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STARCH-PRODUCING *POLYTOMA UVELLA* LACKS SUCROSE AND SUCROSE-METABOLIZING ENZYMES

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Key Word Index—*Polytoma uvella*; Chlorophyceae; alga; sucrose; sucrose synthetase; sucrose phosphate synthetase; enzymes.

Abstract—The absence of sucrose, sucrose synthetase and sucrose phosphate synthetase has been demonstrated by chromatographic, enzymatic and radioisotopic methods in the starch-storing, colourless chlorophyte, *Polytoma uvella*. The significance of this is discussed with respect to starch metabolism.

INTRODUCTION

In higher plant tissues sucrose, through its cleavage by sucrose synthetase (EC 2.4.1.13), is believed to play an important role in providing sugar nucleotides for starch synthesis[1–4]. In our continuing studies of starch metabolism in *Polytoma uvella*[5–7], it was pertinent to investigate whether some of the starch precursor molecules in *P. uvella* could arise in a manner similar to that indicated above for higher plants.

Sucrose and sucrose-metabolizing enzymes have been shown to be present in some starch-producing photosynthetic algae[8–10]. However, there is little evidence to indicate the presence or absence of sucrose and its metabolizing enzymes in non-photosynthetic starch-storing Chlorophyceae. The experimental data reported in this paper show the lack of sucrose, sucrose synthetase and sucrose phosphate synthetase (EC 2.4.1.14) in *P. uvella*, a non-photosynthetic chlorophyte[11, 12]. The results are particularly interesting in that the observed absence of sucrose synthetase would suggest that the precursor moieties for starch synthesis in *P. uvella* are provided by a different mechanism than indicated for higher plants.

RESULTS AND DISCUSSION

When extracts of *P. uvella* were analysed for sucrose by PC no sucrose was detected in extracts of cultures of different ages. The standard sucrose sample co-chromatographed with the *P. uvella* extracts was clearly visible when the chromatographic papers were treated with the three sugar-detecting reagents described in the Experimental. Similarly, no sucrose was detected in the cell extracts of cultures at different stages of growth when these extracts were subjected to the Boehringer sucrose–glucose test.

In our experiments we found that the Boehringer enzymatic test normally was not sensitive enough to measure concentrations below 5 μg sucrose. PC also had a similar drawback. If sucrose was present and was rapidly turned over in *P. uvella* cells, the concentration of sucrose in the extracts may have been small enough not to have been detected by the above two methods. Therefore, in order to confirm the PC and the Boehringer sucrose–glucose test results, cell extracts from *P. uvella* grown in the presence of [U-^{14}C]acetate for different periods of time were subjected to 2D-TLC. Autoradiograms prepared from the developed plates showed that in none of the extracts was radioactivity associated with the sucrose marker (e.g. Fig. 1).

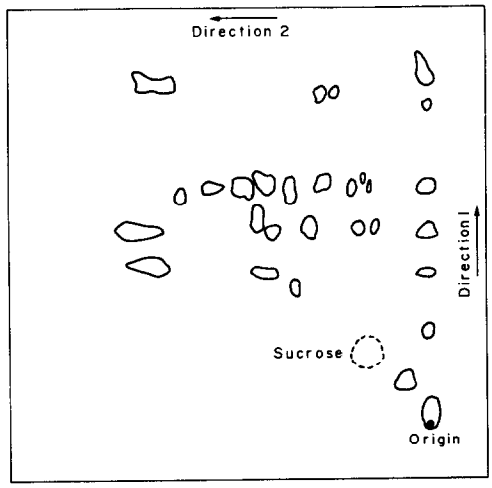

Fig. 1. Tracing of the autoradiogram of the 2D-TLC of the alcohol soluble products from *P. uvella* grown in the presence of [U-^{14}C]acetate for 72 hr. The areas delineated by continuous lines correspond to the radioactive spots. The dotted circle shows the position of unlabelled sucrose added as an int. standard. No attempt was made to identify the labelled products.

