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THE *b.e.* AND *Q* TYPES OF 1,4-α-D-GLUCAN: 1,4-α-D-GLUCAN-6-GLUCOSYLTRANSFERASE ISOZYMES IN ALGAE

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Key Word Index—*Anacystis nidulans; Cyanidium caldarium; Rhodymenia pertusa; Chlorella pyrenoidosa;* algae; branching glucosyltransferase isozymes; polyacrylamide gel electrophoresis, agarose gel; crossed-immunoelectrophoresis; biphyletic evolution of isozymes.

Abstract—The branching isozymes of the red alga, *Rhodymenia pertusa* are of two types: *Q*, which can branch, via the synthesis of α-1,6-glucosyl linkages, linear amyloses to amylopectin; and *b.e.*, which can further branch the amylopectin formed to the more highly-branched floridean starch. Using the technique of tandem crossed-immunoelectrophoresis, it is shown that the *Q* branching isozyme of the red alga is more closely related to the *b.e.* type of branching isozymes of *Anacystis nidulans* and *Cyanidium caldarium* than it is to the exclusively *Q* types of branching isozymes found in *Chlorella pyrenoidosa* and other chlorophytes. The possibility of a biphyletic evolution of the red and the green algae from blue–green ancestral forms is discussed.

INTRODUCTION

Multiple molecular forms (isozymes) of glucosyltransferases exist in algae [1, 2]. These isozymes form two main groups: those responsible for the synthesis of α-1,4-glucosyl linkages (the phosphorylases, EC 2.4.1.1, and the synthetases, EC 2.4.1.11), and the branching enzymes, EC 2.4.1.18, which form α-1,6-glucosyl bonds between already-linked α-1,4 chains.

The storage glucans of algae form a spectrum of basically α-1,4-linked glucose chains with and without branched α-1,6-chains [3]. Hence, the differences between amylose, amylopectin, floridean starch and phytoglycogen actually reflect the degree of branching in the glucan and are mainly due to the activities of the branching isozymes.

Differences in the activities of the branching enzymes in plants were first detected by Lavintman [4]. She distinguished two types of branching enzymes in maize, one which branched linear maltosaccharides such as amylose to amylopectin, and one which inserted further branched α-1,6 linkages into amylopectin, forming highly branched glucans such as phytoglycogens. The mechanism of this branching action in higher plants was recently elucidated by Whelan and co-workers [5, 6], and appears to be identical for algae as well [7].

Black *et al.* [8] described the action of both types of branching enzymes in corn, and used the nomenclature of '*Q* enzyme' for the branching enzymes that could form amylopectin, and 'branching enzyme (*b.e.*)' for the type that could further branch amylopectin. Recently, Boyer and Preiss [9, 10] found at least three branching isozymes in *Zea mays* kernels, each with the capability of branching α-1,4-linked linear glucans to various degrees.

Fredrick [11] first reported the presence of multiple molecular forms of branching enzyme in prokaryotic and eukaryotic algae. Some of these isozymes, apparently in primitive algae such as the cyanobacteria, had both types of activities and were able to form highly branched phytoglycogens such as are found in the 'α-granules' of blue–green algae [12]. The only other group forming phytoglycogens as the sole storage glucan is also a prokaryotic group, the bacteria [13].

The branching isozymes of the enigmatic thermophilic alga *Cyanidium caldarium* are also of the *b.e.* type [14]. However, in the red alga, *Rhodymenia pertusa*, both types of isozymes are present [15]. One of these isozymes has exclusively the *Q* type of activity in this red alga. In chlorophytes, only the *Q* types have been reported [16, 17].

The *b.e.* isozymes of *Cyanidium* and *Rhodymenia* are immunochemically identical with those of prokaryotic blue–green algae [18]. Even the *Q* isozyme found in the red alga appeared to be closely related, immunologically, to its *b.e.* isozymes, possibly being a *charge* isomer of the *b.e.* type and differing by the substitution of only a single amino acid residue [19].

Lindblom [20] reported that crossed-immunoelectrophoresis techniques were much more sensitive for the study of proteins of closely related strains of the alga, *Spirulina*, than the disc polyacrylamide gel methods used by Derbyshire and Whitton [21]. Since the *Q* enzymes of green algae differed from the *Q* enzyme of the red alga, *Rhodymenia pertusa*, a study of these branching isozymes in these algae by the more sensitive tandem crossed-immunoelectrophoresis technique [22] was indicated.

