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Patterns of Urease Synthesis in Developing Soybeans[1]

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ABSTRACT

An examination of *in vivo* polysome-bound activity indicates that soybean (*Glycine max*, cv. Prize) seed urease is synthesized on large polysomes ($n \geq 15$). *In vitro* urease synthesis is directed by a large RNA (3,000–3,300 nucleotides). Urease synthesis occurs throughout the normal protein biosynthetic phase of the developing seed. Surprisingly, the activity/antigen ratios of urease increase throughout development. Urease appears to be in a more highly polymerized state in mature beans *versus* beans in early development.

During the 55 days from pollination to maturity, urease specific antigen (antigen *versus* total seed protein) is greatest on the 20th day, representing 0.6% of total extractable protein. Its synthesis proceeds until the end of the protein biosynthetic phase, approximately day 40. In contrast, the appearance of urease enzyme activity lags that of antigen during early development (11–20 days) and plateaus in late development. Mixing experiments suggest no role for putative urease inhibitors or activators during development. However, several electrophoretically slow migrating forms are unique to the urease of mature beans. It is not known if these are more active species.

An active urease species exhibits an RNAse-sensitive cosedimentation with a heavy polyribosome class ($n \geq 15$). Polyadenylated RNA, size-fractionated to 3,000 to 3,300 bases, directed the synthesis *in vitro* of a major translational product electrophoretically and immunologically similar to the *in vivo*-synthesized urease subunit.

Many species of the Leguminosae have high levels of urease activity in their seeds. Extractable soybean seed protein is at least 0.2% urease (18), whereas urease comprises 0.15% of the dry weight of the Jack bean (23). It has been suggested that urease is of potential value as a marker in genetic transformation of plant cells (19). Since urease has a large subunit size (93.5 kd) (18) and is found in relatively high abundance in soybeans, its mRNA ought to be extractable in enriched form from developing seeds. An RNA fraction sufficiently enriched in urease template can be used to construct urease cDNA clones to use as probes for urease genomic clones. In this paper we discuss some aspects of urease synthesis and maturation in the developing seeds. These preliminary studies are necessary for the isolation of urease mRNA.

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MATERIALS AND METHODS

Biological Material. Soybeans (*Glycine max*, var. Prize) were purchased from Burpee Seed Company, Clinton, IA. Nodulated soybean plants were grown under high pressure sodium lamps at a photosynthetically active light intensity of 649 ± 27 $\mu E/m^2 \cdot s$ at plant height. Photoperiod was 12 h light (25 ± 1°C) to 12 h dark (20 ± 1°C). The age of immature pods, expressed as time (days) from pollination, was determined from pod thickness. We observed a linear relationship between pod thickness and age till 30 d; older pods were staged by tagging individual flowers upon first blooming. Pollination was considered to have occurred on the day before flower opening. Multiple determinations, *i.e.* dry weight, total protein, urease specific activity, and specific antigen, were made on individual beans.

Preparation of Crude Bean Extracts. Staged seeds were removed from the pod and immediately immersed in liquid N_2. Beans were stored at −70°C for 1 to 30 d. Individual beans were lyophilized, weighed, and then powdered in a mortar and pestle. Twenty-five to 100 mg bean meal was ground to a homogeneous slurry with 10 volumes of extract buffer (24 mM Tris barbital [pH 8.6], 1 mM EDTA, 0.01% calcium lactate [w/v], 0.02% sodium azide [w/v]). The slurry was centrifuged twice in an Eppendorf (Brinkmann Instruments, Westbury, NY) microfuge (10 min each time) and the resulting supernatant was used for the determination of urease specific activity and urease specific antigen, as described below.

Urease Enzyme Assay. Urease activity in crude extracts of developing or mature beans was assayed in 50-ml flasks with a 0.9 × 2 cm center well containing a fluted square (1.8 cm × 1.8 cm) of Whatman 3 MM filter paper impregnated with 50 μl 9 M monoethanolamine. A 1-ml reaction mix (pH 7.0) contained 100 to 200 μg bean protein, 1 mg gelatin, 100 μmol Tris maleate, 1 μmol EDTA, 1 μmol β-mercaptoethanol, and 500 μmol [^{14}C]urea (12,000–13,000 dpm/μmol). Flasks were sealed with serum stoppers, and, after incubation times of 0.5 to 1.5 h at 30°C, individual reactions were stopped by the injection of 0.5 ml 2 N H_2SO_4 through the stopper. After another 0.5 h, the filter papers were placed in scintillation vials containing 4 g PPO and 0.4 g POPOP/liter of a toluene (2 parts)/95% ethanol (1 part) mixture. To correct for quenching, cpm were converted to dpm by the external standard ratio method.

