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MULTIPLE FORMS OF STARCH PHOSPHORYLASE FROM BANANA LEAVES

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Key Word Index—*Musa paradisiaca*; Musaceae; banana; multiple forms; starch phosphorylase.

Abstract—Two forms (A and B) of starch phosphorylase were found in the mature banana leaf by polyacrylamide gel electrophoresis and DEAE-cellulose chromatography. The young leaf contained only form A and the appearance of form B with leaf development was accompanied by a decrease in the content of form A. At a later stage of leaf maturity only form B could be found. The MWs of forms A and B were 450000, and 220000 respectively.

INTRODUCTION

Multiple forms of starch phosphorylase (SP) have been demonstrated in tubers [1–3], seeds [4, 5], and leaves [2, 6–8]. To understand starch metabolism in the banana plant, the multiplicity of SPs has been studied in banana fruit [9] and in the present work SPs from banana leaves have been studied as a function of leaf development.

RESULTS AND DISCUSSION

By polyacrylamide gel electrophoresis it was shown that young banana leaf contained one form of SP (form A) and in mature leaf an additional form of SP (form B) was found. The amount of form A diminished with maturity of leaf and only form B could be found in fully mature leaves.

The enzyme activities as well as the interconversion of the two forms of the enzyme were not affected by 10 mM concentration each of Mg^{2+}, Mn^{2+}, Ca^{2+}, K^{+}, AMP and dithiothreitol individually or when AMP and Mg^{2+} were present together. The interconversion of the two forms was also not affected when either of the forms of the enzyme was incubated for 6 hr at 2–5° with ATP and Mg^{2+}; ATP, Mg^{2+} and dithiothreitol; or ATP and Ca^{2+}.

One peak of SP, eluted in 0.25 M NaCl, was obtained on DEAE-celluose chromatography of the 0–90% $(NH_4)_2SO_4$ fraction from the young leaf. In contrast, the mature leaf yielded two peaks of SP eluted in 0.25 M NaCl (form A) and 0.35 M NaCl (form B). At a later stage of maturity only one peak of SP (form B), eluted in 0.35 M NaCl, was obtained.

The pattern of multiple forms of SP remained unchanged whether the entire fractionation of the enzyme from young and mature leaves was completed the same day (5 hr) or in 3 days. It could also been seen that there was little change in total SP activity during the leaf development (Table 1).

The MWs of forms A and B were 450000 and 220000 respectively as determined by gel filtration on Sephadex G-200, employing ferritin, catalase and aldolase as reference proteins.

The reason for the multiplicity of SP in banana leaves is not clear. The possibility of the formation of the multiple forms due to proteolysis appears remote since the isoenzymic pattern remains unchanged when the fractionation period of the enzyme is reduced. Also no intermediate forms of SP were detected during the development of leaf. Gerbrandy *et al.* [10] recently reported the conversion of one form of SP into another during storage of potato enzyme and attributed this to proteolysis. On storage of the purified banana enzyme preparation from young leaf at 4°, a slow conversion of form A into form B was observed. The MW of form A was higher than that of form B, and was of a similar magnitude to that reported for one of the potato SP isoenzymes [3]. The MW of form B, 220000, was just half of form A and was of similar magnitude as reported for other SPs [11, 12]. It is possible that two molecules of form B are linked together by a weak physical bond, which is broken on storage.

Table 1. Starch phosphorylase from young and mature banana leaves

Fraction	Young leaf		Mature leaf	
	Activity (nkats/g tissue)	Sp. act. (nkats/mg protein)	Activity (nkats/g tissue)	Sp. act. (nkats/mg protein)
1. Initial extract (1600 g supernatant)	3.83	0.26	4.00	0.21
2. 0–90% $(NH_4)_2SO_4$	2.33	0.17	2.28	0.13
3. DEAE-cellulose chromatography				
Phosphorylase A (0.25 M NaCl)	1.67	2.78	0.17	0.08
Phosphorylase B (0.35 M NaCl)	0	0	1.17	2.92

In contrast to banana leaves, the number of isoenzymes of SP in *Xanthium* leaves decreases from 4 to 1 with the maturity of leaves [8]. It was of interest to note different isoenzymic pattern of SP in different parts of the banana plant. Whereas only two forms of the enzyme were found in developing banana leaves, three forms of the enzyme have been reported in mature banana fruits [9]. Multiple forms of SP during banana leaf development may be dependent on the physiological state of the leaf. A change in ionic concentration or other factors during leaf development may cause conversion of form A into form B.

EXPERIMENTAL

Leaf tissue. Banana leaves were collected immediately before use from plants grown in the departmental garden. Young leaf was tender and preceded the flower. Mature leaf was slightly rigid, while at a later stage of maturity the leaf was completely rigid.

Enzyme preparation. After removing midribs, 10 g of leaf tissue was cut into small pieces and blended for 30 sec at low speed and 50 sec at high speed with 90 ml of the medium consisting of 0.01 M Tris–HCl buffer pH 7.5, 0.02 M 2-mercaptoethanol and 0.05% Triton X-100. Homogenate was filtered through 2 layers of muslin and the vol made up to 100 ml. Homogenate was centrifuged at 1600 *g* for 30 min to yield a clear supernatant which contained all the SP activity of the homogenate. This supernatant was used in experiments on electrophoresis. For DEAE-cellulose chromatography, the supernatant protein was precipitated by adding $(NH_4)_2SO_4$ to 90% sat. The ppt. was suspended in buffer A (0.01 M Tris–HCl buffer pH 7.5 containing 5 mM 2-mercaptoethanol) using a pestle and mortar and centrifuged at 15000 *g* for 30 min. The clear supernatant containing the enzyme activity was desalted by Sephadex G-25 filtration, and loaded onto a DEAE-cellulose column (5 mg protein/ml bed vol), previously equilibrated with buffer A. The column was washed with 3 bed vols of the above buffer and the enzyme eluted using linear NaCl gradient.

Electrophoresis. Polyacrylamide gel electrophoresis was carried out according to ref. [13], but without SDS and employing 7% gel. Tris–HCl (0.05 M, pH 7.5) was used as electrode and gel buffer. Electrophoresis was done at 4–5° by passing 8 mA current per tube. The time of run was 3 hr, which was predetermined using bromophenol blue as a front indicator. After electrophoresis, gels were taken out and incubated for 3 hr in a mixture consisting of 0.03% soluble starch (freshly prepared), 0.02 M NaF, 0.02 M Tris–maleate buffer pH 6.0, and 5 mM glucose-1-phosphate. Gels were stained using I_2 reagent [14].

Enzyme assay. SP was assayed in the direction of polysaccharide synthesis as described in ref. [15], but Tris–maleate buffer, pH 6 was used instead of citrate buffer, pH 6.2.

MW determination. A Sephadex G-200 column (1.4 × 40 cm) was chosen and equilibrated with buffer A. SP form A, obtained after concentration of DEAE-cellulose eluates from young banana leaves, was layered on top of Sephadex G-200 column and subsequently chromatographed using buffer A. Fractions (1 ml) were collected and flow rate was 2 ml/hr. The same column was earlier calibrated employing ferritin (MW 540000), catalase (MW 240000) and aldolase (MW 147000) as standard proteins. The MW of the SP was calculated by the method of ref. [16].

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