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Wheat Storage Proteins[1]

ISOLATION AND CHARACTERIZATION OF THE GLIADIN MESSENGER RNAs

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ABSTRACT

A total RNA extract was prepared from developing wheat seeds using guanidine-HCl to eliminate endogenous RNase activity. The RNA preparation, substantially free of protein, carbohydrate and DNA, was chromatographed on either a poly uridylic acid-agarose or poly guanylic acid-agarose column to yield a gliadin-enriched mRNA fraction. Only slight differences were observed for the products synthesized in a wheat germ cell-free translation system when either poly adenylic acid-enriched or cytosine-rich RNA was used as a template. These results are consistent with the high proline content of the gliadins and indicate that a large proportion of the mRNA activity in these RNA preparations is directed toward gliadin synthesis. After a second affinity chromatography step, the gliadin-enriched mRNA fraction was fractionated by two cycles on sucrose-density gradient centrifugation under denaturing conditions. The RNA sedimented as a broad band with a peak at 14S and a shoulder at the 11S region of the sucrose gradient. RNA from the peak 14S fraction translated predominantly the two major gliadin polypeptides which had molecular weights of 34,000 and 36,000. Analysis of the 14S RNA by methylmercury hydroxide-agarose gel electrophoresis revealed the presence of a predominant RNA species with a molecular size of 415,000 (1,200 nucleotides).

The gliadins, the prolamin fraction of wheat, are the major storage proteins of endosperm tissue. These proteins are typically characterized by their solubility in alcohol-H_2O solutions, high content of glutamine and proline, and low levels of acidic and basic amino acids (reviewed in Ref. 9). The gliadins can be separated in acidic buffers into four electrophoretic classes, α, β, γ, and ω gliadins in the order of decreasing mobility. Despite the similarity of the different gliadins with respect to their amino acid composition and N-terminal sequence of amino acids, these polypeptides are markedly heterogeneous (3, 17). Mol wts of the α, β, and γ gliadins range from 28 to 46 kd, while the ω-types have sizes between 46 and 70 kd (2). More than 30 species can be resolved by successive isoelectric focusing and aluminum lactate electrophoresis (20). The molecular basis for the gliadin diversity is probably the result of gene duplication and divergence (14).

During seed development, 12 to 15 d past anthesis, synthesis of most, if not all, species of the gliadins begins (13). Poly(A)[4]-enriched RNA fractions have been isolated from endosperm tissue via extraction from both free and membrane-bound polysomes (7). Based on the alcohol solubility, immunological relatedness, and size of the *in vitro* synthesized products, membrane associated poly(A)-enriched RNA possesses the coding capacity for gliadin biosynthesis. Here we report on a purification procedure for the isolation of gliadin mRNAs. The procedure utilizes guanidine-HCl as a protein denaturant to prepare highly intact RNA from whole developing seeds, a tissue enriched in RNase activity (7). Subsequent purification steps include affinity chromatography on either poly(U)- or poly(G)-agarose and sucrose density-gradient centrifugation under denaturing conditions. The size and purity of the gliadin mRNAs have been studied by methylmercury hydroxide gel electrophoresis. The results suggest that the major mRNA species, present in 20- to 25-d-old developing wheat seeds, are those which encode the 34 to 36 kd polypeptides.

MATERIALS AND METHODS

Plant Material. Developing wheat seeds (*Triticum aestivum* var. Cheyenne) were harvested 20 or 25 d past anthesis and stored frozen according to Greene (7).

Isolation of Polysomal Poly(A)-Enriched RNA. Total polysomes were isolated from developing wheat seeds according to Larkins *et al.* (11) with slight modifications (16). The polysome pellets were resuspended in 10 mM Hepes-KOH, pH 7.5, 1 mM Na_2EDTA, 100 mM NaCl, 1.0% SDS, and 50 μg/ml of proteinase K. After incubation at room temperature for 15 min, a poly(A)-enriched fraction was obtained by poly(U)-agarose chromatography as described below.

