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PHOTOSYNTHETIC CO_2 INCORPORATION BY ISOLATED LEAF CELL PROTOPLASTS*

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SUMMARY

The photosynthetic $^{14}CO_2$ fixation by isolated sunflower leaf protoplasts has been investigated and the incorporation pattern has been compared with that of intact sunflower leaves. Dramatic differences have been observed only in respect to the intermediates of the photorespiratory pathway. Protoplasts accumulate glycolate, while intact leaves metabolize this compound into glyoxylate, glycine and serine. The results are discussed in comparison with the glycolate excretion by green algae. The investigations are being continued with C_4 plant protoplasts.

INTRODUCTION

Isolated protoplasts from higher plant leaves have been used successfully in morphogenetic experiments[1,2], for the creation of haploid plants[3] and vegetative hybrids[4], and for the study of the infection mechanism with plant viruses[5]. However, biochemical investigations of the primary carbon metabolism have not been studied so far. The existence of differentiated cells with special biochemical abilities unlike single-cell organisms may justify strong efforts in the preparation of intact protoplasts. It was the intention of the present paper to isolate intact protoplasts with photosynthetic CO_2 incorporation abilities and producing ^{14}C distribution patterns analogous to those of the intact leaves.

MATERIALS AND METHODS

Sunflower leaves have been chosen in respect of their high photorespiratory activity. 3–4-week-old leaves were cut into small strips and incubated for 4 h with a medium containing 0.5% pectinase, 5% cellulase and 20% sucrose, pH 5.4, according to Power and Cocking[6]. During this procedure the material was illuminated with about $4.4 \cdot 10^4$ ergs $\cdot cm^{-2} \cdot s^{-1}$ (fluorescent tubes) and aerated with CO_2-enriched air (1% CO_2). The proceeding technique was adopted from Power and Cocking[6]. Finally, the protoplast preparation was purified by density gradient centrifugation, using a Ludox (DuPont) density gradient. The protoplast fraction

*Preliminary results of a part of the thesis of H.-P. Mühlbach.

accumulated at a density of 1.075 and proved free of intact cells. The yield of intact protoplasts, estimated by chlorophyll determination, was 8–10%.

The photosynthetic experiments were performed in White's medium containing 20% sucrose instead of glucose, pH being 7.4. The protoplast suspension was preilluminated for 10 min with an incandescent lamp (300 W). Then 100 μCi $NaH^{14}CO_3$ (spec. act. 59 Ci/mole) were added and the illumination was continued for another 10 min. The light intensity was adjusted to certain values by wire screens. After 10 min the cell material was killed using boiling ethanol. Aliquots were counted (Beckman DPM 100) for total incorporation rates. In order to avoid difficulties in the chromatographic procedure, the solutes dissolved in distilled water were fractionated using ion-exchange columns (Dowex 1 and Dowex 50). The neutral fraction, containing the added sucrose, was discarded; the combined fractions from the columns were two-dimensionally separated by thin-layer electrophoresis and chromatography[7].

RESULTS AND DISCUSSION

Fig. 1 shows the incorporation rates of ^{14}C in relation to the light intensity. It appears that light intensities higher than $5 \cdot 10^5$ ergs $\cdot cm^{-2} \cdot s^{-1}$ cause light damage. The optimal light intensity may be in good agreement with the maximum sunlight intensity to which the mesophyll cells of the sunflower are exposed.

A comparison is shown in Fig. 2 for the distribution patterns of the CO_2 incorporated into the intermediates. As can be seen from the chromatograms, the distribution patterns of whole leaves and isolated protoplasts are very similar, with the exception that glycolate is accumulated in the protoplast experiment. In the whole leaves glycolate may be transferred into glycine and serine by photorespiration according to Tolbert[8]. The additional existence of labelled glyoxylate supports this

