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EFFECT OF AMITROLE ON BIOSYNTHESIS OF PHOSPHORYLASES IN DIFFERENT ALGAE*

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Abstract—Biosynthesis of the slower anodic-moving phosphorylase isozyme of the blue-green alga, *Oscillatoria princeps* can be completely suppressed by the herbicide, amitrole. The compound is without effect on the faster anodic-moving isozyme. It has been found that amitrole also suppresses the formation of the isozyme in *Chlorella pyrenoidosa*, while having no effect on the faster isozyme of this alga. In *Cyanidium caldarium*, amitrole likewise does not affect the biosynthesis of the faster anodic-moving isozyme. The anomalous behaviour of the faster isozyme of Chlorella in utilizing both glucose-1-phosphate and ADPG is similar to that previously reported in the other green alga, *Spirogyra*. It may represent a transformation of phosphorylase into a synthetase type enzyme.

INTRODUCTION

THE THREE groups of enzymes responsible for the formation of *storage* polyglucosides in algae, have been shown to be closely related with possible evolutionary derivation from each other or from a common ancestral type.[1] Of these three groups, all of which exist in two or more molecular species,[2,3] the phosphorylases (alpha-1,4-glucan: *ortho*-phosphate glucosyltransferases, E.C. 2.4.1.1) exhibit some suggestive intergroup variations.[4,5]

Phosphorylases exist in two *polymeric* forms in certain animal tissues: phosphorylase *a*, which is a tetrameric form active without added adenosine monophosphate (AMP), and phosphorylase *b*, a dimeric form of the enzyme which requires AMP for full activity.[6,7] The two forms are interconvertible.[7] It is debatable whether these polymeric molecular forms of the enzyme should be classed as *isozymes*.[5] Recently, isozymic forms of phosphorylase other than polymeric varieties, have been reported in animals and plants through use of electrophoretic techniques.[2,3,8–11]

* Part IV in the series "Glucosyltransferase Isozymes in Algae"; for Part III see *Phytochem.* **7**, 1573 (1968).

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At least two molecular forms of the enzyme have been detected in both the blue-green and the green algae, but not in red algae.[4,5] In the blue-green alga, *Oscillatoria princeps*, the two forms of phosphorylase detected by polyacrylamide gel electrophoresis, have been shown to exhibit sensitivity to AMP similar to the polymeric *a* and *b* enzymes in animal tissues, but not to be interconvertible.[2] The red alga, *Rhodymenia pertusa* and the enigmatic hot-springs alga, *Cyanidium caldarium*, have been shown to possess only one phosphorylase.[5,12]

The phosphorylase isozymes of *O. princeps* appear to be derived from separate biosynthetic pathways. For example, if the alga is cultured in the presence of the herbicide, 3-amino-1,2,4-triazole (amitrole) a complete suppression of the biosynthesis of the slower anodic-moving isozyme (a_1) occurs, but the phytotoxic compound does not appear to interfere with the formation of the similar-acting but faster anodic-moving phosphorylase (a_2).[13,14]

The most interesting variation in the algal phosphorylase isozymes has been found in the green alga, *Spirogyra setiformis*. In this alga, the a_2 isozyme exhibits properties strongly indicative of a possible evolutionary transformation into the nucleotide-glucosyltransferases (synthetases) (E.C. 2.4.1.11).[15,16]

Since amitrole effectively suppressed the biosynthesis of the a_1 isozyme in Oscillatoria, it seemed possible that the use of this chemical might offer a means for differentiating between the phosphorylase isozymes of other algae. Of great interest was the possibility that the use of amitrole might aid in studies yielding chemotaxonomic data for the classification of the enigmatic alga, *C. caldarium*. This alga has been often classed as a "bleached" form of the green alga, *Chlorella*.[17]

RESULTS

Amitrole appears to suppress the formation of the a_1 phosphorylase isozyme in *Oscillatoria* and *Chlorella*, but not in *Cyanidium*. Figure 1 shows the protein-stained polyacrylamide gels after electrophoresis of the fractionated extracts from *Oscillatoria princeps* (A and B), *Chlorella pyrenoidosa* (C and D) and *Cyanidium caldarium* (E). Note that amitrole-grown cultures of these algae do not contain the a_1 phosphorylase isozyme (B and D). *Cyanidium* normally possesses only the a_2 enzyme (E). It is apparent that the herbicide has no effect on the formation of the other related polyglucoside-synthesizing enzymes, the synthetases (a_3 and a_4) and the branching enzymes (a_5).

