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Isolation of Polyribosomes and Messenger RNA Active in *in Vitro* Synthesis of Soybean Seed Proteins

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ABSTRACT

Polyribosome preparations containing low proportions of monosomes to polyribosomes have been isolated from developing seeds of *Glycine max* L. Merrill using a high pH-high KCl buffer. The polyribosomes were functional in *in vitro* protein synthesis reactions using wheat germ 23,000g supernatant preparations. Results of experiments using aurintricarboxylic acid indicated that most or all of the amino acid incorporation *in vitro* resulted from the completion of nascent polypeptides associated with the isolated polyribosmes. RNA purified from polyribosome preparations by affinity chromatography on oligo(dT)-cellulose was also active *in vitro*, and had different Mg and K requirements for translation than did the polyribosomes. Translation of oligo(dT)-cellulose-purified mRNA was inhibited by the addition of 7-methylguanosine 5'-phosphate, suggesting that soybean mRNAs are "capped" at their 5' ends. Some, but not all, of the products of these reactions were identical in electrophoretic mobility to radioactive polypeptides of storage proteins produced in soybean cotyledons grown in culture.

Little is known about the molecular mechanisms which control the synthesis of storage proteins in developing legume seeds. A large amount of research relating to the characterization of these proteins has been done (see ref. 4 for a review), and Hill and Breidenbach (8) elucidated the temporal sequence for the deposition of soybean (*Glycine max*, L. Merr.) storage proteins. Sun *et al.* (25) studied storage protein synthesis in developing seeds of *Phaseolus vulgaris* L. and reported the *in vitro* synthesis of globulin G1 ("legumin"). We have initiated a similar study of storage protein biosynthesis in developing soybean seeds. Here we report the isolation of polyribosomes from soybean seeds, and the isolation of the mRNAs from polyribosomes, both of which are functional in *in vitro* protein synthesis reactions. This system may be useful in subsequent studies to identify the factors which influence protein levels in legume seeds and the mechanisms which control gene expression in higher plants.

MATERIALS AND METHODS

Extraction of Polyribosomes. Polyribosomes were isolated from developing soybean seeds (cv. Provar), grown in a greenhouse (20), by modifying the methods of Jackson and Larkins (9) and Larkins *et al.* (14). Seeds (<150 mg each, or 150-250 mg each) were frozen with liquid N_2 and used immediately or kept on dry ice until needed. The frozen seeds were powdered in either a cold mortar or a Sorvall Omni-Mixer[1] (Ivan Sorvall, Inc. Norwalk, Conn.), 5 volumes of grinding buffer were added, and the mixture was allowed to thaw and warm to 5 C. (All subsequent procedures were done at this temperature.) The grinding buffer contained 0.2 M Tris-HCl (pH 8) (at room temperature), 0.2 M sucrose, 400 mM KCl, 50 mM $MgCl_2$, and 5 mM dithioerythritol. The mixture was centrifuged at 1,000g for 5 min and the SN[2] was centrifuged at 10,000g for 15 min. The resultant SN was the source of "free" or unbound polyribosomes. The "membrane-bound" polyribosomes were dissociated from the membranes by suspending the pellet of the 10,000g centrifugation in grinding buffer that contained 1% (v/v) Triton X-100. Insoluble material was removed by centrifugation at 10,000g. The supernatants containing the free or membrane-bound polyribosomes were layered on 3 to 6 ml of 1.75 M sucrose in 40 mM Tris-HCl (pH 8), 20 mM KCl, and 1 mM $MgCl_2$, and centrifuged at 36,000 rpm in the Spinco No. 40 rotor for 90 min, or at 27,000 rpm for 150 min in the Spinco No. 30 rotor. After the SN was aspirated, the pellet was rinsed briefly with boiled, deionized H_2O, and then resuspended in a small volume of 40 mM Tris-HCl (pH 8), 20 mM KCl, and 1 mM $MgCl_2$ (suspension buffer). If necessary, the resuspended pellets were clarified by low speed centrifugation. For sucrose gradient analysis 2 to 10 A_{260} units of polyribosomes were applied to 12.5 to 50% linear sucrose gradients prepared in 40 mM Tris-HCl (pH 8), 20 mM KCl, and 10 mM $MgCl_2$ and centrifuged in the Beckman SW 27.1 rotor at 26,000 rpm for 1.75 hr. Gradients were monitored at 254 nm with an ISCO density gradient analyzer (model 640 with UA-5 monitor). When polyribosomes were to be used in *in vitro* protein synthesis reactions they were generally pelleted a second time through 1.75 M sucrose instead of being sedimented in sucrose gradients. These preparations had 240:260:280 nm ratios close to 1.4:2:1.2.

