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STORAGE GLUCAN AND GLUCOSYLTRANSFERASE ISOZYMES OF *CYANIDIOSCHYZON MEROLAE*: A PRIMITIVE EUKARYOTE

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Key Word Index—*Cyanidium caldarium*; *Cyanidioschyzon merolae*; Cyanidiophyceae; Cyanidiaceae; amylopectin; Floridean starch; phytoglycogen; phosphorylases; synthases; branching isozymes.

Abstract—*Cyanidioschyzon merolae*, a primitive eukaryotic alga isolated from supposedly pure cultures of the thermoacidophilic alga, *Cyanidium caldarium*, has many of the characteristics of such prokaryotes as bacteria and the cyanobacteria. *Cyanidioschyzon* appears to have even more of these prokaryotic features than does *Cyanidium*. *Cyanidioschyzon* divides by binary fission as do most bacteria. Its thylakoids are arranged along the periphery of the cell, like the cyanobacteria. Its formation of storage glucan, as well as the type of sugar formed is more like that of the blue–green algae rather than that of the red algae. *Cyanidioschyzon merolae* may be much more primitive than *Cyanidium caldarium*, and could be the *most* primitive eukaryotic cell.

INTRODUCTION

Cyanidium caldarium appears to be an important focus of research for two main groups in biology today: the endosymbiontists who claim the alga as an endocyanome [1], and the traditional biologists who view this alga either as a primitive red alga [2] or as a transitionary organism resulting from many accumulated mutations in prokaryotic ancestors [3].

This eukaryotic alga has long been a source of controversy with regard to its taxonomy [3, 4]. Its importance can be seen by the unusual amount of attention that has been given to this small, inconspicuous cell living in the run-off of acid hot springs [5].

Recently, what were thought to be uni-algal populations of *Cyanidium caldarium* have been found to contain three related, but different, thermoacidophilic algae [6, 7]. Two of these algae are spherical in shape but differ in size [8]. The third is claviform in shape (Fig. 1). While all three algae live at the same acid pHs and elevated temperatures, there are many biochemical as well as ultrastructural differences [8–10]. A new classification scheme has been proposed for these three algae [11].

The club-like alga, tentatively named *Cyanidioschyzon merolae* [11], appears to be the most primitive of the three and may reflect a true transitionary phase between the prokaryotic cyanobacterial cell and the eukaryotic red algae.

Differences between the two spherical forms have been reported [9], particularly with regard to the storage glucans formed and the action of the glucosyltransferase isoenzymes causing their biosynthesis. The smaller of the two spherical thermoacidophiles appears to be *Cyanidium caldarium* and to possess many of the characteristics of blue–green algae [9]; the larger cell is more like the unicellular red algae, forming a storage glucan akin to Floridean starch. The third type that we report upon here appears to be even more primitive than *Cyanidium caldarium* and is probably *most* closely related to prokaryotic algae.

Fig. 1. *Cyanidioschyzon merolae* is shown at × 33 500. Note the claviform appearance of the alga, with peripheral thylakoids (T) and the diffuse nuclear area (N). The numerous black particles are probably ribosomes.

RESULTS

The glucan formed by *Cyanidioschyzon merolae* when isolated from disrupted cells (Fig. 2) was water soluble

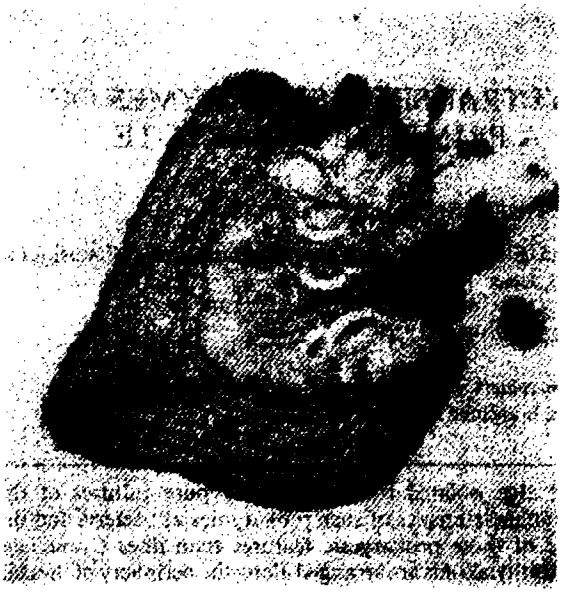

Fig. 2. Storage glucan (SG) granules in cytoplasm of *Cyanidioschyzon merolae*. Note that the storage glucan is formed in the cytoplasm as in red algae and not in the chloroplast. Green algae form their glucans *within* the chloroplast and between the thylakoids (× 38 500).

and gave a deep red coloured iodine complex. Its absorption maximum was at 420 nm. In this respect it was very similar to the alpha particles isolated from the blue-green alga, *Nostoc muscorum* [12]. The storage glucan from this alga was also quite similar in its biochemical properties to that isolated from the smaller spherical thermoacidophilic alga, *Cyanidium caldarium*. The storage glucan differed markedly from that formed by the larger spherical alga (tentatively named *Galdieria sulphuraria*). A comparison of the properties of these storage glucans is shown in Table 1.

