory mechanisms control its expression on plant tissues, especially in leaves and seeds, and what metabolic roles does this enzyme have in these tissues? A role for PPDK in control of stomatal opening has been suggested (Das and Raghavendra, 1974; Schnable, 1981). It has been proposed that in seeds PPDK may be involved in amino acid interconversions (Meyer, et al., 1982).

The sensitive method of protein immunoprecipitation has been helpful in beginning to answer some of these questions. PPDK protein was isolated from maize and purified according to the method of Sugiyama (1973). The purified PPDK was used to prepare antibody in rabbit, and this antiserum was then used as a sensitive probe to demonstrate the presence of PPDK polypeptide in wheat leaves and seeds (Aoyagi and Bassham, 1983). The wheat enzyme not only cross-reacts with maize antibody to an extent indicating considerable similarity, but also has a similar subunit molecular weight of about 94 kdaltons (Fig. 10). Further studies indicate that the enzyme in wheat leaves, although present in only about 1 % the amount in maize tissues, has regulatory properties similar to the maize enzyme (Aoyagi and Bassham, 1984). The antibody probe demonstrated the presence of the enzyme in a variety of C_3 seeds and leaves, though in some cases the molecular weight seems to be slightly different. PPDK was detected in seeds of pea, green bean,and castor bean, in immature plum fruit, and in leaves of wheat, sunflower, and spinach (older

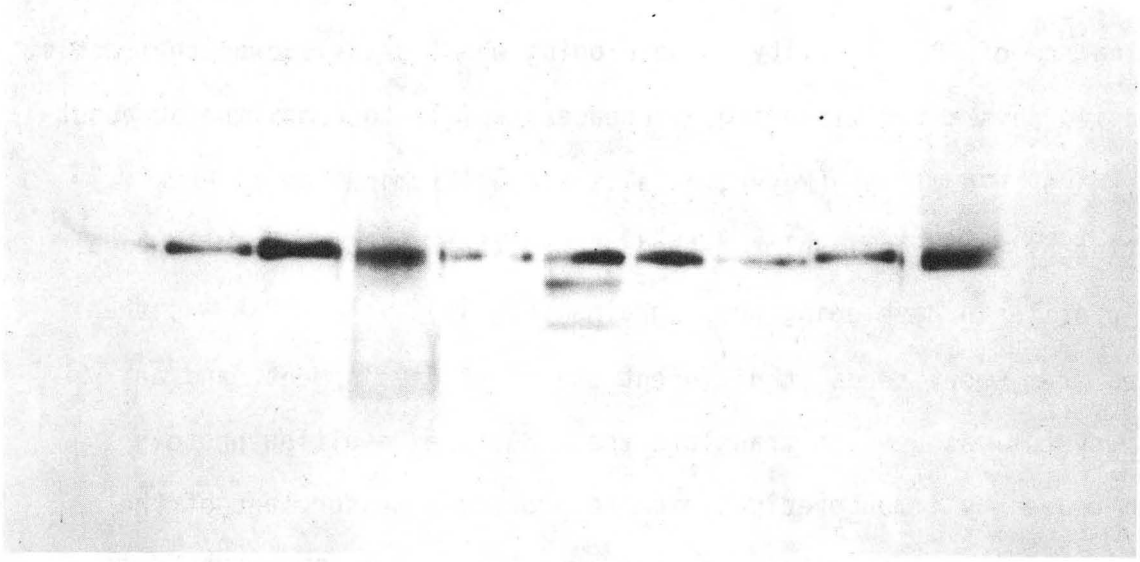

Figure 10. PPDK polypeptide from maize and wheat tissues. (Aoyagi and Bassham, 1983)

leaves only). PPDK was also detected in maize kernels and husk, but not in maize silk or root.

