Phytochemistry, 1967, Vol. 6, pp. 1041 to 1046. Pergamon Press Ltd. Printed in England

GLUCOSYLTRANSFERASE ISOZYMES IN ALGAE*

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(*Received* 22 *February* 1967)

Abstract—Purified phosphorylase preparations from representative members of three algal Groups were subjected to two-dimensional electrophoresis on polyacrylamide gel. Three groups of closely associated isozymes were found in each preparation. Two isozymes of the conventional phosphorylase type were present in preparations from blue-green and from green algae; only a single enzyme appeared in red algal preparations. Two isozymes of the glucosyltransferase type, utilizing either UDPG or ADPG as substrate, were present in all three groups of algae. Each preparation examined by this orthacryl gel technique also showed the presence of two or three isozymes capable of "branching" linear polyglucosides.

INTRODUCTION

PURIFIED preparations of algal phosphorylase, when subjected to electrophoresis on 7% polyacrylamide gels using the *disc* technique of Ornstein,[1] yielded five closely associated enzymatic proteins ("isozymes"?).[2, 3]

Fredrick[3] has shown that the two slower anodic-moving proteins are "conventional" phosphorylases (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.I.I). These enzymes (Fig. 1) a_1 and a_2, are related to each other similar to the animal phosphorylases "a" and "b".[4]

In recent studies, proteins a_3 and a_4 have been shown to be glucosyltransferases which use UDPG *or* ADPG for substrates.[5] These two enzymes, (UDP-glucose: α-1,4-glucan α-4-glucosyltransferase, EC 2.4.I.II), are present in blue-green, red and green algae. In all cases either UDPG or ADPG can be utilized by these enzymes as substrates.[3, 4, 5]

Protein a_5 (see Fig. 1), has been reported to be capable of synthesizing α-1,6 linkages between glucosyl residues, and thereby can convert *linear* maltodextrins to *branched* sugars.[2, 3, 5] This "branching" or "Q" enzyme is α-1,4-glucan:1,4-glucan-6-glucosyltransferase.

The recent reports of the intimate association of polysaccharide synthesizing and degrading enzymes with the polymers they act upon[6, 7] have raised serious doubts whether the five polyglucoside-metabolizing proteins reported in algal preparations are really "isozymes" in the classical sense of the Markert definition.[8]

* Supported in part by a research grant from the Dodge Institute for Advanced Studies, Boston, Mass. and Miami, Fla.

[1] L. ORNSTEIN, In *Gel Electrophoresis* (Edited by J. F. FREDRICK), p. 321. New York Academy of Sciences, New York (1964).
[2] J. F. FREDRICK, *Phytochem.* **1**, 153 (1962).
[3] J. F. FREDRICK, *Phytochem.* **2**, 413 (1963).
[4] J. F. FREDRICK, *Phyton (Buenos Aires)* **21**, 85 (1964).
[5] J. F. FREDRICK, In *Second Conference On Multiple Molecular Forms of Enzymes* (Edited by E. VESELL), New York Academy of Sciences, New York (1967) In press.
[6] Z. SELINGER and M. SCHRAMM, *Biochem. Biophys. Res. Commun.* **12**, 208 (1963).
[7] D. J. LUCK, *J. Biophys. Biochem. Cytol.* **10**, 195 (1961).
[8] C. L. MARKERT and F. MØLLER, *Proc. Natl Acad. Sci. U.S.* **45**, 753 (1959).

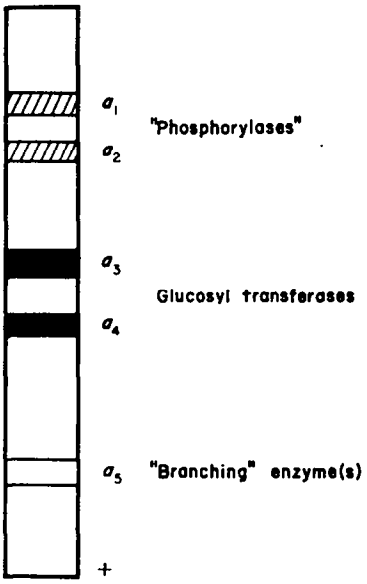

FIG. 1. THE FIVE GENERAL "ISOZYMES" SEPARATED BY DISC ELECTROPHORESIS FROM THE PURIFIED "PHOSPHORYLASE" PREPARATIONS OF ALGAE. THE ANODE (+) IS INDICATED, AND THE ENZYMES ARE GROUPED AS "PHOSPHORYLASES" (a_1 AND a_2), GLUCOSYL-TRANSFERASES (a_3 AND a_4), AND BRANCHING ENZYME(S), a_5.

