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POSSIBLE COMMON ORIGIN OF GLUCOSYLTRANSFERASES IN *OSCILLATORIA PRINCEPS*

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Key Word Index—*Oscillatoria princeps*; Cyanophyceae; blue-green alga; glucosyltransferases; storage polyglucoside; immunoelectrophoresis; evolution.

Abstract—Gel electrophoresis of the glucosyltransferases of the blue-green alga, *Oscillatoria princeps*, followed by immunodiffusion against anti-phosphorylase rabbit serum, showed cross-reactions of the two phosphorylase isozymes and the two synthetase isozymes of the alga. Weak cross-reactions were also obtained with the branching isozymes. Apparent extensive similarities in the structure of the phosphorylases and the synthetases were indicated. However, only partial structural similarities between the two groups of α-1,4-glucosidic bond formers and the branching isozymes exist as indicated by the weak immunological reactions obtained and the formation of "spurs" on the immunoprecipitin lines. If the synthesis of α-1,4-glucosidic linkages and the formation of α-1,6 cross linkages were at one time due to the bifunctional action of a single catalytic protein, then the separation of these two enzymatic activities took place prior to the derivation of the synthetases from the phosphorylases.

INTRODUCTION

The formation of storage glucans in the algae is the end-result of the activities of three groups of glucosyltransferases: *phosphorylases* (E.C. 2.4.1.1), *synthetases* (E.C. 2.4.1.11) and branching enzymes (E.C. 2.4.1.18). These enzymes have been shown to exist in multiple molecular forms (isozymes) in these thallophytes [1, 2]. Together, the enzymes cooperatively form a "system" which probably conferred survival advantage upon those primitive cells possessing it. Since carbohydrates were undoubtedly the first respirable substrates of cells [3], those first cellular structures with the ability to form storage polysaccharides and thereby *store* energy-rich glucans, were no longer dependent upon the dwindling concentrations of hexose monomers present in the primeval melange. These cells thus became independent and were ready to proceed with the next step in cellular evolution, the eukaryotic structure.

Amino acid sequence analyses of the sites phosphorylated in both phosphorylase and synthetase, showed that the hexapeptides at these sites were identical in the enzymes [4, 5]. Further evidence from serological studies supported the structural similarity of these two enzymes [6].

Among the Cyanophyceae, there is immunochemical evidence that the phosphorylases and branching enzymes may be serologically related as well [7]. Based on this evidence, a common ancestral origin for the three groups of enzymes in the algae has been proposed [8, 9]. This hypothesis explored the possibility that the precursor catalytic protein combined the abilities to form α-1,4-glucosidic linkages (giving rise to such 'linear' glucans as the amyloses), and to synthesize α-1,6 branch points (leading to the formation of amylopectins and phytoglycogens).

RESULTS

The immunoelectrophoresis of the glucosyltransferases of *Oscillatoria princeps* on glass slides, has suggested the possible common origin of these three groups of enzymes. In addition, it is possible to use these results for inferences as to the time sequences involved in the fractionation of the original catalytic protein.

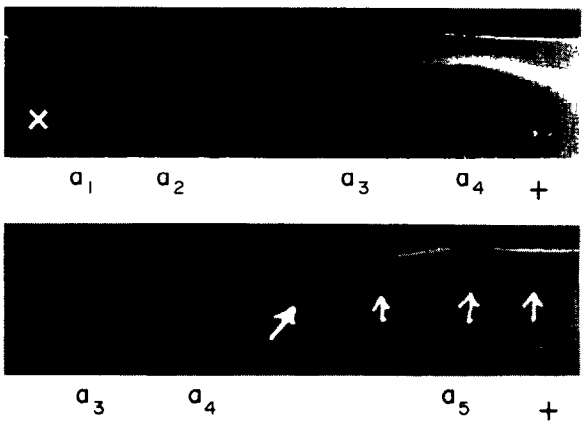

Fig. 1. Immunoelectrophoresis of glucosyltransferases of *Oscillatoria princeps*. *Top*, gel segment from origin (×) through synthetase isozymes. Trough at top contains rabbit anti-phosphorylase serum. a_1, a_2 are phosphorylase isozymes; a_3, a_4 are synthetase isozymes. Note coalescence of precipitin lines between these two groups of glucosyltransferases. Anode (+) is to the right. *Bottom*, gel segment from the synthetase isozymes (a_3, a_4) through the branching isozymes 'region', a_5. There are three faint immunoprecipitates formed in this a_5 region with the antiphosphorylase serum (small arrows). The arrow points to the spur between the precipitin line of the a_4 synthetase and the a_5 region.

Figure 1 shows the immunoprecipitates obtained using rabbit serum containing antiphosphorylase a_2 antibodies. Note the strong reactions obtained, not only with the two phosphorylase isozymes, as would be expected, but also with the two synthetase isozymes present in this alga (top, Fig. 1). There appears to be fusion of the immunoprecipitin lines between the phosphorylases and synthetases. Weaker, but nonetheless, definitive reactions were obtained in the 'a_5 region' (an area which contains the branching isozymes of this alga) to the antiphosphorylase a_2 rabbit serum (bottom, Fig. 1). However, there appears to be a "spur" formed between the immunoprecipitin lines of the synthetases and the branching isozymes region (arrow).

