
Photosynthesis

Carbon Metabolism

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1. Introduction

Photosynthetic organisms capture light energy which they use to form ATP and reducing power. Knowledge of the mechanisms involved in this process was surveyed last year by J. AMESZ. This year we shall examine carbon metabolism during photosynthesis with emphasis on the literature published since the reviews in Volume 34 of *Fortschritte der Botanik*.

It is now generally agreed that all photosynthetic organisms possess the RPP cycle (Calvin cycle) for the reductive assimilation of CO₂. Recent research has concentrated on regulatory aspects of this cycle, particularly at the level of the individual enzymes and also in relation to the movement of metabolites between the chloroplast and cytoplasm. In addition, the loss of considerable amounts of fixed carbon during photorespiration is a problem being extensively studied. Indeed, a group of plants which do not lose carbon from photorespiration is currently the subject of intensive research and the observations reported so far not only explain how photorespired CO₂ is retained, but also clearly demonstrate that the metabolism of carbon compounds during photosynthesis involves metabolic pathways which can differ in importance quite significantly from one type of photosynthetic tissue to another.

2. Enzymology of the Reductive Pentose Phosphate Cycle

The photosynthetic assimilation of CO₂ is catalysed by the twelve enzymes of the RPP cycle. Initially, RuDP is carboxylated to produce two molecules of PGA in an irreversible, rate-limiting reaction (BASSHAM and KRAUSE) catalysed by RuDP carboxylase. A presumed 6C intermediate, 2-carboxi-3-ketoribitol-1,5-diP, was recently synthesised by SIEGEL and LANE and shown to be most probably involved in the reaction.

In the past the observed affinity of isolated RuDP carboxylase for CO₂ was much lower than the affinity of chloroplasts for CO₂. Although the enzyme affinity was later shown to be increased by high concentrations of Mg²⁺ (BASSHAM et al., 3) or by the presence of fructose-6-P (BUCHANAN and SCHÜRMANN, 1), values reported were still only one-quarter that of the chloroplast. Studies by BAHR and JENSEN (1, 2)

Abbreviations: GAP, glyceraldehyde-3-P; PEP, P-enolpyruvate; PGA, 3-P-glycerate; RPP, reductive pentose phosphate; RuDP, ribulose-1,5-diP.

have now confirmed that the enzymes from spinach and maize have affinities for CO_2 equal to that of the chloroplast, but are unstable and rapidly convert to forms with low affinity following isolation. A relatively high affinity was also recently observed for the spinach enzyme at pH 9 by LYTTLETON.

The instability of RuDP carboxylase and the effects of Mg^{2+} and fructose-6-P may be related to the complex subunit structure of this protein; the entire molecule possibly consists of eight large and eight small subunits (see review by SIEGEL et al.). Kinetic (NISHIMURA et al.) and immunological (GRAY and KEKWICK; NISHIMURA and AKAZAWA, 1) experiments indicate that the larger subunit contains the catalytic site for RuDP and CO_2 , while the regulatory influence of Mg^{2+} is effected through the smaller subunit. NISHIMURA and AKAZAWA (2) are now able to reconstitute the spinach enzyme from the constituent subunits and show partial recovery of specific activity.

Other experiments have emphasised the somewhat unusual physical characteristics of RuDP carboxylase. Thus KWOK and WILDMAN found that the substrate RuDP greatly increased the solubility and altered the tertiary structure of the crystalline enzyme from tobacco. In addition, in at least one procaryotic organism RuDP carboxylase was found associated with polyhedral inclusions which are often observed in chemototrophic bacteria and blue-green algae (SHIVELY et al.).