Raymond has recently devised a method for two dimensional polyacrylamide gel electrophoresis, called *orthacryl* gel electrophoresis.[9, 10] The technique is essentially that of carrying out electrophoresis in two different concentrations of polyacrylamide gel orthogonal to each other. Proteins which assume positions on a theoretical straight line passing through the origin of the original gel strip are of identical molecular size. Proteins falling on a curve tangent to the original gel strip are polymeric with regard to each other.

RESULTS

Figures 2–4 show the resulting patterns obtained after application of orthacryl gel electrophoresis to "purified" phosphorylase preparations from three different algae. Figure 2 shows the results using phosphorylase isolated from extracts of the blue-green alga, *Oscillatoria princeps*. Figure 3 shows the pattern obtained from extracts of the red alga, *Rhodymenia pertusa*, while Fig. 4 shows the results from phosphorylase preparations of *Spirogyra setiformis*, a green alga.

In all cases, enzymes a_3 and a_4 fall on a theoretical line (dotted) passing through the origin of the original vertical gel strip. But, nowhere do these enzymes appear to be related to either a_1, a_2 or a_5.

From the positions of enzymes a_1 and a_2 in Figs. 2 and 4, it is probable that they lie on a curve tangent to the original vertical gel position.

[9] S. RAYMOND, In *Gel Electrophoresis* (Edited by J. F. FREDRICK), p. 350. New York Academy of Sciences, New York (1964).
[10] S. RAYMOND and M. NAKAMICHI, *Anal. Biochem.* 7, 225 (1964).

The a_5 enzyme, which separated in the disc technique using 7% polyacrylamide gel as one homogeneous protein (Fig. 1), appears to separate in the orthacryl gels as two or more dis-

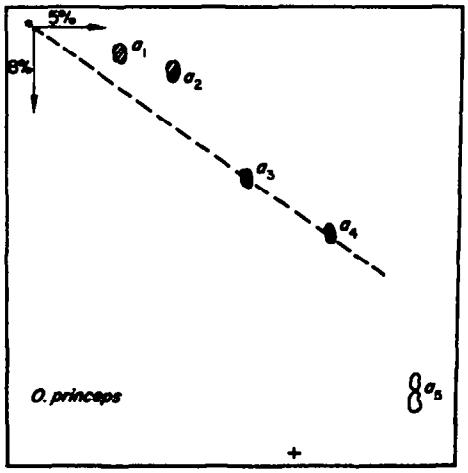

FIG. 2. ORTHACRYL GEL PATTERN OBTAINED FROM THE BLUE-GREEN ALGA, *Oscillatoria princeps*. THE ASTERISK (*) INDICATES THE ORIGIN OF THE PROTEINS ON THE ORIGINAL VERTICAL STRIP. NOTE THAT a_3 AND a_4 FALL ON THE THEORETICAL LINE (DOTTED) WHICH PASSES THROUGH THE ORIGIN.

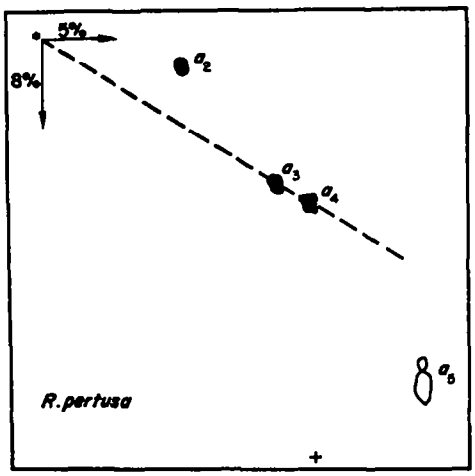

FIG. 3. ORTHACRYL PATTERN OBTAINED FROM THE RED ALGA, *Rhodymenia pertusa*. NOTE THAT a_3 AND a_4 FALL ON THE DOTTED LINE THROUGH THE ORIGIN (*). THERE IS ONLY ONE FORM OF "PHOSPHORYLASE" (a_2) PRESENT IN THIS ALGA. BUT, NOTE THAT "BRANCHING" ENZYME, a_5, HAS TWO FRAGMENTS IN THE 8% GEL, BOTH OF WHICH SHOW RAPID ANODIC (+) MOVEMENT.

crete proteins. Note that in *O. princeps* (Fig. 2) and in *R. pertusa* (Fig. 3), this protein has two components, while in the green alga, *S. setiformis* (Fig. 4), three components seem to be derived from this enzyme. All components in each case branched linear polyglucosides, the only requisite being that α-1,4-linkages be present in the linear chain.