An examination of PPDK activity in developing wheat seeds showed that activity is low a few days after flowering, increases rapidly to a maximum at about 25 days and then declines (Meyer, et al.,1982). Examination of PPDK polypeptide by the protein blot method showed that its amount followed a similar profile in developing wheat grains (Fig 11). Total mRNA was then extracted from wheat seeds at different stages of development, and an in vitro system was used to translate the mRNA. The resulting protein was then probed by immunoprecipitation to provide a measurement of the PPDK mRNA present in the tissue at different ages. The PPDK mRNA profile agreed with the presence of PPDK in the seed tissue (Fig. 12), indicating that the level of PPDK protein and activity may be controlled by gene transcription (Aogagi, et al., 1984). It appears that the level of this enzyme is controlled by degree of gene expression in accordance with the physiological needs of the tissue. Studies with fixation of $^{14}CO_2$ by seed tissue during short periods of photosynthesis suggest that the PEP formed by the PPDK reaction is carboxylated to give oxaloacetate. The reactions may be involved in the conversion of alanine to C_4- and especially C_5-amino acids in the developing seed.

Expression of PEPC isoforms

PEPC appears as several isoforms in green cells (Ting and Osmond, 1973). The presence of each of these is dependent on developmental stage of the plant, whether C_4 metabolism is present or not (Goatly and Smith, 1974)

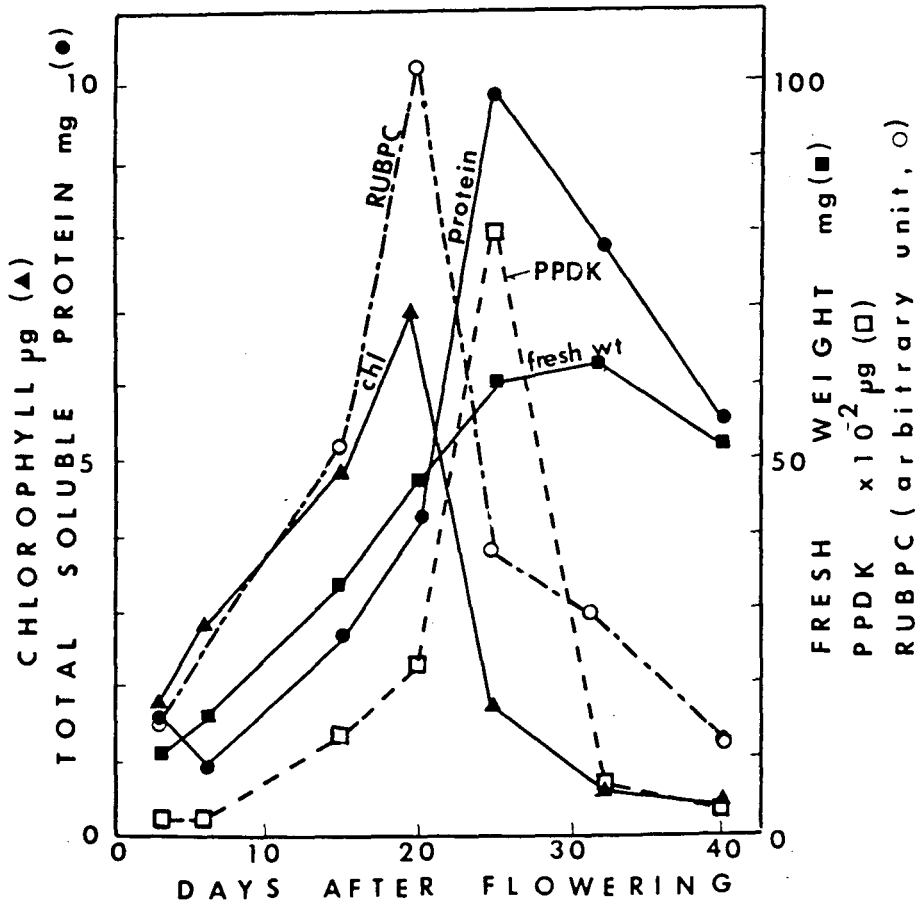

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Figure 11. PPDK profile in developing wheat seeds. (Aoyagi et al., 1984)

