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A study of maturation events in jackbeans (*Canavalia ensiformis*)

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Maturation events have been studied in developing jackbean (*Canavalia ensiformis*) cotyledons by using a combination of analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, overlays with ^{125}I-concanavalin A (Con A) and the use of anti-(Con A) after Western transfer. The number of polypeptides recognized by ^{125}I-Con A varies during maturation, until at maturity only one remains. Several molecular forms of the lectin occur during development; one, corresponding to M_r 33000 and found only in immature seeds, interacts with ^{125}I-Con A, suggesting that it is glycosylated.

Jackbeans (*Canavalia ensiformis*) at maturity contain high levels of Con A in the cotyledons of the seed. Although the structural features of Con A are well-established, little is known of its function. Recently we have been studying the role of lectins through their interactions with other seed components (Bowles & Marcus, 1981; Miller & Bowles, 1982). In mature jackbeans we found only a single polypeptide that bound ^{125}I-Con A after lectin overlays of denatured proteins separated by SDS/polyacrylamide-gel electrophoresis (Bowles *et al.*, 1982, 1983). This lack of abundant glycoproteins in mature seeds led us to study the potential transient occurrence of Con A-binding polypeptides at other stages in the development of the cotyledons. Results described in here suggest that glycosylation of jackbean components varies during maturation and that a transient form of Con A, present only in immature seeds, may itself be glycosylated.

Methods

Jackbeans, obtained from EMBRAPA, Rio de Janiero, Brazil, were germinated and grown to maturity and flowering under greenhouse conditions at the John Innes Institute. Seed was harvested throughout maturation up to 81 dpa, at which time the dry seeds weighed ~1.8g each. After removal from the pods the seed was quick-frozen in liquid N_2, weighed and stored at −70°C until analysis. Seed extracts were made by homogenization of the tissue in 10mM-potassium phosphate (pH 7.2)/145mM-NaCl (fresh weight/buffer ratio, 1g:10ml), sonication (Dawe Microprobe, setting 6, 3 × 10s) of the homogenate and centrifugation at 1000*g* for 5min at room temperature. The post-1000*g* supernatant was used for preparation of gel samples, which were then subjected to polyacrylamide-gel electrophoresis in the presence of SDS and under reducing conditions (Bowles & Marcus, 1981). ^{125}I-Con A lectin overlays, in the presence or absence of methyl α-D-mannoside, Western transfer and use of monospecific rabbit anti-(Con A)/goat anti-(rabbit IgG) serum, with subsequent peroxidase/anti-peroxidase staining, were carried out as described previously (Bowles *et al.*, 1983; Gershoni & Palade, 1983).

Results

Results given in Fig. 1(*a*) show the existent polypeptides in developing jackbeans revealed by Coomassie Blue staining after their separation by polyacrylamide-gel electrophoresis in the presence of SDS and under reducing conditions. Fig. 1(*b*) shows autoradiographs of the gels shown in Fig. 1(*a*) after ^{125}I-Con A overlays were carried out. Equivalent samples after electrophoresis and transfer on to nitrocellulose were probed with monospecific antiserum to Con A. Fig. 1(*c*) shows Con A and polypeptides in the developing seeds that are antigenically related to the lectin and recognized by the antiserum after their denaturation. Tracks 1–4 include analysis of seeds 8–25dpa,

Abbreviations used: Con A, concanavalin A; endo H, endo-β-*N*-acetylglucosaminidase H; dpa, days post-anthesis; SDS, sodium dodecyl sulphate.

