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Distribution of Cytokinin-active Ribonucleosides in Wheat Germ tRNA Species[1]

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ABSTRACT

The distribution of cytokinin activity in wheat (*Triticum aestivum*) germ tRNA fractionated by BD-cellulose and RPC-5 chromatography has been examined. As in other organisms, the cytokinin moieties in wheat germ tRNA appear to be restricted to tRNA species that would be expected to respond to codons beginning with U. Only a few of the wheat germ tRNA species in this coding group actually contain cytokinin modifications. Cytokinin activity was associated with isoaccepting $tRNA^{Ser}$ species and with a minor $tRNA^{Leu}$ species from wheat germ. All other wheat germ tRNA species corresponding to codons beginning with U were devoid of cytokinin activity in the tobacco callus bioassay.

The cytokinin N^6-(Δ^2-isopentenyl)adenosine (i^6A)[4] and structurally related compounds occur as constituents of tRNA molecules in virtually all organisms examined to date (20). Studies with a number of microbial and animal systems have established that the cytokinin moieties in tRNA are restricted to tRNA species that respond to codons beginning with U, where they occur once per molecule at the position adjacent to the 3'-end of the anticodon (12, 20). Because not all tRNA species within the U-group necessarily contain a cytokinin modification, the distribution of cytokinin moieties with respect to particular tRNA species may vary in different organisms.

The cytokinin modifications of plant tRNA species are of particular interest in view of the hormonal function of cytokinins in plant systems. Cytokinin-active ribonucleosides have been isolated and identified from hydrolysates of a number of plant tRNA preparations (5, 6, 13, 22). In addition to i^6A, plant tRNA preparations typically contain ribosyl-*cis*-zeatin (io^6A) and the 2-methylthio derivatives of these two compounds (ms^2io^6A and ms^2i^6A). Trace amounts of the *trans*-isomers of io^6A and ms^2io^6A have also been reported to be present (3, 21, 22). Information concerning the distribution of cytokinins with respect to particular plant tRNA species is much more limited. Ribosyl-*cis*-zeatin has been identified as a constituent of a minor $tRNA^{Leu}$ species from peas (8), but the distribution of cytokinin moieties in other tRNA species from this source was not examined.

The cytokinin-active ribonucleosides present in wheat germ tRNA were identified by Burrows *et al.* (5) as io^6A, i^6A, ms^2io^6A, and ms^2i^6A. The distribution of cytokinins in wheat germ tRNA species has been investigated in the work reported here. Our results indicate that the cytokinin moieties in wheat germ tRNA occur in only a few tRNA species within the U-group.

MATERIALS AND METHODS

Wheat germ was obtained from Biobin Corporation, Monticello, Ill. ^{14}C-Amino-acids were purchased from New England Nuclear. BD-Cellulose was prepared as described by Gillam *et al.* (10). The RPC-5 column packing material was purchased from Astro Enterprises, Inc., Powell, Tenn.

Isolation of Wheat Germ tRNA. Wheat germ tRNA was prepared essentially as described by Burrows *et al.* (5) except that the phenol treatment following DEAE-cellulose chromatography was omitted. The yield was about 1 mg tRNA/g raw wheat germ.

Chromatographic Fractionation of Wheat Germ tRNA. BD-Cellulose chromatography was based on procedures described by Gillam *et al.* (10). Details of BD-cellulose chromatographic procedures are given in the legends to the figures. In contrast to the results obtained with yeast tRNA (10), the resolution of wheat germ tRNA species on unbuffered BD-cellulose columns was improved by the omission of $MgCl_2$ from the salt gradient.

RPC-5 chromatography was based on procedures described by Pearson *et al.* (18). Transfer RNA samples were usually chromatographed on an RPC-5 column (1.27 × 97 cm, packed at 150 p.s.i.) equilibrated with 10 mM Na-acetate (pH 5.0) containing 10 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 0.45 M NaCl. The tRNA samples were applied in 10 ml of equilibrating solution and eluted with a 1,920-ml linear salt gradient (0.45–0.85 M NaCl) in the same buffer solution. The column was maintained at 37 C and 60 p.s.i. during chromatography. Fractions (12 ml) were collected at a flow rate of about 1 ml/min. Exceptions to these conditions are noted in the legends to the appropriate figures.

