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GLUCOSYLTRANSFERASE ISOZYMES FORMING STORAGE GLUCAN IN *PROCHLORON*, A PROKARYOTIC GREEN ALGA

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Key Word Index—*Prochloron*; prokaryotic algae; storage glucan biosynthesis; phosphorylases; synthases; branching enzymes; glucosyltransferase isozymes; evolution.

Abstract—Since the prokaryotic, green marine alga *Prochloron* has not, as yet, been cultured, lyophilized cells were used in a microadaptation of polyacrylamide gel electrophoresis (PAGE) in order to isolate the glucosyltransferase isozymes. The pattern obtained with these capillary gels was identical with those of cyanophytes. Besides two phosphorylase and synthase isozymes, three branching isozymes of the *b.e.* type were found to be present.

INTRODUCTION

The storage glucan of *Prochloron* is a highly branched phytoglycogen which seems to be identical with that of cyanophytes such as *Nostoc* and *Oscillatoria*. In addition, the *Prochloron* glucan also contains a second component which appears to be a short-chain amylose [1]. Such a component is unique to this prokaryotic alga and has never been reported present in any other type of prokaryotic cell, bacteria or cyanobacteria [2].

It has been impossible, to date, to culture *Prochloron* in the laboratory [3]. Therefore, supplies of the alga have been scarce, and studies have been entirely dependent upon fresh or lyophilized material. Because of this it was necessary to adapt the technique of PAGE [4] to a micro scale by using capillary columns for the separation of the glucosyltransferase isozymes. Such columns can maintain small amounts of migrating substances in concentrations high enough to be detected [5].

RESULTS

The glucosyltransferase isozymes of *Prochloron* were resolved using the microelectrophoresis technique on 5 and 8% polyacrylamide gels. The patterns obtained are shown in Fig. 1. Two phosphorylase isozymes and two synthase isozymes were separated on the 5% gel. However, the branching isozymes were only resolved on the 8% gel. The phosphorylase isozymes were histochemically localized by incubation of the microgel columns in a buffered glucose-1-phosphate substrate using maltohexaose as a primer. It was not possible, due to the limited quantity of material, to determine if either of the phosphorylase isozymes were primer independent.

During the application of the procedure to visualize the phosphorylases, a non-glucan forming band appeared (see Fig. 1) between the synthase isozymes and the branching isozymes region. This band, while rapidly hydrolysing the Cori ester, did not form any detectable polysaccharide which stained with iodine. Both synthases were equally active on adenosine diphosphoglucose and uridine diphosphoglucose. The three branching isozymes, resolved on the 8% polyacrylamide gel, were active on amylose and amylopectin, forming bands after incubation in these substrates which stained red-violet with iodine. All three isozymes appeared to be of the *b.e.* type rather than of the *Q* type.

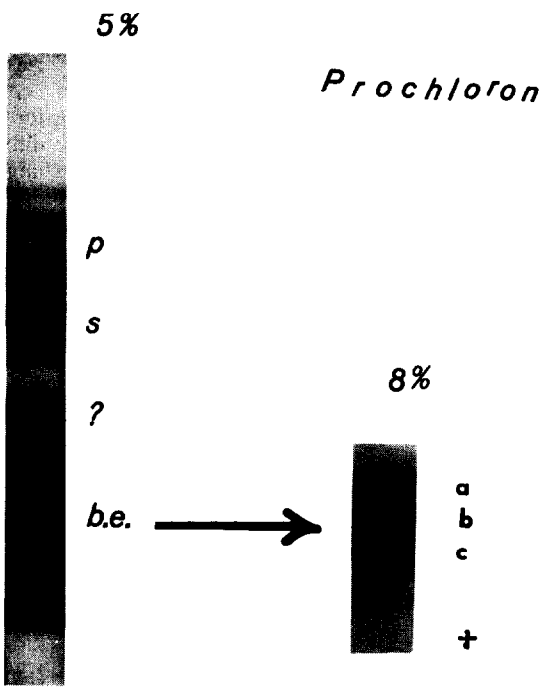

Fig. 1. Separation of storage-glucan forming glucosyltransferases of *Prochloron* by microelectrophoresis on polyacrylamide gel. On 5% gel, the phosphorylase isozymes (*p*) and the synthase isozymes (*s*) are separated. The branching isozymes (*b.e.*) migrate together in the 5% gel, but are separated into 3 isozymes on the 8% gel (a, b, c). The protein band on the 5% gel between the synthases and the branching isozymes area ('?') appears to hydrolyse the Cori ester, and is probably a phosphatase. The anode (+) is to the bottom of the gel columns (*ca* 2.5 ×).

DISCUSSION

Despite the presence of chlorophyll *b* and the lack of biliproteins, there is little doubt that *Prochloron* is a prokaryotic alga [3]. The glucosyltransferase isozymes pattern of *Prochloron* appears to be, from these limited studies, identical with those of blue-green algae, and hence, lends support for placing *Prochloron* with the Prokaryota.

In terms of traditional evolution, it is more probable that the Chlorophyceae evolved from such an alga than from a Cyanophycean ancestor which lacked chlorophyll *b* and contained phycobilins. *Prochloron* has been accepted by those who favor the endosymbiont hypothesis as a possible precursor of the green algal chloroplast [6].

Support for both types of evolution can be found in the fact that *Prochloron* is the only prokaryote whose storage glucan contains amylose, a glucan component of chlorophytes and the higher green plants. The presence of amylose is enigmatic, particularly as the amylose appears to be of a short-chain type [1]. All three branching isozymes of *Prochloron* are of the *b.e.* kind. These would rapidly branch any amylose (and amylopectin) to phytoglycogen.

Prochloron seems to be the only prokaryotic alga which forms amylose. In this respect, *Prochloron* resembles the chlorophytes and higher green plants whose starch is an intimate mixture of amylose and the moderately branched glucan, amylopectin. In higher plants such as maize, the idea of compartmentation has been proposed to explain the biosynthesis of amylose and amylopectin in the same granule, without the conversion of the amylose to amylopectin or phytoglycogen [7]. This compartmentation may involve a fixed spatial location for the enzymes (as complexes) so they are not able to diffuse throughout the cell and thereby, cause the branching of the amylose [8].

Another problem involves the status of phosphorylase. There are two phosphorylase isozymes present in *Prochloron* (Fig. 1). This enzyme's physiological role remains undefined with regard to its participation in the synthesis–degradation of glucans [9].

EXPERIMENTAL

Lyophilized *Prochloron* cells were ground in a Radnoti micro tissue grinder with 1.5 ml cold bicarbonate buffer at pH 6.8 [10]. The homogenate was centrifuged and the supernatant treated with solid $(NH_4)_2SO_4$ to precipitate the glucosyltransferases [10]. The ppt. was suspended in 2 ml deionized water and cooled rapidly to 5°. The opalescent soln was subjected to molecular washing through a Pellicon nmwl 10,000 type PTGC membrane (Millipore Corp., Bedford, MA) to remove the salts. The retentate was taken up in 2 ml of 0.01 M Tris–HCl buffer at pH 6.8.

Capillary tubes (Kimax 51 glass) of 33 mm length and 1 mm i.d. were coated with 0.1% methyl cellulose and dried overnight at 90°. The tubes were filled with either 5 or 8% polyacrylamide and stored at 4° until used. The retentate (500 nl) was carefully layered onto the gel. Electrophoresis was carried out at 0.5° using 0.01 M Tris–HCl buffer (pH 6.8) for 2 hr at 2.5 V/tube [11].

The gels were either stained for protein, or histochemically treated to visualize the isozymes [10].

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