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Isolation and Characterization of Messenger RNAs for Seed Lectin and Kunitz Trypsin Inhibitor in Soybeans

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ABSTRACT

The mRNAs for seed lectin and Kunitz trypsin inhibitor of soybean have been highly enriched by immunoadsorption of the polysomes synthesizing these proteins. Polysomes isolated from developing seed of variety Williams were incubated with monospecific rabbit antibodies produced against lectin subunits or trypsin inhibitor protein. The polysomal mixture was passed over a column containing goat anti-rabbit antibodies bound to Sepharose. Bound polysomes were eluted and the mRNA was selected by passage over oligo(dT)-cellulose. Lectin complementary DNA hybridized to an 1150-nucleotide message and trypsin inhibitor complementary DNA hybridized to a 770-nucleotide message in blotting experiments using total poly(A) RNA. Translation of soybean lectin mRNA using a rabbit reticulocyte lysate yielded a major polypeptide of 32,300 whereas the molecular weight for purified lectin subunits was 30,000. Trypsin inhibitor mRNA directed the synthesis of a 23,800-dalton polypeptide as compared to 21,500 daltons for trypsin inhibitor marker protein. Lectin specific polysomes could not be obtained from a soybean variety which lacks detectable lectin protein whereas trypsin inhibitor-specific polysomes were bound by immunoselection. These results confirmed the specificity of the immunoadsorption procedure and strongly indicated that the lectinless variety was deficient or substantially reduced in functional lectin mRNA.

Attempts to understand the molecular-genetic control of protein accumulation in developing seeds has been an area of intensive research in recent years (5–7, 31). To date, investigations have concentrated upon characterizations of mRNAs for the major storage protein components of the seed such as zein in maize (14, 32) and globulin-1 protein of *Phaseolus vulgaris* (11). The present report describes the preparative isolation and initial characterization of the mRNA for SBL[1] and SBTI, each of which account for only a few per cent of the total seed protein. These two proteins are particularly attractive as marker proteins for studying the molecular control of seed protein synthesis as both are purified easily and well characterized biochemically.

SBL is a glycoprotein capable of agglutinating erythrocytes and it comprises 0.5 to 5% of the total protein in defatted meal depending upon the soybean variety used (24). Native lectin is a 120,000 mol wt tetramer composed of four 30,000-dalton subunits (18) each of which presumably contains a carbohydrate side chain of 2,000 daltons (17). There are two types of lectin subunits with slightly different electrophoretic mobilities as determined by discontinuous polyacrylamide gels containing SDS (19). The Kunitz trypsin inhibitor of soybean is one of the best characterized of all plant proteinase inhibitors. It is a single polypeptide chain of 21,500 containing two disulfide bridges (30). While there are other small mol wt trypsin inhibitors in soybean seeds, SBTI constitutes a major proportion of the trypsin inhibitor activity. The physiological roles of lectins and trypsin inhibitors in plants remain obscure.

In addition to substantial biochemical data for these two seed proteins, lectin and trypsin inhibitor have been defined by classical genetic analyses and soybean varieties which lack detectable SBL or SBTI protein have been found (21, 22, 24). It is the aim of this report and other studies in progress to determine what molecular defects cause the inherited absence of SBL or SBTI protein in certain soybean varieties. Such knowledge will help elucidate the normal functioning and expression of plant genes. As an initial step toward this objective, I have developed modifications to a polysome immunoadsorption procedure which have allowed the isolation of highly enriched mRNAs for lectin and Kunitz trypsin inhibitor. A preliminary report has been published (33). The selected mRNAs were analyzed by their activities in a rabbit reticulocyte cell free translation system and by hybridization of labeled SBL or SBTI cDNA to gel blots of total poly(A) RNA.

MATERIALS AND METHODS

Growth of Plants. Soybeans of variety Williams (maturity group III) were grown under standard field or greenhouse conditions. Greenhouse-grown plants were supplemented weekly with a soluble fertilizer and trace element mix. A combination of fresh weight and seed color characteristics were employed to developmentally stage the seed used for polysome isolations. Under both greenhouse and field growth conditions Williams seed attain a maximum fresh weight of 500 to 700 mg/seed before dehydration. Seed harvested at 100 to 300 mg fresh weight/seed during the early to midmaturation stage had green cotyledons and green embryo axes and seed coats. Seed were frozen at −70 C and stored at this temperature until used. During the latter phase of seed maturation, Chl was lost and Williams seed at maturity were yellow. Seed of variety Sooty (maturity Group IV) were likewise harvested during the mid-maturation stage at 100 to 200 mg/seed before the loss of Chl from the embryo axes and cotyledons and before development of the pigment which gives Sooty a black seed coat.

