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STORAGE GLUCAN BIOSYNTHESIS IN *CYANIDIUM, CHLORELLA* AND *PROTOTHECA*: EVIDENCE FOR ENDOSYMBIOSIS?

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Key Word Index—*Chlorella pyrenoidosa*; *Cyanidium caldarium*; *Prototheca zopfii*; glucosyltransferase isozymes; storage glucan; chloroplasts; endosymbiosis.

Abstract—The storage glucans of *Chlorella pyrenoidosa* and *Prototheca zopfii* are identical and consist of a linear polyglucan akin to amylose and a branched amylopectin component. The branched glucans of these algae differ markedly from that formed by the hot-springs alga, *Cyanidium caldarium*. The more highly branched *Cyanidium* glucan appears to be formed by branching glucosyltransferases which are different from those of the other two algae. The relevance of the data to the possibility of *Cyanidium* being a *Prototheca*-like *Chlorella* that has acquired symbiotic Cyanobacteria as chloroplasts is discussed.

INTRODUCTION

The similarities in the life cycle, morphology and intracellular organization of the enigmatic thermophilic alga, *Cyanidium caldarium* with chlorophytes of the genus *Chlorella*, led Allen [1] to suggest that the alga be renamed *Chlorella caldaria* and be classified as a chlorophyte. The isolation by Sorokin [2] of a high temperature strain of *Chlorella pyrenoidosa* has added to the properties these two algae seem to have in common. The high temperature strain of *Chlorella* was reported to have an optimum growth temperature of *ca* 40° [3], while that for *Cyanidium* was observed to be 45° [4].

Cyanidium had been proposed by Klein and Cronquist [5] as a transition form between Cyanobacteria and red algae or between Cyanobacteria and green algae. The accumulation of biochemical data as to its storage glucan and the three groups of glucosyltransferases responsible for its formation, the phosphorylases (EC 2.4.1.1), the synthetases (EC 2.4.1.11) and the branching enzymes (EC 2.4.1.18), led Fredrick to consider it as an extant relic intermediate between the blue-green algae and the red algae. As such, it would form the only 'bridge' between prokaryote and eukaryote [6].

However, the striking similarities of *Cyanidium* and *Chlorella* have prompted endosymbiont proponents [7] to suggest that *Cyanidium* was actually a colorless *Chlorella*, probably a *Prototheca*, that had acquired blue-green algae, and that these symbiotic Cyanobacteria served as the functional chloroplasts for this thermophilic alga.

Prototheca has long been classified as a 'colorless' *Chlorella* [8, 9]. *Prototheca zopfii* was reported by Barker [10] to accumulate a 'starch-like' glucan as its storage product. Since the alga has been observed to be devoid of chloroplasts or plastid-like structures, the possibility of it entering into a symbiotic relationship with photosynthetic unicellular algae should be explored.

The glucosyltransferases of several algae have been studied, as well as the storage glucans formed by these enzymes [11–13]. It was felt that a study of these isozymes and the resulting polyglucosides formed in the various algae might aid in clarifying the relationship between *Cyanidium*, *Prototheca* and *Chlorella*.

RESULTS

Two dimensional polyacrylamide gel electrophoresis revealed similar, if not identical, patterns of the glucosyltransferase isozymes in the high temperature strain of *Chlorella pyrenoidosa* and *Prototheca zopfii* (Fig. 1). There appeared to be three branching isozymes in both

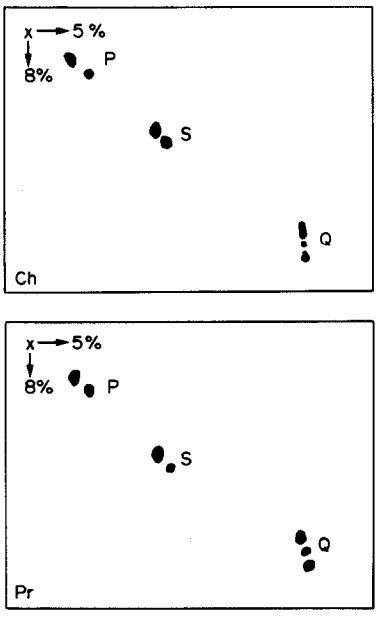

Fig. 1. 2-D Polyacrylamide gel separation of the glucosyltransferase isozymes of *Chlorella* and *Prototheca*. P, Phosphorylase isozymes; S, synthetase isozymes; Q, branching isozymes; x, origin of horizontal electrophoresis. Anodes are to the right and bottom of each diagram. *Ch*, *Chlorella pyrenoidosa*; *Pr*, *Prototheca zopfii*. The *Cyanidium caldarium* separation has been published by Fredrick [11].

algae. All were of the *Q* type and were able to form amylopectin from amylose. However, these branching isozymes were inactive with amylopectin and could not insert further branched chains into this glucan.

The storage glucan formed by the high temperature strain of *Chlorella pyrenoidosa* was identical with that of the colorless alga, *Prototheca zopfii*. It consisted of a starch made up of an unbranched, linear component, amylose, and a branched component, amylopectin. Both amylopectins showed maximum absorption of their iodine complexes at *ca* 580–590 nm (Fig. 2). If this is compared with the single component glucan formed by *Cyanidium caldarium* (Fig. 2), it can be seen that the glucan of *Cyanidium* was more highly branched, showing a maximum absorption for the iodine complex at *ca* 540 nm.

