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STORAGE POLYGLUCAN-SYNTHESIZING ISOZYME PATTERNS IN THE CYANOPHYCEAE[1]

JEROME F. FREDRICK

Research Laboratories, the Dodge Chemical Company, Bronx, N.Y. 10469, U.S.A.

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Abstract—Patterns of the polyglucoside-synthesizing isozymes responsible for the formation of storage glucans in the algae, obtained by gel electrophoresis on polyacrylamide, are strongly suggestive of a line of evolution of the red algae from a blue-green ancestral type, possibly through *Cyanidium caldarium* which appears to be an extant 'bridge' form. The fate of the three groups of isozymes is traced in three Cyanophytes: *Oscillatoria*, *Nostoc* and *Gloeocapsa* through *Cyanidium* and to *Rhodymenia*. The diminution of the a_1-phosphorylase isozyme can be followed in an unbroken path from the blue-greens to the reds. At the same time, the data suggest that the Chlorophytes were derived from the blue-greens with no involvement of the red algae in that line of descent.

INTRODUCTION

OF THE various techniques which have been applied in chemotaxonomy, perhaps the one offering the greatest potential is that of polyacrylamide gel electrophoresis. By this technique, which comprises the *disc* method of Ornstein[2] or the *vertical slab* method of Raymond,[3] extremely minute differences in plant proteins from closely related organisms have been resolved.[4] Derbyshire and Whitton[5] have been able to detect reproducible differences among the non-catalytic proteins of even closely related blue-green algae.

Fredrick, using both methods[6–9] with particular emphasis on the two-dimensional, or *orthogonal* method of Raymond,[10] has been able to detect variations in the isozymes involved in storage sugar synthesis in Cyanophytes, Rhodophytes and Chlorophytes.[11,12] This method has yielded valuable data with regard to the proper classification of the puzzling hot-springs alga, *Cyanidium caldarium*.[13] This alga had been classified by various investigators as a blue-green,[14] a red[15] and a green alga.[16] The recent polyacrylamide gel investigations of the polyglucoside-synthesizing isozymes of *Cyanidium* show patterns which are

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indicative of the alga possibly being a true transition form between the Cyanophyceae and the Rhodophyceae.[13] This had been previously suggested by Klein and Cronquist.[17]

Three main groups of isozymes are involved with the synthesis of storage glucans in algae. These are two phosphorylases (E.C. 2.4.1.1), two ADP/UDP-glucosyltransferases (E.C. 2.4.1.11), and two or more branching isozymes (E.C. 2.4.1.18). All of these isozymes show group-to-group variations among the blue-green, red, and the green algae.[8,9] Recently, it has been found that the so-called 'branching' isozymes which form alpha-1:6-glycosyl bonds in amylopectins and phytoglycogens, may be further subdivided into *two* groups. One type is capable of branching only linear amyloses to form amylopectins. This group behaves exactly like the classical *Q-enzyme* which had been described previously in higher plants[18] and in algae.[19] The other type of branching enzyme is capable, in addition to branching amylose, of introducing further alpha-1:6-linkages into an already-branched polymer such as amylopectin, and of converting branched structures into *more* highly branched sugars such as phytoglycogen.[20,21] These isozymes have been designated as *BE* as a means of differentiating them from the *Q-type* of branching isozymes.

It was found that the blue-green alga, *Oscillatoria princeps* and the hot-springs alga, *Cyanidium caldarium* possess this *dual*-action or *BE* type of branching isozyme. Of the three branching isozymes in the red alga, *Rhodymenia pertusa*, two seem to be conventional *Q*-types, while the other one appears to be a *BE* type.[22] The two green algae used in the previous studies, *Chlorella pyrenoidosa* and *Spirogyra setiformis* possessed only *Q* type branching isozymes.[22]

A more detailed study of these isozymes was needed, particularly with regard to the suggestive evidence obtained that *Cyanidium* might well be a 'bridge' form between the Cyanophyceae and the Rhodophyceae. At the same time, it was decided that a more intensive study of the gel patterns of the phosphorylases and the transferases was warranted, in view of their possible involvement in the evolution of the green algae from ancestral blue-green types.