Preparation of Aminoacyl-tRNA Synthetases. All steps were performed at 4 C. Wheat germ (50 g) was suspended in 150 ml of 50 mM Tris-HCl (pH 7.5) containing 50 mM KCl and 10 mM $MgCl_2$. The suspension was centrifuged immediately (2,000*g*, 5

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[4] Abbreviations: i^6A: N^6-(Δ^2-isopentenyl)adenosine; io^6A and ribosyl-zeatin: N^6-(4-hydroxy-3-methyl-2-butenyl)adenosine; ms^2i^6A: 2-methylthio-N^6-(Δ^2-isopentenyl)adenosine; ms^2io^6A: ribosyl-2-methylthiozeatin; KE: kinetin equivalents.

min) and the supernatant discarded. The pellet was resuspended in 100 ml of the same buffer and homogenized in a Sorvall Omni-Mixer at a setting of 3 for three 1-min periods separated by 1-min cooling periods. The homogenate was centrifuged at 10,400*g* for 30 min. The supernatant recovered from this step was recentrifuged at 27,000*g* for 1 hr and the pellets discarded. The high speed supernatant was adjusted to pH 7.5 with 2 M NH_4OH, and a sufficient volume of 10% (w/v) streptomycin sulfate was added dropwise with stirring to give a final concentration of 2% in the supernatant solution. The resulting suspension was allowed to stand in the cold for 15 min and then centrifuged at 27,000*g* for 30 min. The supernatant was recovered, avoiding any suspended material at the bottom of the tube, and made to 75% saturation with solid ammonium sulfate. The precipitated protein was recovered in one tube by repeated centrifugation at 27,000*g* for 8 min. The resulting pellet was suspended in 5 ml of 10 mM KH_2PO_4 buffer (pH 7.5) containing 1 mM EDTA and 1 mM β-mercaptoethanol and centrifuged at 27,000*g* for 30 min. The supernatant (7 ml) was desalted on a Sephadex G-25 column (140-ml bed volume) equilibrated with the same buffer. The column eluate was collected in 7-ml fractions, and the five fractions containing the highest concentration of protein were pooled, mixed with an equal volume of glycerol, and stored at −20 C. Under these conditions, the crude synthetase preparation could be stored for several months without significant loss of activity. For use in amino acid acceptor assays, the synthetase preparation was diluted 1:1 with 8 mM DTT immediately before use.

Amino Acid Acceptor Assays. All assays were carried out in a final reaction volume of 0.1 ml containing (except as noted) 50 mM HEPES (pH 8.0), 10 mM $MgCl_2$, 5 mM ATP, 1 mM DTT (derived from the diluted synthetase preparation), and 0.1 μCi of the appropriate ^{14}C-labeled amino acid. The exceptions to these reaction conditions were the leucine acceptor assays, which required 10 mM ATP for optimal charging, and the cysteine acceptor assays, which required 3 mM DTT. Each reaction volume was prepared from 50 μl of an assay mix (containing sufficient buffer, $MgCl_2$, ATP, and ^{14}C-labeled amino acid to give the final concentrations specified above), 25 μl of the appropriate tRNA fraction, and 25 μl of the aminoacyl-tRNA synthetase preparation diluted with DTT as described above. (The cysteine assay mix contained, in addition to the components indicated above, sufficient DTT to adjust the DTT concentration of the final reaction volume to 3 mM. This value includes the DTT contributed by the diluted synthetase preparation.) ATP was neutralized (pH 7.5) before addition to the assay mixes. The reactions were incubated at 30 C for periods varying from 45 to 60 min depending on the assay. At the end of the incubation period, 50-μl aliquots were removed and applied to Whatman 3MM filter paper discs (2.3-cm diameter). (In the case of tryptophan acceptor assays, it was necessary to use Whatman GF/C glass fiber discs due to the high blanks encountered with the filter paper discs.) The discs were immediately put into cold 10% (w/v) trichloroacetic acid (10 ml/disc), and washed as described previously (10). The dry discs were placed in scintillation vials with 5 ml of a toluene-based scintillation fluid (Omnifluor, New England Nuclear) and counted in a Packard model 2405 scintillation counter. Counting efficiencies were determined by applying known amounts of ^{14}C-amino-acids to the filter paper discs.

