Phytochemistry, 1975, Vol. 14, pp. 1911–1913. Pergamon Press. Printed in England.

EFFECT OF ENVIRONMENT pH ON STORAGE GLUCAN SYNTHESIS IN THREE THERMOPHILIC ALGAE

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(*Received* 20 *November* 1974)

Key Word Index—*Cyanidium caldarium*; *Oscillatoria princeps*; *Plectonema nostocorum*; Cyanophyceae; storage glucans; glucosyltransferase isozymes; biosynthesis; polyacrylamide gel electrophoresis.

Abstract—*Plectonema nostocorum*, a thermophilic cyanophyte which lives under alkaline conditions at pHs approaching 13, forms a storage glucan showing a maximum absorption of its iodine complex almost identical with that of another thermophilic cyanophyte, *Oscillatoria princeps*, which exists at a more neutral pH, and with that of the acidophilic thermophile, *Cyanidium caldarium*. Gel electrophoretic patterns of the storage glucan-forming isozymes of *Plectonema* do not differ essentially from those of *Oscillatoria*. The a_2 phosphorylase isozyme appears to be primer-independent, and resembles the a_2 isozymes of both *Oscillatoria* and *Cyanidium*. The isozymes responsible for forming α-1,6-glucosidic branched linkages in *Plectonema* are of the *b.e.* type (able to further branch amylopectin), rather than of the *Q* type (able to branch amylose only to amylopectin).

INTRODUCTION

The formation of storage glucans in algae appears to be the result of the interaction of three groups of enzymes [1–3]: phosphorylases (E.C. 2.4.1.1), synthetases (E.C. 2.4.1.11) and branching enzymes (E.C. 2.4.1.18). Both phosphorylases and synthetases form α-1,4-glucosyl linkages, while branching enzyme forms α-1,6-branched points in the storage polyglucosides. All three groups of enzymes have been shown to exist in algae in multiple molecular forms (isozymes), and seem to be related in an evolutionary sense [4–8].

Carbohydrates form universal respirable substrates in cells [9]; the polymeric or "storage" forms undoubtedly offered some evolutionary advantage. Probably those primitive cells capable of synthesizing storage sugars were selected during the course of evolution from among the early cell types. It also seems probable that because of the geochemistry of this planet, these primordial cells first appeared in thermal environments [10]. It might be of value to study the biosynthesis of storage glucans in algae living under [illegible] The algae living in hot springs and their effluents would appear to offer ideal organisms for this.

Recently, it has been shown that some differences exist in the polyglucoside-forming isozymes of thermophilic and mesophilic algae, particularly in the a_2 phosphorylase isozymes [11]. Whether these differences are directly related to the temperature conditions of the particular ecological niche, or to other factors, is not certain. Because of the considerable variation in hydrogen ion concentration in thermal pools and springs, Brock and Brock suggested that the pH of the immediate environment might be an important factor in the biological isolation of the different specie of thermophilic algae [12]. Three thermophilic algae seem to be ideally suited for such studies. *Cyanidium caldarium*, a primitive eukaryotic alga, and the subject of controversy with regard to its taxonomic position [9,13,15,16], lives under extreme acid conditions [12,14]. It can be successfully grown at a pH of 2 and lower, and is normally found in acid hot-springs where the pH is 1–3 [17]. *Oscillatoria princeps* is a cyanophyte which lives in media where the pHs are

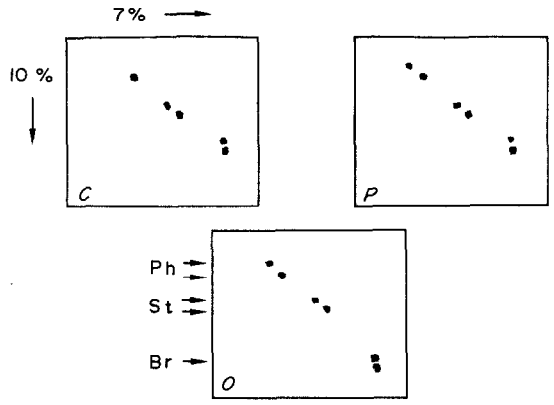

Fig. 1. Electrophoresis of polyglucoside-synthesising enzymes from *Cyanidium* (*C*), *Plectonema* (*P*) and *Oscillatoria* (*O*) on polyacrylamide gels by the orthogonal slab method [27–30].

more or less in the "neutral" range of 6–8. *Plectonema nostocorum* is a basophilic cyanophyte which tolerates a pH of at least 13 [18].

Since the storage glucans formed in algae reflect, in a biochemical sense, the end-products of the interactions of the three groups of polyglucoside-synthesizing isozymes, the isolation and properties of these glucans might possibly give some indication of the patterns of these enzymes with regard to their adaptation to different environmental pH's.