Figure 2 shows the results of incubating the gels in two different substrates. The gels in columns A and B when incubated in glucose-1-phosphate, show the presence of the phosphorylase isozymes a_1 and a_2 in normally cultured algae (A), and the complete absence of the a_1 isozyme in the amitrole-cultured plants (B). Note that *Cyanidium* exhibits no change in the a_2 isozyme when cultured in the presence or in the absence of amitrole.

The gels shown in columns C and D of Fig. 2 were incubated with adenosine diphosphoglucose (ADPG). Note that *Oscillatoria* and *Cyanidium* contain two synthetase isozymes (a_3 and a_4). The *Chlorella* gels show three reactive bands. Two of these are at the normal positions of the synthetase isozymes, but in addition, note that isozyme a_2 also can utilize ADPG as substrate. This a_2 isozyme appears to be active on both the Cori ester and ADPG,

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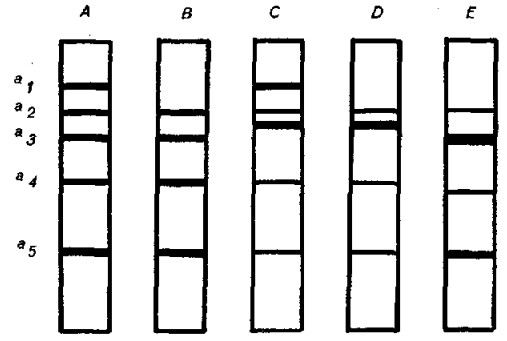

FIG. 1. EFFECT OF AMITROLE ON THE BIOSYNTHESIS OF PHOSPHORYLASE ISOZYMES IN THREE DIFFERENT ALGAE.

The gels were stained with amido-black after electrophoresis. A, *Oscillatoria princeps* grown in normal culture medium; B, same as A but with amitrole added to medium; C, *Chlorella pyrenoidosa* grown in normal medium; D, same as C but with amitrole added to medium; E, *Cyanidium caldarium* grown in normal medium (the pattern with this alga is the same with amitrole). a_1 and a_2 are phosphorylase isozymes; a_3 and a_4 are synthetase isozymes; a_5 represents branching isozymes. The anode is at the bottom of the columns.

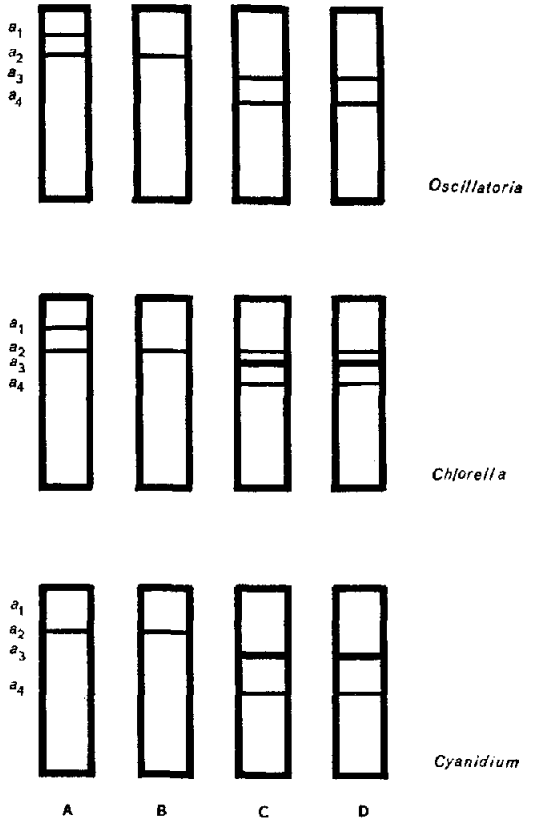

FIG. 2. POLYACRYLAMIDE GELS INCUBATED WITH VARIOUS SUBSTRATES AFTER ELECTROPHORESIS. A and B, glucose-1-phosphate; C and D, adenosine diphosphoglucose. B and D gels are of extracts from amitrole-treated cultures of the algae. Isozyme positions like designations in Fig. 1. Anode to the bottom. See text.