Production of Monospecific Antibodies. Soybean lectin was purified from either whole or defatted meal of Williams mature seed by affinity chromatography using *N*-acetyl-D-galactosamine coupled to Sepharose (1). Soybean trypsin inhibitor was obtained commercially (type 1-S, Sigma Chemical Co.). Both proteins were homogeneous as determined by SDS/polyacrylamide gel electrophoresis and Coomassie blue staining (13). Two to four mg antigen

[1] Abbreviations: SBL, soybean lectin; SBTI, soybean trypsin inhibitor; PBS, phosphate-buffered saline (0.009 M phosphate, 0.123 M NaCl [pH 7.2]); TNT, 50 mM Tris, 100 mM NaCl, 0.1% Triton (pH 7.6)); IgG, γ-globulin; GAR, goat anti-rabbit antibodies; cDNA complementary DNA; DPT-paper, diazophenylthioether-paper.

prepared in 0.1% SDS and 50% Freund's complete adjuvant were injected per rabbit. Immunizations were made at weekly intervals for 4 weeks and then once every 4 to 6 weeks thereafter. After the rabbits were producing immune sera, bleedings of 40 ml/rabbit were made at not less than biweekly intervals. Immune sera to native SBL protein were prepared in the same manner as above but with the omission of SDS.

Lectin or trypsin inhibitor protein was coupled to CNBr-activated Sepharose 4B by standard methods (28, and Pharmacia Technical Bulletin "Affinity Chromatography"). Lectin was heat-treated in the presence of 0.1% SDS prior to coupling. Monospecific antibodies were purified from immune sera by ammonium sulfate fractionation (23) and affinity chromatography on a column containing 1 g appropriate antigen-Sepharose matrix. The sample was applied and nonspecifically bound material was removed by two cycles of washings with 0.1 M Na acetate, 1 M NaCl (pH 4.8), and 0.1 M Na_2CO_3, 1 M NaCl (pH 7.6), as described by Shapiro *et al.* (28). Bound antibody was eluted with 0.5 M acetic acid (pH 2.5). Pooled antibody fractions were adjusted immediately to pH 7.0 by dropwise addition of 2.5 M NH_4OH. The antibody was then dialyzed overnight against 2 × 1 liter changes of PBS, centrifuged to remove any precipitate, aliquoted appropriately, and stored frozen.

Nonimmune rabbit immunoglobulin was purified from sera by ammonium sulfate fractionation and filtration on Sephacryl S-200. Serum from goats immunized against rabbit IgG was obtained commercially (Grand Island Biological Co.) and the goat anti-rabbit antibodies were isolated by ammonium sulfate fractionation and affinity chromatography on a rabbit IgG-Sepharose column. The purified goat anti-rabbit antibodies were coupled to CNBr-activated Sepharose 4B by standard procedures.

Polysome Isolation and Immunoadsorption. All centrifugations, column runs, and other procedures were performed at 4 C. Buffers and glassware were autoclaved and RNase-free techniques were employed. Total polysomes were isolated essentially according to the method of Jackson and Larkins (12). Preparations were routinely made by extracting 36 g frozen whole seed (seed coat plus embryo) in buffer containing 0.5% Na deoxycholate and 2% Triton X-100. The postmitochondrial supernatant was further clarified by addition of several detergents (Tween 40, Brij 35, Nonidet P-40) to 1% final concentration and was used to fill 38-ml tubes containing 10 ml 50% (w/w) sucrose cushion. The polysomes were pelleted through the sucrose layer for 15 h at 28,000 rpm in a Beckman type 30 rotor. The pelleted material was resuspended by gentle homogenization in a total volume of 18 to 20 ml of the same buffer as used for preparing the sucrose cushion (see reference cited above) and was clarified by centrifugation at 3000*g* for 5 min.