Isolation of Total RNA. All operations were carried out at room temperature unless otherwise specified. A total RNA extract was isolated from 20- or 25-d-old developing wheat seeds using guanidine-HCl according to a modification of the procedures of Chirgwin *et al.* (4) and Cox (6). Twenty-five g of frozen seeds were added to 250 ml of 5 M guanidine-HCl, 10 mM Tris-HCl, pH 8.5, 5 mM EGTA, 0.1% laurylsarcosine, 100 mM 2-mercaptoethanol, and 0.2 ml of antifoam and immediately homogenized in an Omni-Mixer[5] (Sorvall) at top speed for 45 to 60 s. The homogenate was stirred for an additional 10 min and then centrifuged at 25,000*g* (10 min at 0°C). The RNA was then collected from the supernatant fluid by two cycles of ethanol precipitations (4). The RNA pellets were then extracted twice with ethanol to remove excess guanidinium salts and contaminating pigments, and dried under nitrogen. At this stage, the RNA preparation was heavily contaminated with polysaccharides, mainly starch. The bulk of this polysaccharide material was removed by selectively extracting

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[4] Abbreviations: poly(A), polyadenylic acid; poly(U), polyuridylic acid; poly(G), polyguanylic acid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

[5] Reference to a product or company does not imply approval by the United States Department of Agriculture to the exclusion of others which may also be suitable.

the RNA three times with 15 ml of sterile H_2O (4). The pooled supernatant fractions were then clarified of any particulate material by centrifuging at 15,000*g* for 20 min. The RNA was then precipitated by overnight incubation at 4°C in 2.5 M LiCl. The RNA was collected by centrifugation at 20,000*g* (15 min, 0°C), resuspended in sterile H_2O, and reprecipitated in the presence of 150 mM sodium acetate, pH 5.0, and 70% ethanol.

Isolation of mRNA. Before each RNA fractionation by affinity chromatography, the poly(U)- and poly(G)-agarose columns were washed with 30 column volumes of loading buffer (10 mM Tris-HCl, pH 7.4, 0.4% SDS, 200 mM NaCl) and then with 30 column volumes of elution buffer (10 mM Tris-HCl, pH 7.4, 0.2% SDS, and 90% deionized formamide) to remove endogenous oligonucleotides from these affinity resins. The RNA was resuspended in loading buffer without NaCl at 1 to 2 mg/ml, heated to 70°C for 5 min and then rapidly cooled on ice. The solution was then made 200 mM NaCl, diluted 3-fold with loading buffer, and then passed three times through either a poly(U)- or poly(G)-agarose column (100 OD_{260} units/ml of resin). After the column was washed with 10 column volumes of loading buffer, the bound mRNA was recovered with 3 column volumes of elution buffer. RNA was then precipitated by overnight incubation at −20°C in the presence of 150 mM sodium acetate, pH 5.0, and 70% ethanol. The enriched mRNA fraction was then fractionated on the affinity resin a second time to minimize rRNA contamination.

Sucrose Density-Gradient Centrifugation. The enriched mRNA preparation was further purified by dimethylsulfoxide-formamide-sucrose density gradient centrifugation (15). Portions of highly enriched mRNA (about 100 μg) were layered on 5 to 20% (w/v) sucrose density gradients and centrifuged in a SW 65L rotor at 330,000*g* (22 h at 20°C). The gradients were monitored at 260 nm, and 0.125- or 0.18-ml fractions were collected. The RNA was precipitated by overnight incubation at −20°C in 150 mM sodium acetate, pH 5.0, and 70% ethanol.

***In Vitro* Protein Translation Assay.** Protein synthesis components were isolated from commercial wheat germ (General Mills) as described by Marcu and Dudock (12) and stored in liquid N_2. The S30 fraction was treated with micrococcal nuclease before each use (16, 18). The translation reaction mixture contained in a total volume of 50 μl, 10 μl of S-30 fraction 25 mM Hepes-KOH pH 7.6, 2.9 mM magnesium acetate, 110 mM potassium acetate, 4 mM DTT, 0.6 μg deacylated tRNA from wheat germ, 40 μg/ml spermine, 2.5 mM ATP, 0.3 mM GTP, 5 mM P-enolpyruvate, 0.5 mM each of 19 unlabeled amino acids, 20 μg of pyruvate kinase, and 5 μCi [^{3}H]leucine (50 Ci/mmol). The reaction was initiated by the addition of 0.1 to 1.0 μg of RNA and incubated at 28°C for 60 min. The amount of [^{3}H]leucine incorporated into protein was measured by TCA precipitation as described previously (16).