RESULTS

The results of the crossed-immunoelectrophoresis using the tandem antigen technique are shown in the

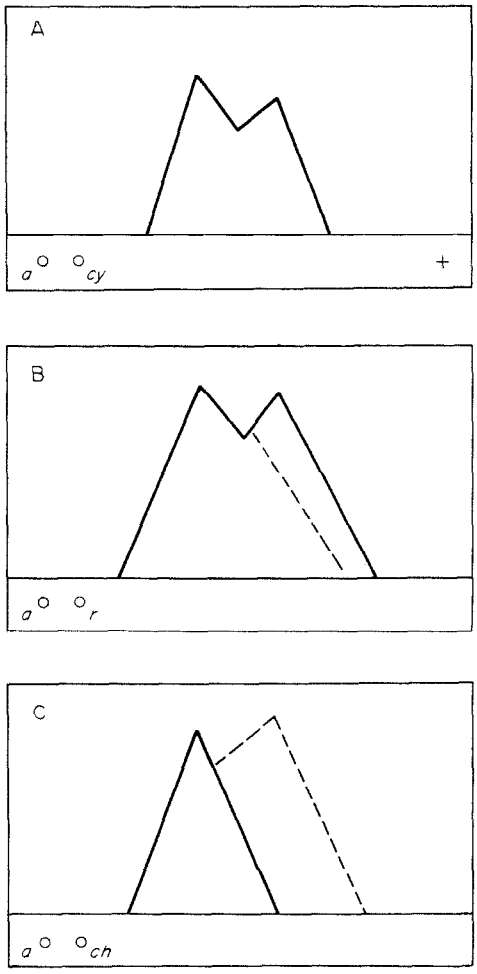

Fig. 1. Three types of tandem crossed-immunoelectrophoresis patterns obtained with branching isozymes of algae as antigens. The bottom strip of each diagram shows the two wells containing the branching isozymes of the various algae which were used as antigens. Electrophoresis in this strip of 1% agarose was carried out with the anode (+) to the right. The rest of the plate was poured with agarose containing antibodies to the most anodal-moving branching isozyme obtained from the polyacrylamide gel electrophoresis of extracts of *Anacystis nidulans*. A shows the pattern obtained using the *Anacystis* antigen (*a*) and the *Cyanidium* antigen (*cy*). B shows the pattern when *Rhodymenia* antigen was used (*r*). The dotted line represents weak spur formation. C shows the pattern when *Chlorella* antigen (*ch*) was used. The black line shows heavy precipitin reactions and pronounced spur formation. In all cases, after the horizontal electrophoresis (bottom strip), immunoelectrophoresis into the top part of the plate took place with the anode at the top of each diagram (see text for explanation of patterns).

three diagrams of Fig. 1. In all cases, the antigens were the most anodal-moving isozymes obtained after 2-D polyacrylamide gel electrophoresis. The antigens were placed in the wells shown, 8 mm, apart and electrophoresis carried out in the agarose with the anode (+) to the right. The rest of the 1% agarose gel used in the upper part of each plate contained the sera of rabbits with antibodies produced against the most anodal-moving branching isozyme of the blue-green alga, *Anacystis nidulans*. The diagrams shown in Fig. 1 represent the three types of immunological reactions observed.

In Fig. 1A, the complete fusion of the precipitin lines of the *Anacystis* and *Cyanidium* branching isozymes antigens, is shown. There was no spur formation.

Fig. 1B shows the results when the *Q* branching isozyme of *Rhodymenia pertusa* was used as antigen. There was a very slight spur formed, with a parallel displacement (shown by the dotted line). However, there was almost complete fusion of the heavy precipitin lines.

Fig. 1C shows the pronounced spur formation when the *Q* isozyme from the green alga *Chlorella pyrenoidosa* was used as antigen in the same system. Note also the weak reaction of the antigen (dotted line) with the antibodies from the *Anacystis* anodal-moving *b.e.* isozyme.

The results obtained with various other combinations of algal branching isozyme antigens are summarized in Table 1.

DISCUSSION

The immunological relationships between the branching isozymes of the cyanophyte, *Anacystis nidulans* and the enigmatic alga, *Cyanidium caldarium*, as well as the red alga, *Rhodymenia pertusa*, strongly suggest that these algae are closely related in an evolutionary sense (Fig. 1). Extensive similarities in their other glucosyltransferases, the phosphorylases and the synthetases, have also been reported [23, 24].

It is of interest that, even though the *Q* isozyme of *Rhodymenia* is only able to branch amylose to amylopectin, and thereby resembles the *Q* enzymes of chlorophytes [23], it nonetheless, differs immunologically from the branching isozymes of these green algae, and resembles more the *b.e.* types found in the more primitive blue-green algae (Table 1).