Antibody (either anti-subunit SBL or anti-SBTI) was added at the ratio of 2.5 to 3.0 $\mu g/A_{260}$ polysomes and buffer was added, if necessary, to adjust the polysomal A_{260}/ml value to approximately 24 A/ml. The mixture was incubated for 1.5 h at 4 C with occasional, gentle shaking. The polysomal incubation mixture was divided into half and each aliquot (usually 350–450 A_{260} units) was applied to a 1 × 10 cm column containing 1 g of goat anti-rabbit-Sepharose beads (GAR-Sepharose) equilibrated in 40 mM Tris, 280 mM KCl, 5 mM $MgCl_2$, 50 μg/ml heparin (pH 7.5). The sample was pumped onto the column at a flow rate of approximately 8 to 10 ml/h. The effluent was saved and repassed over the column. After the sample was applied the column remained undisturbed overnight or was washed at the slow flow rate with 30 ml equilibration buffer. The column was then washed sequentially with the following buffers at a flow rate of 50 ml/h: 30 ml equilibration buffer; 30 ml 2% Triton, 0.5% Na deoxycholate in equilibration buffer; and 100 ml buffer without detergents. The bound polysomes were dissociated by 20 mM EDTA in 40 mM Tris-HCl (pH 7.5) (27). The column effluent was measured for A_{260} and the fractions above the EDTA background absorbance were pooled and frozen at −70 C until used. The GAR-Sepharose matrix was regenerated by sequential washings with 0.5 M acetic acid; 0.1 M Na acetate, 1 M NaCl (pH 4.8); 0.1 M Na_2CO_3, 1 M NaCl (pH 7.6); PBS; and equilibration buffer. The GAR-Sepharose was regenerated in the column after each use. After four to five uses the matrix was removed from the column and thoroughly cleaned by performing the washes on a sintered glass filter.

Isolation and Analyses of mRNA from Immunoadsorbed Polysomes. The EDTA-dissociated polysomes were adjusted to 0.3 M potassium acetate (pH 6.0), and were precipitated by addition of 2 volumes ethanol and storage at −20 C for 16 h. Sodium acetate should not be used in order to avoid precipitation of sodium EDTA from the 67% ethanol solution. The precipitate was collected by centrifugation at 65000*g* for 1.5 h, dried under N_2 gas, redissolved in a minimal volume of Tris-HCl, 1 mM EDTA, 0.1% SDS (pH 7.6). Poly(A) mRNA was selected by two passages over 0.5 g oligo(dT)-cellulose (type T_2 Collaborative Research) according to the procedure of Rozek *et al.* (25).

In vitro translation was performed with a rabbit reticulocyte lysate (New England Nuclear) using [^{3}H]leucine label. Analysis of labeled translation products was made with 12% polyacrylamide gels containing SDS (13). Gels were dried and prepared for fluorography according to Bonner and Laskey (8). Indirect immunoprecipitations of translation products were performed using monospecific rabbit antibodies and goat anti-rabbit unfractionated serum as described in the legend to Figure 4.

Labeled cDNA was transcribed from the SBL or SBTI poly(A) mRNA using avian reverse transcriptase, oligo(dT) primer, and high specific activity [^{32}P]dCTP (2000–3000 Ci/mmol) as described (16). Reaction mixes using 0.25 μg mRNA were labeled to specific activities near 5×10^8 cpm/μg with 11 to 20% efficiency of transcription of the template mass. Agar gels (1%) containing methylmercury hydroxide were run according to Bailey and Davidson (4). Diazophenylthioether cellulose paper (DPT-paper) was prepared according to the procedure of B. Seed (footnote 51 in ref. 3). Transfer of RNA from gels to DPT-paper (Northern blotting) and hybridizations with ^{32}P-labeled probes were performed as described by Alwine *et al.* (2, 3).

RESULTS

Polysome Isolation and Immunoadsorption. The immunological isolation of specific polysomes can be achieved if antibodies to purified protein interact with nascent polypeptides attached to the polyribosomes. In immunoprecipitation, a second antibody (anti-antibody) is used to precipitate the antibody-polyribosome complex (26, 28). More recently, an immunoadsorption procedure utilizing anti-antibody coupled to *p*-aminobenzyl-cellulose has been used to adsorb antibody-tagged specific polysomes. After batch-washing of the matrix, the bound polysomes were dissociated with buffer containing 20 mM EDTA (27). In adapting this procedure, I have found that Sepharose is an appropriate support for the anti-antibody (in this case goat anti-rabbit) and that application of polysomes, washings, and elution can be conveniently performed in a manner analogous to standard column techniques for antigen-antibody purification by affinity chromatography.