Gel Electrophoresis. Products from the *in vitro* translation assays were routinely analyzed by SDS-PAGE, as described previously (16). After electrophoresis, the gel was immersed in 10% (w/v) TCA for at least 1 h and then prepared for fluorography by using either PPO-dimethyl sulfoxide (3) or En^{3}Hance (New England Nuclear).

The RNA species were analyzed by agarose gel electrophoresis under denaturing conditions (1) using 2-mm-thick slab gels containing 1.5% agarose, and 10 mM methylmercuric hydroxide (Alfa Div., Ventron Corp).

RESULTS

Optimal Conditions for the Wheat Germ Translation Assay. It is well established that there is not always a correlation between optimal conditions for the incorporation of amino acids into acid-precipitable material and optimal synthesis of polypeptides (5). This is particularly true for the wheat germ translation system which is notorious for terminating polypeptides prematurely during protein synthesis. Suboptimal conditions may be responsible for the relatively low amounts of *in vitro* synthesized gliadins observed in an earlier study (7). Conditions for the maximal synthesis of gliadin polypeptides were then sought. For this study, poly(A)-enriched RNA as isolated from a total polysome preparation was used as the mRNA source.

At the optimal Mg^{2+} and K^+ levels (7), the products ranged in size from 13 to 70 kd (Fig. 1b). Most of the polypeptides can be assigned to three size classes of 42 to 46 kd, 28 to 38 kd, and 13 to 20 kd. The majority of the polypeptides of the two higher molecular classes, especially the 34 and 36 kd polypeptides, are gliadins as evidenced by their immunoprecipitation by antibodies raised against authentic A-gliadin (7). Two of the major polypeptide bands with mol wts 34 and 36 kd (as indicated by arrows) are similar in size to the polypeptides of A-gliadin (Fig. 1f). The slight size difference of about 2 kd suggests a precursor-product relationship (7).

The presence of polypeptide bands in the 13 to 20 kd class, especially the major band at about 19 kd, is inconsistent with the polypeptide patterns observed during the *in vivo* accumulation of proteins during seed development (13). During this period of active protein synthesis, the majority of the polypeptides belong to the 30 to 38 kd and 42 to 46 kd classes, in agreement with our *in vitro* translation data. Except for an accumulation of a single band at 12.5 kd during the latter stages of seed development, no apparent changes in polypeptide levels below 30 kd are observed (13).

Several conditions, *e.g.* tRNA limitation and state of mRNA structure, may account for the *in vitro* synthesis of these small polypeptides, and were further investigated. Inasmuch as the gliadins possess a high percentage of proline and glutamine resi-

