Phytochemistry, 1971, Vol. 10, pp. 579 to 584. Pergamon Press. Printed in England.

CHANGES IN THE PHOSPHATE COMPOUNDS IN STRAWBERRY LEAVES DURING A DARK–LIGHT–DARK TRANSITION IN RELATION TO SUCROSE BIOSYNTHESIS

F. A. ISHERWOOD and R. R. SELVENDRAN

A.R.C. Food Research Institute, Colney Lane, Norwich

(*Received* 6 *May* 1970)

Abstract—The concentrations of phosphate esters, nucleotides and sugars in strawberry leaves were measured during transition from dark to light to dark again. The period in the light varied from 2 to 30 min. The rapid changes in the concentration of some of the phosphate compounds which occurred within the first few minutes were consistent with the operation of the photosynthetic carbon reduction cycle of Bassham and Calvin. A study of those intermediates which were likely to be involved in sucrose synthesis indicated that uridine diphosphate glucose and fructose-6-phosphate were the most probable precursors of sucrose.

INTRODUCTION

IN THE present study, an analysis was made of the changes in concentration of those intermediates of the photosynthetic carbon reduction cycle which were likely to be concerned in the synthesis of sucrose[1–9] when the leaves were given a short exposure to light; a brief exposure to light was chosen because the concentrations of the initial phosphorylated intermediates were principally affected without major changes in the rest of the system. The results of this analysis were compared with those which might be expected from the operation of the cycle.

The extraction technique and analytical method used[10] enabled one to identify the phosphate compounds present and because other interfering solutes were eliminated, also allowed the individual compounds to be separated and measured either chromatographically or enzymically with greater certainty than earlier methods.

The experiments described involve a dark–light–dark transition, the CO_2 remaining at normal atmospheric level and the temperature about 20°. Under these conditions, sucrose is rapidly synthesized in leaves in the light.

RESULTS

The two sets of experiments are described separately since the first set covered a single period of 30 min in the light and the main object was to determine the type of change which

[1] C. E. CARDINI, L. F. LELOIR and J. CHIRIBOGA, *J. Biol. Chem.* **214**, 149 (1955).
[2] L. F. LELOIR and C. E. CARDINI, *J. Biol. Chem.* **214**, 157 (1955).
[3] J. F. TURNER, *Biochem. J.* **67**, 450 (1957).
[4] N. C. GANGULI and W. Z. HASSID, *Plant Physiol.* **32**, 54 (1957).
[5] E. S. ROREM, H. G. WALKER JR. and R. M. MCCREADY, *Plant Physiol.* **35**, 269 (1960).
[6] J. V. DUTTON, A. CARRUTHERS and J. F. T. OLDFIELD, *Biochem. J.* **81**, 266 (1961).
[7] M. D. HATCH, J. A. SACHER and K. T. GLASZIOU, *Plant Physiol.* **38**, 338 (1963).
[8] C. R. STOCKING, G. R. WILLIAMS and A. ONGUN, *Biochim. Biophys. Res. Commun.* **10**, 416 (1963).
[9] I. F. BIRD, H. K. PORTER and C. R. STOCKING, *Biochim. Biophys. Acta* **100**, 366 (1965).
[10] F. A. ISHERWOOD and F. C. BARRETT, *Biochem. J.* **104**, 922 (1967).

occurred, while the second set covered periods of 2, 15 and 30 min in the light and the object was to determine the rate at which the changes occurred.

First Set of Experiments

The changes in the concentration of glucose-6-phosphate, 3-phosphoglycerate, UDP-glucose, fructose-6-phosphate, ATP, glucose-1-phosphate, and fructose-1,6-diphosphate are shown in Fig. 1a and those for the sugars in Fig. 1b.

