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GLUCAN AND GLUCOSYLTRANSFERASE ISOZYMES OF *GLAUCOCYSTIS NOSTOCHINEARUM*

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Key Word Index—*Glaucocystis nostochinearum*; Chlorophyceae; storage glucan; glucosyltransferases; isozymes; chloroplasts; endosymbionts; Cyanophyceae.

Abstract—The storage glucan of the alga, *Glaucocystis nostochinearum* was isolated in dimethyl sulfoxide. The absorption spectrum of its iodine complex was identical with those of other green algae but differed from that of blue–green algae. It was similar to amylopectin, and was much less branched than the phytoglycogen of Cyanophytes. The pattern of glycosyltransferase isozymes involved in the synthesis of this glucan (phosphorylases, synthetases and branching isozymes) was similar to those of Chlorophytes. The branching isozymes of this alga were typical Chlorophycean 'Q' enzymes and could only insert branch linkages into linear amylose-like substrates; they were unable to further branch amylopectins, as can the branching isozymes of blue–green algae. If the plastids of this alga are endosymbiotic blue–green algae, then they have lost the ability to form highly branched glucans typical of Cyanophytes.

INTRODUCTION

The evolution of the eukaryotic green algae from the prokaryotic blue–green algae, seems to have followed a separate path from that of the red algae [1–3]. In view of the recently accumulated data, both morphological studies (ultrastructural) and biochemical studies (isozyme analysis and immunodiffusion), the Rhodophyceae may have evolved from the Cyanophyceae with the hot-springs alga, *Cyanidium caldarium* as a transitional or extant intermediate form [4–6].

Glaucocystis nostochinearum is a controversial alga which has been of great interest to both endosymbiont proponents and traditional evolutionists [7–12], and which may be an intermediate form between the Cyanophyceae and the Chlorophyceae. This alga, classified as a member of the Chlorococcales, has chloroplasts which have been termed, 'cyanelles'. First named by Fritsch [13] because he believed them to be acquired blue–green algae, these 'cyanelles' have been cited by endosymbiont proponents as an example of the ability of the eukaryotic cell to acquire plastids [7, 8].

Without entering into the endosymbiont–traditional arguments as to the origin of plastids, it seems probable that if these 'cyanelles' are indeed Cyanophytes acquired by the eukaryote, then the analysis of the isozymes involved in the synthesis of storage glucan in *Glaucocystis* should give some indication of the proposed relationship of these organelles to blue–green algae. The isozyme patterns of blue–green and green algae on polyacrylamide gels are quite different [2, 3, 14]. At the same time, the isolation and study of the storage glucan of this alga might indicate whether this green alga forms typical Chlorophycean 'starch' or the more highly-branched glucan found in prokaryotic algae [14].

RESULTS

The glucan isolated from *G. nostochinearum* forms a deep violet complex with iodine which shows maximum absorption at 580 nm. In this respect, it is identical with the amylopectin of the Chlorophyte, *Spirogyra setiformis*. It differs, however, from the glucan of the Cyanophyte, *Oscillatoria princeps* which shows a maximum absorption of its iodine complex at 550 nm.

Fig. 1 shows the glucosyltransferase isozymes pattern from *Glaucocystis after* 2D polyacrylamide gel electrophoresis. There are two phosphorylase isozymes, two synthetase isozymes and two branching isozymes. The pattern is identical with those of the Chlorophytes, *Spirogyra setiformis* and *Chlorella pyrenoidosa*.

The branching isozymes of *Glaucocystis* were able to insert α-1,6-glucosyl branch points into linear amyloses, but were unable to insert further α-1,6 linkages into moderately-branched amylopectins. In this respect, these branching isozymes appear to be classical 'Q' enzymes, typical of green algae.

DISCUSSION

G. nostochinearum forms a storage glucan which is indistinguishable from that of green algae. This glucan is the result of the actions of three groups of glucosyltransferases and appears to be identical with the amylopectin formed by the glucosyltransferases of *Spirogyra setiformis*. The glucan is much less branched than that of blue–green algae [15].