While the branching isozymes of both *Chlorella pyrenoidosa* and *Prototheca zopfii* were of the *Q* type, those of *Cyanidium caldarium* were of the *b.e.* type.

DISCUSSION

Storage glucan granules (phytoglycogen) are found scattered throughout the cell and between the thylakoids in Cyanobacteria [14]. In *Cyanidium caldarium* and in the red algae, the branched storage glucan is found in the cytoplasm of the cell too, and never within the chloroplast [15, 16]. In the red algae, the synthetases are present outside the chloroplast, and hence, any photosynthetically-formed glucose must leave the chloroplast in order for it to be polymerized by these enzymes to Floridean starch [16].

Although some substrate-affinity differences were reported between the phosphorylases of *Cyanidium caldarium* and the Emerson strain of *Chlorella pyrenoidosa* [17], the most striking differences in the glucosyltransferases occur in their branching isozymes [18]. In *Chlorella*, the branching isozymes are only able to form amylopectin, while those of *Cyanidium* can further insert α-1,6-branched linkages into the amylopectin structure and form a highly-branched glucan similar to the phytoglycogen of blue-green algae [19]. The branching isozymes detected in the high temperature strain of *Chlorella pyrenoidosa* used in this study appear to be identical with those of the lower temperature Emerson strain [19].

Since *Prototheca zopfii* does not contain any vestige of a chloroplast structure [10], and since there have been no reports of the 'regeneration' of a chloroplast in this alga as contrasted with the situation in glucose-bleached *Chlorella* [20], one must conclude that the loss of its chloroplast also resulted in the concomitant loss of the organellar DNA associated with it. If *Prototheca* were typical of a proto-*Chlorella*, it should be expected that its branching isozymes would be of the *b.e.* type, and that its storage glucan would resemble the more highly branched phytoglycogen found in Cyanobacteria as well as bacteria [21], since these seem to be the more *primitive* evolutionary structures [22].

The branching isozymes of *Prototheca zopfii* seem to be of the more evolutionary advanced *Q* type, and its storage glucan is identical with the starch of chlorophytes and higher plants. Hence, this alga must indeed be a *Chlorella* that has lost its photosynthetic apparatus. The fact that the branching glucosyltransferase isozymes are identical with those found in the chloroplast of *Chlorella*, despite the loss of organellar DNA by this colorless alga, might be indicative of the control by nuclear DNA over the biosynthesis of these isozymes.

Cyanidium caldarium differs markedly (Table 1) from even the high temperature strain of *Chlorella*, as to the storage glucan it forms, the types of branching isozymes present and the site of formation of the storage glucan. Because of these differences, it seems highly unlikely that *Cyanidium* represents a *Prototheca*-like *Chlorella* that has acquired Cyanobacteria and established an endosymbiotic relationship with these blue-green algae so that they now function as chloroplasts for their host.

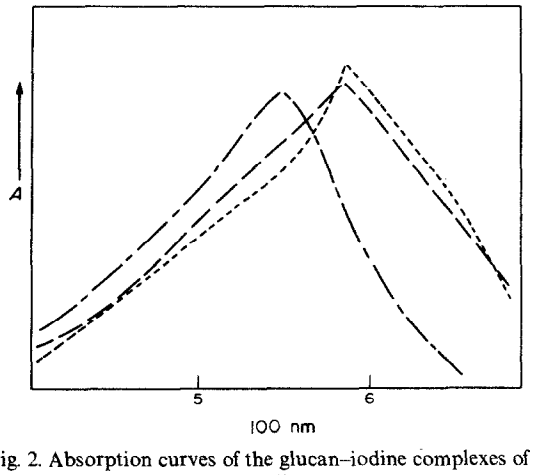

Fig. 2. Absorption curves of the glucan–iodine complexes of the storage glucans. (···), *Chlorella*; (– – –) *Prototheca*; (–·–·–), *Cyanidium*. Increasing absorption (*A*); wavelength × 100 nm.

EXPERIMENTAL

Cyanidium caldarium was grown in a modification of Allen's medium [23]. *Prototheca zopfii* was grown in the medium described in ref. [24]. The high temperature strain of *Chlorella pyrenoidosa* was grown in the modification of Sorokin's original medium made by Cole *et al.* [25].

Thirty day cultures of the algae were centrifuged and the cells extensively washed with deionized H_2O. The cells were macerated and ground with fine quartz sand and extracted with bicarbonate buffer. The glucosyltransferase fraction was obtained via $(NH_4)_2SO_4$ precipitation. The purified glucosyltransferase fraction was subjected to electrophoresis on 5 and 8% polyacrylamide gels using the 2-D isolation techniques. The methods have recently been summarized by Fredrick [26]. Branching glucosyltransferase activity was tested by means of a gel sandwich [27], using amylose and amylopectin as substrates

Table 1. Storage glucan formation in various algae

Alga	Site of glucan formation	Glucan-I Max. absorption (nm)	Branching isozymes Number	*Q*	*BE*
Cyanidium caldarium	Cytoplasm	540	2	+ +	+ +
Chlorella pyrenoidosa	Chloroplast	580			
		610*	3	+ + +	–
Prototheca zopfii	Cytoplasm†	580			
		610	3	+ + +	–

* Amylose component.
† No chloroplast present.

to distinguish between the *b.e.* and *Q* types of branching isozymes. The plyglucans were isolated from extracts of the algae in DMSO by EtOH precipitation in the cold [11]. The iodine complexes of the glucans were formed using the Krisman reagent [28].

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