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A NON-PRIMER REQUIRING α-1,4-GLUCAN PHOSPHORYLASE OF *CYANIDIUM CALDARIUM**

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Key Word Index—*Cyanidium caldarium*; Alga; α-1,4-glucan phosphorylase; storage polyglucans.

Abstract—The enigmatic hot-springs alga, *Cyanidium caldarium* possesses only one type of α-1,4-glucan phosphorylase. This phosphorylase appears to be identical with that of the blue-green alga, *Oscillatoria princeps* and the red alga, *Rhodymenia pertusa*. It is a glycoprotein and is capable of initiating polyglucoside synthesis from glucose-1-phosphate without the addition of primer molecules. It is felt that this adds to the accumulating biochemical evidence showing *Cyanidium* to be a transition form between the prokaryotic blue-green algae and the eukaryotic red algae.

INTRODUCTION

THE ENIGMATIC hot-springs alga, *Cyanidium caldarium* has been claimed by both traditional evolutionists[1-3] and endosymbiont proponents[4,5] as a possible *transition* form leading from the prokaryotic algae to the eukaryotic lines. It shares characteristics of both prokaryotic Cyanophytes and eukaryotic Rhodophytes[6,7] and it has also been classified as a blue-green Chlorophyte.[8]

The fact that it does present a picture of a primitive eukaryote in possessing a lobulated chloroplast with at least eight lamellae and a definitive nucleus,[9] but nonetheless forms a storage polyglucoside identical with that of the Cyanophyte, *Oscillatoria princeps*[10] and thrives under conditions of high temperature, characteristic of prokaryotic bacteria and Cyanophycean algae,[11] has not clarified its taxonomic position.

Some of the less controversial evidence for its taxonomy can be found in a comparison of its polyglucan-synthesizing isozymes. Polyacrylamide gel electrophoresis[12] of these enzymes has shown that *Cyanidium caldarium* contains two synthetases (E.C. 2.4.1.11), two 'true' branching isozymes capable of inserting α-1,6-glucosyl bonds into both amylose and

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amylopectin (E.C. 2.4.1.18) and only *one* phosphorylase (E.C. 2.4.1.1). In this respect, its isozyme pattern is identical with those of blue–green algae with the exception that *two* phosphorylase isozymes are found in Cyanophyceae.[13] The red alga, *Rhodymenia pertusa* also possesses only *one* phosphorylase, while the Chlorophytes, *Spirogyra setiformis* and *Chlorella pyrenoidosa* each contain *two* phosphorylase isozymes.[14] Because of this, and other evidence,[15] schemes of evolution have been proposed from the blue–green algae which are biphyletic,[1,15,16] with one branch leading to the red algae via *Cyanidium caldarium*, and another to the green algae and higher plants through an as yet, unknown intermediate form.

Of the two phosphorylase isozymes present in blue–green and green algae, the a_2 isozyme appears to be a *glycoprotein* and capable of initiating polyglucoside synthesis without the need for maltosaccharide 'primer'.[17] The lone phosphorylase present in the red alga, *Rhodymenia pertusa* appears to be of the same type.[17] Similar phosphorylase isozymes have recently been reported in higher plants such as maize[18] and potato.[19] The potato isozyme, one of five phosphorylases in the tuber, also appears to be a glycoprotein.[19]

This study with *Cyanidium caldarium* indicates that the phosphorylase present in this alga is also a glycoprotein capable of *de novo* polyglucan synthesis from glucose-1-phosphate substrates. This supports the inclusion of this alga as a transition form between the Cyanophyceae and the Rhodophyceae and makes more probable the correctness of a biphyletic origin for red and green algae.

RESULTS

When the cells of *Cyanidium caldarium* were extracted with lithium diiodosalicylate and the resulting purified material subjected to electrophoresis on 7% polyacrylamide gel, a single band was obtained. This band was capable of the formation of an intense blue iodine-staining polyglucan after prolonged incubation of the gel in buffered substrates of glucose-1-phosphate. At the same time, phosphate was liberated and detected via a modified Gomori technique.