The 3 groups of glucosyltransferases exist in at least two molecular forms (isozymes), and the pattern on polyacrylamide gel appears to be the same as those of other Chlorophytes [15, 16]. The branching isozymes

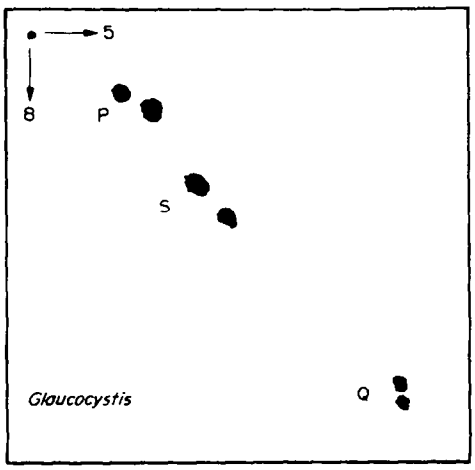

Fig. 1. Two dimensional polyacrylamide gel electrophoresis pattern of the glucosyltransferases of *Glaucocystis nostochinearum*. The arrows indicate the concentrations of gel for each of the dimensional separations. Anodes are to the right and bottom of the figure. Note the two phosphorylases (*P*), two synthetases (*S*) and the two branching isozymes (*Q*).

(Fig. 1) are classical 'Q' enzymes. In this respect, they differ from the branching isozymes of the Cyanophytes [15, 16], all of which are able to form phytoglycogens from amylopectins.

Margulis [7], the leading proponent of the endosymbiotic origin of plastids, has postulated that organelles which have originated as intracellular symbionts and were once free-living, should have counterparts among extant cells, and if these precise extant, morphological and physiological co-descendants of the symbionts cannot be found, the symbionts must have genetic and physiological characteristics, *including the detailed chemical constituents known to be consistent with those generally present in free-living cells.* If the chloroplasts of *G. nostochinearum* were indeed endosymbiotic blue-green algae, then some traces or the original glycosyltransferases should be detected, particularly similar types of branching isozymes. The glucan also should have some resemblance to the glucans synthesized by the glucosyltransferases of blue-green algae. However, this alga resembles the Chlorophytes in both the types of branching isozymes present and the storage glucan formed.

The ultrastructure of the cyanelles of *G. nostochinearum* [9] resembles, to a remarkable degree, the fairly primitive chloroplasts of the alga, *Cyanidium caldarium* [4], itself thought to be a bridge form leading to the Rhodophyceae from a probable Cyanophycean ancestor. Even though Raven [8] suggested that the chloroplasts of *Cyanidium* are symbiotic blue-green algae, recent studies on the RNA and protein synthesis in these chloroplasts indicate that they may, instead, represent an intermediate phase in the evolution of the eukaryotic plastid [17].

Regardless of whether or not *G. nostochinearum* is a missing link between the Cyanophyceae and the Chlorophyceae, the storage glucan it forms is identical with that of typical Chlorophytes and different from that of Cyanophytes. The branching isozymes are 'Q' type typical of green algae and higher plants.

EXPERIMENTAL

G. nostochinearum (Carolina Biological Supply, North Carolina) was grown in a modification of Waris medium [18]: (weight given in mg in 1 l.) KNO_3, 80; $MgSO_4 . 7H_2O$, 16; $(NH_4)_2HPO_4$, 16; $CaSO_4 . 2H_2O$, 40; $FeSO_4 . 7H_2O$, 4; $NaOAc . 3H_2O$, 2; Na_2EDTA, 5; KOH, 2; Beef extract, 0.2; Tryptone, 0.4; Yeast extract, 0.4. The medium was autoclaved for 15 min at 1.4 kg/sq cm. After inoculation with single cells, the 250 ml flasks were illuminated with slim-line cool white fluorescent tubes at *ca* 4400 lx for 16 hr, alternating with an 8 hr dark period. Incubation was at 19°. The algae were harvested after 30 days by centrifugation and washed $\times 3$ with H_2O. They were then macerated with fine quartz sand and extracted. The storage glucan was isolated by extracting the ground algae with cold (5°) DMSO. The extract was centrifuged and the supernatant cooled to 3°. The glucan was pptd by drop-wise addition of EtOH at 0° [5]. The ppt. was collected after 4 hr and dried in the cold. The spectrum of its I_2 complex was obtained as described [5]. The glucosyltransferases were obtained by extracting the macerated cells in the cold with Tris–HCl buffer and the centrifuged extract fractionated with $(NH_4)_2SO_4$ as previously described [5]. The glucosyltransferase fraction was separated in 5% polyacrylamide gel and the sliced gel inserted horizontally into an 8% gel in an E-C 470 Cell and electrophoresis carried out as described [5]. The branching isozymes were tested in a gel 'sandwich' containing either short-chained amylose or moderately-branched amylopectins [15].

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