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Short Communication

Autocatalysis in a Reconstituted Chloroplast System[1]

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ABSTRACT

In whole plants and intact chloroplasts, photosynthesis does not reach its full rate immediately upon illumination but only after a lag which is believed to reflect an autocatalytic increase in the concentration of carbon cycle intermediates. Autocatalysis has now been observed in a reconstituted system containing envelope-free chloroplasts augmented with ferredoxin and other stromal proteins but only catalytic amounts of ATP and NADP. With ribose 5-phosphate as substrate, the CO_2 dependent O_2 evolution recorded for such mixtures implies rates of "endogenous" or ferredoxin-dependent photophosphorylation as high as 360 μmoles of orthophosphate esterified mg^{-1} chlorophyll hr^{-1}.

An essential feature of the Benson-Calvin cycle (5, 16) is its ability to function as an autocatalytic "breeder" reaction, producing more ribulose diphosphate than it consumes. One-sixth of the 3-P-glycerate formed by carboxylation may be regarded as product, the remainder undergoing rearrangement in the reactions which lead to the regeneration of the CO_2 acceptor. In theory, all of the product can also feed back into the cycle and if it does the concentration of the acceptor will double once every five revolutions (14, 16). The autocatalytic aspect of photosynthesis was put forward as an explanation of induction by Osterhout and Haas (13) long before the cycle was formulated. Subsequently, the elucidation of the cycle (5) and the separation of intact function chloroplasts led to direct experimental support of its validity (8, 16).

In the intact isolated chloroplast, autocatalysis is jeopardized by the loss of newly formed products to the external medium but nevertheless the presence of the chloroplast envelope and its impermeability to compounds such as ribulose diphosphate ensures that induction is short unless the export of products is deliberately accelerated by the addition of high concentrations of Pi to the external medium (8, 10, 14, 15). In these and other circumstances the initial lag may be shortened by the addition of those intermediates of the cycle which are known to penetrate the envelope (8, 10, 14–16).

At first, the possibility of observing autocatalysis in a reconstituted system containing only envelope-free chloroplasts seemed remote (17). Clearly, any physical compartmentation consequent upon the presence of the intact envelope would be lost and those photosynthetic products which might otherwise be retained would be free to escape to the external medium. Moreover, although rates as high as 170 μmoles of O_2/mg Chl·hr were obtained by resorting to the use of abnormally high protein/Chl ratios (17), these were only seen in the presence of exogenous metabolites and substrate concentrations of ATP. Since this earlier work, however, the Mg activation of ribulose diP carboxylase in chloroplast extracts has been considerably clarified (3, 12, 14–16) and cycling has been reported in mixtures containing excess protein and 0.5 mM NADP (4). More recently, further minor changes in procedure (made in the light of these advances) have enabled us to obtain good rates of CO_2-dependent O_2 evolution in mixtures containing only catalytic quantities of ATP, NADP, and sugar phosphates. For example, Figure 1 shows the course of CO_2-dependent O_2 evolution following illumination of such a mixture. The maximum rate achieved was 57 μmoles of O_2/mg Chl·hr, and the total evolution (>1.0 μmole) was considerably in excess of that which could result either from the stoichiometric consumption of added hexose phosphate (about 0.1 μmole O_2) or added ATP + NADP (about 0.05 μmole O_2).

In reaction mixtures otherwise identical to those used for Figure 1 but containing substrate concentration of 2 μmoles of ATP and substrate concentrations of either 1 μmole of R5P[2] or 1 μmole of PGA, the maximum rates were 125 and 176 μmoles O_2/mg Chl·hr, respectively. The latter is close to the uncoupled rate of O_2 evolution observed in these mixtures with substrate concentrations of NADP as a Hill oxidant.

Under similar conditions we have also observed what we believe to be the first convincing evidence of autocatalysis in an envelope-free system. Thus, in Figure 2, it is seen that in the absence of ribose-5-P, O_2 evolution (continuous line) and CO_2 fixation (crosses) commenced more or less simultaneously. They then accelerated gradually, and at an equal rate, for several minutes. In the presence of R5P this prolonged induction period was shortened, as it is with intact chloroplasts (6, 14) except that the maximum rate of CO_2 fixation (open circles) was reached almost 3 min before the corresponding maximum for O_2 evolution (continuous trace). This delay in the advent of O_2 evolution can be related, in part, to buildup of 3-P glycerate in the medium but, while present in relative excess, ribose-5-P also competes for ATP and, in so doing, adversely affects the ATP/ADP balance required for optimal O_2 evolution (Lilley and Walker, unpublished).

These results suggest that if sufficiently high concentrations of stromal protein are added to mixtures containing envelope-free chloroplasts (and catalytic quantities of ATP and NADP) the partial reactions of the cycle will commence to operate at

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[2] Abbreviation: R5P: ribose 5-phosphate.