Fractionation of Polyribosome Size Classes. Polyribosomes isolated from developing seeds that weighed 150 to 250 mg each were subjected to sedimentation in sucrose gradients as described above. Fractions containing 1 to 4, 5 to 8, 9 to 12, and >12 ribosomes/messenger RNA were collected, diluted with 1 volume of polyribosome suspension buffer, centrifuged at 26,000 rpm for 3 to 4 hr, and the resultant pellets were suspended in a small volume of buffer. In one experiment the polyribosomes were collected by centrifugation and subjected to a second sucrose gradient centrifugation to verify that the polyribosomes were intact and that the fractions were enriched in the various size classes of polyribosomes.

Partial Purification of Poly(A)-containing RNA. The follow-

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Abbreviations: SN: supernatant; ATA: aurintricarboxylic acid; S-23: 23,000g SN of wheat germ homogenate; PAGE: polyacrylamide gel electrophoresis; m^7G: 7-methylguanosine; $m^7G^5'p$: 7-methylguanosine 5'-phosphate.

ing method for isolating poly(A)-containing RNA was modified from Krystosek *et al.* (13). Polyribosome pellets were suspended in a buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, and 0.5% SDS. After the solution was made 0.5 M with respect to NaCl and heated in a 60 C water bath for 1.5 to 2 min, it was cooled to room temperature and applied immediately to a column (constantly monitored at 260 nm) containing 1 g of oligo(dT)-cellulose (T_2 from Collaborative Research, Inc., Waltham, Mass.) equilibrated in 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.5 M NaCl. After sample application the column was washed with buffer containing 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.5 M NaCl, and 0.5% SDS (SDS was added to the buffer immediately before use). After the A_{260} of the effluent returned to the pre-sample-application level the column was eluted with buffer without NaCl and the 260 nm absorbing material was collected, made 0.2 M with sodium acetate (pH 5.5), and 2 volumes of ethanol were added. The precipitate which formed after standing at least 12 hr at −20 C was collected by centrifugation. The RNA was suspended in glass-distilled H_2O and reprecipitated at least twice with ethanol to remove residual SDS. The final RNA precipitate was dried *in vacuo* and suspended in glass-distilled H_2O and stored frozen. One and one-half to 3% of the A_{260} units of polyribosomes applied to the column eluted in buffer without NaCl and is referred to as poly(A)-containing [poly(A)+] RNA. RNA which did not bind to the column [poly(A)−] was deproteinized by phenol extraction and precipitated with ethanol as described above.

***In Vitro* Protein Synthesis.** Supernatants of the 23,000*g* (S-23) centrifugation of wheat germ extracts were prepared from untoasted wheat germ obtained from Niblack's Inc., Rochester, N.Y., according to the procedures described by Bruening *et al.* (2). Most reactions were done in 50-μl volumes containing 12 mM KCl, 0.4 mM spermidine, 21 mM HEPES (pH 7.5), 0.1 mM $CaCl_2$, 10 μM EDTA, 2 mM dithioerythritol, 80 μM each of GTP and CTP, 1.36 mM ATP, 8.6 mM creatine phosphate, 1 μg creatine phosphokinase (Sigma Chemical Co., St. Louis, Mo.) 40 μM of each of the protein amino acids (except Leu), 4 μM unlabeled Leu, 1 μCi of [^{3}H]Leu (about 40 Ci/mmol, New England Nuclear), and varying amounts of polyribosomes or purified mRNAs. Magnesium acetate and potassium acetate were varied depending upon the RNA added. Reactions were carried out at 23 C for 60 min unless otherwise indicated. Hot trichloroacetic acid-insoluble radioactivity in 10-μl samples was determined using Whatman 3MM filter discs (2).

Extraction of 7S and 11S Proteins from Cotyledons. Proteins were extracted from immature soybean seeds (~200 mg fresh wt/seed) according to the methods of Hill and Breidenbach (7). Aliquots of the 30,000*g* SN were layered on linear 5 to 20% sucrose gradients prepared in the grinding buffer (7) and centrifuged at 26,000 rpm for 40 to 44 hr in the Beckman SW 27.1 rotor. Gradients were monitored at 280 nm with the ISCO monitor and fractions were collected. After protein determinations were made by the Lowry method (18) peak fractions representing the 7S and 11S proteins were frozen.

Tritium-labeled storage proteins were prepared from soybean cotyledons grown in aseptic culture (29) as follows: single cotyledons (20–40 mg each) were incubated in shell vials (1.4 cm diameter) in 0.1 ml of culture medium containing 20 μCi of [^{3}H]Gly (11.4 Ci/mmol, New England Nuclear) for approximately 16 hr. 7S and 11S proteins were isolated as described above.