The *Q* branching isozyme of this rhodophyte is a charge isomer (differing in only a single amino acid residue) of the *b.e.* isozymes also present in this alga [19]. The immunological reactions obtained with the *b.e.* isozymes of *Anacystis* and *Cyanidium* confirm this. There seems to be a much wider evolutionary divergence, on the basis of the immunological reactions obtained in this study, between the *Q* enzyme of this alga and those present in chlorophytes.

If both the red and the green algae were derived from an ancestral blue-green alga [24], it seems apparent from the results of this study that the red algae have not diverged as far as the green algae from the original ancestral type, at least insofar as the branching isozymes involved in storage sugar formation, are concerned.

The apparent lack of any close immunological identity between the *Cyanidium b.e.* antigen and that of *Chlorella* (see Table 1), would seem to exclude *Cyanidium* as a transition form between the blue-green and the green algae. At the same time, the almost complete fusion of the precipitin line of this alga with that of *Anacystis* and *Rhodymenia*

Table 1. Results of tandem crossed-immunoelectrophoresis of antigens of various algae against anacystis antibodies

Algae	Type of branching isozyme used as antigen	Results
Cyanidium	*b.e.*	Complete fusion of heavy precipitin lines with no spurs (similar to pattern A of Fig. 1)
Rhodymenia	*Q*	
Cyanidium	*b.e.*	Pronounced heavy spur formation. Weak precipitin line of *Chlorella* antigen. (similar to pattern C of Fig. 1)
Chlorella	*Q*	
Rhodymenia	*Q*	Weak precipitin line of *Chlorella* antigen. Slight spur formation with parallel displacement (similar to pattern B of Fig. 1)
Chlorella	*Q*	

strengthens the probability of *Cyanidium* as a transition form between the Cyanophyceae and the Rhodophyceae. If this is so, then *Cyanidium caldarium* may represent a true bridge between the prokaryotic blue–green algae and the primitive eukaryotes, the red algae.

The postulated biphyletic evolutionary pathways from the blue–green to the red algae on one hand, and from the blue–green to the green algae on the other [25], appear to be probable as indicated by the results of this study. The green algae may have developed from Cyanophycean ancestors through intermediate forms other than *Cyanidium*. It has been suggested that *Glaucocystis* may be the transition form from the blue–green to the green algae [26]. Recent studies of the proteins and glucosyltransferase isozymes of this alga [27, 28] make such an evolutionary path possible.

EXPERIMENTAL

Cyanidium caldarium, *Chlorella pyrenoidosa*, *Rhodymenia pertusa* and *Anacystis nidulans* were grown in liquid media under conditions previously described [7, 14, 19, 28]. The algae were collected by mild centrifugation, washed ×3 with deionized H_2O, and macerated in bicarbonate buffer with fine quartz sand. The filtered extract was treated in the cold with solid $NH_4(SO_4)_2$ to precipitate the glucosyltransferase fraction. The purified glucosyltransferase fraction thus obtained was subjected to 2-D polyacrylamide gel electrophoresis in gel concns of 5 and 8%. All the methods and procedures have recently been summarized [29].

The most anodic-moving branching isozyme from *Anacystis* obtained after completion of the second-dimensional electrophoretic run was used, without elution from the polyacrylamide gel, to immunize rabbits [30]. The polyacrylamide in the sample served as adjuvant for the procedure [30]. The sera containing the antibodies to the *Anacystis* branching isozyme produced using this method were used in the immunoelectrophoresis procedures described below.

The microadaptation on 5×5 cm plates, of the method of tandem crossed-immunoelectrophoresis described by Kroll [22] was used. The most anodal-moving branching isozyme was eluted from the polyacrylamide gel [24] after the second-dimensional separation of each algal extract, and used as antigen.

Each antigen was placed in wells exactly 8 mm apart in 1% agarose after solidification. The wells were sealed [22] and electrophoresis of each antigen pair carried out for 25 min in the horizontal dimension at 10 V/cm. The rest of the 5×5 cm plate was poured with a 1% agarose soln containing an even suspension of the immune sera. Immunoelectrophoresis was carried out in the cold (6°) for 18 hr at 2 V/cm. The precipitin lines in the pressed plates [22] were stained with Procion blue MRS [29].

Prior to the tandem immunoelectrophoresis, the separated branching isozymes were tested while still in the polyacrylamide gels by incubation with amylose and amylopectin in a gel 'sandwich' [29], to determine the type of action on these polysaccharides by each separated branching isozyme.

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