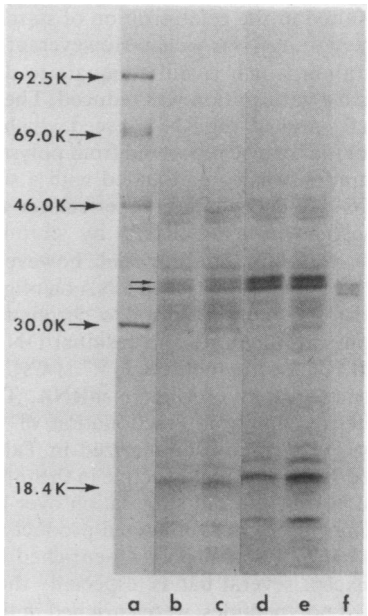

FIG. 1. Products from the *in vitro* translation assay. Poly(A)-enriched RNAs were isolated from polysomes and from a total RNA extract by chromatography on poly(U)-agarose as described in the text. The RNAs were then used in a wheat germ translation assay in the absence (b, d) or presence (c, e) of deacylated tRNA. The synthesized products were resolved by SDS-PAGE and analyzed by fluorography. a, Protein standards, phosphorylase b (92,500), BSA (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lactoglobulin (18,400); b and c, products translated from polysomal poly(A)-enriched RNA; d and e, products translated from poly(A)-enriched RNA as isolated from a total RNA extract; and f, authentic ^{14}C-carboxymethylated A-gliadin. The polypeptides of A-gliadin have mol wts of about 32 and 34 kd under the electrophoretic conditions discussed in the text.

dues (9), the *in vitro* synthesis of the gliadins may be limited by the levels of tRNA for these amino acids. Transfer RNA starvation may induce premature termination of the elongating polypeptide chain during translation and thus cause the production of small polypeptides. To eliminate this possibility, we studied the effect of added deacylated tRNA to our *in vitro* translation assay. Results of this study showed that maximal incorporation of [^{3}H]leucine occurred when 0.6 μg of deacylated tRNA was present in each reaction mixture. At this tRNA level, [^{3}H]-labeled acid precipitable material increased by about 70% (results not shown) and improved the synthetic efficiency of large polypeptide bands (46 kd and larger) without affecting the pattern of smaller ones (Fig. 1c).

Nor was the presence of these small polypeptides the result of an inaccessible RNA template. As shown by Payvar and Schimke (17), the efficiency of conalbumin mRNA translation is poor because of its unique secondary structure. Treatment of the mRNA with methylmercury hydroxide prior to translation, however, elevates the efficiency of conalbumin synthesis to a level consistent with its concentration. Incubation of the wheat mRNA preparation with methylmercury hydroxide prior to translation increased overall protein synthesis by about 20% without affecting the pattern of synthesized polypeptides (results not shown). The results suggest that neither tRNA availability nor mRNA conformation are responsible for the *in vitro* synthesis of small polypeptides.

Extraction of Total RNA from Developing Wheat Seeds. In order to maximize the yield of RNA while minimizing RNA degradation, guanidine-HCl was used as an extractant since it is a potent protein denaturant (6). High concentrations of guanidine-HCl (6 M or higher) during the initial extraction of developing wheat seeds, resulted in the gelatinization of starch granules with subsequent reduction in RNA yields. However, at 5 M guanidine-HCl, a concentration which is still adequate to denature RNase activity (6), starch gelatinization was reduced. The yields of RNA ranged between 0.4 and 1.0 mg RNA/g seed which was more than twice the amount that can be recovered from polysomes. Although the RNA preparation was contaminated with a small amount of carbohydrate, its presence had little effect on the subsequent isolation of poly(A)-enriched RNA by chromatography on poly(U)-agarose. Carbohydrate material, however, did interfere with the isolation of poly(A)-enriched RNA on oligo(dT)-cellulose and, hence, had to be removed prior to chromatography. RNA was purified from carbohydrate and residual DNA by overnight centrifugation of RNA sample through 5.7 M CsCl (4).

Affinity Chromatography of Gliadin mRNA. The recovery of mRNA template activity from fractionation of the total RNA extract on poly(U)-agarose is summarized in Table I. The final poly(A)-enriched RNA was highly active in the *in vitro* translation assays giving an 80- to 140-fold stimulation over background for 1 μg of RNA. The pattern of synthesized products was similiar to that observed when polysomal poly(A)-enriched RNA was used as a template, except several bands, especially the 34 and 36 kd doublet and 46 kd polypeptides, were enriched in the polypeptides synthesized by the total RNA extract. Deacylated tRNA added to the *in vitro* translation assay had little effect on the pattern of polypeptide synthesis (Fig. 1e), although as observed previously it stimulated [^{3}H]leucine incorporation and caused some increase in the synthesis of large polypeptides. These results indicate that the total RNA preparation contains a higher amount of gliadin mRNA than RNA prepared from polysomes.