The activity of RuDP carboxylase is stimulated by fructose-6-P (possibly derived in the chloroplast from a light-activated fructose diphosphate, BUCHANAN and SCHÜRMANN, 1) and strongly inhibited by 6-P-gluconate (CHU and BASSHAM, 1; TABITA and McFADDEN); the latter effect may explain the inactivation of the carboxylation reaction in the dark when 6-P-gluconate levels in the leaf are higher (BASSHAM, 1). Reactivation in the light may involve a separate small protein (WILDNER and CRIDDLE), although an increased level of Mg^{2+} could also explain this phenomenon (WALKER, 1). Interestingly, although 6-P-gluconate inhibits the catalytic activity of the enzyme, this metabolite can activate the enzyme during preincubation (BUCHANAN and SCHÜRMANN, 1; CHU and BASSHAM, 2). Details of these regulatory effects have been recently reviewed (BASSHAM, 2; BUCHANAN and SCHÜRMANN, 2). Activation of RuDP carboxylase is also reported to be a component of the stimulation of CO_2 fixation in isolated chloroplasts by antimycin A (CHAMPIGNY and MIGINIAC-MASLOW; SCHACTER and BASSHAM).

Following carboxylation, the two subsequent steps of the RPP cycle effect the reduction of PGA to GAP utilising ATP and reduced NADP and catalysed by the enzymes PGA kinase and GAP dehydrogenase. A specific chloroplast PGA kinase has been demonstrated (ANDERSON and ADVANI) and shown to be regulated by the extent of phosphorylation of the adenylate pool (PACOLD and ANDERSON). Similar regulation of the cytoplasmic enzyme from other sources has been reported (KRIETSCH and BÜCHER).

The second enzyme, GAP dehydrogenase, presumably uses NADP during photosynthesis, although the isolated enzyme will use NAD as well as NADP. These two activities are probably associated with the same enzyme molecule (VACCHI et al.; MCGOWAN and GIBBS). The chloroplast enzyme may be a modified form of the cytoplasmic NAD-linked GAP dehydrogenase (CERFF and QUAIL). However, MCGOWAN and GIBBS have recently obtained evidence from immunological and isoelectric point data that the structure of the pea chloroplast enzyme is considerably different to that of the pea cytoplasmic enzyme and is therefore unlikely to be a modified form of the cytoplasmic enzyme.

GAP dehydrogenase in chloroplasts is activated upon illumination (ZIEGLER and ZIEGLER). Light activation does not require protein synthesis since only the latter is sensitive to X-rays (SEUBERLING). Rather, the effect of light may be related to the reported *in vitro* activation by reduced dithiols (MÜLLER et al.; ANDERSON and LIM). Indeed, there are indications that the enzymes from spinach (PAWLIZKI and LATZKO) and beans (BRADBEER et al.) gain NADP-linked activity and undergo dissociation in the presence of dithiothreitol. Natural dithiols may activate enzymes *in vivo*; AHMADI and TING have found that the chloroplast NADP-linked malate dehydrogenase is activated by reduced lipoate. Besides dithiols, NADP is also reported to cause subunit dissociation of GAP dehydrogenase, and simultaneously the affinity for NADP increased (PUPILLO and PICCARI).

The remainder of the RPP cycle regenerates RuDP from GAP. This process could be regulated at the steps catalysed by fructose diphosphatase and ribulose-5-P kinase; both of these enzymes are activated during illumination. Light activation of ribulose-5-P kinase (LATZKO et al., 1) has now been examined in intact chloroplasts and suggested to be mediated by a photoproduced reductant (AVRON and GIBBS, 1); in the dark the residual activity remaining could be inhibited by 6-P-gluconate (ANDERSON).

Fructose diphosphatase activation may involve a reduction mediated by ferredoxin (BUCHANAN et al., 1). Alternatively, the greater activity could result from an elevated pH (HELDT et al., 2) and an increased concentration of Mg^{2+} (LIN and NOBEL) induced in the chloroplast stroma upon illumination. These factors may work together to activate the chloroplast fructose diphosphatase, which has ideal kinetic properties for such a response (GARNIER and LATZKO). The Mg^{2+} -induced decrease in the pH optimum recently reported for the crystalline enzyme (EL-BADRY) is also consistent with this proposal.