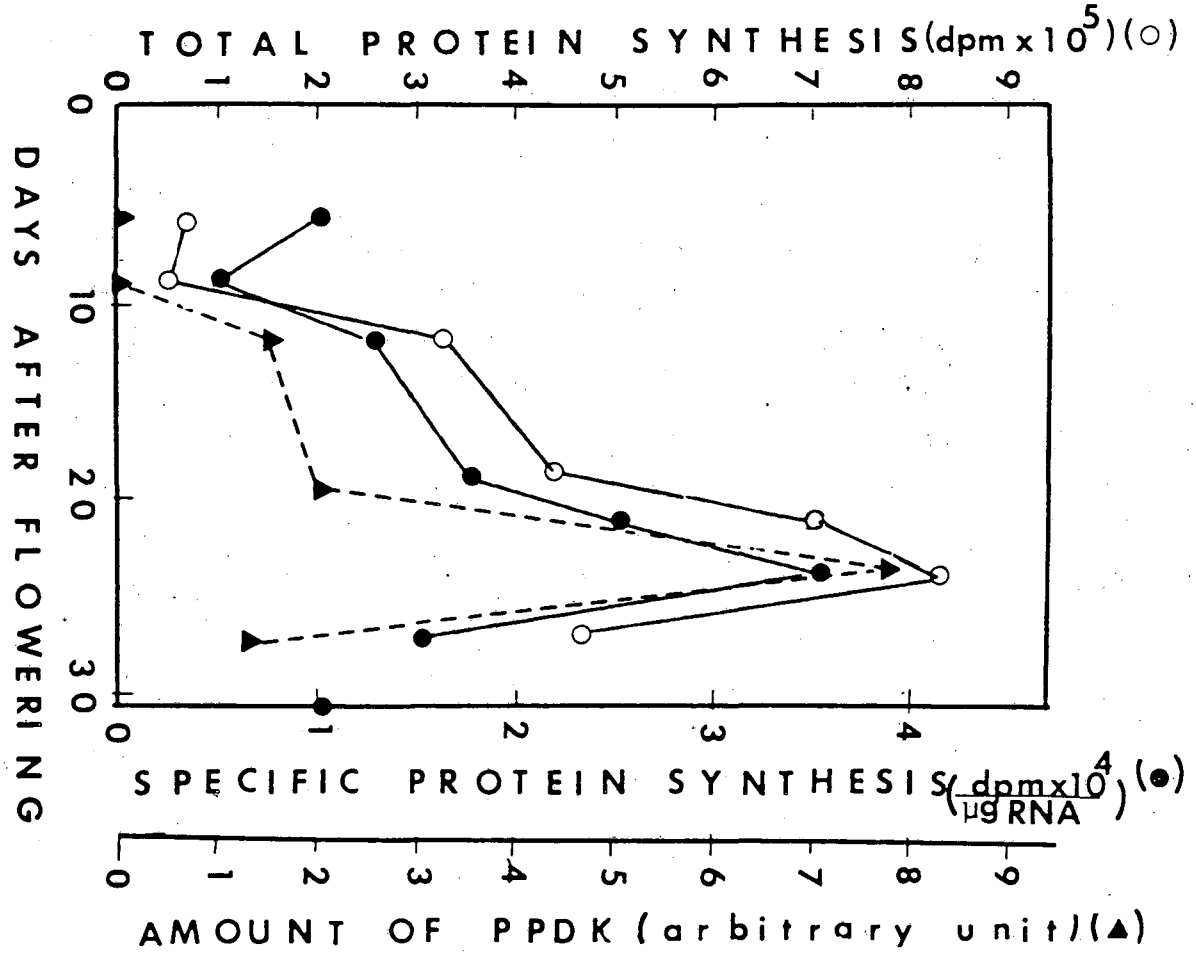

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Figure 12. PPDK mRNA and polypeptide profile in developing wheat seeds.
(Aoyagi & Bassham, 1984)

and type of plant tissue. C_4 photosynthetic CO_2 fixation requires a much higher level of PEPC activity than in normally found in C_3 leaves or in C_4 bundle sheath cells where the reductive pentose phosphate cycle operates. Goatly et al. (1975) suggested that a change in regulatory properties of leaf PEPC activity is one of the first signs of development of the C_4 pathway in sugar cane.

Just as in the case of the physiological dependence of appearance of PK isoforms in C_3 plants this raises important questions about the regulation of gene expression of the PEPC isoforms in plant tissues. Preliminary studies in our laboratory (S. Ruzin) show that in shoot-forming callus tissue of the C_4 dicot, Euphorbia degeneri, the development of the "C_4" isoform of PEPC (Fig. 13) is concommitant with the onset of a $^{14}CO_2$ fixation pattern characteristic of C_4 metabolism. Clearly we need to learn whether there are separate genes for these PEPC isoforms, differential expression of single genes, post translational protein modification of regulatory behaviour (depending perhaps on expression of genes for special regulatory proteins), or other mechanisms. When such knowlege is available, it should be possible to devise strategies for the expression of appropriate amounts of PEPC activity in C_3 cells to impart C_4 characteristics.

