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AN ALGAL α-GLUCAN PHOSPHORYLASE WHICH REQUIRES ADENOSINE-5-PHOSPHATE AS COENZYME

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Abstract—Previous homogeneous preparations of algal α-1,4-glucan:orthophosphate glucosyl transferase (phosphorylase), when subjected to disc electrophoresis on polyacrylamide gel proved to contain at least two fractions which had the ability to synthesize linear glucans from glucosyl phosphate. By the use of a modified Gomori technique, where the phosphate liberated by the enzyme from glucosyl phosphate is precipitated *in situ* as the insoluble calcium salt, converted to the silver salt by treatment with silver nitrate, and this then reduced to black metallic silver by ultra-violet irradiation, it has been possible to further delineate these two phosphorylases. The significance of this situation is explored with a view to the possible similarities existing in these enzymes between plants and animals.

INTRODUCTION

TWO FORMS of phosphorylase (α-1,4-glucan:orthophosphate glucosyl transferase) are known to be present in animals.[1] These forms are different in molecular weight[2] and can be distinguished by their action in the presence and absence of adenosine 5′-phosphate.[3] Studies have shown that the adenosine 5′-phosphate (AMP) requiring form of this enzyme, phosphorylase *b* can be converted to the AMP-insensitive form, phosphorylase *a* by an enzyme which requires adenosine 5′-triphosphate (ATP) and manganese for its activity.[4]

The phosphorylases present in plants do not seem to require AMP for their activity,[5] and hence, it was thought that phosphorylase existed in but one distinct form in plant life. However, it has been reported that algal phosphorylase required manganous ion for optimum activity[6] and that the phosphorylase protein formed a chelate with this metal.[7] It was this phosphorylase protein–manganese chelate which was the "active" form of phosphorylase.

Five proteins having various degrees of linear glucan-synthesizing activity have been isolated from potato extracts by means of paper electrophoresis.[8] Recently, when purified preparations of phosphorylase from the alga, *Oscillatoria princeps*, were subjected to disc electrophoresis in polyacrylamide gels, five distinct proteins were obtained (Fig. 1), two of which exhibited exclusively linear glucan-synthesizing ability.[9] Of interest was the fact that the faster anodic moving fraction was sensitive to AMP.

A modified Gomori technique has been developed for the *in situ* study of the fractions of this algal enzyme on the polyacrylamide gels. This newer technique eliminates to a considerable

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extent, the diffusion of the iodine-stained glucans formed by incubation of the gels in buffered substrates as described by Fredrick.[9] It is the purpose of this paper to report in greater detail on the apparent sensitivity of the algal phosphorylase fraction, a_2 (cf. Fig. 1) to AMP and to manganese.

RESULTS

The hexosyl phosphate when converted to glucan by the phosphorylase in the protein bands on the gel, liberates inorganic phosphate which is immediately precipitated by the calcium in the reaction mixutes (Table 1). This precipitate is converted to the insoluble silver salt which is finally reduced under ultraviolet light to black metallic silver. This is fixed by the thiosulfate solution. Hence, the gels being essentially transparent, show at the site of the enzymatic reaction, a black band of metallic silver (Fig. 2).

TABLE 1. PHOSPHORYLASE ACTIVITY OF FRACTIONS a_1 AND a_2

Solution number*	Fraction a_1	Fraction a_2
I	+	−
II	+	+
III	+	−
IV	+	−

* All solutions contained: 0·05 M Dipotassium D-glucosyl phosphate, 0·03 M Tris–HCl buffer pH 7·2, 0·03 M $CaCl_2$, and 1·0% maltoheptaose.[13] In addition solutions II and III contained 0·003 M maganese chloride, and solutions II and IV 0·001 M adenosine 5′-phosphate.

The results for each solution used for the incubation are tabulated in Table 1. It will be noted that adenosine 5′-phosphate and manganese chloride were varied, the rest of the incubation mixture being constant.

It should be noted that the presence of AMP alone (solution IV) is not sufficient for the synthesis of glucan by fraction a_2. This fraction seems to be dependent on the presence of both the adenosine 5′-phosphate and the manganous ion (solution II). In contrast with this, the activity of fraction a_1 is completely independent of both these components of the incubation mixture.