Reports have implicated a reductive carboxylic acid cycle as an alternative to the RPP cycle for the photoassimilation of CO_2 in some photosynthetic bacteria (BUCHANAN et al., 2). Although feasible, some enzymological criteria (BEUSCHER and GOTTSCHALK; BOTHE et al.) for this scheme may be questioned, and in particular one of these organisms does not lack RuDP carboxylase (TABITA et al.) as previously suspected (BUCHANAN et al., 2). A second proposal for the reductive assimilation of CO_2 , this time involving a direct reduction of CO_2 to formate, was recently advanced to explain the anomalous labelling of some organic acids after $^{14}CO_2$ fixation by intact leaves (KENT; KENT et al.). While the reduction itself was not directly demonstrated, it has been shown possible in an aerobic species of *Pseudomonas* (HÖPNER et al.).

3. Studies with Isolated Chloroplasts

It is now possible to isolate chloroplasts capable of fixing CO_2 at rates comparable with *in vivo* rates of photosynthesis (see GIBBS). The rates of CO_2 fixation observed are very dependent on pH (AVRON and GIBBS, 2) and, although the chloroplast is impermeable to protons, the external pH influences the level of bicarbonate which accumulates in the stroma following the diffusion of CO_2 (WERDAN and HELDT), and probably influences the transport of other metabolites during photosynthesis. The pH of the interior of the chloroplast has also been recognised to influence CO_2 fixation: WERDAN and HELDT have proposed

that the pH rise in the stroma which follows the light-induced transport of protons into the thylakoids (HELDT et al., 2) could act as a CO₂-pump by altering the equilibrium of the reaction catalysed by carbonic anhydrase in favour of bicarbonate accumulation. Carbonic anhydrase is a zinc-containing protein (see POKKER and NG) largely localised in chloroplasts (EVERSON and SLACK; POINCELOT).

Photosynthetic CO₂ fixation by isolated chloroplasts is inhibited by free Mg²⁺ in the reaction medium (AVRON and GIBBS, 2). Substances able to complex Mg²⁺, such as pyrophosphate, will relieve this inhibition (FORTI and ROSA). The level of Mg²⁺ in the reaction medium can also affect product distribution, as shown by BALDREY and COOMBS with their technique of illuminating chloroplasts on filter paper discs.

Pyrophosphate can also influence CO₂ fixation through an effect other than Mg²⁺ chelation: the pyrophosphate may be hydrolysed by pyrophosphatase (released from ruptured chloroplasts) and so act as a source of inorganic phosphate for photosynthesis (SCHWENN et al.). The effects of a protein factor earlier reported to regulate photosynthesis (BASSHAM et al., 1) have now been attributed to pyrophosphatase activity present in this factor (LILLEY et al., 1; LEVINE and BASSHAM).

Illuminated chloroplasts show a delay of several minutes before a steady rate of CO₂ fixation is attained (TURNER et al.). This delay is believed to represent the time required to accumulate intermediates of the RPP cycle (LATZKO and GIBBS; WALKER et al.), and may ultimately relate to a short supply of photosynthetically generated ATP which is reported to be rate limiting for CO₂ fixation (HEBER, 1; HEBER and KIRK). Indeed, rapid attainment of steady state levels has been shown to depend in chloroplasts (SCHÜRMANN et al.) and in *Chlorella* cells (KLOB et al.) on ATP from cyclic photophosphorylation, possibly to boost the level of the CO₂ acceptor RuDP. This, in turn, may be dependent on light activation of ribulose-5-P kinase (LATZKO et al., 1). The actual carboxylation of RuDP is probably not a primary cause of the delay, although light activation of RuDP carboxylase does influence the process (WALKER, 1); results of more recent experiments (LILLEY et al., 2) suggest that the Mg²⁺ activation of RuDP carboxylase (BASSHAM et al., 3) expected following light-induced Mg²⁺ accumulation in the stroma (LIN and NOBEL) occurs more rapidly than the development of a steady rate of photosynthesis.

