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THE α-1,4-GLUCANS OF *PROCHLORON*, A PROKARYOTIC GREEN MARINE ALGA

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Key Word Index—*Prochloron*; prokaryotic alga; storage glucans; phytoglycogen; amylose; chlorophyte chloroplast; endosymbiosis.

Abstract—*Prochloron*, a symbiont found associated with *Lissoclinum patella* (a marine colonial ascidian), was lyophilized and its glucans extracted. The glucans were complexed with *s*-triazine reactive dyes and separated by electrophoresis on cellulose acetate membranes. A highly branched glucan similar to phytoglycogen, and a linear unbranched glucan resembling a short-chain amylose were both detected. This unusual polysaccharide mixture suggests a possible mode of starch biosynthesis in algae in general.

INTRODUCTION

One of the criticisms of the endosymbiont hypothesis to explain the origin of eukaryotic intracellular organelles, has centered upon the necessity to invoke three separate acts of symbiosis to derive the origin of the chloroplasts of rhodophytes, chlorophytes and chromophytes [1]. The relatively primitive chloroplast of unicellular red algae strongly suggests its derivation from blue-green algae. The biochemically and ultrastructurally more complex chloroplast of chlorophytes makes it highly improbable that this Chlorophycean structure was also derived from the same ancestral source.

The discovery by Lewin [2, 3] of a prokaryotic green marine alga, *Prochloron*, was welcomed by endosymbiont proponents as a current example of a possible chlorophyte chloroplast precursor [1]. This alga, definitely prokaryotic in nature, has been shown to contain both chlorophyll *a* and *b*, and to lack red and blue bilin pigments [4].

All members of the Prokaryota, bacteria and blue-green algae (Cyanobacteria), examined thus far, form α-glucans which are invariably highly branched [5, 6]. The bacterial glycogen of *E. coli* and the alpha granules (phytoglycogen) of Cyanophyceae are both highly ramified α-glucans which contain many α-1,6 branched linkages [6]. The α-glucans of eukaryotic algae such as the floridean 'starch' of red algae and the amylopectin component of the starch of chlorophytes and chromophytes contain fewer α-1,6 linkages and hence, are all less branched [7, 8]. In chlorophytes and in chromophytes, this amylopectin component is intimately bound with an unbranched α-glucan called amylose. The mixture is popularly known as 'starch'.

The glucans of the starches of various prokaryotic and eukaryotic algae [9] and yeasts [10] have been separated by electrophoresis of the colored complexes they form with *s*-triazine based reactive dyes. The visible complexes were separated on cellulose acetate membranes in a concentrated borate buffer. The resulting patterns were found to be very similar to those obtained using free-boundary (Tiselius) electrophoresis [10].

RESULTS

The isolated glucans from the lyophilized cells of *Prochloron*, when subjected to this technique, yielded a pattern which strongly suggests that the starch of this alga is a mixture of a highly-branched glucan, similar to phytoglycogen, and an unbranched component resembling amylose (Fig. 1). The branched component of the *Prochloron* starch appears to be much more highly branched, and therefore probably contains more α-1,6 linkages than the amylopectin of green algae such as *Chlorella* (Fig. 1). The absorption maxima of the iodine complex of *Prochloron* starch show peaks at 420 and 610 nm (Fig. 2). This data would corroborate the electrophoresis patterns that the starch of this alga contains two components, one very similar to

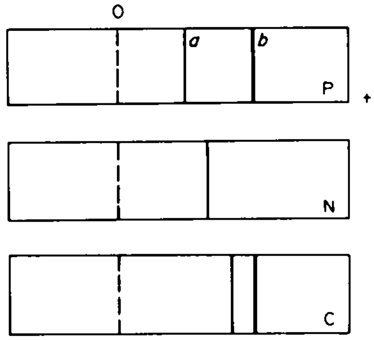

Fig. 1. Membrane electrophoresis of the procion-dyed starches of algae. P, *Prochloron* starch (note the two components, *a* and *b*). The fast anodic-moving component, *b*, appears to be an amylose, similar to that of *Chlorella* starch (C). N, the single component of the starch from the cyanophyte, *Nostoc*. Note that the *a* component of the *Prochloron* starch is at least as highly branched as this phytoglycogen of *Nostoc*. The slower anodic-moving component of *Chlorella* is an amylopectin, and is less branched than either the *a* component of *Prochloron* and the phytoglycogen of *Nostoc*. The anode (+) is to the right of each diagram. O indicates the point of application.