An alternative method of isolating gliadin enriched-mRNAs was by chromatography on poly(G)-agarose. Because the gliadins have a high mole percent of proline residues, their mRNAs are cytosine rich (the codon for proline is -CCX) and hence able to bind to poly(G) (19). The recovery of mRNA activity from poly(G)-agarose, based on the pmol [^{3}H]leucine incorporated/μg RNA was about the same as that obtained from poly(U)-agarose (Table I). Surprisingly, the pattern of synthesized polypeptides obtained by translation of cytosine-rich RNA was similar to that obtained from poly(A)-enriched RNA (Fig. 2). The only major difference was that the poly(A)-enriched RNA had higher capacity to code for polypeptides with mol wts between 19 and 30 kd than cytosine-rich RNA. The results suggest that some enrichment of gliadin mRNA was obtained from poly(G)-agarose chromatography and that the bulk of mRNAs from developing wheat seeds were rich in cytosine nucleotides.

Table I. *Recovery of Poly(A)-Enriched and Cytosine-Rich RNA from Affinity Chromatography*

A total RNA extract was prepared from developing wheat seeds and chromatographed on either a poly(U)- or poly(G)-agarose column as described in the text. Bound RNA was then collected by ethanol precipitation, and their template activity was measured in a mRNA-dependent wheat germ system.

	RNA	Starting Material	[^{3}H]Leucine Incorporated
	mg	*%*	*pmol/μg RNA*
A. Poly(U) chromatography			
1. Total RNA extract	20	100	
2. First pass poly(A)+ RNA	0.4	2.0	14.4
3. Second pass poly(A)+ RNA	0.17	0.8	25.6
B. Poly(G) chromatography			
1. Total RNA extract	30	100	
2. First pass cytosine-rich RNA	0.63	2.1	14.0
3. Second pass cytosine-rich RNA	0.23	0.8	27.2

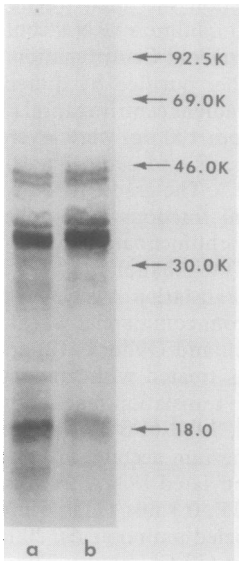

FIG. 2. Products synthesized by either a poly(A)-enriched (a), or cytosine rich RNA (b). The total RNA extract, prepared as described in the text, was chromatographed on either poly(U)- or poly(G)-agarose. Bound RNA was eluted using 90% formamide, precipitated in 70% (v/v) ethanol, and used as a template in the wheat germ translation assay.

Characterization of Gliadin mRNAs. Both poly(A)-enriched and cytosine-rich RNA were further purified by sucrose density-gradient centrifugation under denaturing conditions. Figure 3a depicts the resolution of the different RNA species from cytosine-rich RNA. In addition to the large rRNA peak sedimenting at 25S, a 14S RNA species with a distinct shoulder at 11S predominated. A small peak was also observed at the 9S region of the

