MULTIPLE FORMS OF 1,4-α-GLUCAN PHOSPHORYLASE IN SWEET CORN

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1. Introduction

Phosphorylases (EC 2.4.1.1) are widely distributed in nature and play a major role in the metabolism of starch and glycogen. Isozymic forms of phosphorylase have been reported in a number of plant tissues. Thus, Tsai and Nelson [1, 2] have reported that there are four isozymic forms of phosphorylase in maize, while other reports have described multiple forms of phosphorylase in potato and other plant tissues [3, 4]. The multiple forms of phosphorylase in sweet corn and their possible interrelationships are described in this study.

2. Materials and methods

Sweet corn (su_1 maize) or an isogenic line was a generous gift of Dr. R.G. Creech. This material was frozen in liquid nitrogen immediately after harvesting and was thereafter stored at −70°. The pericarp was removed from 20-day old kernels and the embryo and endosperm tissues separated by dissection. These were then homogenized in 2 v/w of buffer (20 mM sodium fluoride, 50 mM sodium acetate, 0.1 mM dithiothreitol, 10% glycerol, pH 6.5), and centrifuged. The supernatant extracts usually contained about 2 IU/ml of phosphorylase activity as assayed by Lee and Braun [5].

Disc gel electrophoresis was performed with a Buchler disc gel electrophoresis apparatus. The gel buffer system used was based on that described by Davis et al. [6]. The gels were usually 7% acrylamide, and were cast in a buffer containing 30 mM HCl adjusted to pH 7.9 with 2 M Tris base. Samples of the extracts prepared as described above were diluted 5-fold in the stacking gel buffer [6]. This consisted of 0.24 M HCl adjusted to pH 5.8 with 2 M Tris base in 20% glycerol. Bromophenol blue was used as the tracking dye. Diluted extracts (50 μl) were loaded directly on to the gels without sample or spacer gels. The electrophoresis was carried out at 4° at a current of 2 mA per tube.

Phosphorylase activity in the gels was usually detected with an iodine stain. The gels were incubated at room temperature in a solution containing 30 mM glucose 1-phosphate, 50 mM sodium glycerophosphate, pH 6.5, 0.1 mM dithiothreitol, and 1 mg/ml of amylopectin (or 0.1 mg/ml of maltodextrin mixture, DP = 6, Corn Products) as the primer. After incubation for up to 18 hr the gels were rinsed with distilled water and placed in an iodine solution (0.01% iodine, 0.1% potassium iodide, 0.05 N HCl). The polysaccharide synthesized by phosphorylase gave dark purplish-blue bands. An alternate stain, based on the detection of the inorganic phosphate formed [6] was also used. The incubation mixtures contained 30 mM glucose 1-phosphate, 0.2 M calcium chloride, 5% maltodextrin mixture, 50 mM sodium maleate, 0.1 mM dithiothreitol, pH 6.5. Otherwise the staining procedure was carried out as described by Davis et al. [6]. This stain was found to be more sensitive than the iodine stain over the same incubation times, but was more tedious to use.

3. Results and discussion

In previous studies on the purification of 1,4-α-glucan phosphorylase from commercial sweet corn, we had observed that disc gel electrophoresis revealed a single

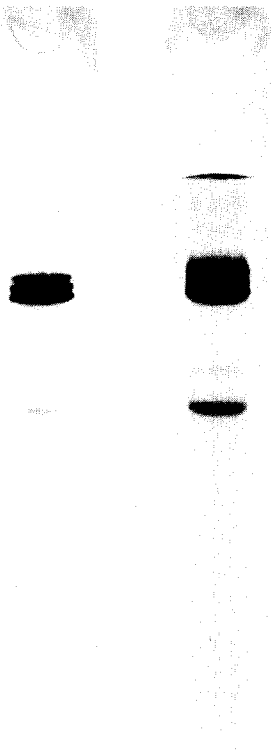

Fig. 1. Polyacrylamide gel electrophoresis of sweet corn endosperm extracts. Left, lightly stained; right, heavily stained.