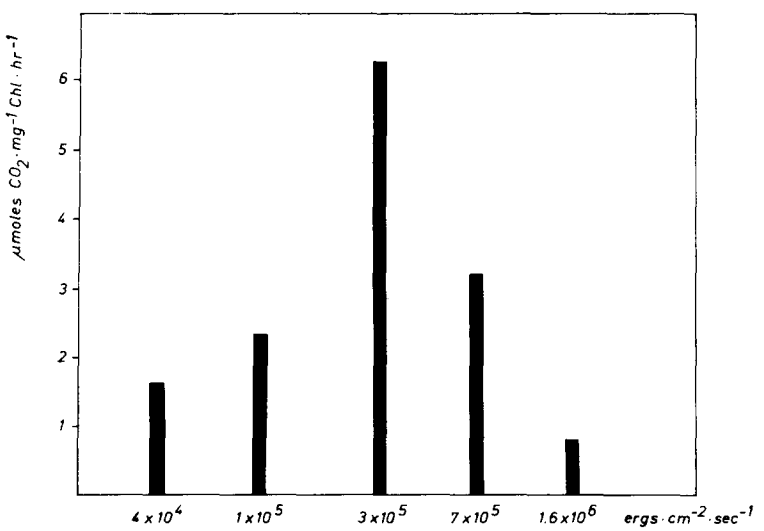

Fig. 1. Total incorporation rates of CO_2 into isolated sunflower leaf protoplasts in relation to the light intensity.

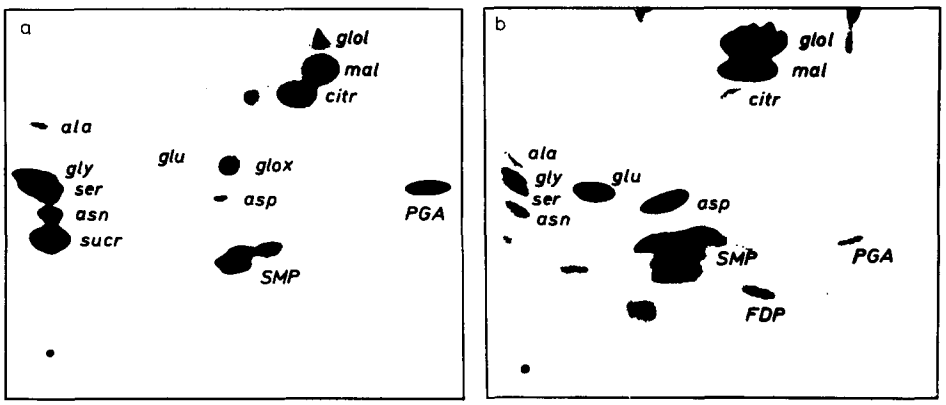

Fig. 2. ^{14}C distribution patterns after 10 min $^{14}CO_2$ incorporation in the light. (a) Whole sunflower leaves, (b) isolated sunflower leaf protoplasts (neutrals discarded). glol = glycolate, citr = citrate, glox = glyoxylate, SMP = sugar monophosphates, PGA = 3-phosphoglyceric acid.

idea. We assume that this effect can be easily explained by the relative low O_2 concentration in the protoplast suspension. If this is true, glycolate excretion by algal suspensions (*cf.* ref. 9) can also be understood by limited photorespiratory activity under O_2-deficient conditions.

The CO_2 incorporation rates into the protoplasts were found to be only approx. 6 μmoles CO_2/h and mg chlorophyll, compared to a value of 300 μmoles CO_2/h and mg chlorophyll in leaves. As protoplasts are stable only in a high concentration of osmotic medium, we assume that the inhibition by these substances (*cf.* refs. 10–12) of a Calvin cycle enzyme is the limiting step of the metabolic chain.

Control experiments were performed by feeding [^{14}C]glycolate to the protoplast suspension. In the absence of O_2 less than 5% of the glycolate were metabolized; by aeration with compressed air after 15 min in the light 25% of the radioactivity was found in glycine, 33% in serine, 10% in glyoxylate, thus showing that photorespiration was the main metabolic pathway for glycolate.

Our experiments demonstrate the usefulness of isolated protoplasts for photosynthetic and photorespiration experiments; they show a number of advantages over whole leaves. The present investigations are being extended to C_4 plants, which contain two distinct kinds of cells (mesophyll and bundle-sheath type) with different chloroplast structures[13] and biochemical abilities[14].

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