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PROTEIN AND ISOZYME PATTERNS OF THE CYANELLES OF *GLAUCOCYSTIS NOSTOCHINEARUM* COMPARED WITH *ANACYSTIS NIDULANS*

JEROME F. FREDRICK
The Research Laboratories, Dodge Chemical Company, Bronx, NY 10469, U.S.A.

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Key Word Index—*Glaucocystis nostochinearum*; Chlorococcales; cyanelles; *Anacystis nidulans*; Chroococcales; water-soluble proteins; glycosyltransferase isozymes; branching isozymes; photosynthetic pigments; polyacrylamide gel electrophoresis.

Abstract—The cyanelles of *Glaucocystis nostochinearum* were isolated after disruption of the algal cells by sonication. The aqueous extracts from these cyanelles were subjected to molecular filtration and electrophoresis on polyacrylamide gels. By comparison with extracts of a unicellular Chroococcalian alga, *Anacystis nidulans* treated in the same way only about half the number of protein bands were found. The proteins were water-soluble with a MW in excess of 10000. Three protein-pigment complexes were detected in *Anacystis*. Two of these (phycoerythrin and phycocyanin) were not present in the cyanelles of *Glaucocystis*. Three branching glucosyltransferase isozymes capable of converting amylopectin to phytoglycogen were present in the Cyanophyte; only two branching isozymes with typical Chlorophycean 'Q' activity were present in the cyanelles of *Glaucocystis*. It seems improbable that the cyanelles of this alga are endosymbiotic blue–green algae; rather, they may represent some intermediate stage in the development of the chloroplast of green algae.

INTRODUCTION

Hall and Claus [1] in their study of the ultrastructure of the Chlorophyte, *Glaucocystis nostochinearum*, concluded that the chloroplasts ('cyanelles') of this alga were actually endosymbiotic blue–green algae of the order Chroococcales. *Glaucocystis* has been viewed by endosymbiont proponents [2, 3] as proof of the ability of eukaryotic cells to acquire prokaryotes as organelles. However, traditional evolutionists regard this alga as a possible step in the evolution of Chlorophyceae from Cyanophycean ancestors [4, 5].

The use of polyacrylamide gel electrophoresis as a posible taxonomic 'tool' for classification of algae, was suggested by Fredrick [6] after his initial discovery of multiple molecular forms of enzymes (isozymes) in blue–green algae. Subsequently, the whole cell proteins of blue–green algae were studied by Derbyshire and Whitton [7] using a discontinuous buffer system in polyacrylamide gels. They found similarities in 20 of the protein bands from a variety of Cyanophytes. Thomas and Brown [8] suggested that *isozyme patterns* on polyacrylamide gels were a valid new taxonomic criterion for the classification of Chlorococcum species. The application of this method to algal taxonomy has recently been reviewed [9].

In a preliminary study, Fredrick [10] found that the storage glucan of *G. nostochinearum* was identical to the amylopectin of Chlorophytes, and that the pattern of glucosyltransferase isozymes involved in the synthesis of the glucan resembled the polyacrylamide gel patterns of the isozymes of typical green algae rather than those of blue–green algae.

In order to study both the protein pattern and the pattern of glucosyltransferases of the 'cyanelles' of *Glaucocystis* and to compare these with those of a unicellular Chroococcalian, typical of a blue–green alga which might have entered into the postulated endosymbiotic relationship with the apochlorotic host [1], a method other than ammonium sulfate precipitation of the protein fractions in the extracts, was devised. This consisted in isolating the cyanelles after disruption of the cells of *Glaucocystis*, extraction of the isolated cyanelles and subjecting the extract to molecular filtration in order to concentrate the proteins and to eliminate some of the lower MW species in the extract. The patterns obtained on polyacrylamide gels, using the discontinuous buffer method [7], for the cyanelles were compared with those of the unicellular Cyanophyte, *Anacystis nidulans*.

RESULTS

The polyacrylamide gel patterns are shown in Fig. 1. The relative positions are shown in Table 1 as R_p values and appeared to be relatively constant in position from gel to gel. The R_p value has been calculated as by Derbyshire and Whitton [7]. The significance of some of the bands is indicated in the Table.