When larger quantities of the various isozymes were isolated by preparative electrophoresis on polyacrylamide gels and the technique of double diffusion used, the similarities and differences obtained on the immunoelectrophoretic slides are more apparent. Figure 2

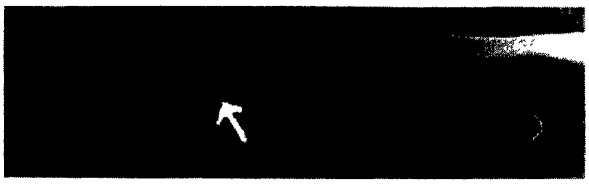

Fig. 2. Immunodiffusion study of the branching enzyme phosphorylase and synthetase of *Oscillatoria*. Antibodies to phosphorylase a_2 isozyme are in the trough at the top of the picture. The well on the left contains pure branching isozyme. The middle well contains pure phosphorylase a_1 isozyme. The well at the right contains pure synthetase a_3 isozyme. Note the coalescence of the arcs of the phosphorylase and synthetase antigens. Note the weak, but persistent arc present at the branching isozyme antigen site. The arrow points to the spur between the precipitin lines of the branching isozyme and the phosphorylase a_1 isozyme.

shows the complete coalescence of the phosphorylase and synthetase precipitin lines (when antiphosphorylase rabbit serum is used). At the same time, while there is a definite reaction of the branching isozymes antigen and the antiphosphorylase serum, a spur is apparent between this antigen and the phosphorylase antigen precipitin lines. This spur was a persistent feature when both phosphorylase and synthetase antigens were used in close proximity to the branching isozymes antigen and was present at all dilutions tested.

DISCUSSION

The role of phosphorylase in the active synthesis of polysaccharides has been in question since the discovery of the nucleoside diphosphoglucose glucosyltransferases [10]. However, the observation that one of the phosphorylase isozymes present in algae, a_2, was capable of the *de novo* synthesis of α-1,4-glucan chains [11] raises the possibility of this enzyme initiating the synthesis of glucan by forming 'primer' glucan which could, in turn, be utilized by the synthetases for the rapid elongation of the α-1,4 chains.

The fact that phosphorylase and synthetase contain identical amino acid sequences in their hexapeptides at their phosphorylated sites points towards a common evolutionary derivation of these two enzymes [4, 5]. Immunological studies further corroborate this possibility [12]. However, the results of immunodiffusion studies must be interpreted carefully. While undoubtedly, as shown for the lysozymes, cross-reactivity is dependent upon amino acid sequence resemblance [13], a recent report indicates that the glycollate oxidizing enzymes of algae can show fully identical antigenic determinants and yet exhibit different electron acceptor specificities [14]. However, in the case of Cyanophycean phosphorylase and synthetase where the end result of the enzymatic actions is the synthesis of α-1,4-glucosidic bonds, the cross-reactivity obtained in this study, plus the amino acid sequence resemblances reported [4, 5], may very well be indicative of a common evolutionary origin.

The weak precipitin lines obtained when the branching isozymes of this alga are tested against antiphosphorylase rabbit serum, suggest that there is only partial immunological similarity in the structure of the branching enzymes and the phosphorylases [15]. Likewise, the persistent spurs obtained when this branching enzyme antigen is placed in adjacent wells to both synthetase and phosphorylase (Figs 1 and 2), suggest that the similarity in structure of the molecule has diverged considerably when compared with the reactions of phosphorylases and synthetases where complete fusion of the immunoprecipitin lines was obtained (Figs 1 and 2).

Hence, if the abilities to form both α-1,4 and α-1,6 linkages originated in a single primordial catalytic protein [9], the bifurcation of these abilities occurred much earlier than the derivation of the synthetases from the phosphorylases.

EXPERIMENTAL

Cultures of *Oscillatoria princeps* were macerated in the cold and extracted with sodium bicarbonate [16]. The extracts were fractionated with $(NH_4)_2SO_4$ and the "phosphorylase fraction" subjected to polyacrylamide gel electrophoresis using the Raymond slab technique in an E-C 470 Vertical Cell [16, 17]. A gel slab 6 mm thick was used for the separation of the isozymes. The areas of the gel containing the separated isozymes were excised and lyophilized, and the isozymes eluted in the cold with Tris–HCl buffer as previously described [18]. The a_2 phosphorylase eluate was used to immunize rabbits [18, 19]. The other eluted isozymes were used as antigens in the immunodiffusion studies. Immunoelectrophoresis of the "phosphorylase fraction" was carried out on microslides coated with 1% agar in 0.01 M Tris–HCl buffer, pH 7.2, at 7 v/cm for 3 hr. The technique was essentially that of Hirschfeld [19]. Antiphosphorylase rabbit serum was added to the trough and the microslides incubated at 25° in a moist chamber for 24 hr. Purified antigens were used in the immunodiffusion experiments. These antigens were the isozymes excised from the gels after preparative polyacrylamide electrophoresis described above. Microslides were used in these studies and the techniques have been described [18].

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