The glucosyltransferases of *Cyanidioschyzon merolae* were studied by PAGE methods. The alga had a pattern of two phosphorylase isoenzymes, two ADPG-UDPG synthases and three branching isoenzymes of the *b.e.* type. None of the branching isoenzymes was of the classical *Q* type. In this respect, the pattern of glucan-synthesizing isoenzymes was more like that of cyanobacteria. The pattern differed from that of the large spherical thermoacidophilic alga *Galdieria*, as well as that of unicellular red algae. These algae have at least one *Q* enzyme among their branching isoenzymes, and form an amylopectin (Floridean starch) storage glucan. *Cyanidioschyzon*, *Cyanidium* and the cyanobacteria form only phytoglycogen, which is more highly ramified than other glucan polymers. Moreover, phytoglycogen has many more alpha-1,6 glycosidic linkages than the other storage glucans. Both *Cyanidioschyzon* and *Cyanidium* have only the *b.e.* type of branching isoenzymes [13, 14].

DISCUSSION

The biosynthesis of storage glucans by a cell obviously confers a selective advantage. Those cells possessing such a mechanism no longer are dependent upon the abiotically-synthesized monosaccharides existing in the primordial 'soup'. Therefore, such cells have a readily-available supply of energy. The prokaryotes, both bacteria and blue-green algae (cyanobacteria), form phytoglycogens (highly-branched storage glucans with many alpha-1,6 linkages). In eukaryotes such as the red algae, the storage glucan is less branched (Floridean starch), and is called amylopectin. The green algae form a mixture of the moderately-branched amylopectin and the relatively unbranched, amylose. Amylose is a 'linear' polymer of glucose and contains mainly alpha-1,4 linkages.

Storage glucan, found in the form of alpha granules in such cyanobacteria as *Nostoc* appears to be a highly branched glucan (Table 1). The storage glucans isolated from *Cyanidium caldarium* [15], as well as that of *Cyanidioschyzon merolae*, are almost indistinguishable from that of the cyanobacteria. It would appear that a more highly branched storage glucan is found in more primitive cells [16].

Storage glucans are formed by the action of three groups of enzymes [16]. Phosphorylases (EC 2.4.1.1) and synthases (EC 2.4.1.11) synthesize alpha-1,4 glycosidic

Table 1. Storage glucans formed in algae

Alga	Iodine colour	Complex max. ab.	Type of glucan
Cyanobacteria			
Nostoc muscorum	red	410 nm	phytoglycogen
Cyanidiophyceae			
Cyanidioschyzon merolae	red	420 nm	phytoglycogen
Cyanidium caldarium	red	440 nm	phytoglycogen
Galdieria sulphuraria	red–violet	540 nm	amylopectin
Rhodophyceae			
Porphyridium purpureum	violet	550 nm	Floridean starch
Glaucosphaera vacuolata	violet	545 nm*	Floridean starch amylose*
Chlorophyceae			
Chlorella pyrenoidosa	blue	550 nm	amyopectin
		610 nm	amylose ('true' starch)

*An amylose component has been isolated in several species of red algae [13].

linkages between glucose residues. It is believed that the phosphorylases break down as well as form such bonds, while the synthases exclusively synthesize alpha-1,4 linkages [17]. Both may be involved in the synthesis of this bond, and hence both may be responsible for formation of amylose-like polymers [18]. The branching enzymes (EC 2.4.1.18) are of two types. The *b.e.* type, as found in the cyanobacteria, *Cyanidioschyzon* and *Cyanidium*, can branch or insert alpha-1,6 glycosidic linkages into amylose and amylopectin to give the more highly branched phytoglycogen [18, 19]. The *Q* type of branching enzyme can only branch amylose and such alpha-1,4 linked linear polymers, and form amylopectin. The *Q* enzyme cannot further branch the amylopectin, which is only moderately branched. Such *Q* enzymes are found in the green algae and, together with their *b.e.* isoenzymes in red algae [18] and the large spherical thermoacidophilic alga, *Galdieria sulphuraria* [14].

It would seem that the three thermoacidophilic algae, proposed as members of the new class of algae, the Cyanidiophyceae [11], show an evolutionary progression from the cyanobacteria through *Cyanidioschyzon merolae* (most primitive), *Cyanidium caldarium* (intermediate), *Galdieria sulphuraria* (like the Rhodophyceae) to the red algae. Perhaps this whole new classification of these *relicts* is more correct than any other, considering the other biochemical properties and ultrastructure of these algae [19–21]. The recently discovered alga, *Nanochlorum eucaryotum*, has been called a green alga with *minimal* eukaryotic features [22]. The properties of that green alga (single mitochondrion, single chloroplast, longitudinal scission reproduction, size, pH 3–9, etc.) are very similar to those we have found in *Cyanidioschyzon merolae* [23]. This may confirm the evolution of the algae along two main lines (biphyletic), with the Cyanidiophyceae leading towards the red algae, and *Prochloron* [24] leading through *Nanochlorum* to the green algae.

EXPERIMENTAL

Cyanidioschyzon merolae was isolated from material collected from the Norris Geyser Basin at Yellowstone National Park, Wyoming, U.S.A. The temperature of the run-off at that point was 40°. Single cells were cloned in Allen's medium [25, 26] and kept at 38° under continuous daylight fluorescent 40 watt tubes. After the cultures were growing vigorously (6–8 weeks), the algae were collected by centrifugation, washed in deionized H_2O and ground with fine sand using 80% dimethyl sulphoxide (DMSO). The ground residue was separated and further extracted twice with DMSO. The glucans were precipitated by adding ice-cold Me_2CO to these DMSO solns at room temps. The glucans were dried *in vacuo* and tested with Krisman's reagent [27].

Polyacrylamide gel electrophoresis (PAGE) was carried out as described [28] and the branching isoenzymes tested on different substrates [28].

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