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GLUCOSYLTRANSFERASE ISOZYMES IN ALGAE—III.

THE POLYGLUCOSIDE AND ENZYMES OF *CYANIDIUM CALDARIUM*

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Abstract—*Cyanidium caldarium* forms a type of storage polyglucoside which resembles that of blue-green and red algae. The storage sugar is a branched polymer of glucose which shows a higher degree of branching than plant amylopectin. Separation of the polyglucoside-synthesizing enzymes of this enigmatic alga on polyacrylamide gel reveals the presence of one phosphorylase, two nucleotide-glucosyltransferase isozymes and two branching isozymes other than Q enzyme. Though the enzyme pattern of the alga has certain similarities to the general distribution of these isozymes in both blue-green and red algae, the mode of action of the branching isozymes, together with the type of storage sugar formed, suggests that the taxonomic classification may fit more appropriately in the Cyanophycean niche.

INTRODUCTION

THE PRESENCE of three groups of enzymes associated with the formation of storage polyglucosides in the algae, has been reported.[1,2] These enzymes, *phosphorylase* (EC 2.4.1.1), *nucleotide-glucosyltransferase* (EC 2.4.1.11) and *branching enzyme* (EC 2.4.1.18), have been shown to exist in two or more isozymic forms in blue-green, red, and green algae.[3,4]

The pattern of these isozymes in the particular alga is reflected in the type of storage sugar formed.[3] For example, the Cyanophyte, *Oscillatoria princeps*, and the Rhodophyte, *Rhodymenia pertusa*, both form a highly branched storage polyglucoside containing many alpha-1,6-glucosyl linkages.[5] However, the Chlorophyte, *Spirogyra setiformis*, forms a "starch" containing *two* polyglucoside components: a moderately branched sugar akin to higher plants' amylopectin, and an exclusively alpha-1,4-glucosyl linked linear polysaccharide of the amylose type.[3-5]

The enigmatic alga, *Cyanidium caldarium*, has been placed into many different taxonomic categories.[6] Recent chemotaxonomic data indicates that its position in phylogenesis may possibly be between the primitive Cyanophyceae and the more evolutionary advanced Rhodophyceae.[7-9] Hirose[9] reported that the cell contents of the alga stained red, red-brown and wine-red with iodine, while Allen[8] reported that the polysaccharide isolated from this

[1] J. F. FREDRICK, *Phyton* **21**, 85 (1964).
[2] J. F. FREDRICK, *Ann. N. Y. Acad. Sci.* 151, 413 (1968).
[3] J. F. FREDRICK, *Physiol. Plantarum* **21**, 176 (1968).
[4] J. F. FREDRICK, *Phytochem.* **6**, 1041 (1967).
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[6] R. M. KLEIN and A. CRONQUIST, *Quart. Rev. Biol.* **42**, 105 (1967).
[7] K. E. NICHOLS and L. BOGORAD, *Botan. Gaz.* **124**, 85 (1962).
[8] M. B. ALLEN, *Arch. Mikrobiol.* **32**, 270 (1959).
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alga stained blue with iodine solution. Kylin[10] observed that when Rhodophycean "floridean starch" was treated with iodine, an array of colours ranging from yellow, brown, red-violet to blue, was obtained.

The isolated polyglucoside of the Cyanophyte, *Oscillatoria*, shows a red to violet color when treated with iodine.[11,12] A detailed study of the storage sugar together with the synthesizing isozymes pattern in *Cyanidium* seemed to be warranted in view of the "transitional" position this alga undoubtedly occupies.

RESULTS

Figure 1 shows the iodine-complex absorption spectra of the polyglucoside isolated from *Cyanidium*, together with that of *Oscillatoria* and the amylopectin fraction from the green alga, *Spirogyra*. The maximum absorption for the *Cyanidium* complex (540 nm) is very much like that of the *Oscillatoria* sugar (550 nm). It differs markedly from the amylopectin fraction of *Spirogyra* starch (580 nm).