To assay urease activity in polysome fractions, the following modifications were made. The center well contained 50 μl 9 M monoethanolamine and no filter paper square. The reaction mix was made 0.02% in sodium azide and contained 0.1 to 0.5 ml of polysome fraction (0.3–1.5 A_{260} units). Blanks contained water or gradient buffers (containing 12.5 or 50% sucrose) in place of polysome fractions. After 2- to 7-d incubations at 30°C, reactions were stopped by the injection of 0.5 ml 2 N H_2SO_4. After 3 h the monoethanolamine was pipetted directly into scintillation vials. In control experiments, 0.5 ml gradient buffer containing 50, 31, or 12.5% sucrose inhibited urease activity in the 1-ml reaction mix by 21, 9, and 0%, respectively. Urease activity measured on

polysome profiles was not corrected for sucrose inhibition. Azide (0.02%, w/v) did not reduce blanks containing gradient buffer plus sucrose. Thus bacterial urease did not appear to contribute to urease measurements.

A unit of urease will hydrolyze 1 μmol urea in 1 min at 30°C and pH 7.0.

Rocket Immunoelectrophoresis. Urease antigen was determined as described previously (20). Agarose (1%, w/v) contained extract buffer and 2.9 μg/ml 'dispecific' antiurease antibodies, purified as previously described (18). Electrophoresis was for 16 h at 30 v/cm gel length. Rocket height was a linear function of urease antigen in the range 0.02 to 0.5 μg.

After electrophoresis gels were pressed under a 3-cm stack of Whatman 3 MM filter paper and, after 1 hr, dried in a microwave oven for 2 min. The gel was both fixed and stained by immersing 1 h in Crowle's double stain (8) (0.25% [w/v] crocein scarlet, 0.015% [w/v] Coomassie Brilliant Blue, 5% [v/v] acetic acid, and 3% [w/v] TCA) and destained for 2 hr in 1% acetic acid.

Determination of Protein in Crude Extracts. Fifty μl of crude extract was boiled for 10 min after the addition of 2 ml 10% (w/v) TCA. Protein was pelleted by centrifugation and dissolved in 0.5 ml 10% NaOH. Aliquots of 10 to 100 μl were taken to 200 μl with NaOH and 0.8 ml microbiuret reagent (17) (0.25% [w/v] $CuSO_4 \cdot 5H_2O$, 1.4–1.5% [w/v] NH_3 4.4 N NaOH) was added. *A* at 300 nm was determined and converted to protein concentration by reference to a standard curve equating A_{300} and known quantities of BSA prepared in an identical manner.

Determination of Total Protein per Bean. Ground lyophilized bean was boiled 20 min in 5 ml 10% (w/v) NaOH/100 mg bean meal. After standing 18 h at room temperature, H_2O_2 (30%, 0.05 vol) was added, the mixture was boiled for 10 min, and centrifuged at top speed for 10 min in a clinical centrifuge. The volume of the supernatant was measured and an aliquot was mixed with an equal volume of 2 M Na_2SO_3. After standing for 4 h, 20 to 60 μl aliquots were taken to 200 μl with 5% NaOH, 1 M Na_2SO_3 and, after the addition of 0.8 ml microbiuret solution, the *A* at 300 nm was determined. Blanks contained 200 μl 5% NaOH, 1 M Na_2SO_3, and 0.8 microbiuret solution.

Total protein per bean was calculated by multiplying the protein yield per weighed quantity of bean meal by the total dry (lyophilized) weight of the original bean.

Preparation of Soluble Soybean Polysomes. 'Soluble' polysomes were isolated from developing or germinating soybean seeds using the high salt, alkaline buffers employed by Beachy *et al.* (3) for developing soybeans. This procedure results in a mixture of both free and membrane-bound polysomes (3). Heparin (Sigma) (500 mg/L) and cycloheximide (Sigma) (1 mM) were added to the grinding buffer while 100 mg/L heparin was added to the suspension, pelleting and sucrose gradient buffers. Glassware, mortar, and pestle were dry-heated at 165°C overnight. Glassware for RNA solutions was coated with dichlorodimethylsilane. Polypropylene centrifuge tubes were soaked in 1 N NaOH overnight and rinsed extensively with boiled, deionized H_2O before use.

Mature soybeans were sterilized by soaking for 3 min in a 1:1 mixture of 95% ethanol and bleach (5.25% NaOCl). After thorough rinsing in sterile, distilled H_2O, the beans were placed on three layers of autoclaved wet Whatman 3 MM in 10-cm glass Petri dishes (5–7 beans per dish). After dark incubation at 28°C for 5 to 7 d, the coats were removed from uncontaminated seeds with emergent radicles. Developing beans were frozen in liquid N_2 immediately after picking and stored at −70°C.

Germinating or developing seed (10 g) was ground to a homogeneous slurry in a mortar with 1 or 2.5 vol grinding buffer, respectively. The slurry plus mortar washings was centrifuged twice at 10,000*g* for 15 min and the supernatant layered over 8 ml pelleting buffer. After spinning 97 min at 40,000 rpm in a Ti 50 rotor, both layers were aspirated and the pellet and tube rinsed twice with 2.5 ml suspension buffer. Pellets were resuspended (0.6 ml/10 g seed) and cleared (1,000*g*, 5 min). Before the clearing spin, half of the preparation was incubated at 37°C for 30 min in heparin-free suspension buffer containing 150 μg/ml RNase A. Cleared preparations were spun through a 15-ml linear 12.5 to 50% sucrose gradient at 25,000 rpm for 90 min in a SW 27.1 rotor. One ml fractions, hand-collected from the bottom of the tube, were assayed for urease activity, as described above, and *A* at 254 nm was determined on 0.1 ml aliquots. In some experiments, polysome gradients were monitored and fractionated with a gradient fractionator (model 640; Instrumentation Specialties Co., Lincoln, NE).