DISCUSSION

The conversion of animal phosphorylase *b* to phosphorylase *a* is brought about by the enzyme phosphorylase kinase in the presence of ATP and manganous ions.[4] At the same time, the molecular weight of the phosphorylase was observed to increase to approximately twice that of the AMP-inactive form (phosphorylase *b*) as was reported by Keller and Cori.[2] It was postulated that AMP reacted with the converting enzyme (the kinase), and not with the phosphorylase, *per se*.[4]

The evidence presented in this study, that previously homogeneous plant phosphorylase preparations contain at least two phosphorylase enzymes (a_1 and a_2), and that one requires the presence of both AMP and manganese for glucan synthesis, seems to be at variance with data accumulated on animal phosphorylases. However, the possibility must be considered

FIG. 1. POLYACRYLAMIDE GEL COLUMN SHOWING FIVE DISTINCT FRACTIONS SEPARATED BY DISC ELECTROPHORESIS FROM AN HOMOGENEOUS ALGAL PHOSPHORYLASE PREPARATION. The bands have been stained with the protein dye, naphthalene black. The anode and cathode are indicated by the plus and minus signs. Bands a_1 and a_2 have exclusively linear polyglucoside-synthesizing ability.

[facing page 414]

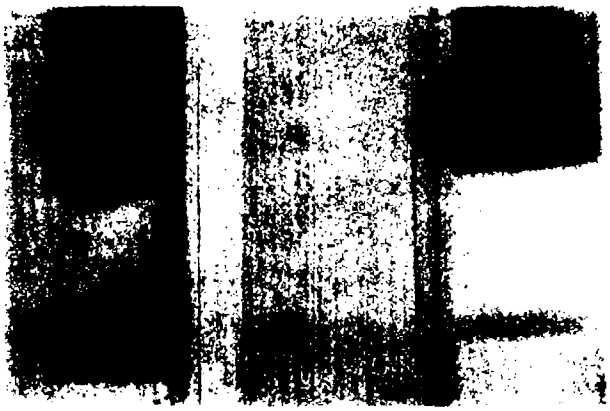

FIG. 2. FRACTIONS a_1 and a_2 ON POLYACRYLAMIDE GELS AFTER INCUBATION IN PRESENCE (A) AND ABSENCE (B) OF ADENOSINE 5′-PHOSPHATE AND MANGANESE ION. Note that fraction a_2 is AMP and manganese dependent.

in view of the contention that the bound manganese can be easily removed from the phosphorylase protein,[6,7] that the subjection of the purified phosphorylase preparation to a potential gradient might conceivably cause the "degradation" of a portion of the original chelate (or "active" phosphorylase). Hence, the presence of two bands of phosphorylase protein on these polyacrylamide gels could be the result of this phenomenon, caused by the very act of electrophoresis, although no such event happened when these extracts were subjected to paper electrophoresis.[10]

Otherwise, the interpretation of the data would make it logical to postulate the presence of an AMP-manganese-sensitive form of plant phosphorylase, which apparently has a lower molecular weight witnessed by the fact that the "sieving" effect of the polyacrylamide gel allows lower molecular weight fractions to migrate more rapidly towards the anode.[11]

If fraction a_2 is an artefact, then the situation in plants is different with regard to this important enzyme, from that reported in animals. Further studies are in progress along these lines.

EXPERIMENTAL

Extracts were prepared from cultures of *Oscillatoria princeps* and the phosphorylase in these fractions isolated as previously described.[7,12] The purified phosphorylase was subjected to electrophoresis on polyacrylamide gel columns at 5° as described.[9] "Map" columns were stained with naphthalene black and were used to locate the bands on the unstained columns.

Unstained columns were removed immediately from the disc electrophoresis apparatus at the end of the run. The gel was separated from the glass support under ice-cold water. The gels were incubated in 75 mm × 10 mm O.D. test-tubes in the solutions described in Table 1. The gels were placed into the tubes so that the anodic ends were downward. The pH of each incubation solution was adjusted to 7·2 prior to addition to the gels. The tubes were incubated at 30° for 2 hr.

At the end of this time, the incubation solutions were decanted from the tubes and the tubes rinsed with cold tap-water. A 1% silver nitrate solution was added to each tube and the tubes placed 12 in. away from a Raymaster TF-8 ultraviolet lamp for 20–30 min. At the end of this time, the silver nitrate solution was poured out and the gels rinsed with running cold tap-water for 3 min. A 2% sodium thiosulfate solution was added to each tube and the tubes allowed to stand at room temperature for one half hour. The gels were stored in the same tubes in a solution of 1% sodium thiosulfate. Gels stored in this fashion will retain the black metallic silver bands for at least 1 week and can be studied in the tubes.

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