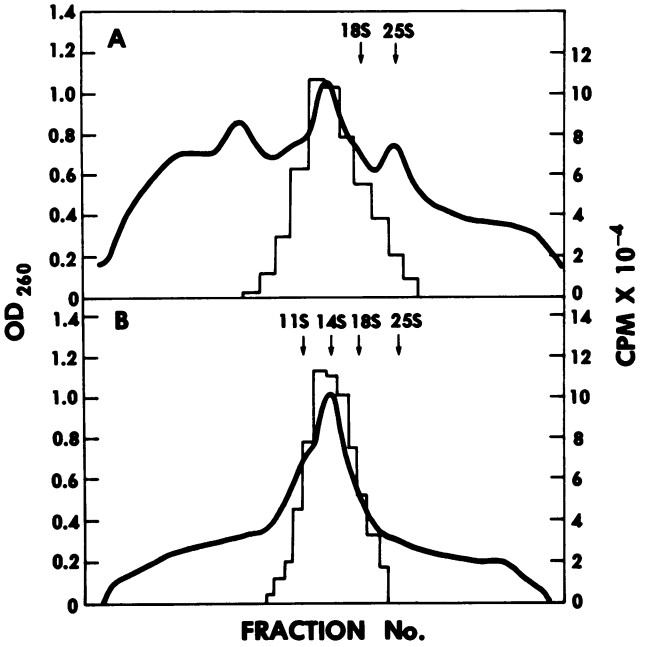

FIG. 3. Profiles of cytosine-rich RNA as resolved by sucrose density-gradient centrifugation under denaturing conditions. Cytosine-rich RNA, as isolated by two cycles of affinity chromatography on poly(G)-agarose, was heat treated and layered on a 5 to 20% (w/v) sucrose density-gradient which was prepared in 95% dimethylsulfoxide, 4% formamide, and 1% buffer containing 1 M Tris-HCl, pH 7.4, 1 M LiCl, and 100 mM EDTA. The sucrose density-gradients were then centrifuged in a SW 65L rotor at 62.5K rpm for 22 h at 20°C. The resolved RNA species were monitored at 260 nm (A) and 0.18-ml fractions were collected. RNA from each fraction was collected by ethanol precipitation and used in the *in vitro* translation assay (bar graph). Peak fractions (between 11S and 18S region) were pooled and recentrifuged on a second sucrose density gradient (B).

gradient but had no template activity in the *in vitro* translation assay. Presumably, chains of 9S poly(G) were released from poly(G)-agarose during the elution of mRNA with 90% formamide. A 9S peak was also observed in gradients containing poly(A)-enriched RNA isolated on poly(U)-agarose but not with oligo(dT)-cellulose. Gradient profiles of poly(A)-enriched RNA were similar to the profile depicted in Fig. 3a.

The RNA from the different fractions of the sucrose density-gradient were then collected by ethanol precipitation and used as a template in the *in vitro* translation assay. Analysis by SDS-PAGE and fluorography indicated that the 14S RNA was rich in the coding capacity for the 28 to 38 kd and 42 to 46 kd polypeptides (results not shown). These presumptive gliadin mRNAs were then purified by a second sucrose density gradient centrifugation (Fig. 3b). A single major peak sedimenting at 14S was observed, although a shoulder at 11S was also present. The band width of the 14S RNA peak was broad, reflecting its heterogeneous mRNA composition (see below). The mRNA template activity, *i.e.* [^{3}H]-leucine incorporation, largely corresponded with the peak of RNA.

Figure 4 shows the pattern of polypeptides synthesized by the different fractions of the second sucrose density-gradient. The heterogeneous character of the 14S RNA was reflected in the wide size range of polypeptides synthesized from 15 to 65 kd. The predominant polypeptides synthesized by the peak 14S RNA fractions, however, were the putative gliadins (28 to 46 kd), of which the 34 and 36 kd polypeptides were the most abundant (Fig. 4). The 11S RNA, which was a distinct shoulder on the 14S peak, preferentially encodes the polypeptides of 20 kd and smaller. The decreased synthesis of these small mol wt polypeptides by

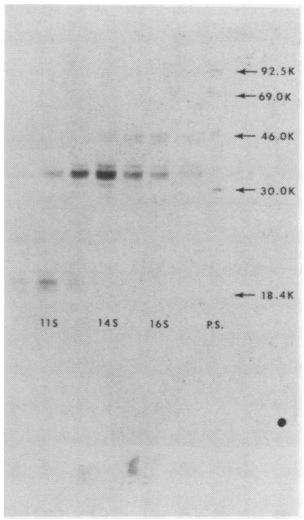

FIG. 4. Products from the *in vitro* translation assay using fractionated RNA from the second sucrose density-gradient. Translation products synthesized by RNA sedimenting between 11S and 18S of the sucrose density-gradient (Fig. 3B) were analyzed by SDS-PAGE and fluorography. The peak fraction of the 14S RNA synthesized mainly the 34 and 36 kd gliadins, although longer exposures to x-ray film revealed the presence of a number of minor polypeptides with mol wts ranging between 20 and 45 kd. P.S., ^{14}C-protein standards.

