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Effect of monensin on intracellular transport and posttranslational processing of 11 S globulin precursors in developing pumpkin cotyledons

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We examined the effect of monensin on the biosynthesis of 11 S globulin, a major storage protein in pumpkin cotyledons. In the developing cotyledons, proglobulin, a precursor of 11 S globulin, is synthesized on the endoplasmic reticulum (ER) and transported to vacuoles, where it is converted to the mature 11 S globulin molecule by the action of a thiol protease [(1985) Plant Physiol. 77, 747–752; (1987) Plant Physiol. 85, 440–445]. The developing pumpkin cotyledons pretreated with the carboxylic ionophore monensin (10^{-6} M) were pulse-chase labeled with [^{35}S]methionine, and subsequently, vacuoles were isolated from the tissues. Although labeled proglobulin was detected in the isolated vacuoles, the conversion of proglobulin to 11 S globulin was strongly inhibited. However, monensin was shown not to inhibit the in vitro processing of proglobulin by vacuolar protease, whose optimum pH was ~ 5.0. It appears, therefore, that monensin inhibits the posttranslational proteolytic cleavage of proglobulin and not its intracellular transport from the ER to the vacuoles. This inhibition may be due to an elevation of vacuolar pH by monensin.

11 S globulin; Monensin; Posttranslational processing; Protein transport; Vacuole; (*Cucurbita* sp).

1. INTRODUCTION

Pumpkin 11 S globulin is the unglycosylated seed protein which accumulates in protein bodies, which are formed from vacuoles by budding during seed development [3]. The precursor molecule, proglobulin (α and β), is synthesized in the rough endoplasmic reticulum (rER) and transported to vacuoles via dense vesicles [1,4]. We have shown that proglobulin is subsequently posttranslationally cleaved between asparagine and glycine residues by a vacuolar thiol protease to produce the mature form of 11 S globulin consisting of γ- and δ-chains linked together by a disulfide bond [2,5,6].

It has been well established that monensin, a carboxylic ionophore, permits transmembrane exchange between protons and monovalent cations and often affects intracellular transport [7]. The Golgi complex is one of the primary sites of action of monensin [7]. Treatment of french bean with monensin interferes with the terminal glycosylation and transport of phytohemagglutinin [8,9] and phaseolin [10], implicating the involvement of Golgi complex during the biosynthetic process of these substances. It has also been reported recently that monensin inhibits the posttranslational processing of rice lectin [9], concanavalin A [11] and glutelin [12] precursors. However, the mode of action of monensin is not firmly established and it is not known whether monensin interferes with the intracellular transport of precursor proteins to vacuoles or causes the inactivation of processing enzymes in the vacuoles.

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In the present study, we have examined the involvement of the Golgi complex in the biosynthesis of unglycosylated 11 S globulin in pumpkin seeds, as well as the effect of monensin on the intracellular transport and posttranslational processing of proglobulin is reported.

2. MATERIALS AND METHODS

2.1. *Plant materials*

Developing seeds of pumpkin (*Cucurbita* sp. cv Kurokawa Amakuri Nankin), grown during the summer season of 1985, were used 35–40 days after fertilization.

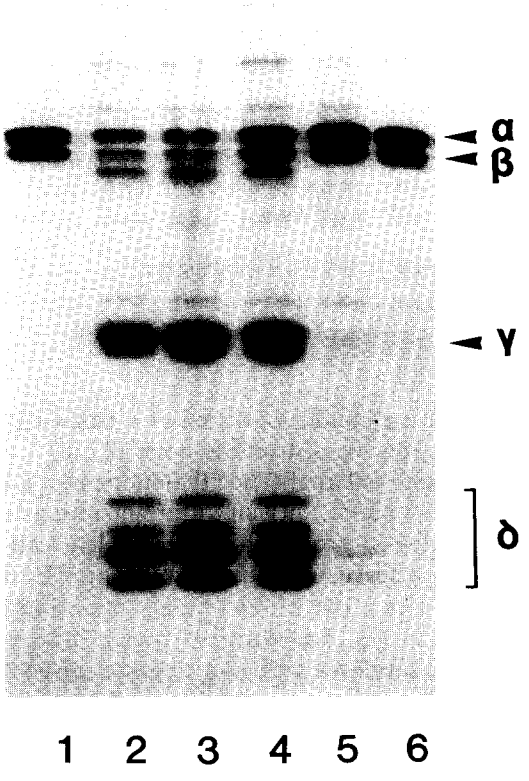

Fig.1. Effect of monensin on in vitro posttranslational processing of proglobulin. Developing pumpkin cotyledons were pretreated with monensin for 1 h at various concentrations (lanes): (1 and 2) 0 M; (3) 10^{-8} M; (4) 10^{-7} M; (5) 10^{-6} M; (6) 10^{-5} M. After the pretreatment, the cotyledons were pulse-labeled with [^{35}S]methionine for 15 min and chased in the presence of the appropriate concentrations of monensin for 10 min (lane 1) or 2 h (lanes 2–6). Homogenates of cotyledons were subjected to SDS-PAGE and fluorography. α, β, proglobulin. γ, δ, constituent polypeptide chains of pumpkin 11 S globulin.