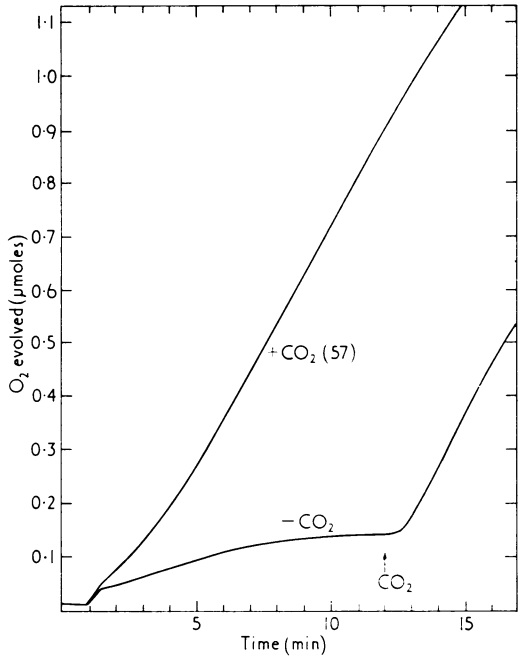

FIG. 1. CO_2-dependent O_2 evolution in a reconstituted chloroplast system containing catalytic ATP and catalytic hexose phosphate. Envelope-free chloroplasts and chloroplast extracts were prepared from intact spinach chloroplasts as previously described (12). The volume of chloroplast extract was decreased to about one-third by dialysis (Sartorius membrane filter) for 1 hr against dilute (one-tenth) resuspending medium prior to use. Assays (for details of illumination and O_2 measurement see ref. 7) were carried out at 20 C in 1 ml of resuspending medium containing 0.33 M sorbitol, 50 mM HEPES, 1 mM EDTA, and 10 mM KCl adjusted to pH 7.9 with KOH. In addition each mixture contained 110 units of catalase (Sigma), 0.1 μmole of ATP, 0.1 μmole of NADP, 90 μg, 7.5 nmoles of ferredoxin, 0.05 μmole of fructose-6-P, 0.05 μmole of fructose-1,6-diP, 2 μmoles of Pi, 5 μmoles of $MgCl_2$, envelope-free chloroplasts (100 μg of Chl), and chloroplast extract (soluble protein from chloroplasts containing 600 μg of Chl). One, as indicated, also contained 10 μmoles of $NaHCO_3$.

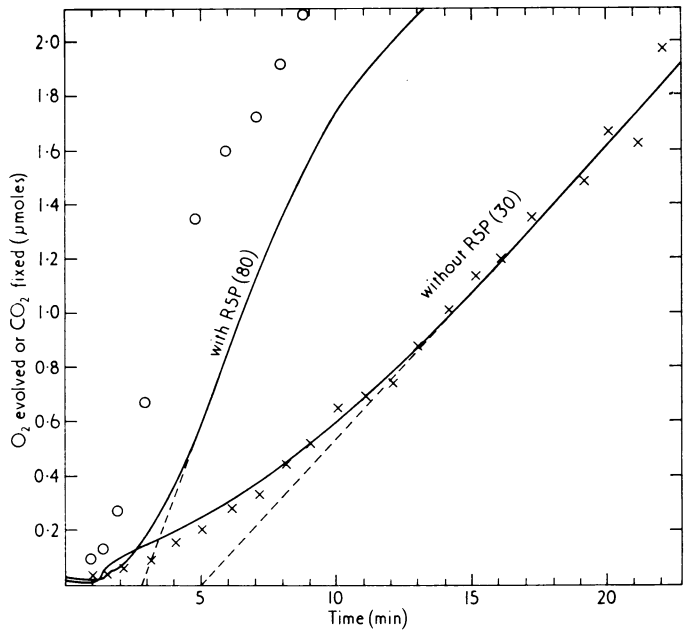

FIG. 2. Autocatalysis in a reconstituted chloroplast system. Experimental details and concentrations as for Fig. 1 except that the assay volumes were increased to 2 ml and the concentration of stromal protein was doubled. Both mixtures contained 18 μmoles of $NaHCO_3$ and the hexose phosphate was omitted. R5P (1 μmole) was added to one mixture as indicated. Immediately prior to illumination $NaH^{14}CO_3$ (2 μmoles 120 μCi) was added to both and simultaneous measurements of O_2 and CO_2 carried out as previously described (6, 12). The continuous lines are O_2 electrode traces, the circles (+R5P) and the crosses (−R5P) record $^{14}CO_2$ fixed into acid stable products. Maximal rates are given in parentheses as μmoles CO_2 or O_2/mg^{-1} Chl·hr^{-1}.