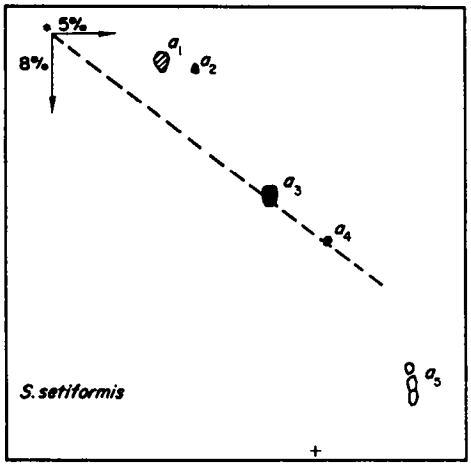

FIG. 4. ORTHACRYL PATTERN OBTAINED FROM THE GREEN ALGA, *Spirogyra setiformis*. HERE THE TANGENTIAL POSITIONS OF THE "PHOSPHORYLASES", a_1 AND a_2 CAN BE SEEN MORE CLEARLY. ENZYME a_5 HAS DIVIDED INTO THREE FRAGMENTS IN THE 8% GEL.

DISCUSSION

There seems to be little doubt that the enzymes involved in the synthesis and degradation of polyglucosides are closely associated, not only with each other, but also with the products or substrates of their actions.[6,7] It would appear that a similarity exists in the molecular weights and structures of these enzymes, permitting them to migrate together through various pore sizes of polyacrylamide gel.[5]

However, insofar as these algal enzymes are concerned, the intimate mixture of five "isozymes" previously reported,[2–5] is actually a *close association* of two groups of isozymes containing two enzymes each, and a third group which may contain two or more similar enzymes. Proteins a_3 and a_4 are identical in molecular size (cf. Figs. 2–4). Raymond has shown that, using the orthacryl technique, those proteins falling on a theoretical straight line through the origin are identical in molecular size.[9] This has since been applied to enzymes such as the lactic dehydrogenase isozymes.[10]

Isozymes a_3 and a_4 also show the same preferences for the substrates, UDPG or ADPG. Both isozymes are capable of utilizing either diphosphoglucose ribonucleotide.[5] This property has also been reported for the starch granule-bound glucosyltransferase of the maize embryo, where the Michaelis constants for UDPG and ADPG are identical.[11]

The two slower anodic moving enzymes, a_1 and a_2, appear to be related to each other much as the animal phosphorylases *a* and *b* with respect to their activity in the presence or absence of AMP.[12] It seems that their relationship with the animal phosphorylases is even closer. After orthacryl gel electrophoresis, they appear to lie on a curve tangent to their original vertical gel strip positions (Figs. 2 and 4). They have much the same properties as the polymeric proteins reported in blood. This would fit in with previous data collected for animal phosphorylases. For example, the work of Krebs and his group[13] as well as the recent report by Livanova *et al.*[14] indicate that phosphorylase *b* is composed of sub-units of 125,000 mole-

[11] T. AKATSUKA and O. E. NELSON, *J. Biol. Chem.* **241**, 2280 (1966).
[12] E. G. KREBS and E. H. FISCHER, *Biochim. Biophys. Acta* **20**, 150 (1956).
[13] E. G. KREBS and E. H. FISCHER, *Ann. N.Y. Acad. Sci.* **88**, 378 (1960).
[14] N. B. LIVANOVA, A. D. MOROZKIN, V. Y. PIKHELGAS and V. O. SHPIKITER, *Biokhimiya* **31**, 194 (1966).

cular weight, while phosphorylase *a* contains subunits arranged in a molecular weight of 250,000. Hence, the animal enzymes appear to be polymeric; the algal enzymes a_1 and a_2 may well be related in the same fashion.

The fastest anodic moving fraction, a_5 (see Fig. 1), although moving as a single protein in the 5% and 7% polyacrylamide gels, appears to split into two or three proteins when electrophoresis takes place at the same pH but in a gel concentration of 8% polyacrylamide (cf. Figs. 2–4). This fraction, a_5, when separated in 7% polyacrylamide by the disc method, was reported as synthesizing α-1:6 linkages between glucosyl chains linked in α-1:4 bonds, thereby "branching" linear maltodextrins.[3,5] Particularly, the action of a_5 was shown to convert amylose to an amylopectin-like polyglucoside.[5] Its unexpected splitting into two and three fractions during orthacryl gel electrophoresis indicates that this "branching" enzyme may therefore be composed of two or more isozymes.