The specificity of polysome binding to GAR-Sepharose is illustrated in Figure 1. No absorbance above the background reading contributed by the 20 mM EDTA elution buffer was detected in instances where polysomes incubated without added antibody or with control IgG were passed over the matrix (Fig. 1, a and b). Figure 1c illustrates that there is additional but much lower recovery of bound polysomes obtained when effluent polysomes are passed through a second column. Antibodies made against native SBL protein were not as effective in binding polysomes as were the antibodies generated using 0.1% SDS to denature the

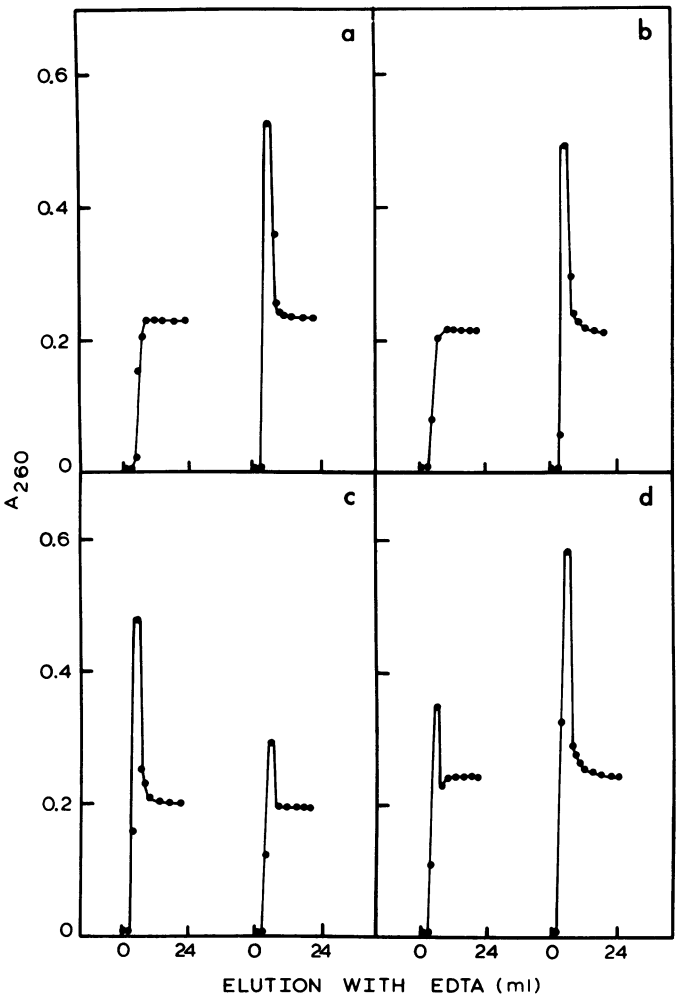

FIG. 1. Dissociation with EDTA of bound polysomes from columns containing 1 g GAR-Sepharose. Background absorbance at 260 due to EDTA in the elution buffer is approximately 0.22. In all cases (a–d), 300 to 500 A_{260} units polysomes were used and unbound polysomes from the first column (on the left) were treated as described and reapplied to a second GAR-Sepharose column whose elution profile is depicted on the right. (a) Polysomes were incubated without addition of antibody and were applied to GAR-Sepharose. Unbound polysomes were incubated with anti-subunit SBL antibodies and were applied to a second column. (b) Polysomes were incubated with nonimmune (control) rabbit IGg and were applied to GAR-Sepharose. Unbound polysomes were incubated with anti-subunit SBL and were applied to a second column. (c) Polysomes were incubated with anti-subunit SBL and applied to GAR-Sepharose. The unbound polysomes were applied without additional antibody to a second GAR-Sepharose column. (d) Polysomes were incubated with 2 mg anti-native SBL antibodies and applied to GAR-Sepharose. Unbound polysomes were incubated with 1.2 mg anti-subunit SBL and were applied to a second column. In all cases (a–d), only one passage of the polysome sample was made through each GAR-Sepharose column.

SBL antigen (Fig. 1d).

Resuspended polysomal pellets were incubated with monospecific antibody and were used directly for immunoadsorption without removal of the unreacted antibody. Polysomes can be separated from unreacted antibody by sedimenting them through sucrose (10). Although removal of excess antibody can lower the amount of anti-antibody needed for immunoprecipitation or immunoadsorption, no substantial increase in recovery of SBL-specific polysomes from GAR-Sepharose columns was achieved by this procedure and it was not used routinely.

During initial experiments, developing Williams seed were individually weighed and divided into groups of 100 to 149, 150 to 199, 200 to 249, and 250 to 300 mg fresh weight before freezing. High yields of total polysomes were obtained from all the weight ranges, and SBL- and SBTI-specific polysomes were isolated by immunoadsorption from all of these weight ranges of Williams seed. In contrast, polysomes extracted from the variety Sooty (one of several varieties which do not produce detectable lectin protein in the seed) did not bind to GAR-Sepharose columns when incubated with anti-subunit SBL antibody (Fig. 2, a and c). SBTI-specific polysomes were selected from Sooty seed of the same developmental stage (Fig. 2d). The results of the immunoadsorption procedure strongly indicate that functional mRNA for lectin synthesis is absent or substantially reduced in Sooty midmaturation seed.