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The experimental data for the detection of sucrose synthetase and sucrose phosphate synthetase by two assay methods are shown in Table 1. They show no enzymatic activity for either sucrose synthetase or sucrose phosphate synthetase in partially purified extracts of *P. uvella* cultures. It is possible that the concentration of the two enzymes in *P. uvella* extracts may have been too small to have been detected by the assay methods employed. To examine this possibility, the combined extracts from comparable weights of *P. uvella* and bean leaves were analysed for the two sucrose-metabolizing enzymes and the results compared with the data from the bean leaf extracts alone. The absence of any significant difference between the two results (Table 1) demonstrates the lack of sucrose synthetase and sucrose phosphate synthetase in *P. uvella*. Thus the lack of the two sucrose-metabolizing enzymes shows that *P. uvella* is unable to metabolize sucrose. This has been confirmed by our other studies, in which it has been observed that *P. uvella* is unable to grow in media containing sucrose (unpublished work).

Sucrose is a normal constituent of Chlorophyceae[11]. Recent studies[9, 10] have shown the presence of sucrose, sucrose synthetase and sucrose phosphate synthetase in photosynthetic *Dunaliella*, a phytoflagellate belonging to the same order, Volvocales, as *Polytoma*. The loss of the photosynthetic apparatus may have resulted in the inability of *Polytoma* to metabolize sucrose. However, in higher plants, a large number of non-photosynthetic tissues are able to metabolize sucrose[3, 4, 13–15]. Also, sucrose synthetase and sucrose phosphate synthetase are believed to be confined to the cytoplasm[10]. The inability of *Polytoma* to metabolize sucrose is probably due to the nature of its carbon source. *Polytoma* is an acetate-requiring heterotrophic flagellate which uses acetate carbon rather than sugar carbon to manufacture its stored glucosidic polymer. The precursor moieties for starch synthesis probably arise from the metabolism of citrate via the glyoxylate cycle. The glyoxylate cycle is present in *Polytomella caeca* [16], a colourless acetate requiring alga closely related to *Polytoma*[11].

Because of the absence of sucrose, a sucrose–starch interconversion system is absent in *P. uvella*. Therefore, unlike the higher plants where sucrose synthetase has been implicated in supplying the sugar nucleotide for starch synthesis through cleavage of sucrose[2, 3], the precursor molecules for starch synthesis in *P. uvella* probably arise by a different pathway. Both glucose-1-phosphate and the sugar nucleotides have been demonstrated in *Polytoma*[17, 18]. Whether glucose-1-phosphate is the primary metabolite for starch synthesis is not yet clear. However, our previous studies[5–7], where an important role for phosphorylase (EC 2.4.1.1) has been suggested would tend to support glucose-1-phosphate as the major metabolite. The presence of sugar nucleotides and the glycosyl transferase [5, 6, 18] also suggests an alternate mechanism for starch biogenesis. The action of sugar nucleotide pyrophosphorylase can result in the formation of sugar nucleotides. Also, adenosine diphosphate glucose pyrophosphorylase (EC 2.7.7.b) has been reported to play an important regulatory role in starch synthesis in a number of plant tissues[19]. Therefore, in order to assess the importance of the glucosyl transferase enzyme(s) in *P. uvella* it is necessary to test for the activities of the sugar nucleotide pyrophosphorylases. This is also pertinent since a regulatory role in starch synthesis for adenosine diphosphate glucose pyrophosphorylase in *Chlorella pyrenoidosa* has been suggested [20].

EXPERIMENTAL

Culturing and harvesting of P. uvella *cells.* The culturing and the maintenance of the algal cells was the same as used in previous expts[5, 7]. Working cultures were obtained by adding 2 ml stock culture to 150 ml medium in 250 ml Erlenmeyer flasks. For analysis, cells from cultures (4–6 l.)