RNA of increasing sedimentation values suggests that our wheat germ cell-free synthesis assay translated mRNA with high fidelity, *i.e.* without significant premature termination of polypeptide synthesis.

Based on the levels of the synthesized products in the *in vitro* translation assay, the gliadin mRNAs, especially those which encode the 34 and 36 kd polypeptides, should be the major RNA species present in the peak 14S RNA fraction. To determine the levels of the gliadin mRNAs, we analyzed the RNA from the different fractions of the sucrose density-gradient by agarose gel electrophoresis in the presence of 10 mM methylmercury hydroxide (1).

The RNA from the peak 14S RNA fraction was resolved basically into a single major band with several minor smaller mol wt species (Fig. 5d). This major band was also detected in 11S and 16S regions of the sucrose gradient (Fig. 5, c and e). The diffusiveness of the RNA band reflects not only the heterogeneous makeup of RNAs which encodes the 28 to 36 kd gliadins but also the variable lengths of poly(A)-tail (Ref. 8 for discussion). Based on the comparison of the mobilities of RNA standards on methylmercuric hydroxide-agarose gels, this major RNA species is about 1,200 bases in average length (Fig. 6). Five other RNA bands were also observed on this gel. The 11S region of the sucrose gradient contained at least two other RNAs containing 830 and 660 bases (Fig. 5c), whereas two larger bands of 1,500 and 1,430 bases as well as a trace of 18S rRNA were present in the 16S region (Fig. 5e).

DISCUSSION

The extraction of polysomes from plant tissues, a method introduced by Larkins *et al.* (11), has been a useful starting point in the isolation of mRNA. Although the resulting mRNA fractions are quite active in the *in vitro* translation assays, the method is somewhat laborious for large scale operations and does not yield the quantitative recovery of RNA. In addition, the initial collection and storage conditions of the plant tissue are critical factors in the integrity of the polysomes (11).

In this study, we defined the conditions for the extraction of

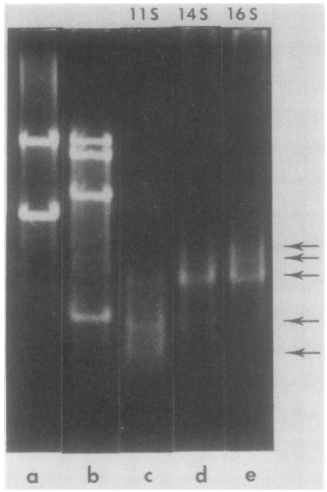

FIG. 5. Resolution of the RNA species by methylmercury hydroxide agarose gel electrophoresis. The RNA from several fractions of the second sucrose density-gradient centrifugation were resolved on 2% agarose gels in the presence of 10 mM methylmercury hydroxide. a, Wheat rRNA prepared by guanidine-HCl extraction (2 μg), 25S, and 18S; b, Bromegrass mosaic virus RNA (1 μg); c, RNA from 11S region of sucrose gradient (1 μg); d, 14S RNA (1 μg); e, 16S RNA (1 μg). The middle arrow indicates the predominant RNA species observed in the peak 14S fraction, while the remaining arrows indicate other RNA species visualized by ethidium bromide staining.

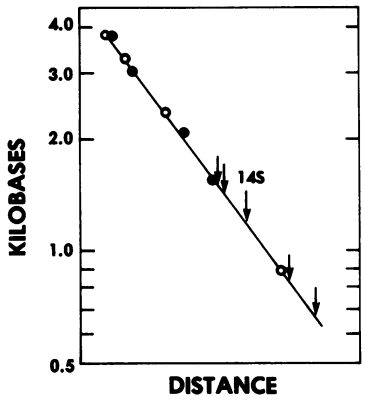

FIG. 6. Molecular size determination of the RNA species as resolved by methylmercury hydroxide agarose-gel electrophoresis. The mobilities of the various RNA species were compared to wheat 25S and 18S rRNAs (●), *Bacillus subtilis* 23S and 16S rRNAs; (○), the four components of Bromegrass mosaic virus RNA. Under our conditions, the mol wts of the Bromegrass mosaic virus RNA components were estimated to be 1.3, 1.1, 0.8, and 0.3 md, assuming an average residue mass of 344. These values were slightly higher than the original estimates of Lane and Kaesberg (10) whose determinations were performed under nondenaturing conditions. Arrows indicate the size of the RNA species observed in Fig. 5. The middle arrow depicts the predominant RNA band (1,200 bases) observed in the peak 14S RNA fraction.