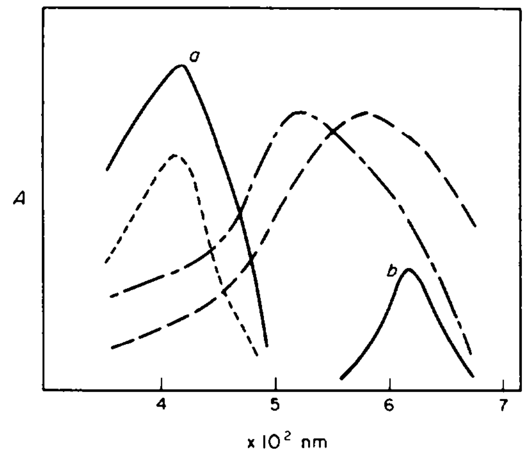

Fig. 2. Absorption spectra of iodine complexes of algal starches. The *Prochloron* starch (——) is made up of two glucans, *a* and *b*. The starch of *Nostoc* (----), a phytoglycogen, shows a maximum absorption similar with that of *Prochloron* (*a*). The amylopectin component of *Chlorella* starch (· - ---·--) shows a maximum absorption at 540 nm, while the amylose component (———) shows a peak at about 600 nm. The *b* component of *Prochloron* starch appears to be an amylose, with a maximum absorption at about 610 nm.

phytoglycogen in structure, and the other akin to amylose. These results were essentially the same with the various samples of *Prochloron* collected from different sites in the Pacific.

DISCUSSION

Prochloron would appear to be a prokaryotic alga [2]. Its unusual photosynthetic pigment pattern of chlorophylls *a* and *b* and its lack of phycobilins, however, tend to resemble eukaryotic chlorophytes rather than prokaryotic cyanophytes [4]. Because of this, Lewin had suggested a separate division of the Prokaryota for this apparently facultative symbiont [3].

The glucans of this alga present an unusual pattern, also. All of the prokaryotes form highly-branched α-glucans which are glycogen-like in structure [11, 12]. One of the glucans of *Prochloron* is indeed very similar to these prokaryotic glucans, and is highly branched (Figs. 1 and 2). However, the finding of a second glucan component of this alga's starch, a linear unbranched amylose, is quite different from any prokaryotic starch. Hence, the 'starch' of *Prochloron* appears to consist of phytoglycogen and amylose.

While amylose is the common component of chlorophyte (and higher plant) starches (Fig. 2), the branched component of these starches is always a moderately-branched amylopectin (Fig. 1). Amylose has never been reported as a constituent of the storage glucans of any other prokaryotes [11, 13]. However, recent studies by McCracken [14] indicate that amylose may be a component of the floridean starch of primitive unicellular red algae. This raises an interesting point for the fundamental problem, as yet unsolved [14], of starch biosynthesis. Amylose is believed to be formed through one of two possible mechanisms. Its presence in starch may be the result of debranching of glycogen, or it is the glucan first formed and then, part of it is branched to form the amylopectin component of starch [15, 16].

In the prokaryotic cyanophytes, *Nostoc* [17], *Oscillatoria* [18] and *Anacystis* [19], no unbranched α-glucan has been detected. The same is also true for the controversial alga, *Cyanidium caldarium*, regarded as a transition form between the prokaryotic blue-green algae and the eukaryotic red algae [20, 21]. If then, the α-glucans are used for chemotaxonomic purposes, it would be necessary to conclude that the mode of biosynthesis of these glucans is different for cyanophytes than it is for *Prochloron*, and that glycogen-like α-glucans are indeed synthesized first in these blue-green algae. The same considerations obviously pertain to *Cyanidium caldarium*, despite its definitive eukaryotic characteristics.