The recoveries of SBL- and SBTI-specific RNAs from immunoadsorption and oligo(dT)-cellulose chromatography are shown in Table I. Total polysomal RNA was obtained at 0.7 to 1.0 mg RNA/g fresh weight seed. Immunoadsorbed polysomes accounted for 0.4 to 0.9% total amount of polysomal RNA (usually between 14–18 mg) applied to each GAR-Sepharose column. This recovery is reasonable based on the fact that SBL and SBTI each account for several per cent of the total seed protein. The choice of using 2.5 to 3.0 μg specific antibody/A_{260} polysomes was based on work of Groner *et al.* (10) who had used immunoadsorption to isolate the mRNA for lysozyme, a protein comprising 2 to 3% total protein synthesized by chicken oviduct. It is possible that higher

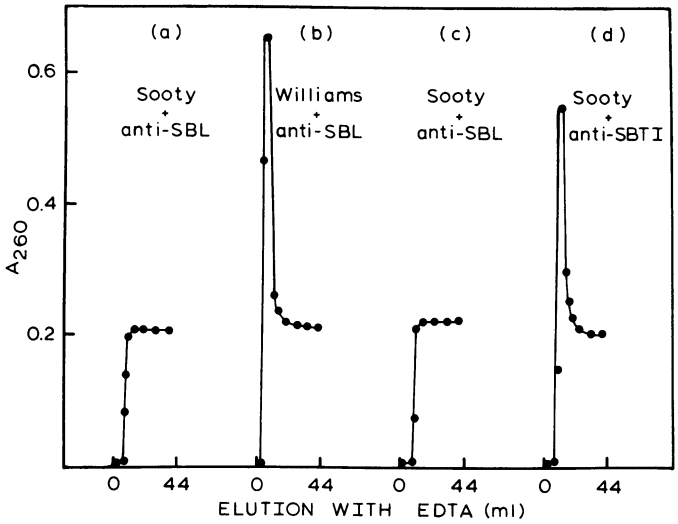

FIG. 2. Comparative binding to GAR-Sepharose of polysomes isolated from Sooty midmaturation seed of 100 to 200 mg fresh weight/seed. (a) Sooty polysomes (340 A_{260}) + anti-subunit SBL antibody; (b) polysomes from Williams seed (540 A_{260}) + anti-subunit SBL; (c) Sooty polysomes (460 A_{260}) + anti-subunit SBL; and (d) Sooty polysomes (230 A_{260}) + anti-trypsin inhibitor antibody. The same lectin antibody preparation was used in a, b, and c.

Table I. *Recovery of Lectin and Trypsin Inhibitor mRNAs from Polysome Immunoadsorption and Oligo(dT)-Cellulose Chromatography*

Fraction	Lectin		Trypsin Inhibitor	
	RNA	% Total	RNA	% Total
	mg		*mg*	
Polysomal RNA[a]	96.4	100	82.9	100
Immunoadsorbed RNA	0.624	0.65	0.55	0.66
Poly(A) mRNA	0.0035	0.0036	0.0035	0.0042

[a] The polysomal RNA was extracted from 126 g and 90 g seed for the SBL and SBTI polysome preparations, respectively.

concentrations of anti-SBL or anti-SBTI would result in higher recovery of immunoadsorbed polysomes. Approximately 0.6 to 0.7% of the immunoadsorbed RNA was obtained as poly(A) RNA after two selections on oligo(dT)-cellulose (Table I). In summary, 3 to 4 μg specific mRNA were extracted from each 100 g mid-maturation seed.

Analysis of Selected mRNAs. The mRNAs were analyzed by *in vitro* translation using a rabbit reticulocyte lysate. The results of Figure 3 demonstrate that the messages direct the synthesis of specific protein products as compared to translational products from total mRNA. Translation of trypsin inhibitor mRNA resulted in a predominant labeled polypeptide of 23,800 which sometimes appeared as a doublet band. Purified SBTI marker protein (21,500) also appeared as two closely migrating bands in some gels. The reason for this minor heterogeneity in both purified and *in vitro* translated trypsin inhibitor protein is unknown. Lectin mRNA directed the synthesis of a major polypeptide at 32,300 apparent mol wt and a less prominently labeled polypeptide of about 29,000. Thus, the predominant *in vitro* labeled products from both the enriched trypsin inhibitor and lectin messages migrate slower than nonlabeled SBTI and SBL subunits. Indirect immunoprecipitations of the translation products with anti-SBL or anti-SBTI (Fig. 4) confirm that the major labeled polypeptides from the selected messages are trypsin inhibitor and lectin. The minor polypeptide synthesized by SBL mRNA was also immunoprecipitable by anti-subunit SBL antibody and showed some variation in apparent mol wt from 25,500 to 29,000.