The protein pattern of *Anacystis* shows a total of 19 visibly distinct bands (*a*), while that of the *Glaucocystis* cyanelles shows only 9 bands (*f*). Three colored bands were observed on the unstained *Anacystis* gel. The band at R_p 0.41 was a pink–orange color, the one at R_p 0.81 was bright blue and that at R_p 0.93 was light green in color (Table 1). Only the light green band was present in the cyanelles gel. These bands stained with the protein stain used and probably represented protein–pigment complexes.

Table 1. Mobilities and significance of proteins* of *Anacystis nidulans* and cyanelles of *Glaucocystis nostochinearum*

R_p		
Anacystis	Cyanelles	Band content
0.06	0.06	phosphorylase isozyme
0.10	0.10	phosphorylase isozyme
0.13		
0.15		
0.19		
0.23		
	0.25	
0.30		
0.32	0.33	synthetase isozyme
0.35	0.35	synthetase isozyme
0.41		phycoerythrin (?)
0.53	0.55	phosphatase–amylase
0.59		
0.63	0.63	branching isozyme
0.65	0.66	branching isozyme
0.68		branching isozyme
0.73		
0.81		phycocyanin (?)
0.86		
0.94	0.93	chlorophyll (?)

* Water-soluble, MW 10000 or greater.

After incubation with glucose-1-phosphate, a band appeared in both the *Anacystis* and cyanelles gels at R_p 0.53 and 0.55. These bands did not form any I_2-stainable material as compared with the phosphorylase isozyme bands at R_p 0.06 and 0.10 (Fig. 1, *b* and *g*). Similar bands at R_p 0.53–0.55 were observed when the gels were incubated after the incorporation of amylose (*d* and *i*) and amylopectin (*e* and *j*). The bands probably represented a phosphatase–amylase complex not completely resolved in the polyacrylamide gel concentration used (Table 1).

The gel from *Anacystis* and from the cyanelles, when incubated with ADPG, revealed two synthetase isozymes (Fig. 1, *c* and *h*, Table 1).

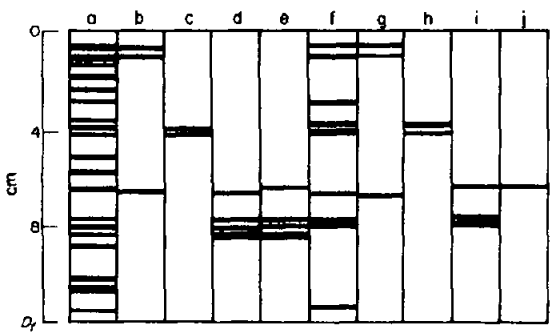

Fig. 1. Polyacylamide gel patterns of proteins of *Anacystis nidulans* (*a–e*) and cyanelles of *Glaucocystis nostochinearum* (*f–j*). Anode is to the bottom of the figure. *a* and *f*, protein stain; *b* and *g*, phosphorylase stain; *c* and *h*, synthetase stain; *d* and *i*, with amylose incorporated into gel (branching enzyme visualization, Q—enzymes); *e* and *j*, amylopectin incorporated into gel (see text). Scale at the left is in cm D_f, dye front; O, origin of proteins.

Apart from the smaller number of proteins present in the cyanelles gel (Fig. 1, *f*), the most significant difference was in the bands at R_ps 0.63–0.68. *Anacystis* showed 3 bands which exhibited activity on both amylose and amylopectin substrates (Fig. 1, *d* and *e*), while the cyanelles gel showed only two bands active on amylose (Fig. 1, *i*) but not on amylopectin (Fig. 1, *j*).

DISCUSSION

The endosymbiotic origin of plastids has attracted much attention since the recent revision by Margulis [11] of Mereschkowsky's original hypothesis [12]. Of particular interest is the phenomenon of *syncyanosis* (symbiosis between a blue-green alga and another alga) as exemplified by *G. nostochinearum* [1]. This supposed symbiosis between a Chlorococcalian (apochlorotic unicellular green alga) and an Aphanothece-like Chroococcalian unicellular blue-green alga, has been studied morphologically [1] and more recently, biochemically [10]. Whole cell analysis of *Glaucocystis* did not reveal any similarity with regard to its storage glucan and its branching glucosyltransferase isozymes with Cyanophytes [10].