Analysis of Polypeptides. Two to 15 μg of 7S or 11S proteins, or aliquots of the *in vitro* protein synthesis reactions (5–20 μl) were mixed with 1 to 2 volumes of disruption buffer [0.125 M Tris-phosphoric acid (pH 6.7), 2% (w/v) SDS, 1% (w/v) β-mercaptoethanol, 17% (w/v) sucrose, and 0.05% (w/v) bromophenol blue] and were heated in a boiling water bath for 2 to 3 min. Alternatively, samples were treated with RNase and EDTA, heated in the presence of 6 M urea, 1% SDS, and 1% β-mercaptoethanol, as described by Sun *et al.* (25). Samples were applied to 1.25-mm-thick slab gels of 10 or 12% polyacrylamide separation gels (9 cm long) overlaid with 5% polyacrylamide stacking gels (~1 cm long) using the SDS-containing discontinuous buffer system described by Maizel (21). After electrophoresis at 100 v until the dye reached the end of the gel, the gels were impregnated with PPO and exposed to preflashed Kodak RP Royal X-omat film according to the methods for fluorography published by Bonner and Laskey (1) and Laskey and Mills (16), or stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.). The mol wt of the polypeptides were estimated by their electrophoretic migration relative to those of mol wt markers: Cyt *c* (13,000), tobacco mosaic virus (TMV) coat protein (17,500), chymotrypsinogen (25,000), pepsin (34,500), BSA (67,000), and β-glactosidase (130,000).

RESULTS

Isolation of Soybean Polyribosomes. Experiments were done to establish conditions for isolating polyribosomes containing relatively low percentages of monosomes. In general, pH 9 buffers yielded poor polyribosome patterns, and buffers containing high KCl to $MgCl_2$ ratios (greater than 6:1) were better than lower ratios. Polyribosomes prepared from seeds ×1150 mg each consistently yielded polyribosomes with sedimentation characteristics similar to that shown in Figure 1. Treatment with RNase or EDTA resulted in loss of polyribosomes and produced monosomes and subunits (RNase) and smaller aggregates of ~1 to 3 ribosomes (EDTA).

Optimal Conditions for *in Vitro* Protein Synthesis from Polyribosomes. The conditions most favorable for the *in vitro* translation of free soybean polyribosomes were determined using wheat germ S-23. The optimum concentration of Mg^{2+} for translation on polyribosomes was determined by adding

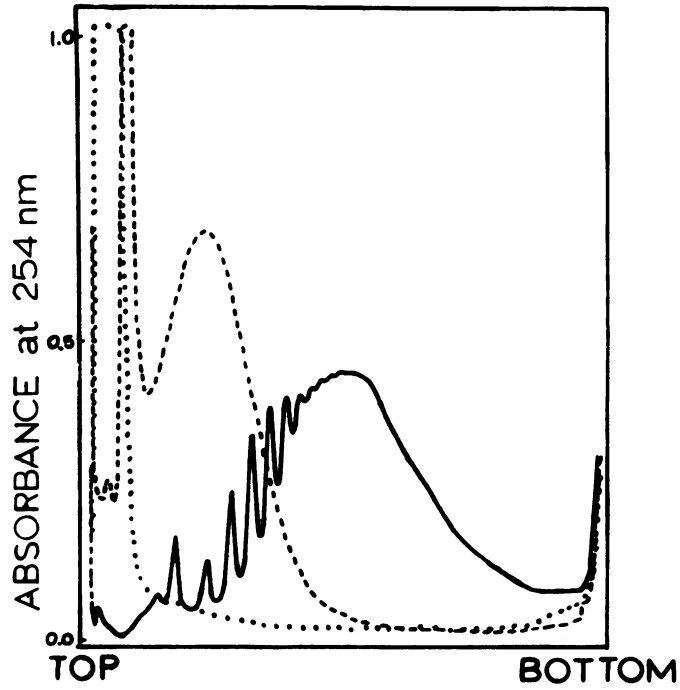

FIG. 1. Sucrose gradient sedimentation of polyribosomes from developing soybean seeds. Approximately 6 A_{260} units of free polyribosomes extracted from soybean seeds (50–150 mg fresh wt/seed) were sedimented on 12.5 to 50% linear sucrose gradients and monitored at 254 nm. Polyribosomes were untreated (——), exposed to 5 μg of pancreatic RNAse at 37 C for 30 min (. . .), or treated with 19 mM EDTA at 0 C for 30 min (- - -).