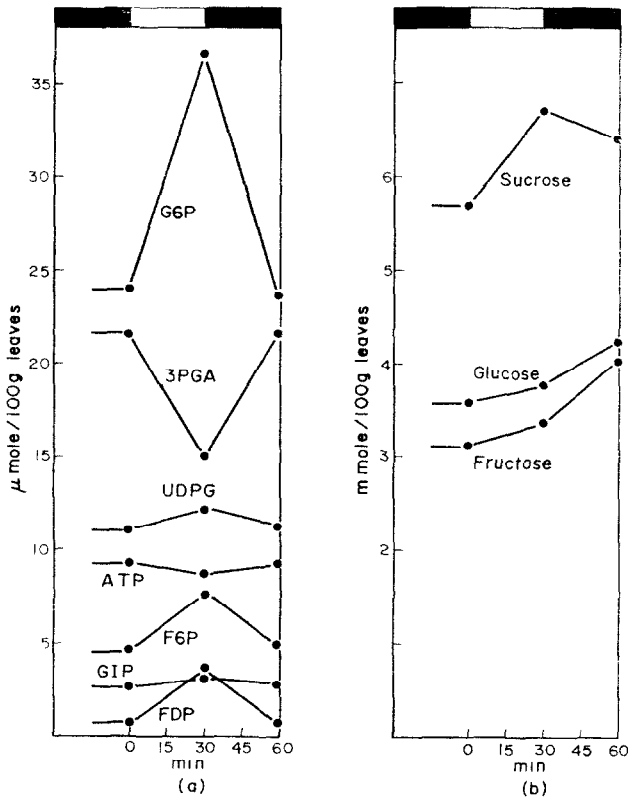

FIG. 1(a). CHANGES IN THE CONCENTRATIONS OF GLUCOSE-6-PHOSPHATE (G6P), 3-PHOSPHOGLYCERATE (3PGA), UDP-GLUCOSE (UDPG), FRUCTOSE-6-PHOSPHATE (F6P), ATP, GLUCOSE-1-PHOSPHATE (G1P) AND FRUCTOSE-1,6-DIPHOSPHATE (FDP) IN STRAWBERRY LEAVES DURING A DARK–LIGHT–DARK TRANSITION. (b) SIMILAR CHANGES IN SUCROSE, GLUCOSE AND FRUCTOSE. PERIOD IN LIGHT 30 min.

Sucrose-6-phosphate (0·34), glucose-1,6-diphosphate (0·81), P_i (850), 5′-UMP (0·65), 3′UMP(2·1), UDP (2 2), CTP (1 9), UTP (3·2) and AMP (0·1) were also measured but showed no significant change. The ADP behaved similarly to the ATP, varying in amount from 9·4 in the dark to 8 8 in the light. The figures are the amounts present in μmoles/100 g fr. wt.

Under the experimental conditions, a carbon fixation rate of about 4700 μmoles of hexose/100 g leaves/hr was observed.

Second Set of Experiments

The change in the same group of compounds for experiments in which the leaves were exposed to light for varying times are given in Fig. 2.

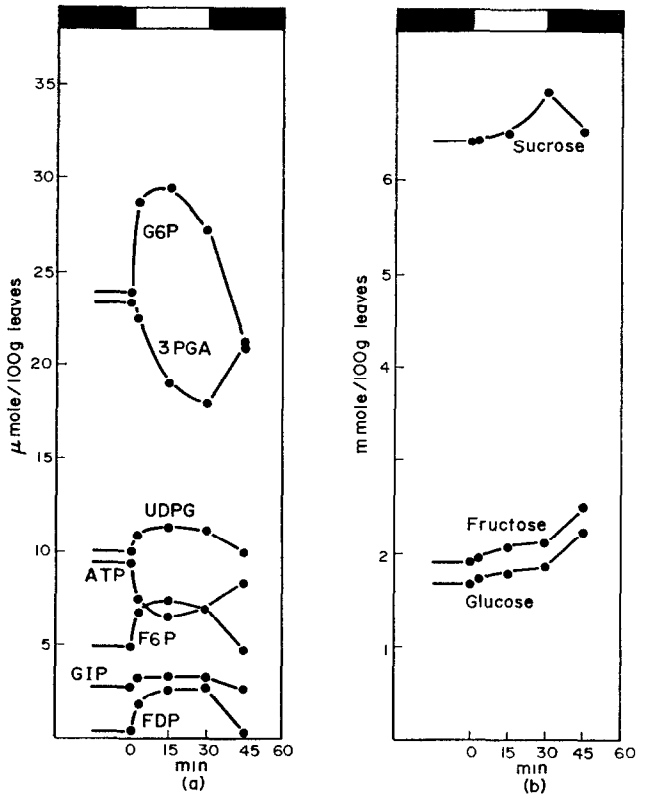

FIG. 2(a) and (b) SEE LEGEND FOR FIG. 1(a) AND (b). PERIOD IN LIGHT 2, 15 AND 30 min.

Sucrose-6-phosphate (0·36), glucose-1,6-diphosphate (0·69), 3′-UMP (2·1), UDP (2·5), CTP (1·7), UTP (3·6) and AMP (0·2) showed no change.

A carbon fixation rate of about 2800 μmoles hexose/100 g leaves/hr was observed.

DISCUSSION

The changes in the concentrations of the photosynthetic intermediates when leaves are subjected to a dark–light–dark transition are the consequence of the interaction of a number of complicated sequences of metabolic reactions both inside the chloroplast and outside in the cytoplasm. In the present study interpretation of the results has been made on the basis that all the reactions considered occurred primarily in the chloroplasts, and that these reactions proceeded rapidly as compared with the rate of movement between the chloroplasts and the cytoplasm. The effect of light was superimposed on the pattern of interaction between the chloroplasts and the cytoplasm and the changes in concentration were the main substance of the present study. It was also assumed that in many cases only part of the total amount of a particular compound in the leaf was present in the chloroplasts, and because of this, the measured change in amount might reflect a large relative change in the chloroplasts.