The present study further indicates that the cyanelles of this alga are probably not blue-green algae. A comparison of the proteins of these cyanelles and those of an unicellular blue-green alga, *A. nidulans*, shows fewer water-soluble proteins with a MW in excess of 10000 in the cyanelles than in the blue-green alga (Fig. 1). These differences are apparent not only insofar as the branching isozymes are concerned, but also in the photosynthetic pigments (Table 1). While phycoerythrin and phycocyanin–protein complexes are present in *Anacystis*, only chlorophyll appears to be present in the cyanelles of *Glaucocystis*.

While it is possible that in a syncyanosis-type of relationship, some of the functions of the endosymbiont are eventually relinquished to the host [13], those enzymes involved in the synthesis of storage glucan appear to be retained in the chloroplasts of algae. The presence of the three groups of glucosyltransferases in the chloroplasts (cyanelles) of *G. nostochinearum* indicates that this is indeed the case.

The branching isozymes of the cyanelles of *Glaucocystis* can convert amyloses to moderately-branched amylopectins, but are not able to insert additional α-1,6-glucosyl linkages into amylopectin, as can the isozymes of the blue-green alga, *Anacystis*. Hence, the storage glucan of these cyanelles is much less branched than the phytoglycogen-like glucans characterized by Fredrick [14] for *Oscillatoria princeps* and by Chao and Bowen [15] for *Nostoc musorum*. Rather, the cyanelles of *Glaucocystis* exhibit the types of branching isozymes characteristic of Chlorophytes [16] and form amylopectins typical of green algae and higher plants.

Because of the biochemistry of *Glaucocystis* [10], and particularly the similarity of the glucosyltransferase isozymes present in its cyanelles to those of green algae, it is felt that this alga does not typify the *syncyanosis* assigned to it by many investigators [1–3]. Further work on this alga should prove valuable for it may represent a transitional form, as postulated by Klein [4], between the Cyanophyceae and the Chlorophyceae. The ultrastructure of its cyanelles, instead of being viewed as an adaptation of endosymbiotic blue-green algae to a

host [1], may prove to be a *primitive* stage along the line of development from the unorganized thylakoids of Cyanophytes to the compact structure of the Chlorophycean chloroplast.

EXPERIMENTAL

Cells from a 30-day culture of *G. nostochinearum*, grown on the medium described in ref. [10], were collected by centrifugation, washed twice with 60 mM Tris–HCl buffer (pH 8.1) containing 0.2 M sorbitol and 10 mM KCl. The drained cells were suspended in 5 ml of the buffer and cooled to 5°. All further operations were performed at this temp. The suspended cells were disrupted with a Polytron PT 10/ST homogenizer for exactly 20 sec. The resulting brei was centrifuged at 1000 *g* for 1 min to remove the cell debris. The supernatant was centrifuged for 10 min at 1000 *g*. The pellet contained mostly intact cyanelles, and was washed twice with Tris–HCl buffer alone. It was drained and ground with fine quartz sand. The slurry was extracted with 5 ml of the Tris–HCl buffer and the extract centrifuged at 1000 *g* for 3 min. The supernatant was filtered through a Pellicon Type PTGC membrane with a *nmwl* of 10000 for 45 min. At the end of this time, the retentate showed an increase in protein concn of *ca* ×20. Cells from a 32 day culture of *A. nidulans* were disrupted as described above, and the extract was subjected to the same treatment. Electrophoresis of the retentates was carried out on polyacrylamide gels using the discontinuous buffer technique described [7] but using the E-C 470 Vertical Cell so that multiple separations of the same retentates were available. The gels were sectioned vertically and a parallel section stained with Procion Blue MRS for protein [17]. Other parallel sections were incubated in various substrates and stained histochemically for phosphorylase, synthetase and branching enzyme action [16]. Branching enzyme action was tested on two substrates, amylose and amylopectin [18].

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