RESULTS

The electrophoretic patterns of the polyglucosidesynthesizing isozymes are shown in Fig. 1. The overall similarity of the patterns of the three Cyanophytes used in this study, is apparent. But, there is a difference in the *absolute* concentration of the a_1 phosphorylase isozyme in the patterns. While this isozyme appears to be present in almost equal concentration as the a_2 phosphorylase isozyme in *Oscillatoria princeps*, it seems to be much diminished in quantity in *Nostoc muscorum* and in *Gloeocapsa dimidiata*.

The apparent identity of the isozymic pattern of *Cyanidium caldarium* and of *Gloeocapsa dimidiata* would seem to indicate a definite relationship between those two algae, except that the a_1 phosphorylase isozyme is not present in *Cyanidium caldarium*. It should be noted that *Rhodymenia pertusa* is also deficient in this isozyme.

Insofar as the ADP/UDP-glucosyltransferase isozymes, the patterns of all five algae are

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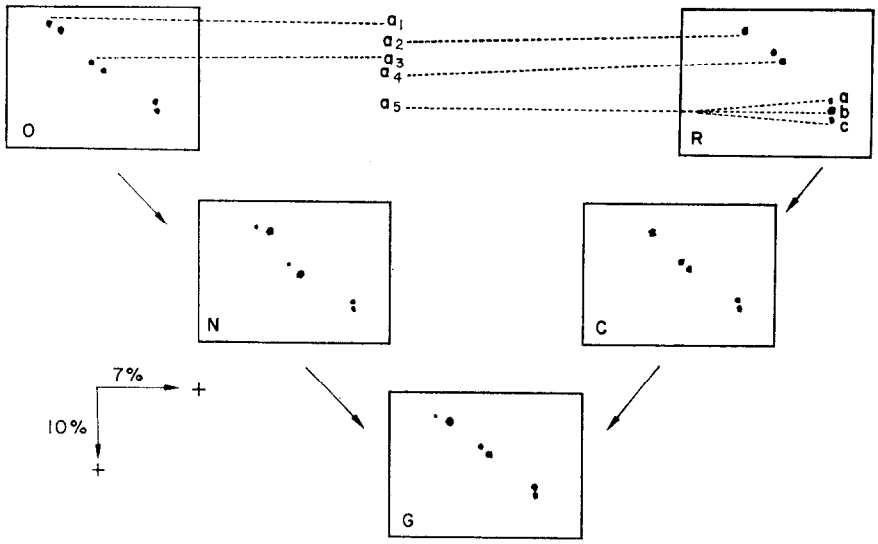

FIG. 1. ORTHOGONAL POLYACRYLAMIDE GEL PATTERNS OF STORAGE GLUCAN-SYNTHESIZING ISOZYMES OF ALGAE.
The concentrations of gel and the anodal (+) migrations are indicated by the arrows at the bottom left of the figure. a_1 and a_2 indicate the phosphorylase isozymes; a_3 and a_4 are ADP/UDP-glucosyltransferase isozymes; a_5 is the area of the branching isozymes. Note that these consist of either two or three isozymes (*a*, *b*, *c* in the *R* pattern). *O* = *Oscillatoria; N* = *Nostoc; G* = *Gloeocapsa; C* = *Cyanidium; R* = *Rhodymenia*. All a_5 isozymes are *BE* types except in the *R* pattern where only *a* is a *BE* type while *b* and *c* are *Q* types. See text.

much the same. *Nostoc muscorum* seems to contain a smaller quantity of the less anodic moving transferase, a_3 than do the other four algae.

The branching isozyme patterns (a_5 area) of the three blue-green algae and of *Cyanidium caldarium* are identical. The biochemical properties of these isozymes were the same; they were capable of branching amylose and of introducing further branching into amylopectin structures. In this respect, they have a dual action, and hence are classified as *BE* type of branching enzymes. However, the Rhodophyte contains three branching isozymes (*a*, *b* and *c*), only one of which (*a*) has the properties of those present in the Cyanophytes and in *Cyanidium caldarium*. The other two branching isozymes (*b*, *c*) are of the *Q* type.

