Phytochemistry, Vol 22, No 5, pp 1155–1157, 1983
Printed in Great Britain

0031-9422/83/051155-03$03 00/0

INTRASPECIES EVOLUTION OF ENZYMIC MECHANISMS ASSOCIATED WITH THE SYNTHESIS OF α-1,4 STORAGE GLUCANS IN TWO THERMOPHILIC–ACIDOPHILIC ALGAE OF *CYANIDIUM* TYPE

JEROME F FREDRICK and JOSEPH SECKBACH*

The Research Laboratories, Dodge Chemical Co , Bronx, NY 10469, U S A , *School for Overseas Students, The Hebrew University, Jerusalem, Israel

(*Received* 16 *August* 1982)

Key Word Index—*Cyanidium caldarium, Glaucosphaera vacuolata, Nostoc muscorum*, Cyanophyceae, α-1,4 storage glucans, phytoglycogen, floridean starch, glucosyltransferase isozymes, thermoacidophilic algae, eukaryotic algae, endosymbiosis, evolution

Abstract—*Cyanidium caldarium* is an enigmatic eukaryotic alga which is both acidophilic and thermophilic. Its taxonomic position has been in doubt and, hence, it has been classified with practically every algal group, including prokaryotic blue-green algae It has recently been found that supposedly axenic cultures of *Cyanidium caldarium* actually were mixtures of two or more similar thermoacidophilic algae. The α-1,4 glucans, which are formed by these algae, form a spectrum of storage glucans which is suggestive of a possible orderly evolutionary progression from the Cyanobacteria to the Rhodophyceae. The glucosyltransferase isozymes responsible for the biosynthesis of these storage sugars also present suggestive evidence that these algae are 'bridges' linking the blue-greens and the reds thereby forming a continuous line of evidence for this evolutionary pathway

INTRODUCTION

The controversial hot-springs alga, *Cyanidium caldarium*, has been described by endosymbiontists as an endocyanome [1, 2]. This would indicate that a symbiotic relationship was established between some cyanophyte and an apochlorotic pro-eukaryotic algal cell. It has also been proposed that this alga is actually an extant 'fossil' and, therefore, is a transition form between the Cyanobacteria and the red algae [3 5]. Recent biochemical evidence, particularly with regard to the storage glucan that *C. caldarium* forms and the enzymes responsible for its biosynthesis, as well as morphological evidence from electron microscopic studies of the structure of the chloroplast in *Cyanidium*, lend strong support to the concept that this alga is not an endocyanome but rather represents an example of a true transition form between the Cyanobacteria (Cyanophyceae) and the Rhodophyceae [6–8] While the biochemistry of the glucan and the glucosyltransferases associated with its formation and degradation are almost identical with *Nostoc* [9], the morphological data indicates Rhodophycean characteristics [10, 11]

Recently, it has been shown that much of the research carried out with supposedly axenic cultures of *Cyanidium caldarium* may have involved mixed populations of two, and possibly three, related algae [12, 13] These algae have been isolated from different parts of the world [14] The main characteristics of these two algae are shown in Table 1.

From these data, it would appear that the two algae represent examples of successive stages of the intraspecies evolution of morphological characters [15]. It was decided to test whether these changes in characteristics extended to biochemical features as well. Cloned populations of cells started from single isolated cells were used

Table 1 Thermophilic–acidophilic algae related to *C caldarium*

	Cyanidium variety or type	
Structure	Form A* (RK-1)†	Form B* (M-8)†
Cell size (μm)	2.0	4 0
Endospores formed	4	4, 8, 16, 32
Nucleus	+	+
Mitochondrion	1	more than 1
Vacuoles	–	+
Trophism	obligatory autotroph	facultative heterotroph

*Data from ref. [12].
†Data from ref [14]

The storage glucans were complexed with iodine for further characterization after they were isolated from extracts of the algae. The glucosyltransferases were isolated and studied by PAGE methods

RESULTS

The iodine complexes of the storage sugars of both types of *Cyanidium* showed that the smaller alga (form A) formed a type of highly branched α-1,4 glucan that could not be distinguished from that formed by the Cyanobacterium, *N. muscorum* (Fig. 1). The larger alga (form B) synthesized a storage glucan that apparently was not as highly branched as that of *Cyanidium* form A and resembled more the floridean starch of red algae Note that the iodine complex resembles that of the glucan of the red alga, *G vacuolata* (cf curves of absorption spectra in Fig 1)