Implications for possible genetic engineering to increase primary productivity

If the mechanisms controlling the expression of PPDK and PEPC can be discovered and ultimately manipulated, how might such knowledge be used for

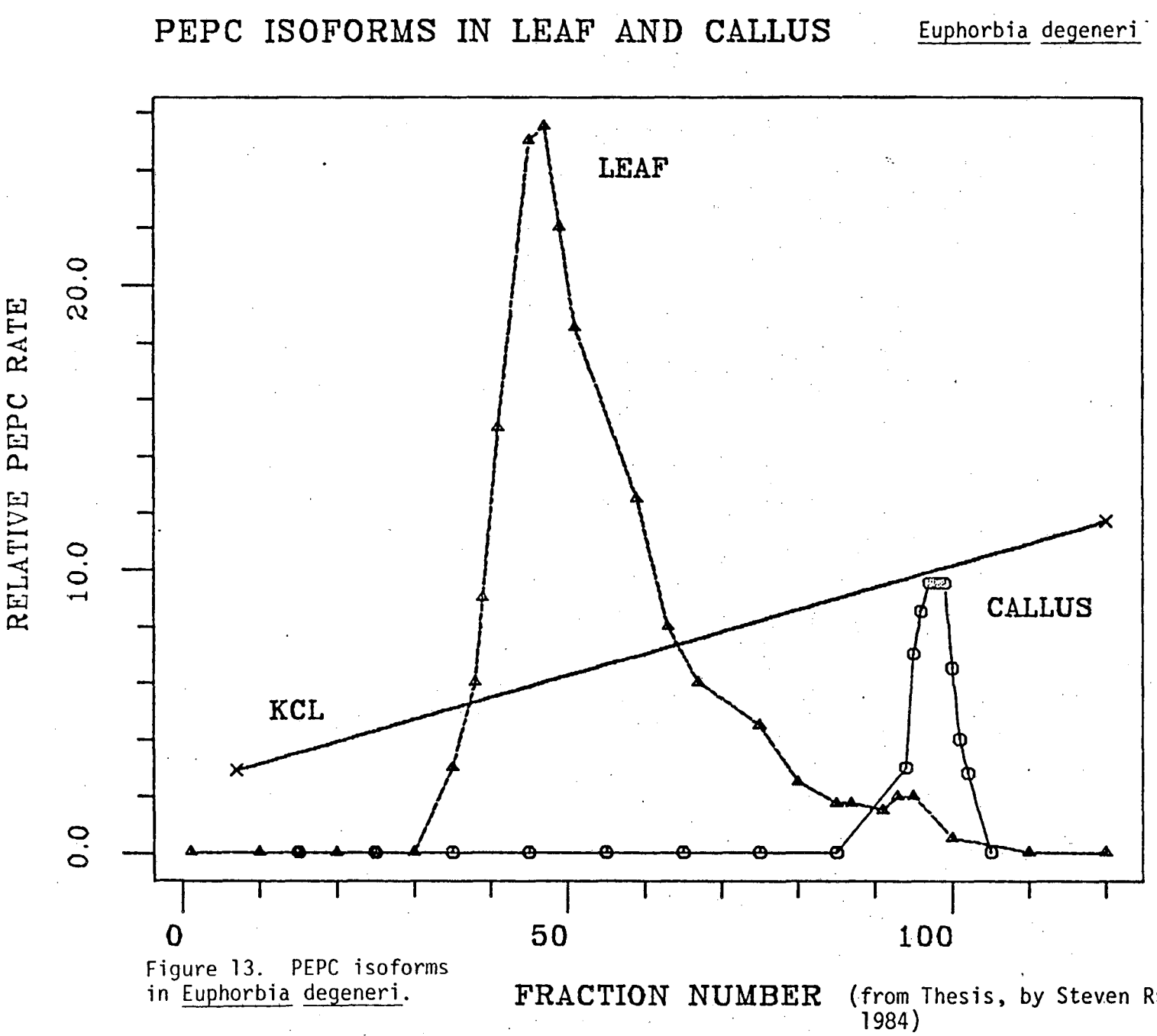

Figure 13. PEPC isoforms in Euphorbia degeneri. (from Thesis, by Steven Ruzin, 1984)