intact RNA from developing wheat seeds using guanidine-HCl. This procedure should be applicable to all plant tissues with only minor variations in the methodology. The total RNA extract, as prepared by guanidine-HCl, shows no evidence of degradation (Fig. 5), although it remains slightly contaminated with polysaccharides. These polysaccharides can be effectively removed by subsequent purification of mRNA by CsCl centrifugation (4) or by cellulose chromatography (15). In spite of the high purity of the final RNA product, the total RNA extract is not active in the *in vitro* translation assay unless further purified by affinity chromatography on either poly(U)- or poly(G)-agarose. Unfractionated guanidine extracted RNA may contain a substance which inhibits protein synthesis. A similar instance of the inhibitory nature of guanidine-HCl-extracted RNA has been reported by others (5).

The major products synthesized by the cell-free translation assay fall into three size classes of 42 to 46 kd, 28 to 38 kd, and 13 to 20 kd. The larger two size classes are consistent with the size of the α, β, and γ gliadins which accumulate during seed development. The synthesis of the 13 to 20 kd polypeptides is puzzling since there is no evidence for the accumulation of these polypeptides during the development of wheat endosperm (13). Their syntheses are not the result of endogenous mRNA activity in the wheat germ extract, since the extract is routinely treated with micrococcal nuclease. Nor is the existence of the 13 to 20 kd polypeptides an artifact of the *in vitro* translation assay arising by premature termination of mRNA translation. Evidence from the sucrose density-gradient centrifugation of mRNA preparations reveals the presence of an 11S RNA that presumably encodes the 13 to 20 kd polypeptides. The *in vitro* synthesis of these small polypeptides may be due to endogenous nicked mRNA, mRNA that encodes a precursor for the 12 kd albumins which increase in levels during the latter stages of seed development (13), or perhaps mRNAs of rapidly turned over polypeptides.

An mRNA preparation enriched for gliadin synthesis can be obtained by chromatography on poly(G)-agarose at about the same efficiency and yield as from poly(U)-agarose (Table I). The gliadin mRNAs are highly enriched for cytosine bases which is consistent with the high proline content of gliadin. Using oligo(dG)-cellulose chromatography, Stuart *et al.* (19) have purified at least two mRNA species that presumably encode for hydroxyproline-rich glycoproteins from carrot roots. In light of the efficient binding of gliadin mRNAs to poly(G)-agarose and their similar template activity as compared to poly(A)-enriched RNA, the results suggest that the final mRNA preparations, isolated from developing wheat, are more than 90% enriched for gliadin synthesis (Table I).

Poly(A)- or cytosine-rich RNA sedimented as a major peak at the 14S region of the sucrose density-gradient, with a definite shoulder at 11S. The 14S RNA was heterogeneous in composition when analyzed by methylmercury agarose gel electrophoresis. The peak fraction of the 14S RNA contains a major RNA band at 1,200 bases and a minor band at 780 bases. The 1,200 base RNA is large enought to contain the structural information for the 34 and 36 kd gliadins, and the level of this band on agarose gels (Fig. 5) correlates well with the level of *in vitro* synthesis for these polypeptides (Fig. 4).

The two major gliadins have an average mol wt of 35 kd. The minimum size required for an mRNA to encode a 35 kd polypeptide would be about 850 nucleotides, assuming an average mol wt of an amino acid to be 117. If our assumptions are valid, the 35 kd gliadin mRNAs contain additional noncoding segments of 350 nucleotides or about 30% of the total base sequence.

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