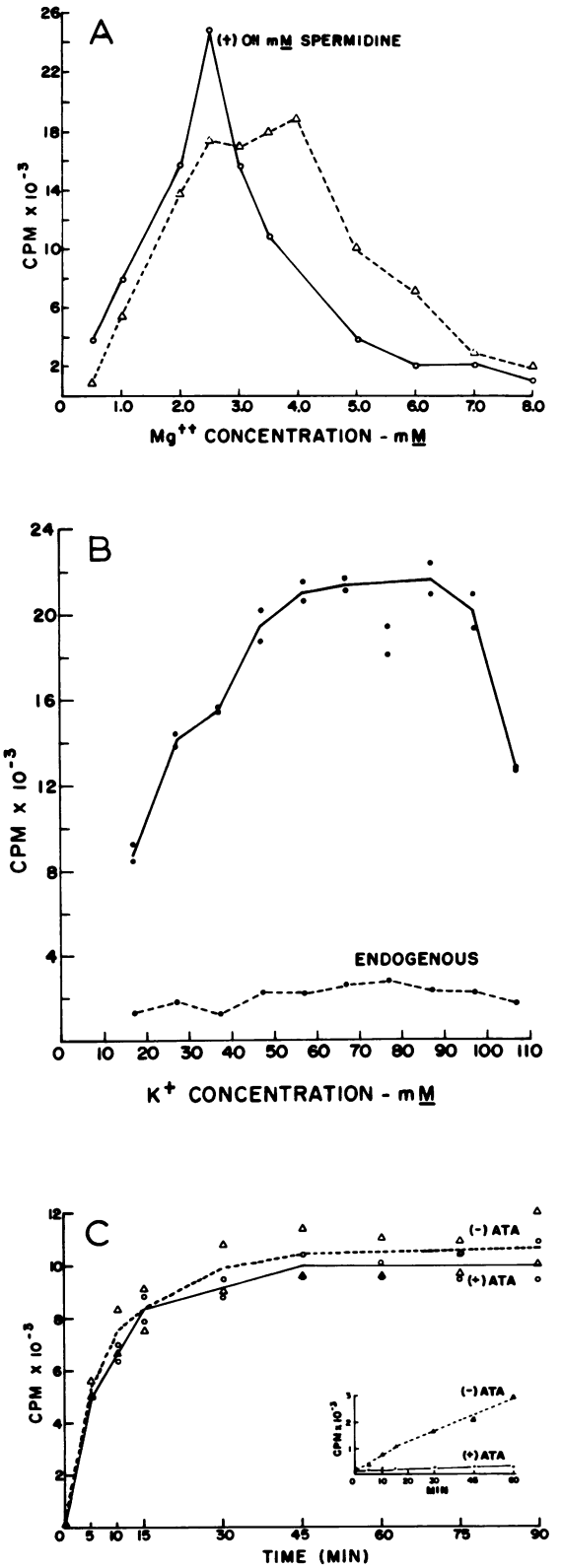

FIG. 2. Effect of Mg^{2+} and K^+ concentrations, and time on *in vitro* protein synthesis from soybean polyribosomes. A: Increasing amounts of magnesium acetate were added to the reaction mixtures containing 0.54 A_{260} units of free polyribosomes in the presence (O——O) or absence (Δ- - -Δ) of 0.4 mM spermidine. B: Increasing amounts of potassium acetate were added to reaction mixtures containing 0.29 A_{260} units of polyribosomes (●——●) or no added polyribosomes (●- - -●). All reaction mixtures contained 2.5 mM Mg^{2+} and 0.4 mM spermidine. C: Radioactivity incorporated by 0.13 A_{260} units of polyribosomes incubated at 30 C for various lengths of time in the presence (O——O) or absence (Δ- - -Δ) of 0.1 mM aurintricarboxylic acid (ATA). Inset: effect of 0.1 mM ATA on radioactivity incorporated in reaction mixtures containing TMV-RNA (0.088 A_{260} units of TMV-RNA was added to a 100-μl reaction mixture). The [^{3}H]Leu incorporated by the wheat germ S-23 reaction mixtures was determined after trichloroacetic acid precipitation. The data in parts A and C have been corrected for radioactivity incorporated in the absence of added RNA (endogenous).

increasing amounts of magnesium acetate in the presence or absence of 0.4 mM spermidine (Fig. 2A). Spermidine lowered the Mg^{2+} optimum (2.5 mM *versus* 4.0 mM without spermidine) and when reaction mixtures contained 2.5 mM Mg^{2+} and 0.4 mM spermidine, the optimum concentration for K^+ was 55 to 85 mM (Fig. 2B). Incorporation was linearly related to the amount of polyribosomes added (data not shown).

To determine whether the incorporation of [^{3}H]Leu was due solely to completion of nascent polypeptides associated with the isolated polyribosomes or to initiation of protein synthesis on soybean mRNA in the wheat S-23 system, a time course of incorporation was done in the presence and absence of 0.1 mM ATA, an inhibitor of initiation events (22). At 23 C [^{3}H]Leu incorporation continued for at least 60 min and ATA had no effect on the reaction (data not shown). At 30 C the reaction was nearly linear for the first 15 min and was complete by 45 min; again ATA had no effect on the reaction (Fig. 2C). ATA was, however, an efficient inhibitor of protein synthesis when TMV-RNA (Fig. 2C, inset) was used as mRNA. The data suggest that only nascent polypeptides were being completed when soybean polyribosomes were translated *in vitro*.