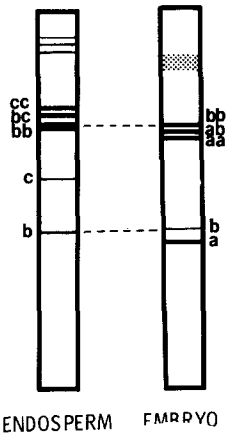

Fig. 2. Diagram of the multiple forms of sweet corn phosphorylase from endosperm and embryo extracts on gel electrophoresis. The diagram represents the patterns observed in 7% gels, and is drawn to scale with the bottom of the gel representing the distance travelled by the dye front. Symbols are given in terms of the proposed monomer–dimer relationship of the multiple forms (see table 1).

major band of phosphorylase activity, but that minor bands could be seen on prolonged incubation of the gels [5]. In this study we have carried out a systematic examination of the presence of multiple forms of phosphorylase in sweet corn. For this study we used an isogenic line of sweet corn, an *su*$_1$ variety supplied by Dr. R.S. Creech. On long incubation of gels of sweet corn extracts multiple forms of phosphorylase were readily observed. Since the genetic composition of the embryo and endosperm of maize are different, the embryo and endosperm extracts were examined separately. An example of the resolution which was achieved is shown in fig. 1, for the endosperm extract. Our overall results are shown diagrammatically in fig. 2. For the endosperm there were eight activity bands. Of these, bb is the major band, and is the fastest migrating of a group of three close bands. This group is followed by two faster moving bands; there are three minor slow moving bands. These patterns were obtained by overnight incubation of the gels. In the embryo a similar pattern is seen, of a group of three closely migrating bands, followed by two faster bands. There also appeared a broad, slow band. These patterns were reproduced a number of times over a period of several months during the storage of the corn at −70°.

The patterns of the endosperm and embryo are similar but not identical. They show only two points of identity, these being the bands marked b and bb (fig. 2). This was established by experiments in which the embryo and endosperm extracts were run side by side in the same gel; this was done by the insertion of a partition of thin plastic in the gel tube to separate the samples. Secondly, electrophoresis of a mixture of the embryo and endosperm extracts showed that the only bands which were superimposed were b and bb. Thirdly, in experiments discussed below, the bands corresponding to b and bb in the endosperm and embryo gave identical plots of log R_m against gel concentration.

The presence of multiple bands on electrophoresis is in itself insufficient proof for the existence of genetically distinct isozymes, since these forms could arise from a number of other reasons, e.g. limited

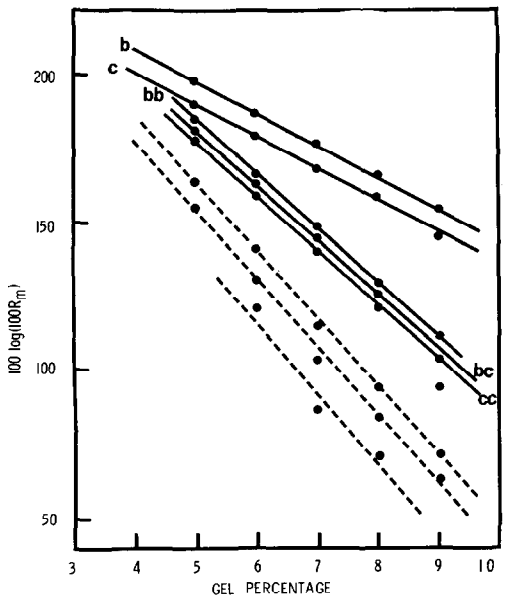

Fig. 3. Plot of log R_m against gel concentration for the multiple forms of phosphorylase in sweet corn endosperm. Symbols are as in accordance with fig. 2. The dotted lines represent the three slowest migrating forms shown in fig. 2.