DISCUSSION

The polyacrylamide gel electrophoresis patterns of the isozymes involved in storage glucan synthesis in the five algae used in this study, can be arranged as in Fig. 1 to present a a suggestive evolutionary sequence. There appears to be no essential difference in the *BE* type branching isozymes of any of the three Cyanophytes. Likewise, the branching isozymes of *Cyanidium caldarium* appear to be identical with those of the three blue-green algae. Recently, this similarity in biochemical properties has been extended to size and molecular weight.[23]

The fate of the a_1 phosphorylase isozyme when traced from *Oscillatoria princeps* through the sequence to the red alga, *Rhodymenia pertusa*, shows a gradual diminution in quantity.

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There is a decrease from *Oscillatoria* to *Nostoc* which is even more pronounced in *Gloeocapsa*. This isozyme is completely lacking in *Cyanidium caldarium* and in *Rhodymenia pertusa*. It has been shown that this isozyme is not dependent upon a cofactor, AMP,[24] and that its biosynthesis is effectively blocked in blue-green algae by amitrole.[25] Green algae also have two phosphorylase isozymes. The formation of the a_1 isozymes of *Chlorella pyrenoidosa* and *Spirogyra setiformis* is also blocked by growing these Chlorophytes in the presence of amitrole.[25] Although two phosphorylase isozymes have been reported in maize[26] and in spinach chloroplasts,[27] no evidence has been presented of the sensitivity of the formation of these isozymes to amitrole in these higher plants. It is interesting to speculate that the presence of *two* phosphorylase isozymes occurs in the blue-green alga, *Oscillatoria princeps*, in the green alga, *Spirogyra setiformis*, and in higher plants such as corn and spinach, but is not found in either the red alga, *Rhodymenia pertusa* or the hotsprings alga, *Cyanidium caldarium*.

These data would seem to lend supporting evidence to the idea that *Cyanidium caldarium* may be a 'bridge' form between the blue-green and the red algae.[17] Further data along these lines has been recently reported by Klein in his 'goodness of fit' hypothesis of analogy/homology when applied to Cyanophyte:Rhodophyte and to Chlorophyte:Rhodophyte. Positive values were overwhelmingly obtained from the comparison of chemical and microstructural characters in the Cyanophyte:Rhodophyte group, while mostly negative values were obtained when Chlorophyte:Rhodophyte characters were compared.[28]

It would seem probable that the blue-green algae gave rise to the red algae with *Cyanidium caldarium* as a probable extant transition form. At the same time, it would seem that the green algae were derived directly from the blue-greens and did not enter into any phylogenetic pathway involving the red algae.[17,28]

EXPERIMENTAL

Oscillatoria princeps, *Nostoc muscorum* and *Gloeocapsa dimidiata* were cultured in Gerloff's modified Chu No. 10 medium.[29] *Cyanidium caldarium* was grown from a culture supplied by L. Bogorad (Harvard University) in a modification of Allen's medium.[30] The red alga was grown in Provasoli's medium as modified by Fries.[31] The algae were harvested, washed in deionized water, macerated with sterile sand in the cold and extracted with dilute sodium bicarbonate solution. The isozymes were isolated by fractionation with iron-free ammonium sulfate as described by Fredrick and Gentile.[32]

Two-dimensional polyacrylamide gel electrophoresis was carried out in an E-C 470 Vertical Cell in the manner previously described.[8] The buffers used were Tris–EDTA–borate and have been described.[8] The polyacrylamide gel concentrations were 7% and 10% (w/v). Pre-runs of all gels used were at 350 V for 1 hr. Electrophoresis of the proteins in neighboring slots on the same gel slab was for 2 hr at 350 V in the horizontal direction, and for 3·5 hr at 400 V in the vertical direction.

Methods for the histochemical detection of the isozymes on the gels were as previously described.[11] Final patterns on duplicate orthogonal gels were stained with amido-black.[8]

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