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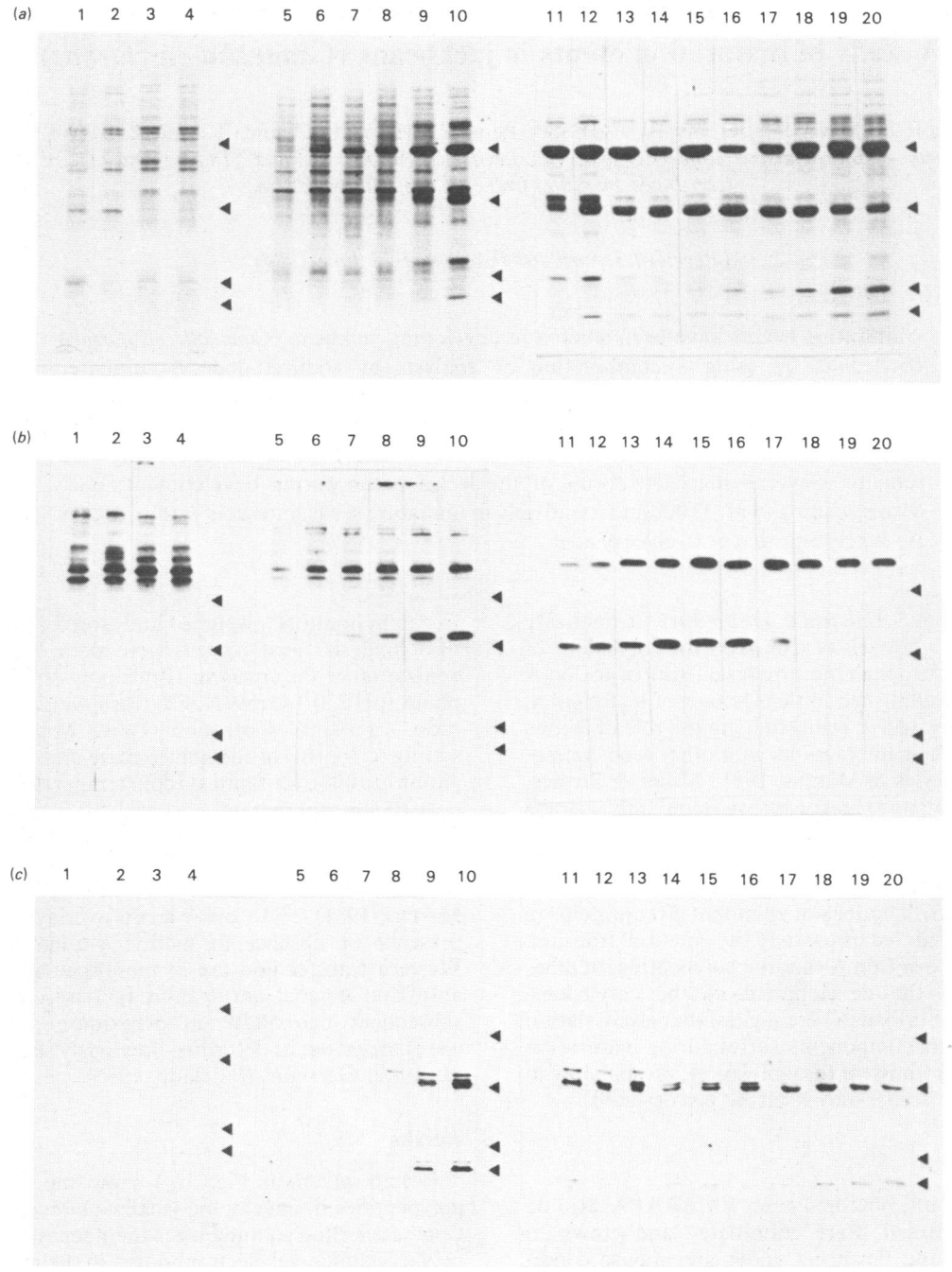

Fig. 1. *Analysis of jackbean maturation*

Extracts from maturing jackbeans were prepared as described in the text. Polypeptides were separated by SDS/polyacrylamide-gel electrophoresis using 10–15% (w/v)-polyacrylamide gradient gels and stained with Coomassie Blue (*a*). Gels were incubated with ^{125}I-Con A, and autoradiographs showing polypeptides recognized by the ^{125}I-lectin are shown (*b*). After electrophoresis of equivalent extracts, polypeptides after Western transfer were probed with anti-(Con A) and antigens revealed with peroxidase (*c*). A 60 μg sample of protein was loaded per track. Fresh weight of seeds analysed: track 1, 3 mg; 2, 44 mg; 3, 150 mg; 4, 161 mg; 5, 200 mg; 6, 300 mg; 7, 400 mg; 8, 550 mg; 9, 700 mg; 10, 1 g; 11, 1.2 g; 12, 1.3 g; 13, 2.0 g; 14, 2.3 g; 15, 2.8 g; 16, 2.5 g; 17, 2.4 g; 18, 2.2 g; 19, 2.0 g; 20, 1.8 g. Arrowheads (◀; top to bottom) depict the M_r of final forms of canavalin subunit (M_r 49500), Con A subunit (M_r 27000) and Con A-related polypeptides (M_r 13000 and 11000).

tracks 5–10 of seeds 26–32 dpa and tracks 11–20 of seeds 33–81 dpa.

Track 20 shows the cotyledon profile at maturity. As shown in Fig. 1(*a*), the most abundant polypeptide in the cotyledons is the subunit of the storage-reserve canavalin, at M_r 49500. The lectin subunit and lectin-related polypeptides of lower M_r were also clearly visible in the Coomassie Blue-stained gel, at M_r 27000, 13000 and 11000. After overlay of the polypeptides in the mature cotyledons with ^{125}I-Con A, only a single polypeptide of M_r 66000 bound the lectin in a carbohydrate-specific (methyl α-D-mannoside-reversible) interaction (Fig. 1*b*, track 20). We have previously characterized this polypeptide as the mannosylated heavy subunit of the tetrameric enzyme α-D-mannosidase (Bowles *et al.*, 1982, 1983). When the polypeptides after Western transfer were probed with anti-(Con A), two main bands were observed in track 20 at M_r 27000 and 11000. A faint band of M_r 9000 was also visible, but the Con A-related polypeptide at M_r 13000 was not revealed by the procedure, suggesting that the antisera raised to native Con A did not recognize that polypeptide after its denaturation. Immunoprecipitates from anti-(Con A)/cotyledon extracts do contain both the Con A-related polypeptides of lower M_r as well as the subunit of M_r 27000 (S. E. Marcus, P. R. Maycox & D. J. Bowles, unpublished work).