Duplicate gels when treated with amido-black and with the periodic acid-Schiff reagent, exhibited staining reactions for both reagents in the same bands. This indicated that both protein and carbohydrate components were present in this single enzymatically active band. Preincubation of the gels with α-amylase destroyed the ability of the band to form iodine-stainable material even after prolonged incubation periods in glucose-1-phosphate. Activity was restored when soluble maltohexaose and maltoheptaose were added to the incubation mixtures.

DISCUSSION

The storage polyglucan formed by *Cyanidium caldarium* is highly branched and appears to be identical with that formed by *Oscillatoria princeps*.[10] It differs from the Floridean 'starch' of the red algae in being more branched.

The branching isozymes of *Cyanidium* also have been shown to be identical in molecular

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weight and other physicochemical properties with those of the Cyanophyte.[20] The present study indicates that the single phosphorylase present in this hot-springs alga is similar to the a_2 phosphorylase isozymes of blue–green, red and green algae[17] in being a glycoprotein capable of forming α-1,4-glucosyl linked polymers from glucose-1-phosphate without the addition of 'primer' molecules. The glucan appears to be an integral part of the enzyme molecule in this phosphorylase isozyme.[17]

Ikan and Seckbach[21] have recently shown that the lipids of *Cyanidium* show relationships to both red and blue–green algae. They propose to solve the anomalous position of this alga by suggesting that it is a blue–green Rhodopyhte. However, despite the fact that the Rhodophytes are thought to be fairly primitive eukaryotic algae because of the thylakoid arrangement in their chloroplasts which resembles the situation in Cyanophytes,[22] the lability of red algae to extremes in temperature would indicate that *Cyanidium* is more primitive an eukaryotic cell in the evolutionary scale than the Rhodophytes. Indeed, if, as has been suggested, thermophily is a *primitive* trait and not an adaptive one,[23] then the thermophilic properties of this alga suggest a closer relationship with the primitive blue–green algae.

Insofar as the similarity in storage polyglucan-forming isozymes is concerned, *Cyanidium caldarium* seems to be a logical occupant for the transition link between the blue–green and the red algae. The a_1 phosphorylase isozyme is not present in *Cyanidium*, nor is it present in Rhodophytes. It is present in both blue–green and green algae, however. This would indicate that *Cyanidium* is more logically in the biphyletic pathway leading to the Rhodophyceae and not a transition form leading to the Chlorophyceae. The persistence of this a_1, primer-requiring phosphorylase isozyme in the Chlorophyceae may be one of the reasons for the two-component 'starch' formed by green algae (a mixture of amylopectin, which is less branched than the sugar of *Cyanidium*, and the unbranched, amylose).

On the basis of these studies, the logical inclusion of *Cyanidium caldarium* in a separate genus as a transition form between the Cyanophyceae and the Rhodophyceae seems fully warranted. It may also prove to be one of the most primitive of eukaryotes.

EXPERIMENTAL

Cyanidium caldarium was grown in Seckbach's modification of Allen's medium,[21] using carbon dioxide gassing. The cells were harvested after 3 weeks by centrifugation and the pellets washed with deionized H_2O to remove traces of soluble nutrients of the medium.

The pellet was suspended in 0·3 M lithium diiodosalicylate and 0·05 M Tris (hydroxymethyl)aminomethane hydrochloride at pH 7·5. The procedure followed was that reported by Marchesi and Andrews[24] for the extraction of glycoprotein from red blood cell membranes, with the exception that after dialysis, the material was suspended in 0·5 M polyvinylpyrrolidone[25] to complex any traces of phenol remaining after the partition.

The concentrated supernatant, after centrifugation, was stored at 4° and subjected to electrophoresis on polyacrylamide gel in an E–C cell as previously described.[12] Gels were run in triplicate and stained for protein with amido-black, for carbohydrate by the periodic acid-Schiff reagent. Enzyme activity and liberation of inorganic phosphate were detected by histochemical procedures previously described.[26,27]

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Detection of polyglucosides was done with Krisman's modified iodine reagent.[28] Where the gels were preincubated with α-amylase, the technique described by Fredrick[17] was used. Lithium diiodosalicylate was purified by recrystallization three times from hot water of the commercially available reagent.[29]

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