Isolation of RNA from Developing Soybeans. Extraction buffer (50 mM Tris-HCl/9.0, 0.15 M NaCl, 5 mM EDTA, 1% [w/v] SDS) was autoclaved and filtered through a 0.22-μm membrane filter (Nalge-Sybron, Rochester, NY). Buffer (200 ml), heated to 60°C in a Waring Blendor (previously rinsed with 0.1 N NaOH and heated to 60°C for 3 h in 0.1% SDS, 1% Na_2EDTA), was agitated and 100 g frozen (10–20 d field grown) beans were immediately added. After 30 s, 200 ml of a 1:1 chloroform:phenol mix (containing 1% [w/v] 8-quinolinol and saturated with 0.1 M Tris-HCl/8.6) was added to the stirring slurry. Agitation was stopped after 30 s, the contents of the blender cooled to 4°C and then centrifuged at 10,000*g* for 15 min. To the separated aqueous phase, powdered NaCl was added to 0.3 M. (In earlier preparations, the organic phase was reextracted with 0.1 M Tris-HCl/8.6, but this reextraction was eliminated in later preparations since it recovered less

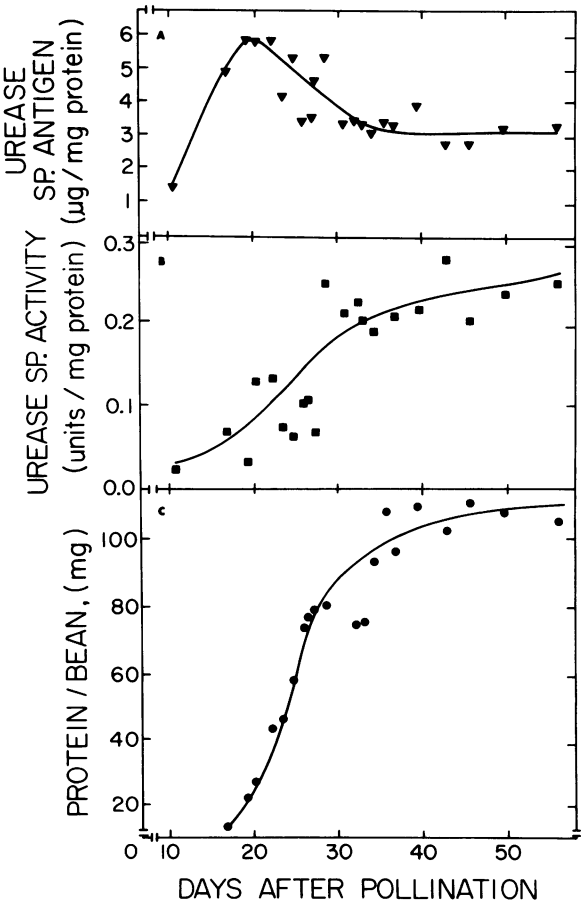

FIG. 1. The appearance of urease antigen and catalytic activity during seed development. Urease antigen (A) and activity (B) were measured in common extracts of staged beans. Each point is the average determination of three individual beans. A portion of each bean was saved for determination of total protein (C).

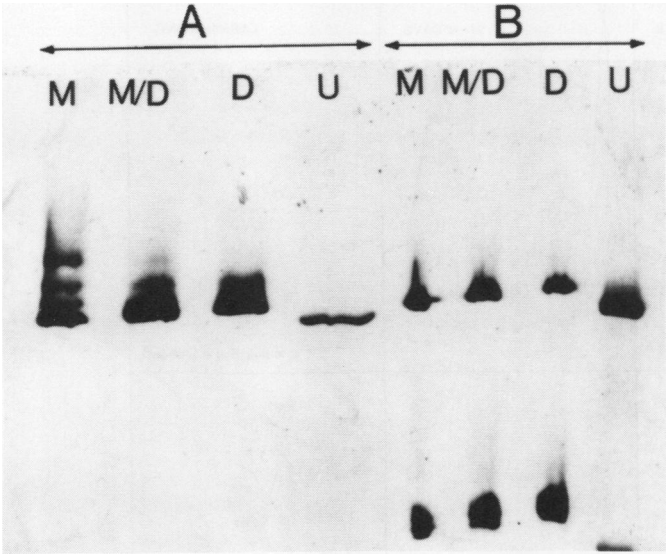

FIG. 2. Active urease species in crude extracts from mature and developing (day 10–20) soybeans. A, mature (M) and developing (D) crude extracts and their 1:1 mixture (C), 380 μg protein in each sample, were subjected to electrophoresis in a 7.5% native acrylamide gel; their active species were detected by a urease activity stain (11). The specific activity of the mature extracts was lowered to that of the developing extract (1.07 units/mg) by the addition of mature extract protein from a urease-negative variety (20). B, extracts were treated with 50% glycerol, 5% DTT for 30 min at 60°C. Combined extracts were mixed after treatment. Purified urease (U) contained approximately 2 μg protein.