Determination of Cytokinin Activity. The cytokinin activities of tRNA and nucleoside fractions were determined in the tobacco callus bioassay (15) as described previously (4). The tRNA samples were recovered from the column eluates by addition of 0.1 volume of 0.6 M $MgCl_2$ and 2.5 volumes of cold 95% (v/v) ethanol (19). The precipitated RNA was allowed to stand for at least 24 hr at −20 C prior to recovery by centrifugation at 27,000*g* for 15 min. This procedure gave essentially quantitative recovery of even very dilute RNA samples. All bioassay samples were acid-hydrolyzed in 5 ml 0.1 N HCl (100 C, 45 min), and the neutralized hydrolysates were incorporated into 100 ml of bioassay medium. The sample size used for bioassay varied from 300 to 15 A_{260} units/sample depending on the purity of the tRNA fraction. (Under the bioassay conditions used here, 50 A_{260} units of crude wheat germ tRNA gave better than 0.5 of the maximum bioassay response, and cytokinin activity could be detected with 10 A_{260} units of the crude tRNA preparation.) Cytokinin activities are expressed as kinetin equivalents (μg KE) defined as the μg of kinetin (6-furfurylaminopurine) required to give the same growth response as the test samples under the specified bioassay conditions.

Analysis of the Cytokinin-active Ribonucleosides Present in Wheat Germ tRNA Fractions. Transfer RNA samples were hydrolyzed to nucleosides with snake venom phosphodiesterase and alkaline phosphatase (11). The lyophilized hydrolysates were fractionated by ethyl acetate extraction and Sephadex LH-20 chromatography (1) as described previously (5). Fractions corresponding to the cytokinin-active ribonucleoside constituents of wheat germ tRNA were recovered from the Sephadex LH-20 columns, evaporated to dryness, and tested for cytokinin activity in the tobacco bioassay as described above.

RESULTS

Fractionation of Crude Wheat Germ tRNA. The crude wheat germ tRNA was fractionated by chromatography on BD-cellulose (Fig. 1). The elution profile for cytokinin activity was compared

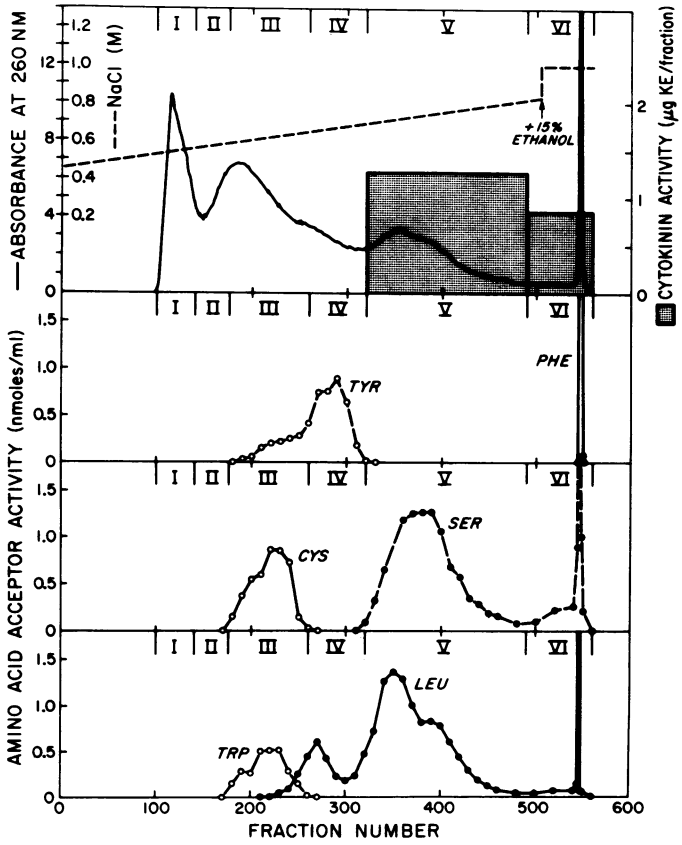

FIG. 1. Chromatography of crude wheat germ tRNA on BD-cellulose. Wheat germ tRNA (36,500 A_{260} units dissolved in 250 ml of 0.45 M NaCl) was applied to an unbuffered BD-cellulose column (3.2 × 103 cm) equilibrated with the same salt solution. The column was eluted with a 12-liter linear salt gradient (0.45 M–0.9 M NaCl). Fractions (20 ml) were collected at a flow rate of about 3 ml/min. The gradient was discontinued at about 0.83 M NaCl and the column purged with 1 M NaCl containing 15% (v/v) ethanol. After determination of amino acid acceptor profiles, the column eluate was pooled into six large fractions (I–VI) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