reasonably good rates even though they depend entirely on the presence of small quantities of endogenous substrate which will persist in the partially dialyzed (see legend to Fig. 1) chloroplast extract. The entire system will then not only cycle (see Fig. 1 and ref. 4) but also accelerate (Fig. 2) in the same autocatalytic fashion as does the intact organelle or the whole plant. In addition, if it is assumed that CO_2-dependent O_2 evolution requires the formation and consumption of 3 molecules of ATP per O_2, then the rate of endogenous photophosphorylation seen in the presence of R5P in Figure 2 must be 240 μmoles/mg Chl·hr. Rates as high as 120 for O_2 evolved (and therefore 360 Pi esterified) have been recorded with different chloroplast preparations under otherwise identical conditions or with catalytic ADP in place of catalytic ATP. These rates are of the same order as those normally observed with pyocyanine (2) and are entirely consistent with earlier reports of fast rates of cyclic and noncyclic photophosphorylation supported by ferredoxin (1). They indicate that it is only necessary to use agents such as pyocyanine (in order to promote rapid rates of ATP formation) if there is insufficient stromal protein (including ferredoxin) in the reaction mixture (*cf.* 2). It may be noted that the total quantity of ferredoxin used in these experiments (exogenous + endogenous = about 108–125 μg/ml) is considerably higher than that normally present in the intact plastid (about 3 μg ferredoxin/100 μg Chl [9]), but that its concentration in a 1- or 2-ml reaction mixture (about 0.01 mM) is only one-tenth of that within the intact chloroplast, assuming a stromal volume of 2.3 μl/100 μg Chl (11).

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LITERATURE CITED

1. ARNON, D. I., H. Y. TSUJIMOTO, AND B. D. MCSWAIN. 1967. Ferredoxin and photosynthetic phosphorylation. Nature 214: 562–566.
2. AVRON, M. AND M. GIBBS. 1974. Carbon dioxide fixation in the light and in the dark by isolated spinach chloroplasts. Plant Physiol. 53: 140–143.
3. BAHR, J. T. AND R. G. JENSEN. 1974. Ribulose diphosphate carboxylase from freshly ruptured spinach chloroplasts having an *in vivo* $Km[CO_2]$.[1] Plant Physiol. 53: 39–44.
4. BASSHAM, J. A., G. LEVINE, AND J. FORGER, III. 1974. Photosynthesis *in vitro*. I. Achievement of high rates. Plant Sci. Lett. 2: 15–21.
5. CALVIN, M. AND J. A. BASSHAM. 1962. The Photosynthesis of Carbon Compounds. Benjamin, New York.
6. COCKBURN, W., D. A. WALKER, AND C. W. BALDRY. 1968. Photosynthesis by isolated chloroplasts. Reversal of orthophosphate inhibition by Calvin cycle intermediates. Biochem. J. 107: 89–95.
7. DELIEU, T. AND D. A. WALKER. 1972. An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. New Phytol. 71: 201–225.
8. GIBBS, M. 1971. Carbohydrate metabolism by chloroplasts. *In:* M. Gibbs, ed. Structure and Function of Chloroplasts. Springer-Verlag, Berlin. pp. 169–214.
9. HASLETT, B. G., R. CAMMACK, AND F. R. WHATLEY. 1973. Quantitative studies on ferredoxin in greening bean leaves. Biochem. J. 136: 697–703.
10. HEBER, U. 1974. Metabolite exchange between chloroplast and cytoplasm. Annu. Rev. Plant Physiol. 25: 393–421.
11. HELDT, H. W., F. SAUER, AND L. RAPLEY. 1971. Differentiation of the permeability properties of the two membranes of the chloroplast envelope. Progress in Photosynthesis, Vol. II. Dr. W. Junk N.V., The Hague. pp. 1345–1355.

12. LILLEY, R. MCC., K. HOLBOROW, AND D. A. WALKER. 1974. Magnesium activation of photosynthetic CO_2 fixation in a reconstituted chloroplast system. New Phytol. 73: 659–664.
13. OSTERHOUT, W. J. V. AND A. R. C. HAAS. 1918. On the dynamics of photosynthesis. J. Gen. Physiol. 1: 1–16.
14. WALKER, D. A. 1973. Photosynthetic induction phenomena and the light activation of ribulose diphosphate carboxylase. New Phytol. 72: 209–235.
15. WALKER, D. A. 1974. Chloroplast and cell—Concerning the movement of certain key metabolites *etc.* across the chloroplast envelope. *In:* D. H. Northcote, ed., Med. Tech. Publ. Int. Rev. Sci. Biochem. Ser. I, Vol. 11. Butterworth, London. pp. 1–49.
16. WALKER, D. A. 1972. Some characteristics of a primary carboxylating mechanism. *In:* Phytochemical Society Symposium on Plant Carbohydrate Biochemistry, Edinburgh (1972). Academic Press, New York. pp. 7–26.
17. WALKER, D. A., A. V. MCCORMICK, AND D. M. STOKES. 1971. CO_2-dependent oxygen evolution by envelope-free chloroplasts. Nature 233: 346–347.