2.2. *Pulse-chase labeling*

Developing cotyledons were soaked in 20 mM Tris-HCl (pH 6.8) containing monensin (10^{-8} to 10^{-5} M) for 1 min and further incubated for 1 h on moistened filter paper in Petri dishes at 25°C. The pretreated cotyledons were pulse-chase labeled with [^{35}S]methionine as described before [1], except that the chase medium contained monensin at the same concentration as the pretreatment step. The labeled tissues were homogenized with buffer containing 10 mM Tris-HCl (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 10% glycerol and 0.001% bromophenol blue in the mortar, centrifuged 10000 × *g* for 10 min at 4°C. The supernatant fraction was subjected to the SDS-PAGE and fluorography.

2.3. *Preparations of vacuoles*

Vacuoles were prepared from pulse-chase labeled or unlabeled-cotyledons of developing seeds essentially as described previously [2,3].

2.4. *In vitro assay for posttranslational processing of proglobulin*

The assay for in vitro proteolytic processing was as described

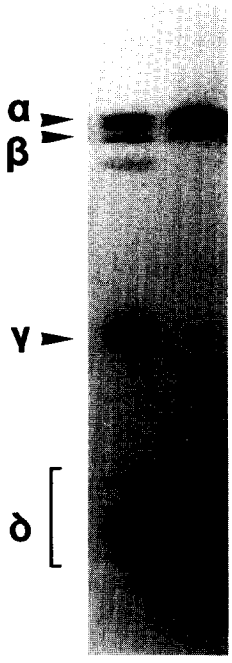

Fig.2. Effect of monensin on intracellular transport of proglobulin to vacuoles and posttranslational processing of proglobulin in vacuoles. Developing cotyledons pretreated with (lane 2) or without (lane 1) 10^{-6} M monensin for 1 h, were pulse-labeled with [^{35}S]methionine for 15 min and chased for 2 h. Vacuoles were isolated from the labeled cotyledons and subjected to SDS-PAGE and fluorography. α, β, proglobulin. γ, δ, constituent polypeptide chains of pumpkin 11 S globulin.

previously [2]. The ER fraction was prepared from pulse-chase labeled developing cotyledons. Labeled proglobulin in the ER fraction was used as a substrate for assaying the proteolytic processing activity. The vacuolar fraction isolated from unlabeled developing cotyledons was used as a crude processing enzyme. The reaction mixture containing labeled proglobulin and the enzyme was incubated for 2 h at 30°C and then subjected to SDS-PAGE and fluorography.

3. RESULTS AND DISCUSSION

Pulse-chase labeling of pumpkin cotyledons revealed that the radioactivity was initially detected in proglobulin α and β (fig.1, lane 1). Labeled proglobulin was proteolytically processed to produce γ and δ chains after a 2 h chase (fig.1, lane 2). A radioactive band just below proglobulin β (shown in lane 2) was detectable in the vacuoles after the chase (fig.2, lane 1), indicating that it was a mature form of another seed protein, not a degradation product of proglobulin. Treatment of pumpkin cotyledons with 10^{-6} M and 10^{-5} M monensin inhibited the conversion of proglobulin to mature globulin even after a 2 h chase (fig.1, lanes 5 and 6). No effect was observed by the treatment with 10^{-8} M and 10^{-7} M monensin (fig.1, lanes 3 and 4). These observations are consistent with those reported by other investigators concerning the biosynthesis of rice lectin [4], concanavalin A [11] and rice glutelin [12].