with the distribution of tRNA species expected to correspond to codons beginning with U (cysteine, leucine, phenylalanine, serine, tryptophan, and tyrosine acceptor activities). As observed with tRNA preparations from other organisms (2, 4, 16, 23), cytokinin-containing tRNA species eluted toward the end of the salt gradient and in the ethanol purge region of the elution profile. The cytokinin activity that eluted with the salt gradient was associated with a region of the elution profile containing major peaks of serine and leucine acceptor activity (pooled fraction V). The material eluted by the ethanol purge (pooled fraction VI) contained all of the phenylalanine acceptor activity as well as serine and leucine acceptor activity. All of the cysteine, tryptophan, and tyrosine acceptor activity as well as a small peak of leucine acceptor activity eluted near the middle of the salt gradient in regions of the elution profile that were devoid of any detectable cytokinin activity.

For comparative purposes, a sample of crude wheat germ tRNA was fractionated by RPC-5 chromatography (Fig. 2). Although this column resolved a number of isoaccepting tRNA species not observed with BD-cellulose chromatography, it did not provide as good an initial separation of the tRNA species corresponding to codons beginning with U as did the BD-cellulose column. Cysteine, phenylalanine, serine, and a portion of the tyrosine acceptor activity together with small peaks of leucine and tryptophan acceptor activity eluted in the cytokinin-active region of the elution profile. A major peak of tryptophan acceptor activity, two peaks of tyrosine acceptor activity, and most of the leucine acceptor activity eluted prior to the cytokinin activity.

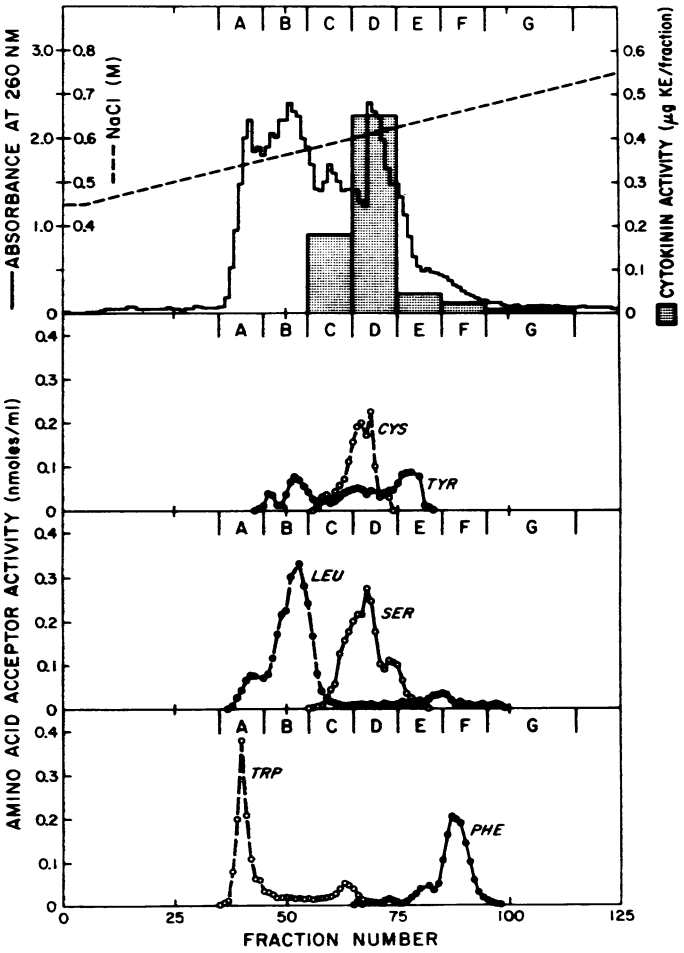

FIG. 2. Fractionation of crude wheat germ tRNA by RPC-5 chromatography. Wheat germ tRNA (1,000 A_{260} units) was fractionated by RPC-5 chromatography as described under "Materials and Methods." After determination of amino acid acceptor profiles, the column eluate was pooled into seven large fractions (A–G) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

