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SHORT COMMUNICATION

DISSOCIATION OF PURIFIED STARCH PHOSPHORYLASE FROM YOUNG BANANA (*MUSA PARADISIACA*) LEAVES

B. VENKAIAH, SADHANA SRIVASTAVA AND ANIL KUMAR

Department of Biotechnology, Devi Ahilya University,
Khandwa Road, Indore—452001

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Purified starch phosphorylase from young banana leaves having the molecular weight 450,000 on storage at 0—4°C gets dissociated into a form having molecular weight 220,000. The dissociation is stimulated by the presence of 10 mM glucose-1-phosphate. No loss of enzyme activity occurs on dissociation. The dissociated enzyme has properties similar to the enzyme having molecular weight 220,000 isolated from mature banana leaves.

Starch phosphorylase (EC 2.4.1.1. α-1, 4-D-glucan: orthophosphate, α-glucosyl transferase), catalyzing the conversion of starch into glucose-1-phosphate in the presence of orthophosphate,

(α-1, 4 D-glucosyl) n+orthophosphate ⇌ (α-1, 4 D-glucosyl) n−1+ glucose-1-phosphate, plays an important role in degradation of starch in plants. Kumar and Sanwal (1977) have shown the presence of two forms of phosphorylase in developing banana leaves depending on the maturity of the leaf. One enzymic form having the molecular weight 450,000, is present in young banana leaves and the other having the molecular weight 220,000 appears on maturity of the leaf with concomitant decrease in the form having molecular weight 450,000. In the fully matured banana leaves, only one form of phosphorylase having molecular weight, 220,000 is found (Kumar and Sanwal 1977). In the present communication, studies have been done on the *in vitro* association and dissociation of these two multiple forms.

Enzyme assay of starch phosphorylase was carried out in the direction of polysaccharide synthesis as described by Green and Stumpf (1942) with some modifications as described by Kumar and Sanwal (1982a).

Starch phosphorylase having molecular weight 450,000 from young banana leaves and having molecular weight 220,000 from mature banana leaves were purified

using identical methodologies as described by Kumar and Sanwal (1981a, 1982a). The purity of the enzyme proteins was checked using native polyacrylamide gel electrophoresis.

Association and dissociation of starch phosphorylase was checked using native polyacrylamide gel electrophoresis method (Davis *et al.*, 1967) with some modifications (Kumar and Sanwal, 1982a). Spacer gel contained 3% acrylamide and 0.75% methylene bisacrylamide in 0.05 M, pH 6.5, sodium citrate buffer. The separating gel contained methylene bisacrylamide (0.2%) and acrylamide (5%) in 0.05 M, pH 7.5 tris-HCl buffer. The electrode buffer was the same as the buffer in the separating gel. A current of 8 mA/tube was applied and the time of electrophoresis was kept 4 hr. The amount of loaded protein was 100 μg. Gels were stained using commassie brilliant blue.

Molecular weight was determined by gel filtration through Sephadex G-200 column (80×1.5 cm) equilibrated with 0.01 M tris-HCl buffer, pH 7.5 containing 5 mM 2-mercaptoethanol. Ferritin (MW 540,000), catalase (MW 240,000) and aldolase (MW 147,000) were used as reference proteins. The flow rate was 10 ml/hr. 5 ml fractions were collected. The molecular weight was calculated by the method of Whitaker (1963).

There was dissociation of young banana leaf enzyme (MW 450,000) into a form having MW 220,000 on storing the purified preparation in 0.01 M tris-HCl buffer, pH 7.5 containing 5 mM 2-mercaptoethanol at 0—4°C for a week. After 15 days, there was complete dissociation into MW 220,000 form. This dissociation was stimulated by the presence of 10 mM glucose-1-phosphate. In the presence of 10 mM glucose-1-phosphate, there was complete dissociation of the multiple form having MW 450,000 into MW 220,000 form within 3 days. There was no reassociation of the dissociated enzyme on dialysis overnight against 0.01 M tris-HCl buffer, pH 7.5 in order to remove glucose-1-phosphate and 2-mercaptoethanol gradually from the enzyme preparation. On the other hand, all the efforts to convert MW 220,000 (isolated from mature banana leaves), into 450,000 form by incubating it for 2 hr in the cold condition with 20 mM concentration of glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, L-tyrosine, DL-tryptophan, succinate, fumarate, oxaloacetate, separately were unsuccessful.

Young leaf starch phosphorylase (MW 450,000) showed equal Vmax of the enzyme with amylose, starch and glycogen as a primer whereas that with amylopectin as a primer was 27% of that with starch as a primer at 5 mM glucose-1-phosphate (Table I). However, the dissociated enzyme as well as the enzyme isolated from the

Table I. Km and Vmax values of starch phosphorylase isolated from young banana leaf (MW 450,000), dissociated enzyme molecule (MW 220,000) and isolated from mature banana leaf (MW, 220.000)

Primer used	Conc. of primer	Conc of G-1-P	MW 450,000*			MW 220,000**			MW 200,000***		
			Km		Vmax (nkats)	Km		Vmax (nkats)	Km		Vmax (nkats)
	(mM)	(mM)	Primer (mM)	G-1-P (mM)		Primer (mM)	G-1-P (mM)		Primer (mM)	G-1-P (mM)	
Starch	2	var.		0.66	0.56		2.1	0.64		1.8	0.57
	var.	5	1.3		0.54	2.05		0.66	2.20		0.54
Amylose	2	var.		0.51	0.57		1.6	0.63		1.4	0.55
	var.	5	2.8		0.56	4.10		0.61	4.15		0.57
Amylo-	2	var.		0.93	0.15		2.0	0.62		1.7	0.56
pectin	var.	5	4.3		0.14	3.52		0.59	3.63		0.55
Glycogen	2	var.		0.82	0.53		2.0	0 19		1.6	0.17
	var.	5	3.8		0.56	3.14		0.20	3.23		0.18

*-young leaf enzyme; **-mature leaf enzyme; ***-dissociated enzyme: var.-variable.

mature leaf showed equal Vmax of the enzyme with amylose, amylopectin and starch as a primer whereas that with glycogen as a primer was 30% of that with starch as primer at 5 mM glucose-1-phosphate. The Km values for primers and glucose-1-phosphate for the dissociated enzyme as well as the enzyme isolated from mature banana leaf were comparable but different from the young leaf enzyme.

The dissociation of the enzymic form having MW 450,000 present in young banana leaves, *in vitro*, gets stimulated by the presence of 10 mM glucose-1-phosphate. It indicates that during maturity of the leaf, glucose-1-phosphate might play an important role in the dissociation of the MW 450,000 enzymic form. The concentration of glucose-1-phosphate might increase in the leaf tissue on maturity compared to very young unfurled light green leaf due to more chlorophyll content which would lead to more photosynthesis. Glucose-1-phosphate is an intermediate in starch biosynthesis. It is also the substrate for ADP glucose synthetase which is regulatory enzyme of starch biosynthesis.

The kinetic characteristics of the enzyme having molecular weight 450,000, were different from that of the dissociated enzyme having molecular weight 220,000. However, the kinetic properties of the dissociated enzymic form were comparable with the enzymic form present in mature banana leaf having the molecular weight, 220,000. The results indicated that the dissociation of MW 450,000 into 220,000 form, may lead to change in the conformation of the enzyme molecule which changes the Vmax of the enzyme with respect to amylopectin and glycogen and also the affinity of the enzyme molecule with respect to various primers and glucose-1-phosphate.

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