than 10% of the RNA found in the first aqueous phase.) After the addition of 2.5 vol 100% ethanol and overnight precipitation at −20°C, the pellet was collected and washed 5 times in 3 M ammonium acetate (pH 5.5), resuspended in 100 ml 0.3 M ammonium acetate, and reprecipitated overnight after the addition of 2.5 vol ethanol. After collecting and drying the pellet, it was dissolved in water to 20 A_{260} units/ml. Approximately 3,000 A_{260} units were obtained at this stage with a 260/280 nm A ratio of 2.2 to 2.3. From this preparation, polyadenylated RNA was isolated by the method of Aviv and Leder (1).

Sucrose Gradient Centrifugation of Polyadenylated RNA. Linear 5% to 20% sucrose gradients in 95% DMSO (v/v) (Fisher Scientific) and 4% formamide (v/v) (Bethesda Research Labs) (6) containing 1 mM EDTA, 10 mM LiCl, and 10 mM Tris-HCl/7.3 were formed in SW 50 polyallomer tubes (Beckman) (4.8 ml total gradient volume). Poly A RNA (125 μg) was prepared for centrifugation by heating at 55°C for 5 min and mixing with nine volumes 99% (v/v) DMSO, 1 mM EDTA/8.0 (final volume, 0.4 ml). Gradients were spun at 45,000 rpm for 28 h at 20°C. The gradients were monitored at 280 nm and 0.3 ml fractions collected on a gradient fractionator.

Electrophoresis Techniques. Horizontal methyl mercury hydroxide agarose gels were run as described by Bailey and Davidson (2). SDS discontinuous slab gels were prepared according to Laemmli (13). Seed protein or rabbit reticulocyte lysate translation reaction was mixed with 1 to 2 volumes of 0.125 M Tris phosphate/6.8, 2% (w/v) SDS, 1% (v/v) β-mercaptoethanol, 17% (w/v) sucrose, and 0.05% (w/v) bromophenol blue (disruption buffer [3]), and boiled for 2 min before applying to gel. After electrophoresis for 3.5 h at constant power (wattage) to produce initially 30 mamp of current, gels were stained in 45% (v/v) methanol, 9.2% (v/v) acetic acid, and 0.25% (w/v) Coomassie Brilliant Blue G-250 for 0.5 h and destained in 7% (v/v) acetic acid containing approximately 0.5% (w/v) DEAE-cellulose. For fluorography, gels were treated with Enhance (New England Nuclear) or prepared by the method of Bonner and Laskey (5). Preflashed (14) x-ray film (Kodak XRP-5) was exposed to dried gels in intensifying casettes kept at −80°C for 1 to 5 d.

Native polyacrylamide gels were run essentially as described above, except that SDS was omitted from all buffers, the separating (lower) gel was 7.5% acrylamide and gels were run 2.5 h at 15 mamp constant current. Samples were mixed with 0.1 vol 50% (v/v) glycerol, 10 mM Tris maleate (pH 7.0) 0.2% (w/v) bromophenol blue. Gels were fixed, stained, and destained as described above or they were treated with a specific activity stain for urease (11).

***In Vitro* Translation.** *In vitro* translations were performed at 30°C for 60 min in a rabbit reticulocyte lysate system (Bethesda Research Labs). Total Mg and K concentrations were 1.17 mM (chloride salt) and 182 mM (48 mM chloride and 134 mM acetate salt), respectively, and were optimized for maximal [^{35}S]methionine incorporation which was determined by pipetting 5 μl translation mix onto a 1.8 × 1.8 cm square of Whatman 3 MM. Filter papers were dropped into cold 10% (w/v) TCA containing 5 mM unlabeled methionine. After two 10-min washes, the filter papers were boiled for 10 min in 5% (w/v) TCA containing 2.5 mM methionine. Following 1-min washes in 95% ethanol (twice) and in acetone (twice) the filters were air dried and counted. Gel analysis of translation products was as described above.

Immunological Techniques. Rabbit antibody to holourease was induced and purified by affinity chromatography as described previously (18). Nickel-free urease immunogen (1.7 mg) was produced by dialysis against 0.1 M sodium acetate, 5 mM EDTA (pH 5.1) for 48 h at 25°C. In our hands, this treatment, which causes a parallel loss of activity and nickel from Jack bean urease (10), caused soybean urease to lose all activity and yielded a heavy precipitate. This was solubilized by boiling 2 min in the presence of 0.1% (w/v) SDS. An equal volume of Freund's complete adjuvant (Miles Laboratories) was added and 1.3 mg protein was injected subcutaneously into a rabbit followed 3 weeks later by injecting the remaining 0.4 mg. After 2 more weeks, serum from this animal precipitated native urease in an agarose double diffusion immunoassay.