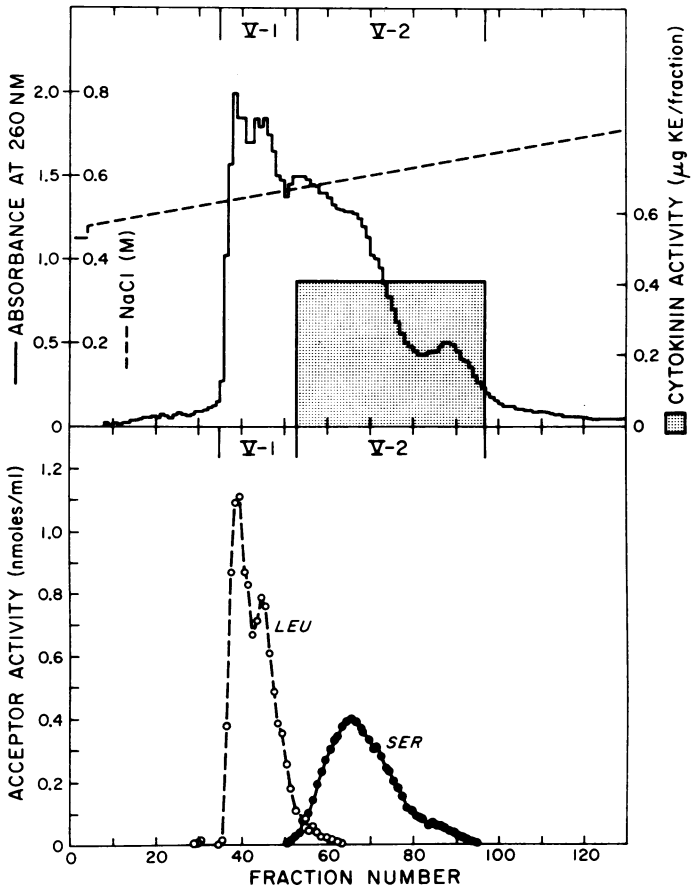

FIG. 3. RPC-5 chromatography of fraction V tRNA. Transfer RNA (1,000 A_{260} units) from pooled fraction V of the BD-cellulose elution profile shown in Figure 1 was chromatographed on an RPC-5 column as described under "Materials and Methods" except that the salt gradient extended from 0.48 M to 0.75 M NaCl. After determining the amino acid acceptor profiles shown, the column eluate was pooled into two large fractions (V-1 and V-2) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

Identification of Cytokinin-containing tRNA Species in BD-Cellulose Fraction V. A sample of the cytokinin-active tRNA preparation recovered from the fraction V of the large scale BD-cellulose fractionation described above was rechromatographed on an RPC-5 column (Fig. 3). The leucine and serine acceptor activities present in the tRNA sample were resolved by the RPC-5 column. Cytokinin activity was associated with only the $tRNA^{Ser}$ containing portion of the elution profile.

The $tRNA^{Ser}$ peaks from two replicate RPC-5 fractionations of fraction V tRNA were combined and rechromatographed on a BD-cellulose column at acid pH (Fig. 4). Under these conditions, the serine acceptor activity was resolved into two peaks, both of which were highly active in the tobacco callus bioassay.

Identification of Cytokinin-containing tRNA Species in BD-Cellulose Fraction VI. A sample of the cytokinin-active tRNA preparation recovered from fraction VI of the large scale BD-cellulose fractionation was also rechromatographed on an RPC-5