In order to determine further the possible inhibitory effect of monensin on the transport of proglobulin to vacuoles, the organelles were isolated. As shown in fig.2, labeled proglobulin was detected in vacuoles isolated from both monensin-treated and untreated (control) cotyledons (lanes 1 and 2). It can be seen that the amount of labeled proglobulin transported to vacuoles was not diminished by monensin treatment (lane 2). The doublet α-subunit bands are occasionally seen depending on the conditions of electrophoresis because of the microheterogeneities of the polypeptides. Moreover, it is worth

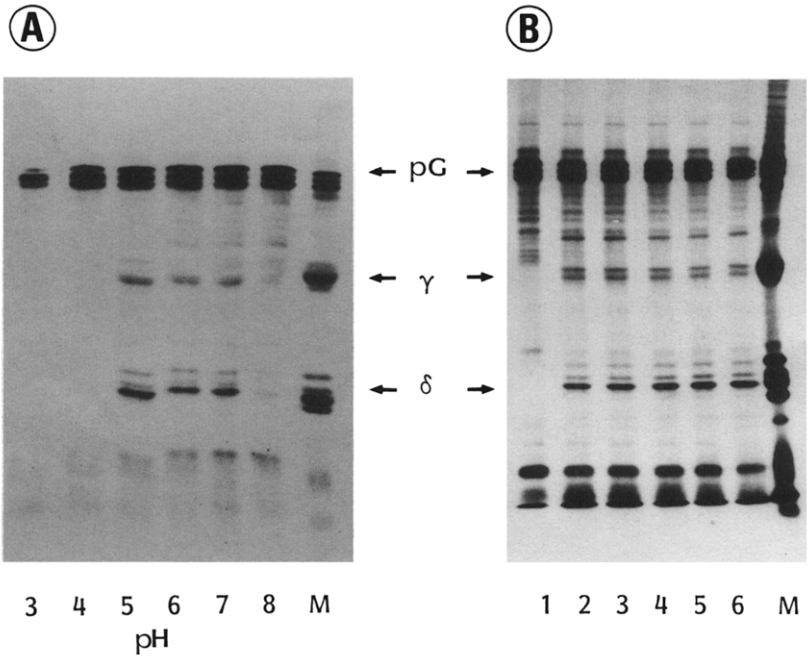

Fig.3. pH dependency (A) and effect of monensin (B) on in vitro proteolytic processing of proglobulin. ER fractions isolated from [^{35}S]methionine-labeled developing cotyledons was incubated with crude processing enzyme for 2 h at 30°C. (A) To determine the optimum pH, 50 mM citrate-phosphate buffer (pH 3, 4, 5, 6 or 7) or 50 mM Tris-HCl (pH 8) was used. (B) Monensin was added to the reaction mixture at a final concentration of 0 (lanes 1 and 2), 10^{-8} M (lane 3), 10^{-7} M (lane 4), 10^{-6} M (lane 5) and 10^{-5} M (lane 6). The reactions were terminated before (lane 1) and after (lanes 2–6) incubation for 2 h at pH 5.0 and 30°C. M, markers for proglobulin (pG) and the constituent polypeptide chains (γ and δ) of 11 S globulin.

noting that proglobulin was clearly detected in the dense vesicles which were isolated from both the monensin-treated as well as the untreated control cotyledons (not shown). Taken together, these findings indicate that monensin does not inhibit the intracellular transport of proglobulin from the ER to vacuoles via dense vesicles. Our results obtained using the unglycosylated seed protein, however, are completely different from those using glycosylated proteins such as phytohemagglutinin [2]. Immunocytochemical studies using concanavalin A have shown that monensin induces the secretion of a component normally destined for deposit in the protein bodies [11].

As shown in fig.2, after a chase incubation for 2 h, mature 11 S globulin (γ and δ) was shown to accumulate in vacuoles isolated from the control cotyledons (lane 1), but not in vacuoles from the monensin-treated tissues (fig.2, lane 2). It was thus suggested that proglobulin transported to vacuoles was not processed to the mature globulin. In order to study the inhibitory mechanism of monensin which behaves as a weak base, the pH dependence of the posttranslational processing enzyme activities was examined (fig.3A). By densitometric analysis, the optimal pH for the in vitro proteolytic processing was observed at pH 5.0, whereas it was decreased to 20% at pH 6.0 and 7.0 and to 10% at pH 8.0.

Results presented in fig.3B show the effect of monensin on the in vitro proteolytic processing of proglobulin. Although monensin inhibited posttranslational processing of proglobulin in vivo, we found no inhibitory effect on the in vitro processing of labeled proglobulin by the vacuolar processing enzyme activity at pH 5.0.

Monensin, as well as other acidotropic weak bases such as chloroquine and NH_4Cl, are known to elevate the pH of the acidic cellular compartments, thereby affecting their biological functions [13]. Since the plant vacuoles are generally in acidic pH [14], we conclude that the inhibitory effect of monensin on the processing of proglobulin to mature 11 S globulin in pumpkin cotyledons is ascribable to the elevated pH of the vacuolar compartment.

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