Immunoprecipitation of translation product was performed by the method of Lingappa *et al.* (15). From 50 μl of translation mix, urease antigen was precipitated by 9 μg purified antibodies or by 5 μl anti nickel-free urease serum. *Staphylococcus aureus* (cowan) cells were from the Enzyme Center, Inc., Boston, MA.

RESULTS AND DISCUSSION

Appearance of Urease Activity and Antigen during Embryo Development. Nodulated soybean plants, cultivated in a growth room under a 12-h photoperiod (25 ± 1°C light and 20 ± 1°C dark) were the source of developing embryos. Prize cultivar yielded mature, dry beans approximately 55 d after pollination. Both urease specific antigen and specific activity were determined in the same crude extract derived from individual beans. Figure 1A demonstrates that urease antigen is first detectable in 11-d-old embryos. The differential synthesis of urease protein (the synthesis of urease *versus* the synthesis of the bulk storage proteins) is greatest during the 11th to 20th d of development. The development of urease enzyme activity (Fig. 1B), however, lags the synthesis of urease protein during the 11th to 20th d. During the 20- to 35-d interval, urease specific antigen levels decline, whereas urease specific activity increases. Urease specific activity is at its highest during late development and in the mature bean, whereas urease specific antigen is at its highest during mid-development (approx. the 20th d). The data points in Figure 1 represent averages of determinations on three different beans; understandably there is some variability inherent in these small sample sizes. Nevertheless, the increasing urease activity: antigen ratio during bean development is evident.

Mixing extracts of early development beans (10–20 d) with

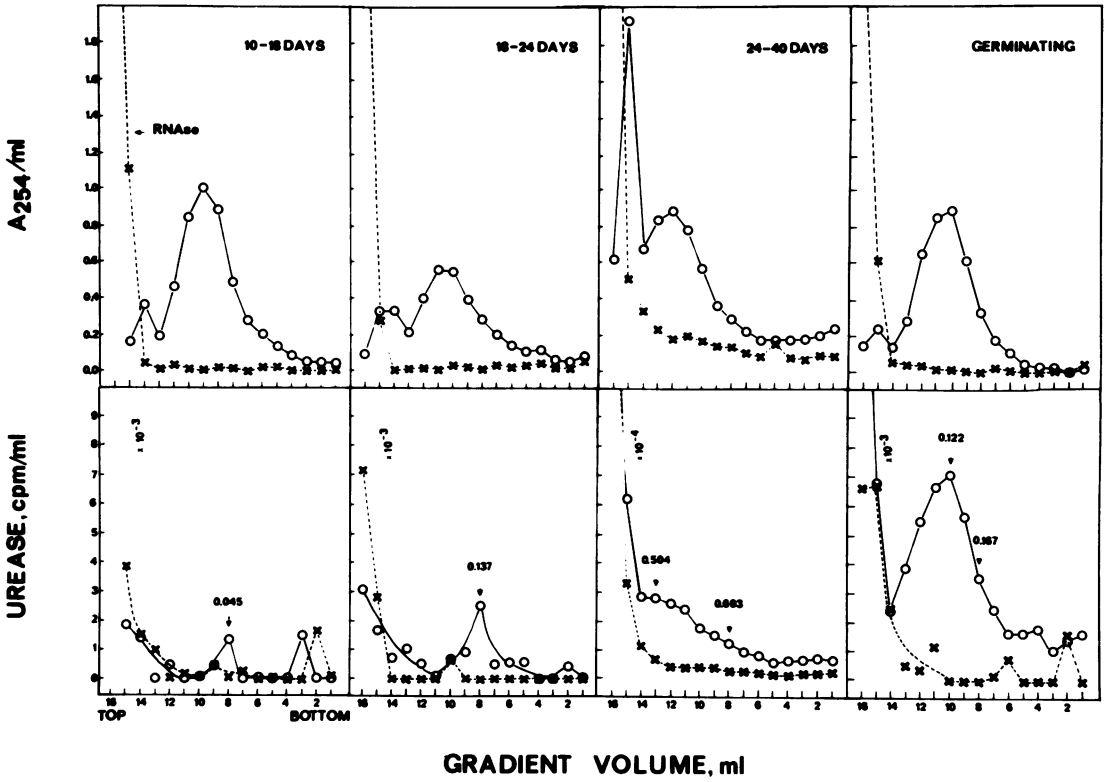

FIG. 3. Polysome profiles in developing and germinating soybean seeds. Polysomes were prepared ("Materials and Methods") from 10 g developing and axenic germinating soybeans. From 1 ml hand-collected fractions aliquots were taken to measure A_{254} or urease activity. RNAse-treated polysomes (×); untreated polysomes (○).