Final forms of the cotyledon proteins, as shown in track 20, became apparent in the Coomassie Blue-stained gels during the mid-stage of development. For example, by the time the seeds had attained a fresh weight of 0.7g (track 9), both the canavalin and Con A subunits were clearly distinguishable. The extent of glycosylation of the cotyledon components, as revealed by ^{125}I-Con A overlays, varied considerably during maturation (Fig. 1*b*). In the early stage (tracks 1–4), up to seven polypeptides, of M_r 66000 and higher, bound ^{125}I-Con A. The mid-stage (tracks 5–10) was characterized by the gradual disappearance of the bulk of ^{125}I-Con A-binding polypeptides of high M_r and the appearance of a new band at M_r 33000. The polypeptide was present for most of the late stage, until finally, as the seeds approached maturity, only the α-D-mannosidase subunit of M_r 66000 remained and was able to bind ^{125}I-Con A. Comparison of data in of Fig. 1(*b*) and 1(*c*) indicates that the polypeptide of M_r 33000 that occured transiently during mid-late stages and bound ^{125}I-Con A in the overlays also interacted with anti-(Con A) after Western transfer. Results from the transfers suggest that a range of molecular forms of Con A exist during maturation; two of those which interact with anti-(Con A) after Western transfer have an M_r higher (33000 and 29500) than that of the intact subunit found in mature seeds. From data shown in Figs. 1(*a*) and 1(*c*), it is also clear that the Con A-related polypeptides of low M_r do not appear concurrently during maturation. From comparison of the Coomassie Blue-stained polypeptides (Fig. 1*a*) and those recognized by anti-(Con A) (Fig. 1*c*), the related polypeptide of M_r 11000 became distinguishable during the mid-stage. Although the polypeptide of M_r 13000 was not recognized in denatured form by the lectin antisera, it became apparent in the Coomassie Blue-stained gel only by the later stages of maturation.

Discussion

Recent studies on several legume species has indicated that storage proteins and lectins undergo a complex series of processing events during their synthesis, transport and packaging into protein bodies (Chrispeels, 1984). In common with secretory proteins of mammalian cells, segregation of seed proteins into the lumen of the endoplasmic reticulum is a co-translational event, as is signal-sequence removal and any core glycosylation that may occur (Bowles, 1982). Post-transitional processing has also been reported, in particular the limited endoproteolysis of polyprotein precursors to give rise to final forms of the seed proteins (Spencer & Higgins, 1980; Turner *et al.*, 1981; Croy *et al.*, 1980; Roberts & Lord, 1981). Of particular relevance are recent studies on pea (*Pisum sativum*) lectin, which have demonstrated a polyprotein form containing both α- and β-subunits (Chrispeels *et al.*, 1982; Higgins *et al.*, 1983*a*,*b*). Endoproteolytic processing appears to be a surprisingly slow process, and low levels of non-processed precursor form of M_r 23000 are maintained throughout cotyledon maturation and are found also in dry seeds. The observations led to the suggestion that, in pea cotyledons, there were several gene products of $M_r \sim 23000$ formed from a small family of very-closely-related lectin genes, and certain products could be processed to low-M_r forms more efficiently than others (Higgins *et al.*, 1983*b*).

In the present study, Con A has been found to exist in several molecular forms, some of which are maintained until seed maturity and others whose transient occurrence suggests they are precursor forms. In the dry seed the lectin consists of an intact subunit, two major polypeptides of lower M_r (13000 and 11000) and minor polypeptides (at M_r 18000 and 8000) (Wang *et al.*, 1971). Although the low-M_r polypeptides are structurally related to the intact subunit, no conversion of the subunit into fragments occurred on seed hydration/germination, nor on prolonged incubation *in vitro* of intact subunit with endogenous jackbean proteinases

(Dalkin & Bowles, 1983; Dalkin *et al.*, 1983). Additional transiently occurring forms of the lectin, recognized by anti-(Con A) after Western transfer, include prominent polypeptides of M_r 33000 and M_r 29500. Lectin overlays of the denatured polypeptides indicated that the M_r-33000 form bound ^{125}I-Con A in a sugar-reversible interaction, suggesting the polypeptide was either mannosylated or glucosylated. Should the Con A-related polypeptide of M_r 33000 prove to be a precursor for any of the final forms of Con A in mature seeds, a deglycosylation event would be involved. There are several precedents for such an event in eukaryotic systems (Herbert & Uhler, 1982; Julius *et al.*, 1984). The use of ^{125}-Con A overlays has also shown that the level of glycosylation changes during jackbean maturation, in that in early/mid stages, many polypeptides in the extracts can bind ^{125}I-Con A, but by maturity only one, the heavy subunit of α-D-mannosidase, remains. Several lines of evidence have indicated that native α-D-mannosidase cannot interact with Con A, but first requires denaturation by SDS into protomers to reveal the endo H-susceptible Con A-binding mannosylated oligosaccharide (Bowles *et al.*, 1983). The lack of glycoprotein receptors to Con A in the mature seed suggests either that the function of the carbohydrate-binding sites of the lectin is related solely to events during cotyledon maturation, or that the glycoconjugate receptors for those sites in the mature seed are not glycoproteins.

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