In a second approach to determine the degree of enrichment obtained by the immunoadsorption procedure and to estimate the message sizes, cDNAs prepared to the selected messages were used to probe total poly(A) RNA bound to DPT-paper (Fig. 5). Direct visualization by ethidium bromide staining shows a small number of moderately abundant to superabundant mRNAs present during the midmaturation phase of soybean embryogenesis (lane 2). Two of the most prevalent of these have sizes of 2.1 and 2.5 kb, and the smaller message at 2.1 kb is the same size as the 18S rRNA. Lectin cDNA gave intense hybridization to an 1150 nucleotide region (lane 3) which is only weakly visible by ethidium bromide staining. The faintly labeled bands represent hybridization between contaminant sequences in the lectin cDNA population and other abundant messages as those at the 2.1 and 2.5 kb positions. The contaminant sequences are overemphasized in this autoradiogram since the probe was labeled to such a high specific activity and since exposure of the main band is well beyond the linear range of the film. The trypsin inhibitor probe hybridized intensely to a 770-nucleotide region (lane 4). The blotting experiments thus indicated that the immunoadsorbed mRNAs were very highly enriched although not homogeneous. Based on translation and blot hybridization autoradiograms, 70% was a conservative estimate for the enrichment of the immunoselected mRNA preparations.

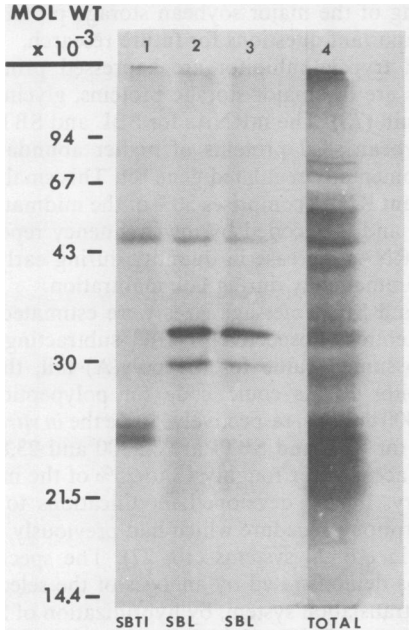

FIG. 3. Analysis of translation products by SDS/polyacrylamide gel electrophoresis and fluorography. From the left, the RNAs translated were (lane 1), 60 ng SBTI mRNA; (lanes 2 and 3), 60 ng SBL mRNA; and (lane 4), 0.5 μg total poly(A) RNA. Mol wt of the marker proteins (phosphorylase *b*, BSA, SBL, SBTI, and lysozyme) are indicated in the margin. The band at approximately 45,000 daltons is a contaminant polypeptide which was present in all assays including globin controls and control assays without exogenous RNA.