The glucosyltransferases of both forms of *C caldarium* appeared to be similar Each contained one phosphorylase (EC 2 4 1 1) isozyme and two synthetase (EC 2 4 1 11) isozymes. Both of the synthetase isozymes were active using ADPG and UDPG

However, the branching isozymes (EC 2.4.1.18) of the smaller alga (form A) were active on both amylose and amylopectin substrates, rapidly branching both substrates to phytoglycogens. Two of the three branching isozymes of the larger variety (form B) were identical with those of the smaller alga The third branching isozyme was definitely of the classical 'Q' variety and, while it could branch amylose to moderately ramified amylopectin, it was unable to introduce further α-1,6 glucosyl linkages into the amylopectin Hence, the larger variety of *C caldarium* (form B), contained two branching isozymes of the b.e type and one isozyme of the Q type

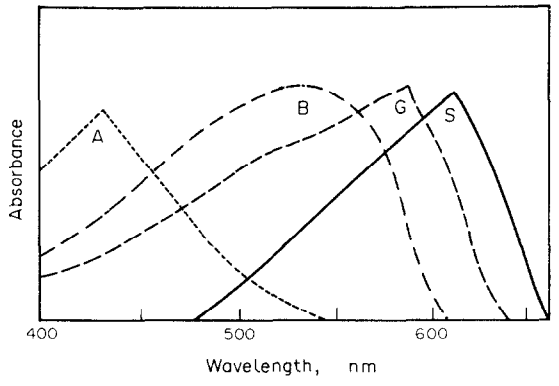

Fig 1 Absorption spectra of glucan–iodine complexes of the storage glucans of *C. caldarium* forms A (----) and B (– –), and *G vacuolata* (– – –) and of the iodine complex of soluble starch (——) (less branched than floridean starch) The A type is a phytoglycogen similar to that of the blue-green alga *N muscorum* [16]

DISCUSSION

The discovery of two, and possibly three, different varieties of *C caldarium* has posed some interesting possibilities for the study of the processes of evolution within an algal group The two forms of *Cyanidium* used in this study are an example of the close relationship of these algae to the Cyanobacteria (for example form A or RK-1) and to the Rhodophyceae (form B or M-8) Besides the similarities in the structure of the storage glucan synthesized by form A *Cyanidium* and that formed by *N muscorum*, and that of type B *Cyanidium* and *G vacuolata*, the types of glucosyltransferase isozymes isolated from these algae after PAGE, are also suggestive of this evolutionary relationship

The presence of linolenic acid in *Cyanidium* B, and in eukaryotic algae, and its absence in *Cyanidium* A and prokaryotic algae [17, 18] would also suggest that the A form of *C caldarium* is the more primitive and, hence, closer to the Cyanobacteria, while the B form of the alga appears to be more evolutionally advanced and similar, therefore, to the Rhodophyceae [17].

It is of interest also that most red algae are found in marine habitats [19] and, hence, must have some tolerance for the higher osmotic pressures of sea water It has been shown that the B form of *C. caldarium* is more salt tolerant than the A form [20] Therefore, this form of *Cyanidium* appears to possess many characteristics of red algae

From the standpoint of traditional evolution, there seems to be little doubt that both of these thermoacidophilic forms of *C caldarium* are closely related They are certainly the only eukaryotic algae found living under these conditions. Even though these varieties of *Cyanidium* differ in cell size (cf Table 1), there really is not too great a morphological difference in these cells Both forms of *Cyanidium* have chloroplasts that are similar in structure and that are surrounded by only one membrane [8, 10] The similarities in most of the glucosyltransferase isozymes responsible for storage glucan synthesis in both algae adds further evidence of a species relationship between these two forms

If the A and B forms of *Cyanidium* reflect the path of evolution within a single species, then they may be representative of the actual evolution of the red algae from an ancestral Cyanophycean (cyanobacterial) type. There is no doubt that the A form is definitely more primitive than the B form and has characteristics of the blue-green algae while the B form is certainly more Rhodophycean in nature

The great overall similarity in the biochemical properties of the glucan formed by the B form of *C caldarium* and that of *G vacuolata*, shown by McCracken *et al* [21] to be a primitive rhodophyte and not an endocyanome, indicates that the biosynthesis of α-1,4 storage glucan was modified as evolution progressed from the A (or more cyanobacterial form) to the B (or more rhodophyte-like form) possibly via changes in one or more of the branching isozymes One of the branching isozymes of the B form of *C caldarium* is definitely of the classical Q type This was shown to occur in another red alga, *Rhodymenia pertusa* [22] The presence of both forms of *C caldarium* indicates a possible pathway for the intraspecies evolution of *C. caldarium* and lends additional evidence to the transitional 'bridge' status of these algae between the Cyanophyceae and the Rhodophyceae