Two recent reports point out the possibility that there may be different "branching" enzymes. Lavintman and Krisman[15] reported a form of this enzyme which could convert amylose *or* amylopectin into phytoglycogen. This would incidate that its action differs from that of the previously reported "Q" enzymes.[16–19] Kjolberg and Manners[20] report an enzyme in yeast which can transfer *branched* as well as linear chains of glucose molecules to a "primer". Perhaps *multiple* branching enzymes would help to explain some of the diverse polyglucosides found in algae.[21] From an evolutionary standpoint, the green algae form a starch which is very similar in composition to that of higher plants, while the red algae synthesize a starch which is devoid of amylose, and which resembles glycogen quite closely.[22] A branched polyglucoside akin to glycogen is also synthesized by the blue-green alga, *Oscillatoria princeps*.[23]

Meeuse and Kreger[24] have shown that, using X-ray diffraction techniques, the basic structure of the algal starches is made up of α-1:4 linkages between glucosyl residues, and hence, any differences involved must be due to the *degree of branching*. Most storage polyglucosides differ only in the length of the unit chain, as reported by Takahashi and Ono.[25]

It would appear therefore that, in general, any differences in the *type* of storage polyglucoside formed is less dependent upon the actions of α-1:4 synthesizing enzymes (such as isozymes a_3 and a_4), and more on the α-1:6 forming enzymes. This situation certainly seems to be the case with the three algae used in this present study. There do exist certain evolutionary differences in the a_1-a_4 enzymes of these three algae, but in general (cf. Figs. 2, 3 and 4), no great interdivisional differences are apparent in these four enzymes.

EXPERIMENTAL

Alkaline extracts were prepared from cultures of *Oscillatoria princeps*, *Spirogyra setiformis* and *Rhodymenia pertusa* and the phosphorylase in the extracts was fractionated with ammonium sulfate and purified until

[15] N. LAVINTMAN and C. R. KRISMAN, *Biochim. Biophys. Acta* **89**, 193 (1964).
[16] S. PEAT, W. J. WHELAN and J. M. BAILEY, *J. Chem. Soc.* 1422 (1953).
[17] S. NUSSENBAUM and W. Z. HASSID, *J. Biol. Chem.* **196**, 785 (1952).
[18] R. AIMI and T. MURAKAMI, *Proc. Crop Sci. Soc. Japan* **26**, 245 (1958).
[19] S. A. BARKER, A. BEBBINGTON and E. J. BOURNE, *J. Chem. Soc.* 4051 (1953).
[20] O. KJOLBERG and D. J. MANNERS, *Biochem. J.* **86**, 10 (1963).
[21] B. J. D. MEEUSE, In *Physiology and Biochemistry of Algae* (Edited by R. A. LEWIN), p. 289. Academic Press, New York (1962).
[22] E. PERCIVAL, In *Comparative Phytochemistry* (Edited by T. SWAIN), p. 151. Academic Press, New York (1966).
[23] J. F. FREDRICK, *Physiol. Plant.* **6**, 100 (1953).
[24] B. J. D. MEEUSE and D. R. KREGER, *Biochem. Biophys. Acta* **35**, 26 (1959).
[25] K. TAKAHASHI and S. ONO, *J. Biochem. (Tokyo)* **59**, 290 (1966).

chromatographically homogeneous.[2,4] Vertical gel electrophoresis was carried out in a cell as described by Raymond.[26] Gels were made up in the following buffer solution, which was also used for the electrophoresis:

2-amino-2-hydroxymethyl propanediol-1,3	40 g
Disodium EDTA	4 g
Boric acid	1·5 g
Water, deionized	to make 4 l.

The pH of the buffer was exactly 9·30.

The first gel contained 7·5 g Cyanogum-41, 0·1 ml tetramethyl-ethylene diamine (TMED) and 100 mg ammonium persulfate in 150 ml of the buffer. This set to a firm gel in 35 min at 23°. After it had set, it was pre-run for 25 min at 250 V, as recommended. At the end of this time, 0·10 ml of the purified phosphorylase was added to each slot of the gel. The samples were run at 250 V for 2½ hr, while water at 10° was circulated through the system to prevent denaturation of the proteins in the gel.

A strip was carefully cut out of this gel of exactly 0·5 cm × 10 cm. This was imbedded lengthwise in the second gel. The second gel consisted of 12·0 g Cyanogum-41, 0·2 ml TMED, 200 mg ammonium persulfate in 150 ml of the same buffer as above. Electrophoresis took place under the same conditions except that the run was extended for 4 hr.

The finished gel slabs were stained with amidoblack[2] or subjected to reaction with substrates as previously described by Fredrick.[5]

[26] S. RAYMOND, *Clin. Chem.* **8**, 455 (1962).