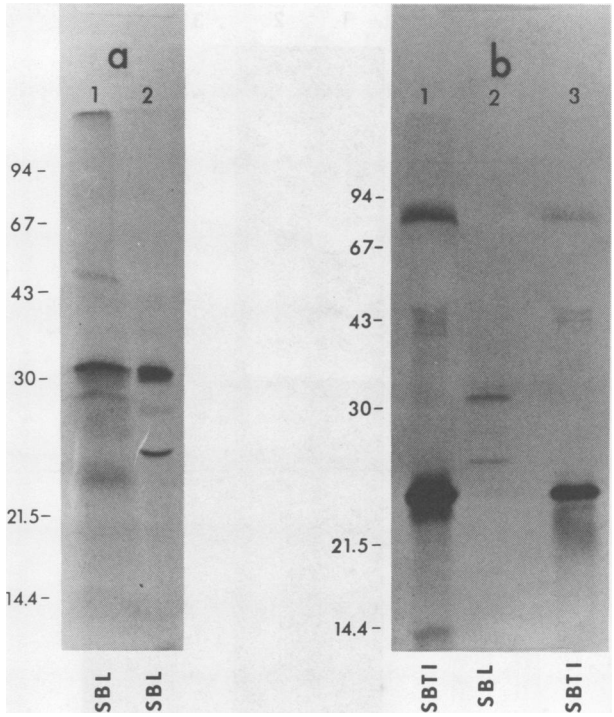

FIG. 4. Indirect immunoprecipitation of translation products. Twenty to fifty μl *in vitro* translation reaction were diluted with 150 μl TNT buffer and approximately 9 μg monospecific SBL or SBTI antibody were added and incubated at 4 C for 3 h. Twenty-five μl of unfractionated goat anti-rabbit serum were added and the incubation was continued for an additional 12 to 16 h. The immunoprecipitates were collected by centrifugation and washed three times with 500 μl TNT. The pellets were dispersed in 10 to 15 μl TNT containing 2 M urea and were dissociated with SDS, mercaptoethanol, and boiling in preparation for loading onto gels. Mol wt $\times 10^3$ for the markers are the same as indicated in Figure 3. (a) Lane 1, labeled products synthesized on by enriched SBL mRNA; lane 2, immunoprecipitation with anti-subunit SBL of translation products synthesized by the enriched SBL mRNA. (b) Lane 1, immunoprecipitation with anti-SBTI of translation products using enriched SBTI message; lane 2, immunoprecipitation with anti-subunit SBL of polypeptides synthesized by enriched SBL mRNA; and lane 3, immunoprecipitation with anti-SBTI of products from total polysomal poly(A) RNA. The polypeptide at 23,800 is intensely labeled in the trypsin inhibitor immunoprecipitates of lanes 1 and 3 of Fig. 4b. Whether the fainter band at 77,000 reflects a real affinity or simply aggregation or nonspecific entrapment in the immunoprecipitates is unknown.

DISCUSSION

The results have demonstrated the usefulness of an immunoadsorption procedure for isolating highly enriched mRNAs for spe-

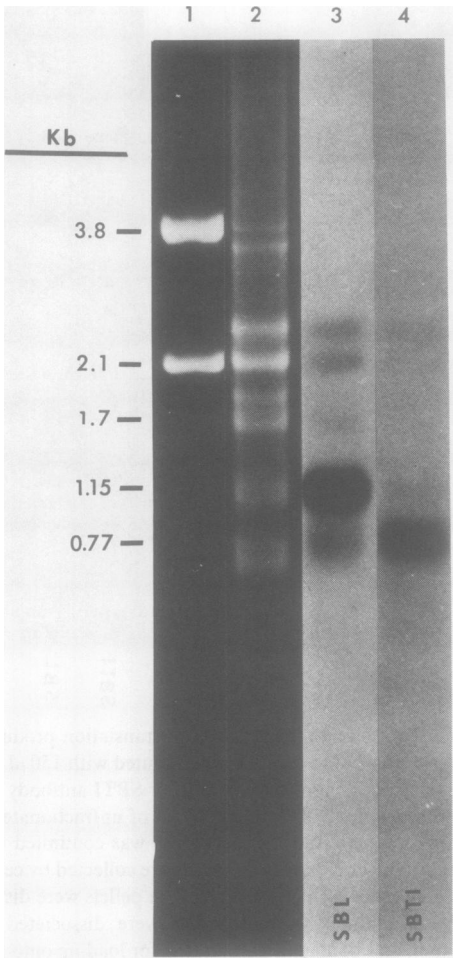

FIG. 5. Estimation of molecular sizes for lectin and trypsin inhibitor mRNAs. Total poly(A) RNA from soybean embryos was electrophoresed on 1% agarose gels in the presence of 5 mM CH_3HgOH and the RNA was transferred to DPT-paper strips. Bands hybridizing with ^{32}P-labeled cDNA prepared using immunoselected SBL or SBTI mRNA were visualized by autoradiography. From left, tracks 1 and 2, direct visualization by ethidium bromide staining of rRNA and total poly(A) mRNA, respectively; track 3, hybridization of [^{32}P]cDNA (1.4×10^6 cpm) prepared from SBL mRNA; and track 4, hybridization of [^{32}P]cDNA (2.1×10^6 cpm) prepared using enriched SBTI mRNA.

cific proteins which constitute only several per cent of the total seed protein. To date, polysome immunoselection procedures have received little use for preparative isolation of plant mRNAs. In this report, goat anti-rabbit antibody coupled to Sepharose has been used in a column procedure as opposed to batch washings of antibody-tagged polysomes adsorbed to an anti-antibody cellulose matrix (10, 27). While the principle is the same, the elution of A_{260} above the EDTA background was easily detected from the GAR-Sepharose columns and the problem of fines generates by alkali-treated cellulose was avoided. Although a large proportion of the anti-antibody is bound to the inside of the Sepharose beads and may be unavailable for interaction with the polysomes, obtaining sufficient anti-antibody for coupling to Sepharose is generally not a limiting step. The limiting steps in selecting specific polysomes by immunoadsorption are obtaining sufficient quantities of the purified protein for the production and immunopurification of monospecific antibodies and isolating polysomes in preparative amounts.