the improvement in the primary productivity of C_3 plants? Could some aspects of C_4 metabolism be usefully imparted to C_3 plants? Would there be any gain in net photosynthesis if, for example, gene expression could be manipulated to increase expression of PPDK (and some decarboxylating enzyme such as malic enzyme) in the chloroplasts and of PEPC in the cytoplasm, thus providing an intracellular CO_2 shuttle (Fig 14)?

Kranz anatomy found in C_4 plant leaves might be essential for the greater efficiency of the primary photosynthetic process in these plants. The mesophyll and bundle sheath cells in the Kranz anatomy are separated by a cell wall (sometimes thick and cometines containing containing suberin) which restricts back-flow of CO_2 and HCO_3^- to plasmadesmata and thus conserves the gradient in CO_2 level between the two cell types created by the C_4 transport (Hatch and Osmond, 1976). Certainly the apparent inverse expression of PPDK and RuBPC small subunit in the mesophyll and bundle sheath cells of C_4 leaves suggests that separation of photosynthetic carbon fixing functions has been advantageous and therefore selected by evolution.

Nevertheless, if there is any substantial limitation to flow of CO_2 from cytoplasm into chloroplasts in C_3 cells during photosynthesis and under conditions favoring photorespiration, carbon shuttling could be advantageous. A possible reason for the higher CO_2 compensation point of isolated spinach cells as compared with Chlorella grown in air has been suggested (Tsuzuki et al.,1981). The absence of carbonic anhydrase in cytoplasm of green cells was considered to be a a possible cause of slower diffusion of CO_2 through the cytoplasm in the larger green cells of leaves, as compared with algal cells. There might therefore be some difference in higher plant cells between