Optimal Conditions for *in Vitro* Protein Synthesis from Purified mRNAs. The polyribosome-associated RNA which bound to oligo(dT)-cellulose [poly(A)+] as well as the RNA which did not bind [poly(A)−] were translated in the S-23 system described above. Using procedures previously outlined Mg^{2+} and K^+ optima were determined in the presence of 0.4 mM spermidine by adding increasing amounts of magnesium acetate or potassium acetate. The data given in Figure 3, A and B show that the optimal concentrations for Mg^{2+} (1.9 mM) and K^+ (105 to 135 mM) for the translation of both poly(A)+ and poly(A)− RNAs are identical. The Mg^{2+} and K^+ and K^+ optima for these RNAs differed from the optima for the translation of soybean polyribosomes (Fig. 2). The products of 12 reactions containing from 55 to 185 mM K^+ were subjected to SDS-PAGE and fluorography; no qualitative differences in the products were observed (data not shown), which is contrary to the translation of rat liver albumin mRNA under varying K^+ concentrations in the wheat germ system (30). Since the Mg^{2+} and K^+ optima for the poly(A)+ and poly(A)− RNAs were similar, and since there were no qualitative differences in the products of the *in vitro* protein synthesis reactions as a result of adding either type of RNA (data not shown), we concluded that the mRNAs of each fraction were probably identical in their coding capacities.

A time course for the translation of poly(A)+ RNA is given in Figure 3C and demonstrates continued mRNA activity through 90 min, in contrast to the translation on soybean polyribosomes (Fig. 2C). The presence of 0.1 mM ATA in the reactions completely inhibited the incorporation of [^{3}H]Leu (data not given), indicating that these reactions required initiation events for the successful incorporation of amino acids.

Comparison of the Polypeptides Synthesized *in Vitro* with Those Found in Soybean Seeds. Polypeptides of 7S and 11S storage proteins isolated from developing seeds were subjected to PAGE in the presence of SDS (Fig. 4). The positions to which several marker proteins migrated are shown. The many lightly stained bands probably reflect the lack of rigorous purification of the 7S and 11S proteins. Thanh and Shibasaki (27, 28) purified the α, α′ and β subunits of soybean 7S

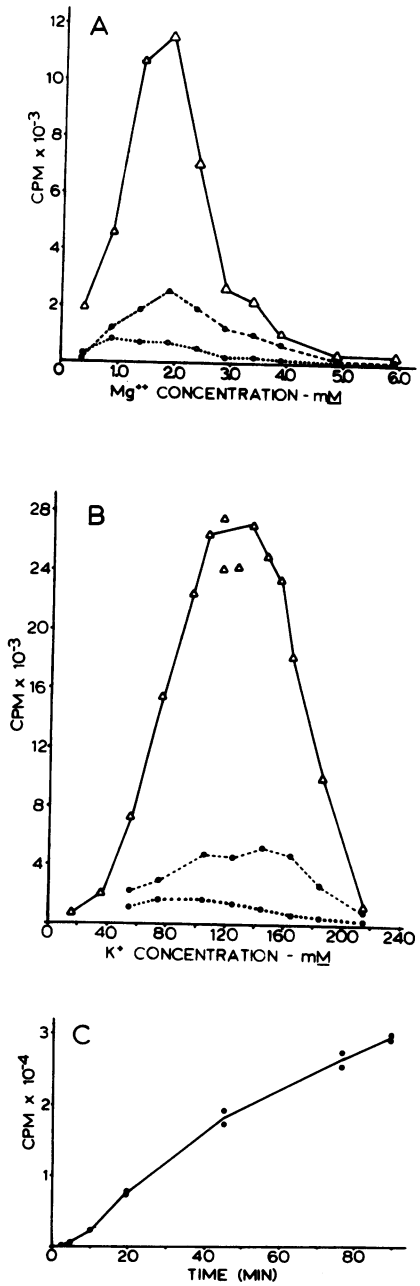

FIG. 3. Effect of Mg^{2+} and K^+ concentrations, and time on *in vitro* protein synthesis directed by RNA extracted from soybean polyribosomes. A: Increasing amounts of magnesium acetate (in the presence of 120 mM potassium acetate) were added to reaction mixtures containing 0.086 A_{260} units of poly(A)− RNA (●---●), 0.0044 A_{260} units poly(A)+ RNA (△——△), or to reaction mixtures without added RNA (○···○). B: Increasing amounts of potassium acetate (in the presence of 1.9 mM magnesium acetate) were added to reaction mixtures containing added RNAs or no added RNA as described in A. C: Radioactivity incorporated by 0.0067 A_{260} units of poly(A) + RNA incubated at 25 C for various lengths of time. The [^{3}H]Leu incorporated in the reaction mixtures was determined after trichloroacetic acid precipitation.

protein (β-conglycinin) to near homogeneity, and they kindly provided us with purified subunits. Direct comparisons of the electrophoretic mobilities of our 7S proteins with the purified α, α′ and β subunits indicated that the major stained bands in our protein preparations co-migrate with the purified subunits (Fig. 4). Comparisons made in 5 or 7.5% polyacrylamide in the presence of 8 M urea and 1% SDS (26) gave similar results. The 11S protein which we isolated was composed of at least four (possibly six) subunits with mol wt of 18,000 to 20,000 and 25,000 to 32,000 (Fig, 4). The approximate mol wt of these stained subunit bands corresponded well with those reported by Draper and Catsimpoolas (5). The [^{3}H]Gly-labeled polypeptides of 7S and 11S proteins isolated from radiolabeled cotyledon cultures had mobilities identical with unlabeled polypeptides from immature seeds (data not shown).