The *Prochloron* starch contains amylose as well as the branched phytoglycogen (Figs. 1 and 2), and in this regard more closely resembles the two-component starch of *Chlorella* (Fig. 1) and other chlorophytes. While it has not been possible, due to the inability of culturing *Prochloron*, to examine the glucosyltransferases involved in the biosynthesis of its starch, such studies most probably would shed some light on the formation of this unusual starch. A study of the debranching enzymes of this alga would be of particular interest in order to ascertain whether the amylose detected in its starch may be an artifact caused by the debranching of a possibly, initially-formed branched glucan such as its phytoglycogen. Such studies might serve to elucidate whether this unusual prokaryotic green alga, with its chlorophyte-like photosynthetic pigments, is indeed an example of the precursor of the Chlorophycean chloroplast.

EXPERIMENTAL

Prochloron was collected from three different sites on Palau, W.C.I., during September 1979. The cells were expressed from *Lissoclinum patella*, washed with sea-water and lyophilized [22]. Detritus constituted less than 1 % of the lyophilized material.

The lyophilized cells were sonicated in boiling water [23] rather than in dimethyl sulfoxide [9]. The polysaccharides were precipitated with ice-cold acetone after filtration and overnight cooling of the filtrate. Complexes of the glucans were formed with the reactive dye, Procion red P-8B (I.C.I., Americas Inc., Wilmington, Delaware) using Fredrick's modification [9] of the technique of Dudman and Bishop [10]. Electrophoresis of the complexes was on cellulose polyacetate membranes (Sepraphore III, Gelman Instrument Co., Ann Arbor, Michigan) using the previously described method [9]. Isolated polysaccharides from *Nostoc muscorum* and *Chlorella pyrenoidosa* were carried through for comparison of mobilities.

Samples of the precipitated polysaccharides were dissolved in deionized water and complexed with iodine using the Krisman reagent and method [24]. The absorption maxima of the iodine complexes were obtained using a Coleman Senior spectrophotometer. The isolated glucans from *Nostoc* and *Chlorella* were treated the same way and their spectra used for comparison.

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REFERENCES

1. Whatley, J. M., John, P. and Whatley, F. R. (1979) *Proc. Roy. Soc. London Ser. B*: **204**, 165.
2. Lewin, R. A. (1976) *Nature* **261**, 697.
3. Lewin, R. A. (1977) *Phycologia* **16**, 217.
4. Lewin, R. A. (1975) *Nature* **256**, 735.
5. Manners, D. J. (1968) in *Control of Glycogen Metabolism* (Whelan, W. J., ed.) pp. 83–100. Academic Press, New York.
6. Fredrick, J. F. (1979) *Phytochemistry* **18**, 1823.
7. Fredrick, J. F. (1971) *Physiologia Plant.* **24**, 55.
8. Antia, N. J., Cheng, J. Y., Foyle, R. A. J. and Percival, E. (1979) *J. Phycol.* **15**, 57.
9. Fredrick, J. F. (1973) *Plant Cell Physiol.* **14**, 1187.
10. Dudman, W. F. and Bishop, C. T. (1968) *Can. J. Chem.* **46**, 3079.
11. Fredrick, J. F. (1980) in *Origin and Evolution of Eukaryotic Intracellular Organelles* (Fredrick, J. F., ed.) in press. New York Academy of Sciences Press, New York City.
12. Fredrick, J. F. (1973) *Ann. N.Y. Acad. Sci.* **210**, 254.
13. Fredrick, J. F. (1971) *Phytochemistry* **10**, 1025.
14. McCracken, D. A. and Cain, J. (1979) *J. Phycol. Suppl.* **15**, 20.
15. Erlander, S. R. (1970) *Starke* **22**, 393.
16. McCracken, D. A. and Dodd, J. L. (1971) *Science* **174**, 419.
17. Chao, L. and Bowen, C. C. (1972) in *Taxonomy and Biology of Blue-Green Algae* (Desikachary, R. V., ed.) pp. 13–17. University of Madras Press, India.
18. Fredrick, J. F. (1968) *Phytochemistry* **7**, 1573.
19. Fredrick, J. F. (1977) *Phytochemistry* **16**, 1571.
20. Fredrick, J. F. (1978) *J. Thermal Biol.* **3**, 1.
21. Fredrick, J. F. (1976) *Plant Cell Physiol.* **17**, 317.
22. Schulz-Baldes, M. and Lewin, R. A. (1976) *Phycologia* **15**, 1.
23. Sheath, R. G., Hellebust, J. A. and Sawa, T. (1979) *Phycologia* **18**, 149.
24. Krisman, C. R. (1962) *Analyt. Biochem.* **4**, 17.