proteolysis or nonspecific aggregation. Thus, both ammonium persulfate [7] and bromophenol blue [8] have been reported to give rise to artefacts on gel electrophoresis. This possibility was eliminated in our experiments by omission of the bromophenol blue, and by experiments in which the gels were pre-treated with 5 mM dithiothreitol or pre-electrophoresed to remove the persulfate. It did not seem likely that the multiple forms which were observed were due to proteolysis, since the material which was used had been immediately frozen on harvesting. In addition, no changes in the levels of activity or in the patterns of multiple forms was observed during storage of the sweet corn for long periods.

Since 20 to 30 kernels were generally used in an experiment, the possibility that the multiple bands were a composite of a genetically heterogeneous population was examined. This was done by examination of the patterns given by the electrophoresis of extracts prepared from individual endosperms and embryos. However, the patterns observed were identical to those obtained with large preparations.

The relationship between the multiple forms of phosphorylase was investigated by the technique of Hedrick and Smith [9]. The migration of a protein on disc gel electrophoresis is a function of both the size and the charge of the protein, and no inferences regarding the charge or size relationships could be made from the electrophoretic patterns which were observed. However, Hedrick and Smith [9] have shown that the charge effects may be negated if the relative mobilities are determined in a series of gels of different acrylamide concentration. The plot of the logarithm of the relative mobility (log R_m) against gel concentration is a linear function, and furthermore, the negative slopes of these plots was found to be a linear function of molecular weight [9]. Thus, a family of isozymes of identical molecular weight but of different charge would yield a family of parallel lines for the plot of log R_m against gel concentration. This technique was therefore used for both the embryo and endosperm extracts. Our results for the endosperm are shown in fig. 3. It is seen that there are three groups of parallel lines, and thus in the endosperm there are three groups of different molecular weights. Similar studies of the embryo extracts showed that these fell into the same molecular weight groups. In addition the bands indicated as b and bb (fig. 2) in the embryo gave coincident plots with the same bands from the endosperm, indicating their identity. It was also noted in a number of such experiments that the differences in the negative slopes was always approximately equal; for example, in one experiment the slopes were found to be 8, 15 and 21. Since the relationship between these slopes and the molecular weight is a linear one, the three groups must differ in molecular weight by the same amount; the simplest explanation for this would be that these groups represent monomer, dimer and trimer, of which the former two account for the major part of the phosphorylase activity. In the following discussion only the proposed monomer and dimer groups will be considered. The distribution of the multiple forms among these two groups is shown in table 1. The monomer group consists of the bands a, b, and c, while the dimer group consists of bands aa, ab, bb, bc and cc.

On the basis of the evidence presented above, the multiple forms of the two lower molecular weight groups may be rationalized in terms of a monomer–dimer relationship. Thus the endosperm has the

Table 1
Proposed relationship of the multiple forms of phosphorylase.

Proposed forms*	Endosperm	Embryo
Dimer		
cc	+	–
bc	+	–
bb	+	+
ab	–	+
aa	–	+
Monomer		
c	+	–
b	+	+
a	–	+

* a, b, c are arranged in the observed order of increasing mobility, while the dimeric forms are arranged in the expected order of mobility.

monomer composition b and c, and the dimers bb, cc and bc, while the embryo has the monomer composition a and b, and the dimers aa, ab and bb. The observed number of multiple forms, and the observed mobilities of the endosperm forms relative to the embryo forms, are entirely consistent with this hypothesis (table 1). While the evidence presented is not conclusive, it suggests that the multiple forms of phosphorylase in sweet corns arise from only three gene products. A parallel example of multiple forms of phosphorylase which have their origin in the formation of hybrid dimers is that of the mammalian phosphorylases [6]. The results presented here give the first indications that such monomer–dimer interactions may also occur with a plant phosphorylase.

Acknowledgements

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