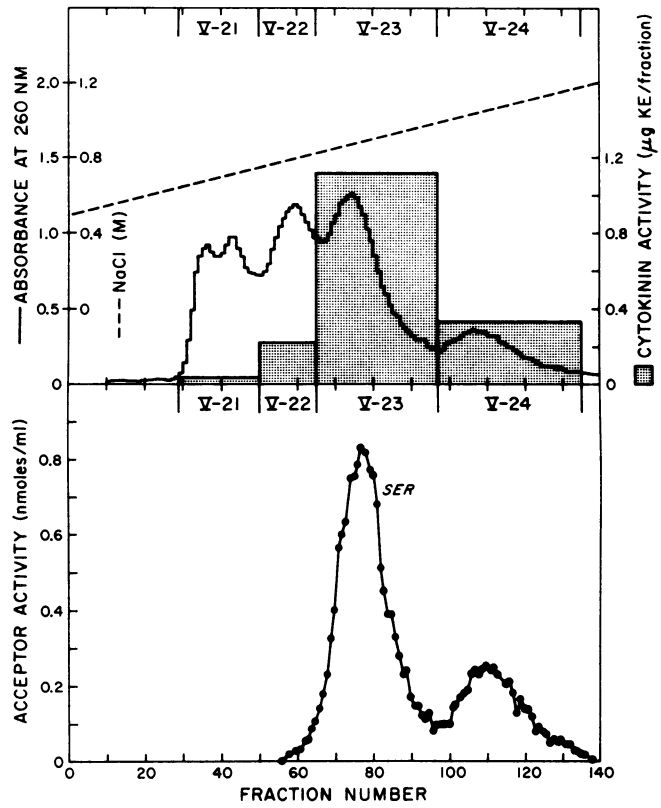

FIG. 4. BD-Cellulose chromatography of fraction V-2 tRNA. Transfer RNA (700 A_{260} units) corresponding to pooled fraction V-2 of the RPC-5 elution profile shown in Figure 3 was applied to a BD-cellulose column (1.5 × 30 cm) equilibrated with 5 mM Na-formate (pH 4.0) containing 0.5 M NaCl. The tRNA sample was applied to the column in 7 ml of the equilibrating buffer. The column was eluted with a 2-liter linear salt gradient (0.5 M–1.5 M NaCl) in the buffer solution described above. Fractions (10 ml) were collected at a flow rate of about 0.7 ml/min. After determining the serine acceptor activity profile, the column eluate was pooled into four large fractions (V-21 to V-24) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

column (Fig. 5). Due to the relatively small amount of material available and the overlapping distribution of phenylalanine, leucine, and serine acceptor activities, the pooled tRNA fractions (VI-1 to VI-4) recovered from the column eluate were further fractionated before testing for cytokinin activity. Rechromatography of Fraction VI-2 on a BD-cellulose column in the presence of dimethylformamide partially resolved two peaks of serine acceptor activity (Fig. 6). Cytokinin activity was associated with both $tRNA^{Ser}$ peaks. The decrease in cytokinin activity in fractions containing the later eluting and larger of the two peaks may be due to the presence of $tRNA^{Ser}$ species that do not contain a cytokinin or contain the less active ms^2io^6A and ms^2i^6A derivatives. Rechromatography of fraction VI-3 on an RPC-5 column at a slightly alkaline pH gave good resolution of the leucine and phenylalanine acceptor activities present in this fraction (Fig. 7). The cytokinin activity chromatographed with the leucine acceptor activity.

Further Purification of Wheat Germ $tRNA^{Cys}$, $tRNA^{Trp}$, and $tRNA^{Tyr}$. Wheat germ $tRNA^{Cys}$ and $tRNA^{Trp}$ were further purified and separated from one another by RPC-5 chromatography of fraction III tRNA from the large scale BD-cellulose fractionation. Wheat germ $tRNA^{Tyr}$ from BD-cellulose fraction IV was purified by the phenoxyacetylation procedure of Gillam *et al.* (9). After removal of the phenoxyacetyltyrosine group, the purified $tRNA^{Tyr}$ was separated into two isoaccepting tRNA species by BD-cellulose chromatography (not shown). Tests of the resulting tRNA preparations in the tobacco callus bioassay confirmed the absence of cytokinin modifications in wheat germ $tRNA^{Cys}$, $tRNA^{Trp}$, and $tRNA^{Tyr}$. Table I summarizes the purity and cytokinin activity data for these tRNA preparations and the partially purified preparations of other wheat germ tRNA species in the U-group.

Analysis of the Cytokinin Composition of Wheat Germ tRNA Fractions. The cytokinin-active tRNA fractions recovered from the large scale BD-cellulose fractionation (Fig. 1) (pooled fractions V and VI) were compared with respect to cytokinin composition. Samples of the tRNA from each fraction were hydrolyzed to nucleosides and the hydrolysates fractionated as described under "Materials and Methods." The distribution of cytokinin activity with respect to individual cytokinin-active ribonucleosides isolated from the hydrolysates by Sephadex LH-20 chromatography is summarized in Table II. All of the cytokinin-active ribonucleosides present in crude wheat germ tRNA were present in each tRNA fraction. In both cases, the major cytokinin-active ribonucleoside present was ribosylzeatin. The minor cytokinin-active ribonucleosides present in wheat germ tRNA (ms^2io^6A, i^6A, and ms^2i^6A) were responsible for a slightly larger fraction of the total cytokinin activity in the tRNA eluted by the ethanol purge (fraction VI) than in the tRNA eluted by the salt gradient (fraction V).