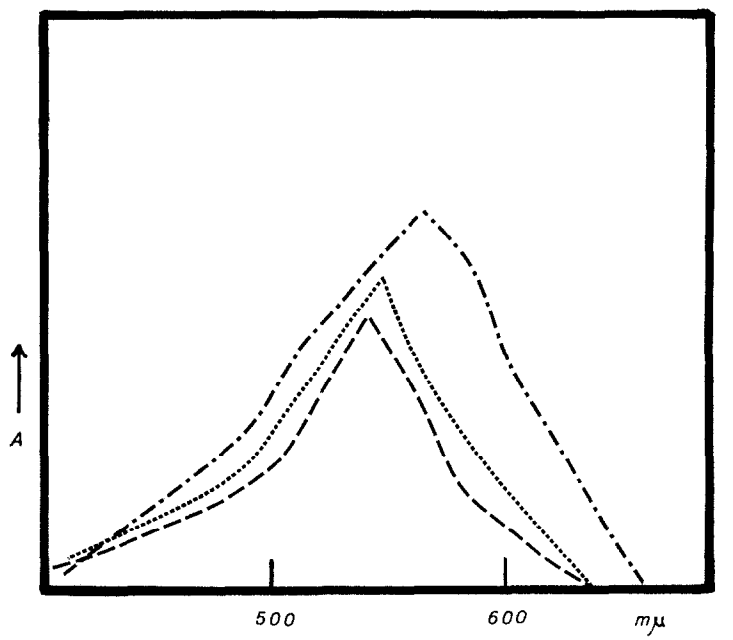

FIG. 1. ABSORPTION SPECTRA OF IODINE COMPLEXES OF: ——— *Cyanidium caldarium* STORAGE POLYSACCHARIDE, ... *Oscillatoria princeps* SUGAR, AND -·-·-· AMYLOPECTIN FRACTION OF *Spirogyra setiformis* "STARCH".

In Figure 2, the enzyme pattern of *Cyanidium* is shown after separation by two-dimensional polyacrylamide gel electrophoresis. Note that only *one* phosphorylase (a_2) is present in the alga. The alga contains *two* nucleotide-glucosyltransferases (a_3-a_4). Note also that separation in 8% polyacrylamide gel has resulted in *two* distinct branching enzymes (a_5).

[10] H. KYLIN, *Förh. Fysiograf. Sällsk. Lund* **13**, 1 (1943).
[11] J. F. FREDRICK, *Physiol. Plantarum* **4**, 621 (1951).
[12] J. F. FREDRICK, *Physiol. Plantarum* **6**, 100 (1953).

Isozymes a_3 and a_4 can utilize UDPG and ADPG for substrates, although preliminary results indicate that ADPG is the more efficient. Isolation of both a_5 isozymes indicates that these two branching enzymes can form alpha-1,6-glucosyl linkages with either amylose or amylopectin.

DISCUSSION

Cyanidium caldarium forms a storage polyglucoside which resembles that of *Oscillatoria* and *Rhodymenia* (Fig. 1). However, the isozyme pattern of the polyglucoside-synthesizing enzymes superficially resembles that of *Rhodymenia* more than that of the blue-green *Oscillatoria* (cf. Fig. 2 with Fig. 3 of reference 4). Both *Rhodymenia* and *Cyanidium* appear to

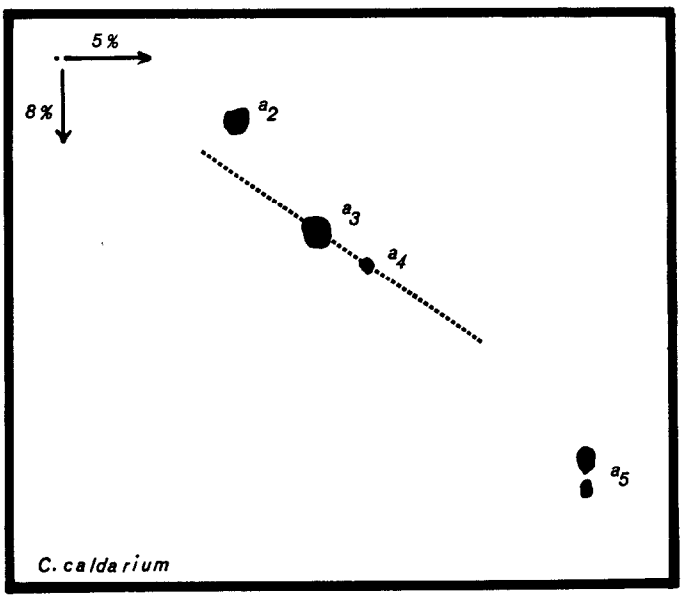

FIG. 2. POLYACRYLAMIDE GEL ELECTROPHORETIC PATTERN OF ISOZYMES OF *Cyanidium*. THE SEPARATION VIA TWO-DIMENSIONAL ELECTROPHORESIS WITH 5% POLYACRYLAMIDE (HORIZONTAL) AND 8% POLYACRYLAMIDE (VERTICAL). Isozymes a_3 and a_4 are on a theoretical straight line which passes through the origin (*) of the first dimension separation. Note that the a_5 protein has separated into two isozymes in the 8% gel. Anodes to the right (horizontal separation) and to the bottom of the figure (vertical separation).