The [^{3}H]Leu-labeled polypeptides synthesized by the *in vitro* translation of polyribosomes and the poly(A)+ RNA from polyribosomes isolated from seeds <150 mg each were compared with the [^{3}H]Gly-labeled polypeptides of isolated 7S and 11S proteins by SDS-PAGE and fluorography. There were at least five polypeptides with similar electrophoretic migrations, including: (*a*) the α′ and α subunits of the 7S protein (Fig. 5, tracks 1, 2, and 3, upper one-fifth); (*b*) one or two bands equivalent to the low mol wt subunits of the 11S protein (designated in Fig. 5 as 11S-1); and (*c*) two or three bands equivalent to the higher mol wt subunits of the 11S protein (designated in Fig. 5 as 11S-2). Free and membrane-bound polyribosomes were equally efficient in translation reactions (*i.e.* radioactivity incorporated per A_{260} of RNA added to the reaction mixture) and the products resulting from the reactions did not differ (data not shown).

Several experiments were done to correlate the synthesis of storage protein polypeptides with various size classes of polyribosomes. Since storage protein synthesis increases (relative to total seed protein synthesis) as the seed matures (ref. 8; our unpublished observations), we isolated polyribosomes from larger immature seeds (150–250 mg fresh wt). As shown in Figure 6 these polyribosomes contained a greater proportion of monosomes than did polyribosomes from seeds less than 150 mg each (Fig. 1). Nevertheless, mRNA with 13 or more polyribosomes were discernible in these patterns. Fractions that contained 1 to 4, 5 to 8, 9 to 12, and >12 ribosomes were taken (see Fig. 6). Polyribosomes collected by centrifugation were added to *in vitro* protein-synthesizing reactions, and the resulting radioactive products were analyzed following SDS-

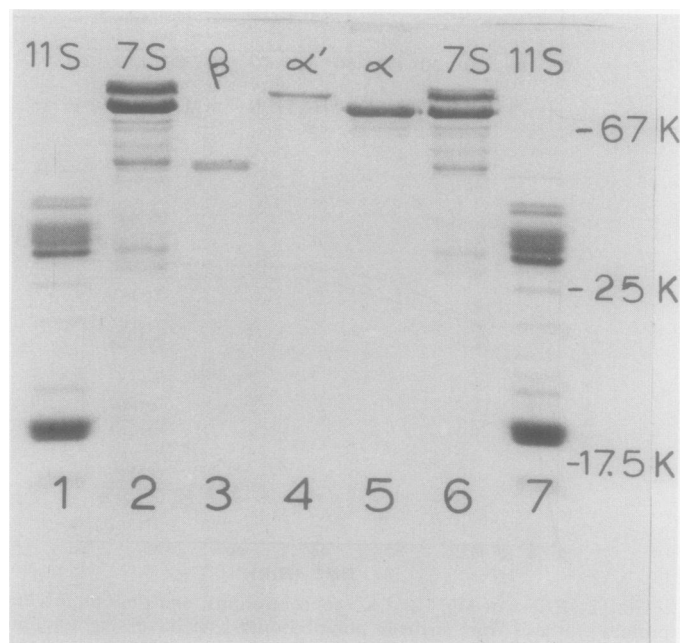

FIG. 4. Polypeptides of soybean storage proteins. Fifteen μg of 7S (tracks 2 and 6) or 11S (tracks 1 and 7) proteins, or 5 μg each of the purified 7S subunits, β (track 3), α′ (track 4), or α (track 5), were treated with SDS and β-mercaptoethanol and electrophoresed on a 5 and 12% polyacrylamide slab gel using the SDS-containing, discontinuous buffer system of Maizel (21). Proteins were stained with Coomassie brilliant blue R-250.