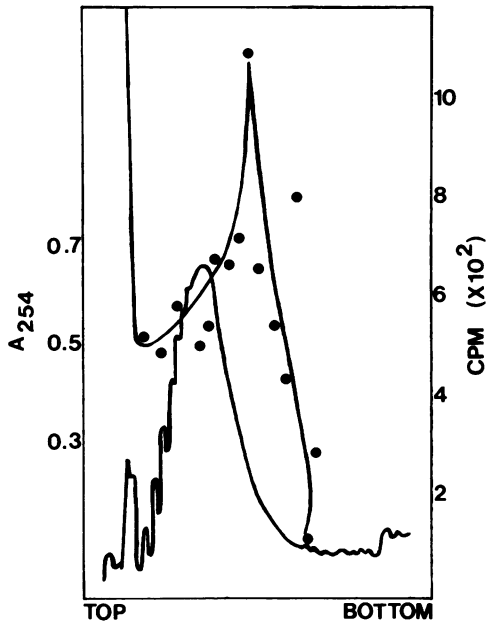

FIG. 4. The appearance of a urease activity peak (●) on a polysome profile from 10- to 24-d developing beans. Urease catalytic activity was determined as described under "Materials and Methods."

those of mature beans resulted in extracts containing the expected intermediate level of urease enzyme activity, which argues against the presence of urease inhibitor(s) or activator(s). To test possible structural changes accompanying the apparent enhancement of urease activity in late development, bean extracts were electrophoresed in 7.5% native polyacrylamide gels. Mature beans consistently showed several slow migrating active species not present in extracts of developing beans. To eliminate the possibility that these heavier species were detected simply because of the higher activity in mature seed extracts, the specific activities of mature and developing seed extracts were equalized by adding protein from a ureaseless bean (cv. Itachi) to mature extracts. Such mixtures result in no alteration of Prize's isozyme pattern (20). In the native gel of Figure 2A several slowly migrating urease species are unique to mature bean extracts. These are likely larger aggregates of urease (7, 12, 18), but we do not know if they are more active forms.

Both mature and developing bean extracts were incubated at 60°C for 0.5 h in 50% (v/v) glycerol and 5 mM DTT. This treatment converts active urease species to two forms (Fig. 2B). Both developing and mature extracts now have identical patterns as shown by comparing the two extracts and a mixture of the two. That the isozyme patterns of mature and developing beans become identical upon treatment suggests that there are no major structural differences between their active urease subunits. Interestingly, urease purified from mature Prize beans also shows two active forms upon heating in DTT and glycerol. However, these species migrate differently from those in both mature and developing extracts. This demonstrates the importance of making the extracts equivalent in protein backgrounds.

Thus, along with increased specific ureolytic activity (activity/antigen) during seed development, active urease holoenzyme becomes more aggregated, possibly explaining urease's apparent activation. However, posttranslational modification of active or inactive urease subunits has not been ruled out.

Urease Synthesis on Polyribosomes from Developing Seeds. Polyribosomes were isolated from soybeans harvested from three developmental intervals (days 10–18, 18–24, and 24–40) and from germinating beans. The extraction yields both free and membrane-bound polysomes (3) (see "Materials and Methods"). The two