have only one phosphyorylase. Both contain two nucleotide-glucosyltransferases which utilize ADPG or UDPG for substrates.[4] However, qualitatively, it appears that isozyme a_4 is reduced in quantity in *Cyanidium* (Fig. 2). This situation resembles that in the green alga, *Spirogyra* where this enzyme also appears to be present in a lesser concentration than its isozymic partner.[4] The apparently reduced concentration of the isozyme in the green alga, may reflect the fact that both a soluble and an insoluble form of the enzyme have been reported in another green alga, *Chlorella*.[13]

The isolated polyglucoside of *Cyanidium* appears to be different from the branched component of *Spirogyra* starch (Fig. 1). The iodine complex of *Cyanidium* starch shows a maximum absorption at 540 nm, while the amylopectin fraction of Spirogyra starch has an iodine complex which absorbs at 580 nm.

[13] J. PREISS and E. GREENBERG, *Arch. Biochem. Biophys.* **118**, 702 (1967).

The polyacrylamide electrophoretic pattern of *Cyanidium* shows *two* branching enzymes to be present (Fig. 2); in *Spirogyra*, three branching isozymes were reported.[3-5] It is significant that the two branching isozymes of *Cyanidium* are different from those of the green alga insofar as their activities are concerned. The branching isozymes of *Spirogyra* and other green algae seem to be of the *Q* enzyme type and cannot branch amylopectin.[4,14] Those of *Cyanidium* resemble the branching isozymes of the blue-green alga, *Oscillatoria*, in that they are active on both amylose and amylopectin, forming the characteristic polyglucoside described above.[5] In this respect, *Cyanidium* resembles the Cyanophyte more than the Chlorophyte.

If the polyglucoside's iodine complex absorption spectrum is used as a rough indicator of the degree of polymerization of the sugar,[15] it would appear that the *Cyanidium* sugar is slightly more branched than that of either *Rhodymenia* or *Oscillatoria*. It is interesting to speculate in this regard that this alga shows a more "primitive" storage sugar than the supposedly more primitive blue-green alga, *Oscillatoria*.[16]

Cyanidium, therefore, exhibits many of the enzymic properties of *Oscillatoria*, particularly with regard to the branching isozymes. In the Rhodophyte, *Rhodymenia*, only one of the branching isozymes can branch both amylose and amylopectin; the other two are active only upon amylose.[4] All in all, it appears that *Cyanidium* is more closely related to the blue-green algae than to any other groups.

EXPERIMENTAL

Wild-type *Cyanidium caldarium* was grown in liquid medium in 5-l. conical flasks with continuous aeration and illumination by means of fluorescent lights. The medium was Bogorad's modification[17] of Allen's media.[8] The modification consisted of supplying iron as the EDTA chelate and substituting ammonium chloride for the sulfate salt. The medium was adjusted to pH 2·5–3·0 with 0·001 M H_2SO_4. Growth took place at room temperature. The cells were harvested by centrifugation after 40 days and washed twice in deionized water.

30 g wet-weight of cells were macerated in a porcelin Coors mortar with Alcoa alumina # A-301. The ground cells were extracted for 6 hr in the cold (8°) with dimethyl sulfoxide (Experimental Drug Grade, Crown-Zellerbach Corp.), and then filtered through Whatman # 41 paper. The filtrate was cooled to 4° and 2 volumes of ice-cold 97% ethanol added. The mixture was allowed to stand in the cold for 4 hr and the precipitate removed by centrifugation. The precipitate was washed twice with cold ethanol and redissolved in dimethyl sulfoxide. The polysaccharide was reprecipitated and washed again as before. The granular precipitate was dried *in vacuo* in the cold. It yielded a pearl-white powder which was readily soluble in cold water to form an opalescent solution. 30 mg were dissolved in 50 ml of water. Aliquots of this solution were treated with Krisman's reagent,[18] and spectral studies performed with a Coleman spectrophotometer. Di-methyl sulfoxide does not affect the maximum absorption of the iodine complex.[19,20]

For the enzyme studies, the ground alga was extracted with Tris-EDTA-Borate buffer, precipitated with ammonium sulfate, and gel electrophoresis carried out in an E—C # 470 Vertical Cell as previously described.[4,5]

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