Possible C_4 metabolism in single cell

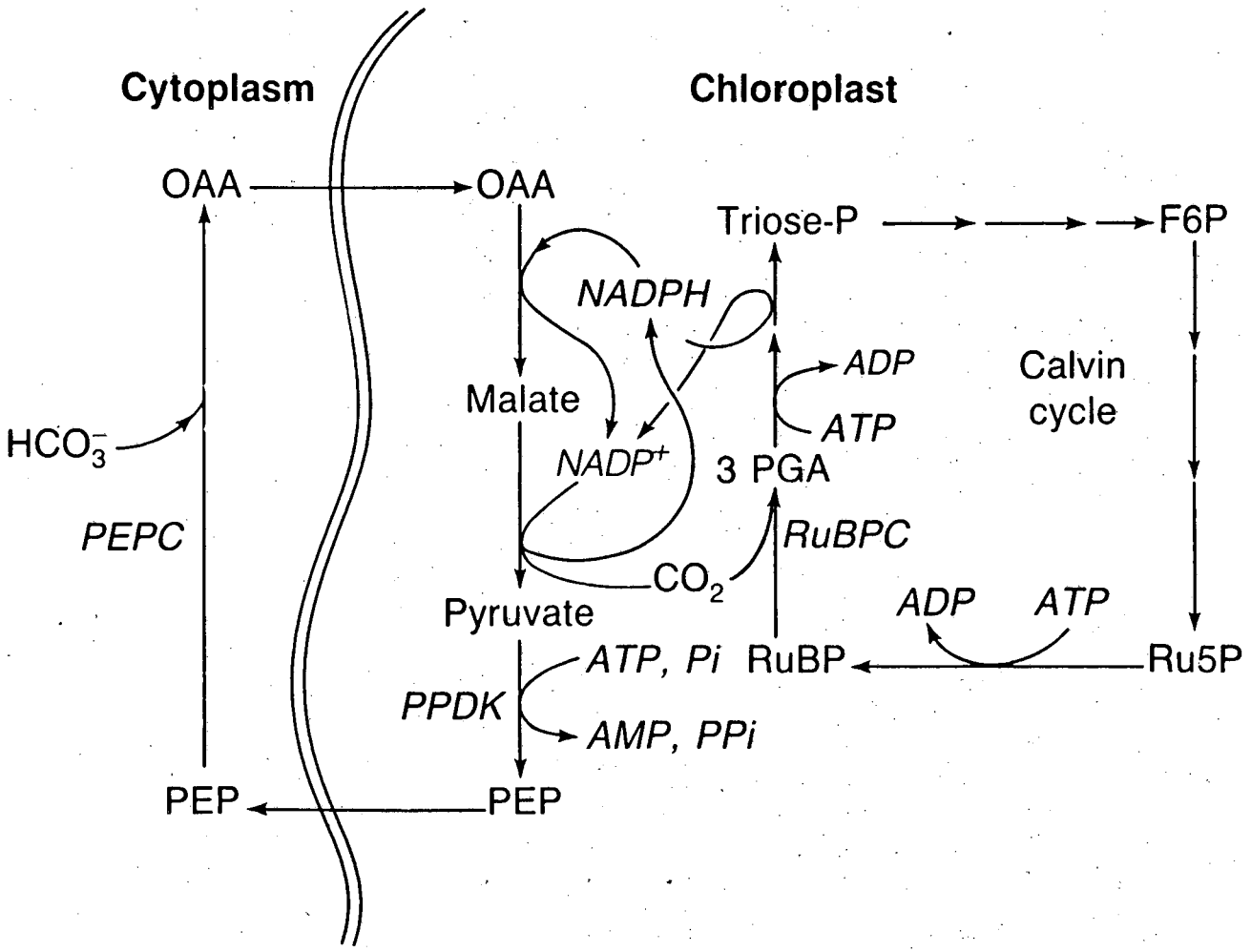

Figure 14. Hypothetical intracellular C_4 pathway

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Abbreviations as in Figure 1

the average CO_2 concentration in the cytoplasm and that in the stroma. If so, a carbon shuttle in such cells could result in an increase in stromal CO_2 level. Even a small increase in stromal CO_2 could be somewhat beneficial in overcoming the negative effects of photorespiration.

Is it possible that some C_3 plants already make limited use of some type of shuttle? There has been considerable research on possible intermediate C_3-C_4 species (for review, see Ray and Black, 1979). Such species may be intermediate between C_4 and C_3 plants by one or more of the several criteria of C_4-ness. These criteria include low CO_2 compensation point, leaf anatomy, insensitivity to O_2 pressure change, increasing net photosynthesis even to high light intensities, $\delta^{13}C$, etc. An enzymatic criterion has been the relative levels of RuBPC and PEPC activities.

In view of the finding that PPDK may be widely distributed in C_3 leaf tissues, it might prove interesting to examine levels of PPDK in these intermediate species, using the sensitive immunoprecipitation method, since some PEPC but perhaps no PPDK is required for non-photosynthetic metabolism in photosynthetic leaf cells. It might be that PPDK presence is in fact a good criterion, since formation of PEP from pyruvate would be required for a shuttle unless a different decarboxylating enzyme such PEP carboxykinase is present in the chloroplasts.

Of particular interest would be plants such as Helianthus annuus (sunflower) that have a response to light intensity and CO_2 concentration intermediate between C_3 and C_4 plants (Hesketh, 1963), but no apparent C_4 leaf anatomy.

Other questions arise with respect to possible intracellular C_4-like metabolism. For example, the ATP used by PPDK in the chloroplasts should be generated by increased cyclic photophosphorylation and the balance between ATP and NADPH production should not change in such a way as to upset delicate regulatory relations in the reductive pentose phosphate cycle. There are other questions of transport and regulation to be considered, and other hypothetical pathways one could propose.

The value of such questions and speculations is to acquaint us with the various kinds of research which must be done before we can hope to improve on nature by tampering with central intermediary metabolism and photosynthesis. Much more must be learned about the expression of specific genes and groups of genes in eukaryotic plant cells as a function of tissue, physiology, and environment before such "engineering" can even be attempted. In the meantime, the science of genetic engineering in plants can advance and receive some encouragement from progress towards other important goals such as increased resistance to disease, drought, cold, and pesticides, and improvements in yields of specific biosynthetic products, athough these achievements will not come easily either.

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