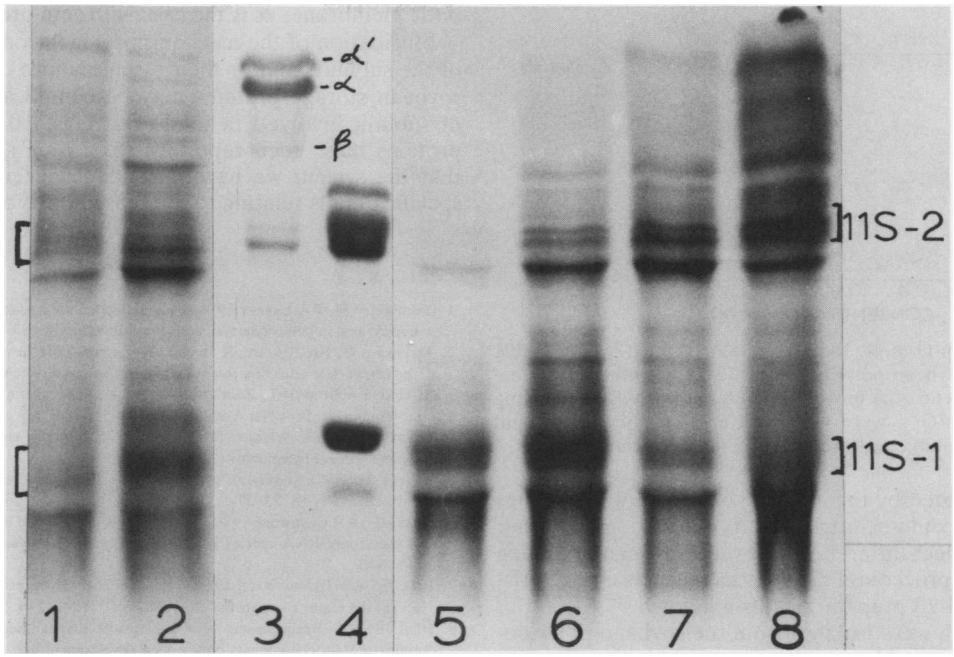

FIG. 5. Comparison of the products of the *in vitro* protein-synthesizing reactions with 7S and 11S proteins. [^{3}H]Leu-labeled polypeptides resulting from the *in vitro* translation of polyribosomes (track 1) or poly(A) + mRNAs (track 2), and [^{3}H]Gly-labeled polypeptides of 7S (track 3) and 11S (track 4) proteins from soybean cotyledons labeled in culture, were electrophoresed as described in Figure 4. Following electrophoresis the gel was prepared for fluorography as described in the text. The x-ray film was exposed for 7 days. Track 5 represents the products from the *in vitro* translation of fractions enriched in polyribosomes containing 1 to 4 ribosomes/mRNA; track 6, 5 to 8 ribosomes/mRNA; track 7, 9 to 12 ribosomes/mRNA; and track 8, >12 ribosomes/mRNA. (See Figure 6 for details of the fractionation).

PAGE and fluorography. The larger polyribosomes gave rise to higher mol wt products (Fig. 5, tracks 5–8). It appears that the lower mol wt subunits (~19,000 mol wt) of the 11S protein are synthesized predominantly on polyribosomes containing 1 to 8 ribosomes. Similarly, the larger 11S protein subunits and the 7S protein subunits are synthesized on larger polyribosomes. In these experiments there was apparent co-migration of six of the *in vitro* synthesized polypeptides with four of the subunits of 11S protein (as described above), and with the α' and α subunits of 7S protein.

The products of reactions to which purified poly(A)+ RNA had been added (Fig. 5, track 2) were compared with those resulting from the translation of polyribosomes (Fig. 5, tracks 1, and 5–8). The similarities in products indicate that the purification of the mRNA did not result in extensive damage to the RNA.

Experiments with m^{7}G and m^{7}G$^{5'}$p. Messenger RNAs from a large number of eukaryotic organisms are known to be "capped" at their 5' ends, *i.e.* they have a terminal m^{7}G$^{5'}$ppp$^{5'}$ N (23): results from several laboratories (17, 24) suggest that the cap may affect the efficiency of translation of mRNA. The *in vitro* translation of capped mRNAs can be inhibited by adding m^{7}G$^{5'}$p to reaction mixtures, but not by adding m^{7}G, and the use of these reagents may suggest whether or not an mRNA or mRNA population is capped (17). We added varying amounts of both chemicals (P-L Biochemicals, Milwaukee, Wis.) to reactions containing poly(A)+ RNA from soybean polyribosomes; m^{7}G$^{5'}$p inhibited the incorporation of [^{3}H]Leu while m^{7}G had no effect (Fig. 7). The results suggest that the soybean mRNAs translated in the wheat germ system had the m^{7}G$^{5'}$p cap.

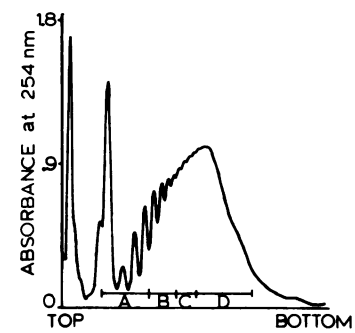

FIG. 6. Fractionation of polyribosomes for *in vitro* translation. Polyribosomes were extracted from seeds 150 to 250 mg each, and subjected to sucrose gradient centrifugation and UV monitoring as described in Figure 1. Fractions A (1–4 ribosomes/mRNA), B (5–8), C (9–12), and D (>12) were collected and polyribosomes were pelleted as described in the text. The products of the *in vitro* translation reactions are shown in Figure 5.

DISCUSSION

Various investigators used the developing seeds of maize (3, 14, 17), *Phaseolus* (25), or *Avena* (19) to study the mechanisms controlling storage protein biosynthesis. Larkins and co-workers (14, 15) and B. Burr and F. A. Burr (personal communication) have partially characterized the mRNA which codes for the synthesis of zein, the major storage protein of maize, and determined several of its physical characteristics, including its sedimentation coefficient, apparent mol wt, and its *in vitro* function. Its purported role in the *opaque-2* mutant of maize has also been investigated (10, 11).