DISCUSSION

The present study has provided the first detailed description of the distribution of cytokinin-active, hypermodified bases in a plant tRNA preparation. The distribution of cytokinin activity in wheat germ tRNA fractions is consistent with the general rule, established in studies of microbial tRNA preparations (2, 4, 17),

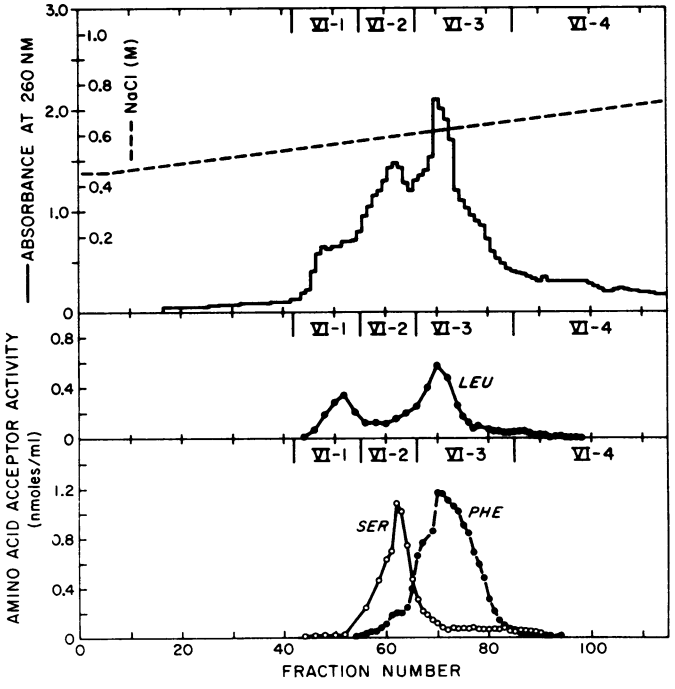

FIG. 5. RPC-5 chromatography of fraction VI tRNA. Transfer RNA (1,000 A_{260} units) from pooled fraction VI of the BD-cellulose elution profile shown in Figure 1 was chromatographed on an RPC-5 column as described under "Materials and Methods." After determining amino acid acceptor activities, the column eluate was pooled into four large fractions (VI-1 to VI-4) as indicated. The RNA was recovered from the pooled fractions as described under "Materials and Methods."

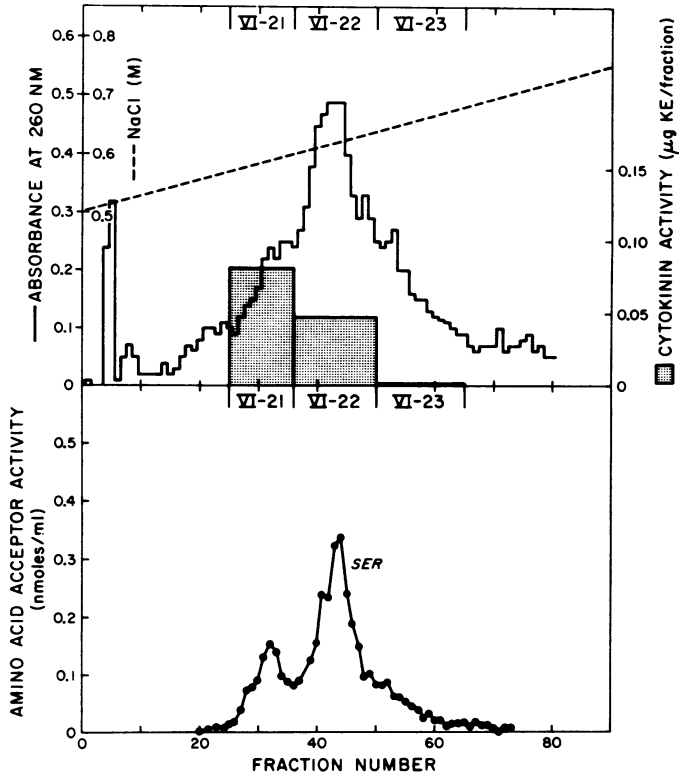

FIG. 6. BD-Cellulose chromatography of fraction VI-2 tRNA. Transfer RNA (120 A_{260} units) from pooled fraction VI-2 of the RPC-5 elution profile shown in Figure 5 was applied to an unbuffered BD-cellulose column (1.5 × 30 cm) equilibrated with 0.5 M NaCl containing 2% (v/v) dimethylformamide. The tRNA sample was applied to the column in 5 ml of the equilibrating solution. The column was eluted with an 1,800-ml linear salt gradient (0.5 M–1.0 M NaCl) containing the same concentration of dimethylformamide. Fractions (10 ml) were collected at a flow rate of about 0.7 ml/min. After determining the serine acceptor activity profile, the column eluate was pooled into three large fractions (VI-21 to VI-23) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