Examination of the results given in Figs. 2a and 2b indicates that rapid changes occurred within 2 min but that afterwards the concentrations changed only slowly. The changes

occurring within 2 min of the transition from dark to light are here assumed to occur wholly within the chloroplasts. There is some evidence from other work to suggest that this is so for experiments with 'intact' isolated chloroplasts[11] and with whole leaves from which the chloroplasts were subsequently separated by a non-aqueous isolation method[12] have shown that rapid changes in the phosphate compounds occur during the first few minutes in the light. The exact length of time before metabolites appear in appreciable concentrations either in the incubating medium or in the cytoplasm is variable and probably reflects the limitations of the methods used. Experiments[8] on the formation of sucrose from $^{14}CO_2$ in tobacco leaves showed that while the chloroplasts contained labelled sucrose almost immediately following exposure to light, an appreciable amount did not appear in the non-chloroplastic components until 1–2 min after $^{14}CO_2$ feeding started.

In the discussion that follows, only the results described in Fig. 2 will be mentioned; those in Fig. 1 are similar but not so detailed.

Changes Within 2 min *After Illumination*

As soon as the leaves kept in the dark were exposed to light, the level of 3-phosphoglycerate fell and that of fructose-1,6-diphosphate rose; the initial rates of change were 0·7 and 1·6 μmoles/min/100 g leaves respectively, and the initial concentrations 24 and 0·5 μmoles/100 g fr. wt. respectively (the figures being calculated in terms of three carbon units). Under the same conditions, 93 μmoles of 3-phosphoglycerate were reduced to sugar (based on sugar estimates).

The amounts of fructose-6-phosphate, glucose-6-phosphate, glucose-1-phosphate and UDP-glucose all increased in light in a parallel manner to that of the fructose-1,6-diphosphate while the level of ATP fell. These changes are consistent with the requirements of the photosynthetic carbon reduction cycle.[13] The ATP is needed together with NADPH for the reduction of 3-phosphoglycerate and for the formation of fructose-1,6-diphosphate. The other phosphorylated sugars are derived from the fructose-1,6-diphosphate.

Formation of sucrose increased rapidly after the first 5 min; the amount of sucrose present in the leaves at the start of the experiment was large and this tended to obscure the changes assumed to be taking place in the concentration in the chloroplasts.

Changes After the First 2 min *of Illumination*

The 3-phosphoglycerate continued to fall during the whole period of illumination (30 min) while the sucrose increased rapidly, particularly towards the end of the period. The glucose-6-phosphate, fructose-6-phosphate and UDP-glucose fell after 15 min in the light whereas the ATP began to rise at this time. In order to interpret these results an estimate of the starch formed is necessary but unfortunately this was not possible.

The most unexpected result was the slight effect the dark–light transition had on the concentration of sucrose-6-phosphate. The very small change in this compound could be due to the fact that only minute traces of free sucrose-6-phosphate are present and one is measuring only the enzyme bound sucrose-6-phosphate (which is presumably easily liberated by acid treatment during the extraction). This would be constant in amount. The relatively

[11] J. A. BASSHAM, M. KIRK and R. C. JENSEN, *Biochim. Biophys Acta* **153**, 211 (1968)
[12] U. V. HEBER, *Biochemistry of Chloroplasts* (edited by T. W. GOODWIN), Vol II, p. 71, Academic Press, London (1967).
[13] J. A. BASSHAM and R. G. JENSEN, *Harvesting the Sun* (edited by A.S. PIETRO, F. A. GREER and T. J ARMY), p. 79, Academic Press, London (1967).

low concentration of sucrose-6-phosphate compared to most of the other sugar phosphates (in the light) adds support to this idea.

Synthesis of Sucrose

It has been shown by *in vitro* experiments[1,2] that the synthesis of sucrose can occur by transfer of glucose from UDP-glucose to either fructose or fructose-6-phosphate. Both synthetase enzymes have been isolated from chloroplasts[9] so that either reaction could be responsible for the formation of sucrose from the intermediates of the photosynthetic carbon reduction cycle.