Three to four μg lectin or trypsin inhibitor-specific poly(A) RNA were routinely isolated from 100 g Williams early to mid-maturation seed (Table I). While SBTI-specific polysomes were obtained from midmaturation seed of the variety Sooty, lectin polysomes were not bound during immunoadsorption. This result indicates that Sooty, a variety which does not express detectable lectin protein, also does not express or has substantially reduced levels of functional lectin mRNA. Recently, solution and blot hybridization experiments using a cloned lectin sequence to compare RNAs from Sooty *versus* a control variety have confirmed this interpretation (Goldberg *et al.*, in preparation).

Immunoselected SBL mRNA directed the synthesis of a major polypeptide of 32,300 which was immunoprecipitable with anti-SBL antibody. A minor polypeptide showing some variation in mol wt from 25,500 to 29,000 was also immunoprecipitable. Whether there are two lectin mRNAs in the preparation or whether there is a precursor-product relationship between the two polypeptides is unclear at this time. The major lectin polypeptide synthesized *in vitro* is larger by several thousand than the 28,000 mol wt value expected for nonglycosylated lectin subunits (17, 18). Trypsin inhibitor polypeptide labeled *in vitro* and immunoprecipitable with anti-SBTI antibody was also about 2,000 daltons larger than SBTI marker protein. These data indicate that processing of nascent polypeptides may be involved during *in vivo* synthesis of lectin and trypsin inhibitor. Polypeptide precursors 2,000 to 3,000 larger than the native proteins have been found for zein (15) and for barley α-amylase (20). Many proteins which are transported through or bound to membranes are synthesized with a peptide "signal" sequence of several thousand daltons at their NH_2 termini (29). The subcellular localizations of trypsin inhibitor and lectin in soybean cotyledons are not precisely known. Total polysomes were used for enriching the SBL and SBTI mRNAs by immunoadsorption. To date, there is no information as to whether lectin and/or trypsin inhibitor are preferentially translated by membrane-bound or free polyribosomes. The sites of synthesis, processing events, and final subcellular localizations of lectin and trypsin inhibitor in seed, particularly as compared to the synthesis and processing of the major soybean storage proteins, are interesting and important questions for future research.

Lectin and trypsin inhibitor are expressed primarily in the cotyledons as are the major storage proteins, glycinin (11S) and and conglycinin (7S). The mRNAs for SBL and SBTI along with those for soybean seed proteins of higher abundance reflect a small developmentally regulated gene set. This small number (7–10) of abundant RNAs comprises 50% of the midmaturation stage mRNA mass and is encoded by low frequency repetitive DNAs (9). These mRNAs increase in quantity during early maturation and decline dramatically during late maturation.

The SBL and SBTI message sizes were estimated to be 1,150 and 770 nucleotides, respectively. After subtracting 100 nucleotides as an assumed value for the poly(A) tail, the lectin and trypsin inhibitor RNAs could code for polypeptides of up to 42,300 or 27,000 daltons, respectively. Since the *in vitro* synthesized polypeptides for SBL and SBTI are 32,300 and 23,800, untranslated regions account for roughly 12 to 25% of the message.

In summary, I have developed modifications to a polysome immunoadsorption procedure which had previously been applied only in certain animal systems (10, 27). The specificity of the procedure was demonstrated by analysis of the selected mRNAs in an *in vitro* translation system, by hybridization of SBL or SBTI cDNA probes to mRNA gel blots, and by immunoselection of lectin polysomes from lectin-positive *versus* lectin-negative varieties. The procedure is potentially applicable for enriching other seed protein mRNAs. More exhaustive attempts to demonstrate the exact degree of enrichment of the preparations could be made but such information is not directly relevant to the original objective. That aim was to obtain highly enriched fractions which could be used for initial characterizations and which could be cloned directly or used for screening shotgun clones. Recently,

SBL cDNA has been used to select a pBR322 hybrid plasmid containing the lectin sequence from a cDNA clone bank constructed with total midmaturation mRNAs (Goldberg *et al.*, in preparation). Using the cloned probe, experiments are in progress to determine the particular molecular events which result in the absence of lectin in some soybean varieties.

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