In this report we discuss the isolation from developing soybeans seeds of polyribosomes and poly(A)-containing mRNA functional in *in vitro* protein synthesis. The optimal conditions for *in vitro* translation of the polyribosomes differed from those reported by Sun *et al.* (25) for polyribosomes from cotyledons of another legume, *P. vulgaris*. For example, the Mg^{2+} optimum for soybean polyribosomes was 2.5 mM in the presence of 0.4 mM spermidine, and 4 mM in its absence, but the Mg^{2+} optimum for *P. vulgaris* polyribosomes was 6 mM. The K^+ optima were also different. In our experiments with the wheat germ S-23 translational system very little reinitiation of protein synthesis occurred on soybean mRNA when polyribosomes were used in the reactions, but Sun *et al.* (25) reported that up to 29% of the

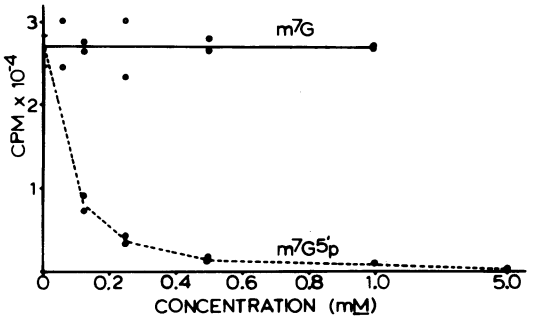

FIG. 7. Effects of $m^7G^{5'}p$ on the translation of poly(A)-containing mRNAs taken from soybean polyribosomes. Protein-synthesizing reaction mixtures with 0.0166 A_{260} units of poly(A) + mRNA and varying amounts of m^7G or $m^7G^{5'}p$ were incubated at 24 C for 75 min and trichloroacetic acid-insoluble radioactivity was then determined.

incorporation stimulated by the addition of bean polyribosomes to wheat germ S-23 could be attributed to reinitiation. Whether these differences reflect differences between soybean and bean polyribosomes or to procedural differences (such as characteristics of the different S-23 preparations) is unknown.

The mRNAs which were isolated from the soybean polyribosomes had very different Mg^{2+} and K^+ optima for their *in vitro* translation than did the polyribosomes, which may help to explain why there was no reinitiation of protein synthesis when polyribosomes were translated *in vitro*. The poly(A)+ mRNA was 100 to 300 times more efficient in *in vitro* protein synthesis reactions (radioactivity incorporated/mg of RNA) than the poly(A)− RNA, which contains most of the ribosomal RNAs. Whether the mRNA activity of the poly(A)-fraction results from failure of the oligo(dT)-cellulose to bind all of the poly(A)+ RNA, because there are poly(A)− mRNAs, or RNAs with very short segments of poly(A) is not known; experiments with mRNAs isolated from pea seedlings (6) and our preliminary findings with soybean RNAs, however, suggest that the latter is the case.

The products of the *in vitro* protein synthesis reactions that were translated from added polyribosomes or from isolated mRNA were nearly identical (Fig. 5, tracks 1 and 2). Coelectrophoresis of *in vitro* translation products with authentic 7S and 11S storage proteins indicated that some peptides of the storage proteins were synthesized *in vitro* (Fig. 5): experiments are underway to verify the identity of the products by peptide fragment analyses. The experiments with fractionated polyribosomes demonstrated that the 11S polypeptides of ~19,000 mol wt (indicated in Fig. 5 as 11S-1) were predominately associated with smaller polyribosomes (1–8 ribosomes/mRNA) whereas the 7S α' and α peptides and the 11S polypeptides of ~29,000 mol wt (indicated in Fig. 5 as 11S-2) were associated predominantly with the larger polyribosomes (8 or more ribosomes/mRNA).

Our results suggest that the mechanism for the deposition of soybean storage protein(s) differs substantially from that of the corn (3, 14) and oat storage proteins (19). For example zein is apparently synthesized on polyribosomes bound to membranes which are closely associated with protein bodies of corn (3, 14). This does not seem to be the case with soybean storage proteins. We could detect no differences in the polypeptides synthesized *in vitro* when either free or membrane-bound polyribosomes were used, but we cannot unequivocally assure that our polyribosome isolation procedure has not caused the removal of ribosomes from membranes, thus making them appear as free polyribosomes. However, photomicrographs of protein bodies in developing soybean seeds (F. Burr, personal communication) do not show ribosomes intimately associated with the protein body membrane, as is the case with zein protein bodies (3).

Elucidation of the mechanisms which control the biosynthesis of the subunits and/or their assembly into complete 7S and 11S soybean storage proteins may be complicated by the numbers of subunit involved [a total of at least 10 subunits of the two proteins have been reported; (5, 12, 27)]. However, we feel that the system we have described will enable us to examine specific details relating to the biosynthesis of these proteins.

LITERATURE CITED

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