In our experiments, it was found that when the transition from dark to light occurred, the concentration of UDP-glucose and fructose-6-phosphate increased to a maximum within about 5 min and then only changed slightly. The amount of sucrose increased at first slowly and then after 10 min, more rapidly. In the dark, the sucrose, the UDP-glucose and fructose-6-phosphate fell to their original levels before illumination. The glucose and fructose increased steadily in light but increased more rapidly in the 15 min in the dark. The increase in the dark is probably due to an accelerated hydrolysis of the sucrose. The change in the UDP-glucose was small ($<15\%$) and unlikely to affect the argument about the synthesis of sucrose because both reactions use UDP-glucose as donor and any variation in concentration, or change in the amount present in the chloroplasts, will have a similar effect on each reaction. The main difference between the reactions concerns the acceptor molecule, fructose or fructose-6-phosphate.

If fructose is the acceptor then the rate of formation should remain largely unchanged during the dark period because the fructose concentration increases slightly (20%) and compensates for the fall in the UDP-glucose.

If fructose-6-phosphate is the acceptor, then the rate of formation should sharply decrease in the dark because the concentration of fructose-6-phosphate falls by 30–40%. In fact if we assume that only about 40% of the total fructose-6-phosphate[12] in the leaf is present in the chloroplasts and that it is this fructose-6-phosphate which is lost, then it follows that very little will remain in the chloroplasts and synthesis of sucrose from this source will be small. Since reactions independent of photosynthesis in which sucrose is hydrolysed to glucose and fructose continue throughout, it is clear that the concentration of sucrose in the leaves will fall. The evidence presented here, therefore, favours the second reaction as the route for the synthesis of sucrose. This is in agreement with conclusions drawn from experiments in which [^{14}C]-glucose and fructose were infiltrated into leaves[14] and from energy considerations based on the equilibrium constants for the two reactions.

EXPERIMENTAL

Plant Material

Mature strawberry leaves (var. Royal Sovereign) were picked from a field near the laboratory, washed, and dried by blotting between filter paper. They were divided into uniform samples of equal weight (60 g) and stored in a dark room for 4 hr at 20°. In the first set of experiments, the leaves were picked early in the season (19 May 1966) and in the second, late in the season (10 August 1966).

Procedure

In the first set of experiments the leaves were divided into four samples (A, B, C and D). Two of the samples (A and B) were dropped into liquid nitrogen simultaneously in the dark at the beginning (0 min). The other two samples (C and D) were exposed to sunlight (30,000 lx at 20°) for 30 min. At the end of the

[14] E. W. PUTMAN and W. Z. HASSID, *J. Biol. Chem.* **207**, 885 (1954).

light period, one sample (C) was dropped into liquid nitrogen. The other sample (D) was removed to the dark room, stored for 30 min in the dark and then dropped into liquid nitrogen.

In the second set of experiments, six samples (A, B, C, D, E and F) were taken. As in the first experiments, the leaves were stored in the dark room for 4 hr at 20°. Samples A and B were simultaneously dropped into liquid nitrogen in the dark (0 min). The other four samples (C, D, E and F) were exposed to sunlight (40,000 lx at 20·5°) for varying periods of time: C for 2 min, D for 15 min, E and F for 30 min and C, D and E were dropped into liquid N_2. F was removed to the dark and left for 15 min before dropping into liquid N_2.

Methods

The phosphate compounds and sugars were extracted and analysed as described earlier.[10,15,16] Estimates of phosphate esters on duplicate samples of leaves (samples A and B) agreed within 5% for all except fructose-1,6-diphosphate (12%) and sucrose-6-phosphate (6%) expressed as a percentage of the mean value.

The concentrations of UDP-glucose, ATP and AMP in the purified strawberry leaf extract were determined enzymically as well as by absorption measurements on the eluate from the anion columns.[16] To allow for the known contamination of the eluates with other nucleotides it was assumed on the basis of a detailed chemical analysis of the peaks that 85% of peak V was UDP, 95% of VI CTP, 85% of VI UTP, 80% of VII ADP, and 85% of IX was ATP.

In the second set of experiments, the rapid formation of a u v. absorbing compound which cochromatographed with 5-UMP on the first anion column was observed to occur during the light period, decreasing in the dark period following. This compound was readily separated from 5-UMP by rechromatography using the formic acid system for elution. The absorption spectra under neutral and acid conditions showed that it was not a nucleotide though it had an absorption maximum at 260 nm in neutral solution. The compound was not adsorbed by poly-*N*-vinylpyrrolidone.

Acknowledgements—We thank Professor H. K. Porter, F.R.S. for reading this paper and for her critical comments on the interpretations of the changes observed during the dark–light–dark transition. One of us (R.R.S.) thanks the managers of the Broodbank Fund, University of Cambridge, for a fellowship. The work described in this paper was carried out at the Low Temperature Research Station, Cambridge.

[15] R. R. SELVENDRAN and F. A. ISHERWOOD, *Biochem. J.* **105**, 723 (1967).
[16] F. A. ISHERWOOD and R. R. SELVENDRAN, *Phytochem.* **9**, 2265 (1970).