RESULTS

The glucan of *Plectonema nostocorum* showed a maximum absorption of its iodine complex at 540 nm. The storage glucans of all three algae exhibited maxima of their iodine complexes in the range of 540–550 nm. The colors of the complexes were all red-violet. The three glucans were readily dispersed in cold water and gave opalescent solutions. None showed any retrogradation even after 24 hr.

When the extract from *Plectonema nostocorum* was fractionated with ammonium sulfate and the polyglucoside-synthesizing enzymes fraction subjected to electrophoresis on 7 and 10% polyacrylamide gels, using the orthogonal slab method, the resulting pattern was identical with that of *Oscillatoria princeps*. The pattern also resembled that obtained from *Cyanidium caldarium*. The faster anodic-moving phosphorylase isozyme exhibited the ability to form amylose without the need for maltoheptaose primer. This isozyme appears to be identical with the a_2 phosphorylase two branching isozymes of *Plectonema* appear to be true branching enzymes (*b.e.*) with the ability to branch amylopectin. In this respect, they were identical in their actions with the branching isozymes of the other two algae (Fig. 1).

DISCUSSION

The occurrence of the three related groups of isozymes involved in storage polyglucoside formation in all algae examined thus far [19], would seem to indicate that these enzymes are a *sine qua non* for both prokaryotic and eukaryotic algae. The continuity of this same group of enzymes in intermediate and higher plant forms [19] points to the natural selection of these enzyme mechanisms for the formation of reserve supplies of readily available chemical energy throughout cellular evolution.

When dealing with the Cyanophyceae, one of the most primitive algal groups, one would expect to detect some changes in these enzymes to accommodate the particular habitat conditions. There are some minor variations in the physical–chemical constants of one of the phosphorylase isozymes, a_2, when the thermophilic and mesophilic Cyanophytes are compared [11]. But, even here, the differences are slight and probably not the result of molecular changes in the enzymes, but rather reflect changes in the chemical kinetics of these reactions at the higher temperatures.

The enigmatic acidophilic alga, *Cyanidium caldarium*, apparently contains the same a_2 phosphorylase isozyme as is present in *Oscillatoria* and *Plectonema*. The fact that this isozyme has been shown to be conjugated with polyhexose, and is a glycoprotein [19], would make it reasonable to expect that under the conditions of the habitat occupied by this alga (high temperature and high acidity), this glycoprotein would show some hydrolysis to yield the a_1 isozyme [20], and that *this* form of phosphorylase should be the one prevalent in this alga. This is not the case, since this a_1 isozyme appears to be absent from *Cyanidium caldarium*.

The storage glucans of all three algae show a remarkable similarity. On the basis of the relationship of the absorption maxima and iodine colors with chain length, it would appear that the structure of these glucans is the same both in size

An interesting observation resulting from this data is that the acidity of the immediate ecological habitat apparently does not affect either the complex kinetics of the isozymes involved in storage glucan synthesis, or the properties of the glucan itself. This would seem to present an example of a series of biochemical mechanisms which were, in all probability, established in the evolutionary progression from primordial to prokaryotic cell, and selected through such transition forms as represented by the primitive eukaryote, *Cyanidium caldarium*, and continued through the next step in cellular evolution, the formation of the extant eukaryotic cell.

EXPERIMENTAL

All algae were grown in 2 l. conical flasks maintained at 45° and illuminated with daylight flourescent tubes. All of the liquid media contained ethylene diamine tetraacetic acid (EDTA) in various sodium salt forms in a final concentration of 0·01 % w/v.

Cyanidium caldarium was grown in a modification of the Allen medium as previously described [23]. The acid form of EDTA was used in this medium, and the pH maintained at 2·5–3·0. *Oscillatoria princeps* was grown in modified Chu no. 10 medium [24] with disodium-EDTA used. The medium was maintained at pH 7·5 [25]. *Plectonema nostocorum* was also grown in the modified Chu no. 10 medium. The use of tetrasodium-EDTA assisted in maintaining the pH of the medium at 12, and in preventing precipitation of cations at the alkaline pH.

The algae were harvested, washed thoroughly to remove traces of the media, and extracts prepared as previously described [26]. The storage glucans were isolated and purified by precipitation with EtOH from cold dimethyl sulfoxide solutions [27]. The glucans were dispersed in cold H_2O and the complexes formed with Krisman's iodine reagent [28]. Absorption spectra were obtained with a Beckman spectrophotometer.

The polyglucoside-synthesizing isozymes were separated in an E-C no. 470 vertical cell on polyacrylamide gels as described [29]. Localization and visualization of the separated isozymes were performed by modified histochemical techniques which have been described [27–30].

Acknowledgements—This research was supported by a grant from The Dodge Institute, Cambridge, MA. The author is indebted to Alan Mark Fredrick for help with the manuscript.

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