Table 1. Sucrose synthetase and sucrose phosphate synthetase assays on partially purified extracts of *P. uvella* cultures of different ages

Sample	Age of culture (hr)	Sucrose synthetase (cpm/10 μl extract)*	Sucrose synthetase (Sp. act. units)†	Sucrose phosphate synthetase (cpm/10 μl extract)*	Sucrose phosphate synthetase (Sp. act. units)†
Control I (bean leaf extract)		3210	0.95	7370	5.80
Control II (mixed bean leaf and *P. uvella* extract)		3280	0.95	7350	5.79
P. uvella	60, 72, 96 and 192	No activity detected		No activity detected	

*As described in ref. [17].
†As described in refs. [21, 22].

at different stages of growth were harvested by centrifugation at 300 *g* for 10 min. The suspension was washed twice in cold Tris–HCl buffer (pH 7.5) and finally resuspended in either pH 7.5 Tris–HCl buffer (for enzyme assay) or in 85% EtOH (for sucrose detection). The cells in the final suspension were broken by exposure to ultrasound at 0° and the homogenate centrifuged at 15 000 *g* for 20 min. The clear, pale-yellow suspension was used for the expts.

For the autoradiography expt, 100 μCi sodium [U-^{14}C]-acetate (New England Nuclear) was added to 25 ml stock medium contained in 50 ml Erylenmeyer flasks. The labelled acetate medium was inoculated with 1.0 ml stock culture. The cells were harvested as indicated above. The final pellet was suspended in 1–2 ml 85% EtOH and exposed to ultrasound.

Detection of sucrose. Three separate methods were utilized to test for sucrose in *P uvella*: (A) PC of the conc alcoholic extracts of *P. uvella* was carried out on Whatman No. 1 chromatographic paper together with known samples of sucrose, glucose and fructose. Descending chromatography was carried out in three different solvent systems: (1) PhOH–H_2O (4:1), (2) BuOH–HOAc–H_2O(6:3:1) and (3) EtOAc–pyridine–H_2O (12:5:4). The sugars were detected by treating the papers separately with (a) EtOH naphthoresorcinol soln[21]; (b) aniline–diphenylamine reagent[22] and (c) $AgNO_3$–NaOH reagent[23]. (B) ^{14}C-labelled extract of *P. uvella*, together with sucrose as reference, was subjected to 2D–TLC (Si gel) in *n*-BuOH–HOAc–H_2O (12:3:5) and PhOH–H_2O (4:1) respectively. After chromatography the TLC plate was exposed to X-ray film. The film was developed after 15 days exposure and the TLC plate sprayed with EtOH naphthoresorcinol reagent to locate the sucrose which was run as an int. standard. (C) The sucrose–glucose test no. 139041 from Boehringer was used for the enzymatic determination of sucrose[9]. The NADPH formed during the reaction was measured at 340 nm. Prior to the analysis, the EtOH extract of *P. uvella* was taken down to dryness and the dried residue suspended in a small vol. of distilled H_2O. After centrifugation at 20 000 *g* for 15 min the supernatant was used in the test.

Enzyme assay. The methods used to assay for sucrose synthetase and sucrose phosphate synthetase were as indicated in refs. [15, 24, 25]. The radioactivity of the labelled sucrose formed, when using the method of Salerno *et al.*[25], was counted in a liquid scintillation counter. To check for the sensitivity of the assay methods for *P. uvella* and as an enzyme control during enzyme assay, crude extracts from comparable weights of *P. uvella* cultures and leaves of 23-day-old greenhouse-grown bean seedlings were mixed and the mixed extract partially purified by the method of ref. [25]. The partially purified extract was analysed for the two sucrose-metabolizing enzymes. For comparison, partially purified bean leaf extracts were also analysed for sucrose synthetase and sucrose phosphate synthetase activities.

Protein estimation. Protein was measured by the Lowry method[26].

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