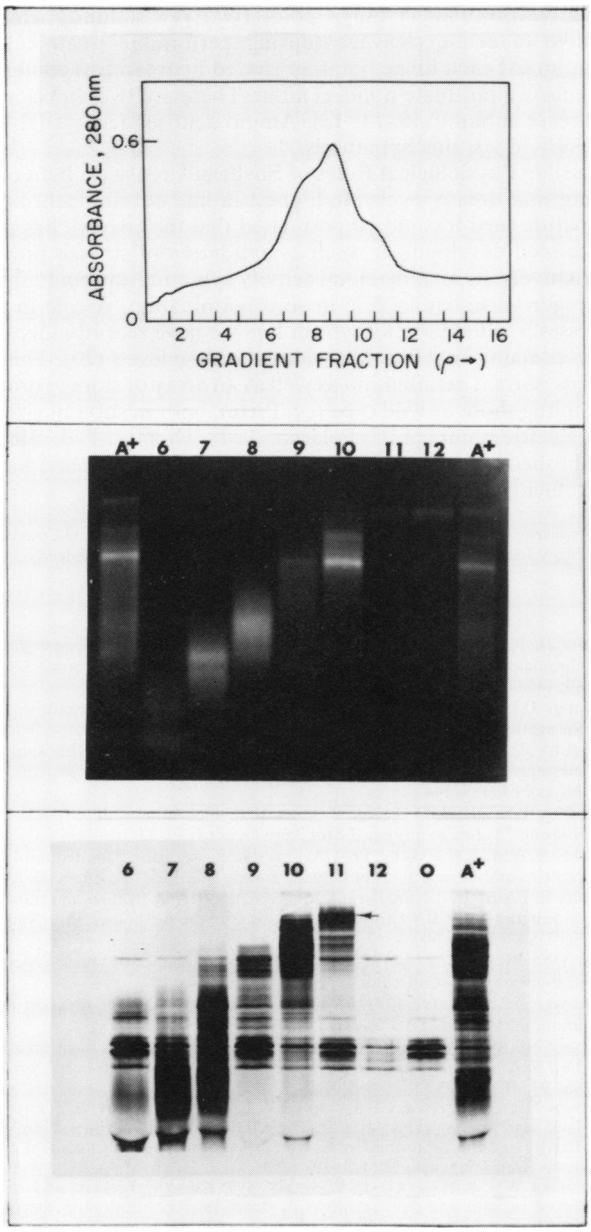

FIG. 5. Translation of size-fractionated polyadenylated RNA. Poly A-containing RNA was fractionated on a DMSO-sucrose gradient (top) and analyzed by electrophoresis in a methyl mercury hydroxide agarose gel (middle). The arrow in fraction 11 indicates the position of *in vivo* urease subunits in the 12.5% polyacrylamide gel (bottom). Background translation products (lane O) and translation products from unfractionated poly A-RNA (A^+) are also shown (bottom panel).

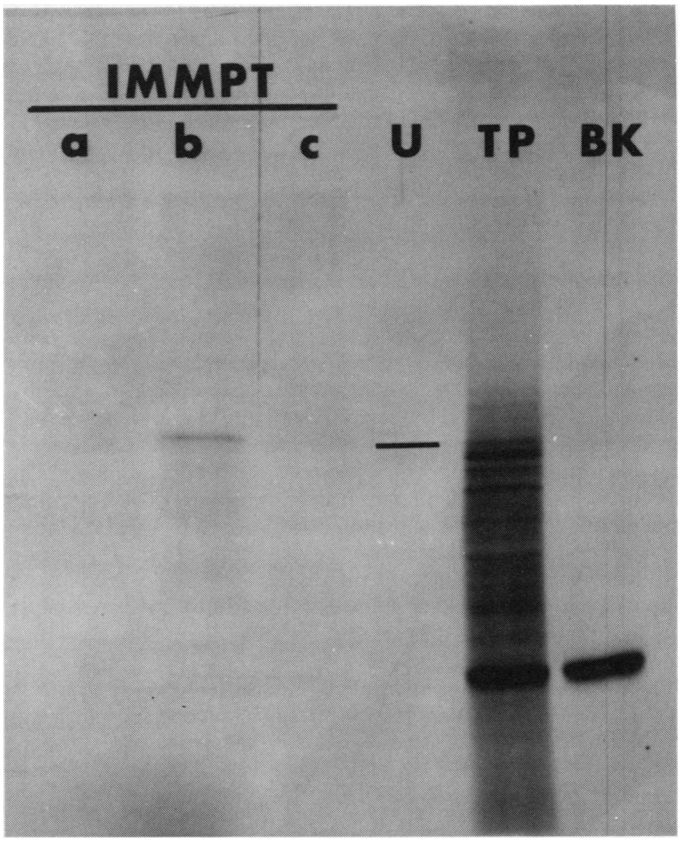

FIG. 6. Immunoprecipitation of urease translation product. Translation products (lane TP) from a heavy size class polyadenylated RNA were immunoprecipitated with antiserum to denatured, nickel-free urease. The species in lane b closely migrated with native urease (indicated by line in lane U) in a 10% SDS polyacrylamide gel. Lane a represents an immunoprecipitation with 15 μg competing *in vivo* urease, whereas 10 μg, nonimmune rabbit IgG was substituted for antiserum in the sample of lane c. Five μl of translation mix was electrophoresed in lanes B1 (no RNA control) and TP and 36 μl translation mix was subjected to each of the three immunoprecipitations.

earliest developmental stages exhibit a small but reproducible peak of urease enzyme activity on the heavy side of the polysome profile (Fig. 3). In RNase-treated polyribosome preparations, the bulk of the urease activity stayed at the top of the gradient. These observations suggest that urease is synthesized on heavy polysomes if the assumption is made that the polysome-bound activity is due to an active nascent urease or to an inactive nascent chain complexing with active completed subunits. When sucrose gradients of polysomes isolated from early embryos (10–24 d) were pumped through a flow cell, the urease peak appeared at approximately the $n = 15$ polysome size class (Fig. 4). From the 93.5 kd size of urease (18) this agrees well with the size of polysomes of other eukaryotic messages. For example, rat liver albumin (mol wt 65,000) is translated on ($n = 9$ to 10) polysomes (22). The α and α′ subunits of the 7S storage protein of soybean, whose precursor forms are 78 and 80 kd, respectively (4), are synthesized on polysomes of $n \geq 13$ (3).

Polysomes from beans in the 24th to 40th days of development and from germinating beans exhibit increasingly congruent urease activity profiles and polysome A_{254} profiles. This probably reflects nonspecific aggregation of free urease to polysomes since late maturation (24–40 d) and germinating beans have high levels of free urease. Indeed, urease activity sedimenting with 24 to 40 d and germinating polysomes represents only 23 to 46% of the total activity on the respective gradients. The arrows on the urease activity profiles indicate the 'specific activity' of urease, in this case expressed as μmol urea hydrolyzed/d/A_{254}. Although not plotted separately, all gradients show a peak of urease specific activity within one or two fractions of the urease peak shown in the first two profiles of Figure 3.