A new and interesting approach to the study of chloroplast metabolism is the development of the reconstituted chloroplast system (STOKES and WALKER). In this system osmotically shocked chloroplasts, supplemented with a soluble fraction derived from chloroplasts and catalytic quantities of cofactors, show light dependent O₂ evolution in the presence of PGA. More recently, BASSHAM et al. (2) have reported *in vivo* rates of CO₂ fixation in a reconstituted system. These authors provide data indicating that high rates of CO₂ fixation are more dependent on a high concentration of soluble chloroplast components in the vicinity of chlorophyll than on an intact chloroplast envelope.

4. Communication between Chloroplast and Cytoplasm

Of the intermediates of the RPP cycle only the pentose monophosphates, triose phosphates and PGA are now believed to pass freely between the

chloroplast and cytoplasm (see WALKER, 2), although the absolute permeability of the chloroplast envelope can clearly be altered (NOBEL). An elegant series of experiments by HELDT and RAPLEY has demonstrated that the triose phosphates and PGA are actively transported by a translocator on the inner membrane (HELDT and SAUER) of the chloroplast envelope. Recently, DOUCE et al. found ATPase activity associated with isolated chloroplast envelopes and speculated that this ATPase may be involved in such active transport.

These observations and others showing accumulation of triose phosphates in the medium of photosynthesising chloroplasts indicate that assimilated carbon can be exported from chloroplasts as triose phosphates. These may then be metabolised to sucrose since sucrose-synthesising enzymes predominate in the cytoplasm (BIRD et al., 3); earlier conclusions to the contrary may have resulted from faults in (BIRD et al., 1) or improper use of (HEBER, 2) the technique of non-aqueous isolation of chloroplasts. The presence of a separate fructose diphosphatase in the cytoplasm of green leaves (LATZKO et al., 3) is consistent with the cytoplasmic location of sucrose biosynthesis. On the other hand, starch is synthesised inside the chloroplast in green leaves (see PREISS and KOSUGE) and is presumably metabolised to sugar phosphates when export from the chloroplast becomes necessary.

In the cytoplasm the complete glycolytic sequence may be restricted during photosynthesis because of inhibition of the phosphofructokinase reaction by ATP (HIRT and TANNER); plant phosphofructokinase is strongly inhibited by free ATP (KELLY and TURNER). However, the latter portion of glycolysis could continue to operate and convert triose phosphates exported from the chloroplast to pyruvate. This pyruvate may enter the tricarboxylic acid cycle which continues to operate in the light (RAVEN; CHAPMAN and GRAHAM), and probably supplies dicarboxylic acid carbon skeletons for the synthesis of amino acids reported to occur in chloroplasts (KIRK and LEECH; MAGALHAES et al.; see GIVAN and LEECH). In fact, these dicarboxylic acids could be actively transported into chloroplasts by a membrane-located dicarboxylate translocator (HELDT and RAPLEY).

In contrast to the free movement of some carbon compounds, photosynthetically generated ATP (HELDT et al., 1) and reduced NADP (ROBINSON and STOCKING) cannot cross the chloroplast envelope. Nevertheless, these substances can be indirectly transferred to the cytoplasm through a shuttle whereby PGA enters the chloroplast and is reduced to triose phosphate, which returns to the cytoplasm where it is converted back to PGA with the production of ATP (HEBER and SANTARIUS; KRAUSE) and reduced NAD (STOCKING and LARSON). Alternatively, reduced NADP may be produced in the cytoplasm when the triose phosphate is directly oxidised to PGA by the non reversible GAP dehydrogenase (KELLY and GIBBS, 2). The properties of this enzyme, particularly the high affinity for NADP (KELLY and GIBBS, 1), seem ideally suited to this role. Reducing equivalents may also be transferred via a malate-oxaloacetate shuttle which can move reducing power either into or out of the chloroplast (HEBER and KIRK).

Two detailed and thorough reviews on the flow of metabolites between the chloroplast and cytoplasm (HEBER, 2; WALKER, 2) are in press at the time of this writing.

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