and can transfer glucosyl residues from both to a maltodextrin chain. This situation is identical with that previously reported in another green alga, *Spirogyra setiformis*.[5]

DISCUSSION

Amitrole repressed the biosynthesis of the a_1 phosphorylase isozymes in blue-green and green algae. It did not affect the formation of a_2 isozymes of either *Oscillatoria* or *Chlorella*. Neither did it have any suppressing influence on the biosynthesis of the lone phosphorylase (a_2) by *Cyanidium caldarium*. These observations would indicate a probable similarity in biosynthetic pathways for these respective isozymes in these three different algae.

It appears that the biosynthetic pathway for isozyme a_1 is different from that for isozyme a_2, since a_1's synthesis is repressed by amitrole while a_2's is not. This would support the thesis that the phosphorylase isozymes are separately coded for by *different* genes.[18] Further evidence of this has been obtained for the multiple phosphorylases of maize seed where a mutation alters the characteristics of endosperm phosphorylases without affecting the embryo phosphorylases.[19]

Evidence was previously presented of the immunological identity of the phosphorylases of such Cyanophytes as *Nostoc*, *Oscillatoria* and *Gleocapsa*.[20] It now appears rather probable that the phosphorylases of Chlorophytes and Cyanophytes are also related, at least insofar as their biosynthetic origin is concerned. Likewise, the phosphorylase of the "transition" alga, *C. caldarium*, also appears to be related to the Cyanophyte and Chlorophyte enzymes. It certainly seems suggestive that these diverse algal groups may have a common evolutionary origin.

Recent analyses of the amino acid sequences of peptides of phosphorylase *b* and of glycogen synthetase showed that the two enzymes had identical amino acid sequences, and hence, might be related in an evolutionary sense.[15,16] In the light of this, the anomalous behaviour of the a_2 isozyme of green algae such as *Spirogyra*[5] and *Chlorella* (Fig. 2 C) in being able to utilize both glucose-1-phosphate *and* ADPG as substrates, assumes importance. If this is not a hybridization effect such as has been reported to occur with certain phosphorylases of animal origin,[21] then it may represent a transformation of the relatively inefficient phosphorylase protein into a highly efficient synthetase protein.[22] Such an event could be the result of duplication of the gene which codes for the a_2 isozyme. The survival of such a duplicated gene would not be influenced by negative selection pressures.[23] The ultimate transition of the protein into a true synthetase could then be expected to occur. This isozyme merits intensive investigation.

It would also seem that the isozymes of *Cyanidium* are more closely related to those of *Oscillatoria* than those of *Chlorella*.

EXPERIMENTAL

Algae were cultured in liquid media with constant illumination at 23–25°. *Oscillatoria princeps* was cultured in Gerloff's modification of Chu No. 10 as previously described.[14] *Cyanidium caldarium* was cultured in Bogorad's modification of Allen's medium.[12] *Chlorella pyrenoidosa* was cultured in Preiss and Greenberg's medium.[24]

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Where amitrole was used, it was purified by repeated crystallizations[25] and dissolved in the culture media to give a final concentration of 0·001 M.

Extracts of the macerated algae were prepared and fractionated with $(NH_4)_2SO_4$ ("Enzyme grade", Mann Research Labs., New York City) as described.[1,4] The purified phosphorylase preparations so obtained were applied to gel slabs of 7% polyacrylamide in an E-C No. 470 Vertical Cell and electrophoresis took place as described.[2] After electrophoresis, the gel slabs were sliced and each vertical strip incubated in buffered substrates of glucose-1-phosphate or ADPG,[5] or stained with amido-black.[2]

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