Identification of the Urease mRNA Size Class and Translation Product. To determine the urease RNA template size poly A-containing RNA was isolated from field-grown 10- to 20-d-old soybeans and fractionated on a 5 to 20% sucrose gradient in 95% DMSO, 4% formamide (6). Figure 5 shows a profile obtained from a typical gradient (top) and the appearance of the fractionated RNA after electrophoresis in methyl mercury hydroxide agarose (middle). When the fractionated RNA was used to direct

protein synthesis *in vitro*, the products displayed in the 12.5% SDS polyacrylamide gel of Figure 5 (bottom panel) were obtained. Translation products in the size class of the holourease subunit are synthesized almost exclusively from the template of fraction 11. The translation product indicated by the arrow in fraction 11 (Fig. 5C) comigrates with the native urease subunit although small differences in migration rates cannot be ruled out on this gel.

An immunological test was performed for the relatedness between the urease subunit synthesized *in vivo* and the *in vitro* translation product with which it closely migrates. Antiserum was raised against urease purified as described previously and freed of nickel by dialysis against EDTA at pH 5.1 (10) (Materials and Methods). This serum specifically immunoprecipitated a translation product which on 10% polyacrylamide gels migrates with or slightly slower than the *in vivo* urease subunit (Fig. 6, lane b). RNA was size-selected such that its putative urease translation product was not the major band, thus providing a more convincing demonstration of the specificity of the immunoprecipitation. The specificity is further demonstrated by the lack of a detectable precipitate when IgG is substituted for antiserum (Fig. 6, lane c) or when 15 μg competing *in vivo* urease is added to the translation products before immunoprecipitation (Fig. 6, lane a). Thus, it appears that the urease subunit is made from a mRNA slightly smaller than the 25S rRNA species (Fig. 5, B and C) which contains about 3,300 nucleotides (16).

In our unpublished experiments, urease template is found in RNA sliced from a region just below and extending into the lower portion of the 25S rRNA band on methyl mercury hydroxide gels. A urease subunit of 93.5 kilodaltons should have a message containing 2,400 coding bases, suggesting that there are 600 to 900 noncoding bases in urease mRNA.

Urease Isozymes. The two forms of urease shown in the extracts of Figure 2B are likely identical to the fast and slow moving urease species reported by Buttery and Buzzel (7) in crude extracts of several soybean cultivars. They reported a genetic control over the propensity to form the fast or slow species although heat treatment or germination seemingly converted the slow form to the fast one (7). It was concluded that the slow species is likely a hexamer and the fast species a trimer. We reported that purified soybean urease can exist as a fast or slow form depending on the salt environment (18). From gel chromatography elution volumes and relative migration rates in native gels of differing acrylamide concentration, we concluded that the fast and slow forms were likely a trimer and hexamer, respectively (18). That the two forms in crude extracts of mature Prize migrate differently from the two forms of purified Prize urease (Fig. 2B) suggests that each form in crude extract differs in charge or conformation from its counterpart in purified (and much more dilute) preparations. Fishbein (11, 12) has reported that purified Jack bean urease can exist in 12 different forms depending on salt concentration, pH, sulfhydryl reagents, hydroxyl compounds such as glycerol, and other factors. These forms represent different states of conformation and aggregation.

The Urease Subunit Size. Staples and Reithel (21) reported that the smallest predicted subunit size of Jack bean urease which gives the best integer composition values for all its amino acids is 30,400 daltons. The number of cyanogen bromide cleavage products was that expected for the methionine residues per 30,000 dalton peptide. Performic acid oxidation of urease produced a 30 kd species on SDS polyacrylamide gels. We have found that soybean and Jack bean ureases share an identical subunit size (93.5 kd) and antigenic determinants and that their amino acid compositions are similar (18). To accommodate the findings of Staples and Reithel (18) with our findings of a 93.5 kd size for urease synthesized *in vivo* and *in vitro* (reported here) we postulate a single polypeptide of 93.5 kd consisting of three contiguous homologous regions, each independently assuming a tertiary structure. Between domains we postulate a hinge point (two per subunit) which is sensitive to acidic cleavage (during performate treatment, for example). At each hinge point, or shared between two contiguous domains, we postulate a nickel atom. There are two nickel atoms per urease subunit (10, 11, 18). Amino acid sequence analysis is necessary to test this hypothesis.

Possible Physiological Roles of Soybean Urease. In light of our finding that urease levels are highest in late development and in the mature seed it could be postulated that the enzyme has a role in late seed development, such as amidation of storage proteins. Alternatively, its maximal total activity at maturation could signify a nitrogen assimilatory role in seed germination. A role in both processes was tested in a soybean line we have recently identified which contains only 0 to 0.2% normal urease levels (20). This line exhibits normal germination as well as an array of storage proteins resembling those of wild type (cv. Prize), both in subunit size and in isoelectric points (J. C. Polacco, A. L. Thomas, P. J. Bledsoe, unpublished results). Thus, a physiological role for urease has yet to be elucidated.

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