Work continues on an unusual third form of *Cyanidium*, isolated by the Italian researchers, from hot-springs in Italy This form, while closely related to the A form, is reported to have characteristics of the B form as well [23] The isolation and cloning of this alga would lend even more support for intraspecies evolution among the Cyanidiophyceae

EXPERIMENTAL

C caldarium, forms A and B were obtained from the University of Naples culture collection Each form was cloned from isolated individual cells and grown in Allen's medium [24] at pH 2 5 and 40° The cultures were continuously illuminated with fluorescent daylight tubes [12] *G vacuolata* was obtained from the University of Texas culture collection (UTEX 1662) This alga was grown in the medium and under the conditions specified by McCracken *et al* [21]

The algae were collected by centrifugation, washed × 3 with deionized H_2O and then macerated with fine quartz sand in boiling H_2O as described by Sheath *et al* [25] using Ramus' method [26] The glucans were ppted from the cooled extracts with Me_2CO or EtOH [25] and were dried *in vacuo* The glucans were complexed with I_2 using Krisman's [27] soln and the absorption spectrum determined in a Coleman spectrophotometer

Extracts of the algae in the cold were prepared as described [28] and the glucosyltransferases ppted with $(NH_4)_2SO_4$ [28] The glucosyltransferases were isolated using PAGE as described and tested with different substrates [28]

Acknowledgement—We wish to thank Professor P DeLuca, Botanical Institute, University of Naples, for making available the cultures of the different forms of *Cyanidium*

REFERENCES

1 Margulis, L (1968) *Science* **161**, 1020
2 Kremer, B P, Feige, G B and Schneider, A W (1978) *Naturwissenschaften* **65**, 217
3. Klein, R M (1970) *Ann N.Y Acad Sci* **175**, 623
4. Fredrick, J F (1977) *Phytochemistry* **16**, 1489
5 Seckbach, J and Fredrick, J F (1981) in *Origin of Life* (Wolman, Y., ed) pp 567–574 D Reidel, Holland.
6 Fredrick, J. F (1981) *Ann. N.Y. Acad Sci* **361**, 426
7 Seckbach, J, Hammerman, I S and Hanania, J (1981) *Ann N Y Acad Sci* **361**, 409
8 Toyama, S. (1980) *Cytologia* **45**, 779
9. Fredrick, J F (1976) *Plant Cell Physiol* **17**, 317
10. Seckbach, J and Fredrick, J F (1980) *Microbios* **29**, 135
11 Kremer, B P (1982) *Br Phycol J* **17**, 51
12. DeLuca, P, Gambardella, R and Merola, A (1979) *Bot Gaz (Chicago)* **140**, 418
13 DeLuca, P and Teddei, R (1976) *Webbia Racc Scr Bot* **30**, 197
14. Nagashima, H. and Fukuda, I (1981) *Jpn J Phycol* **29**, 237
15. Dobzhansky, T (1970) *Genetics of the Evolutionary Process* pp 351–390 Columbia University Press, New York
16 Fredrick, J. F (1980) *Phytochemistry* **19**, 2611
17. Boenzi, D, DeLuca, P and Teddei, R (1977) *G Bot Ital* **111**, 129
18 Moretti, A. and Nazzaro, R. (1980) *Delpinoa* **21**, 1
19 Velde, Van H. H (1977) Ph D Thesis p 7 Free University of Amsterdam.
20 Massacchio, A, Sabato, S., Pinto, G and Teddei, R (1976) *Delpinoa* **18**, 1
21 McCracken, D. A, Nadakavukaren, M J and Cain, J R (1980) *New Phytol.* **86**, 39.
22. Fredrick, J F (1971) *Physiol Plant* **24**, 55
23 DeLuca, P, Moretti, A and Teddei, R (1977) *Delpinoa* **19**, 69
24. Allen, M B. (1959) *Arch. Mikrobiol* **32**, 270
25 Sheath, R G, Hellebust, J and Sawa, T (1979) *Phycologia* **18**, 149
26 Ramus, J (1972) *J Phycol* **8**, 97
27 Krisman, C R. (1962) *Analyt Biochem* **4**, 17
28. Fredrick, J F (1978) in *Handbook of Phycological Methods* (Hellebust, J and Cragie, J, eds) pp 245–253 Cambridge University Press, New York