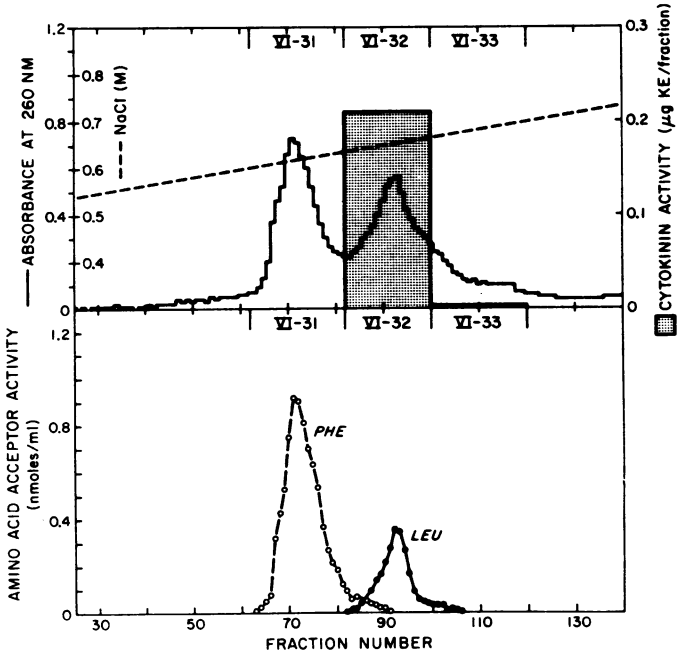

FIG. 7. RPC-5 chromatography of fraction VI-3 tRNA. Transfer RNA (233 A_{260} units) from pooled fraction VI-3 of the RPC-5 elution profile shown in Figure 5 was chromatographed on an RPC-5 column essentially as described under "Materials and Methods" except that the column was equilibrated with 10 mM Tris-HCl (pH 7.5) containing 10 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 0.45 M NaCl. The tRNA was applied to the column in 5 ml of the equilibrating buffer and eluted with a 1,920-ml linear salt gradient (0.5 M–0.75 M NaCl) in the buffer solution described above. After determining the amino acid acceptor activity profiles, the column eluate was pooled into three large fractions (VI-31 to VI-33) as indicated. The RNA was recovered from the pooled fractions and assayed for cytokinin activity as described under "Materials and Methods."

that hypermodified bases of this type occur in only those tRNA species that would be expected to respond to codons beginning with U. In contrast to the results obtained with microbial systems, the cytokinin moieties in wheat germ tRNA are restricted to only a few tRNA species within the U-group.

The cytokinin-containing tRNA species in wheat germ appear to consist of only $tRNA^{Ser}$ species (two major and possibly two minor isoaccepting peaks) and one minor $tRNA^{Leu}$ species. The latter is probably equivalent to $tRNA_6^{Leu}$ from peas, which has been shown to contain ribosylzeatin (8). The identification of these wheat germ tRNA species as cytokinin-containing species is tentative in the sense that complete purification was not achieved. However, the final tRNA preparations tested for cytokinin activity in the tobacco bioassay were free of any significant contamination with other tRNA species in the U-group, and the enrichment in cytokinin activity paralleled the enrichment in amino acid acceptor activity. All other wheat germ tRNA species that would be expected to respond to codons beginning with U ($tRNA^{Cys}$, $tRNA^{Phe}$, $tRNA^{Trp}$, $tRNA^{Tyr}$, and major peaks of $tRNA^{Leu}$) were devoid of cytokinin activity.

Failure to detect cytokinin activity in purified preparations of wheat germ $tRNA^{Phe}$ was expected as this tRNA species is known to contain base Y (rather than a cytokinin-active base) at the position adjacent to the 3'-end of the anticodon (7). The bases located at the corresponding position in wheat germ $tRNA^{Cys}$, $tRNA^{Trp}$, and $tRNA^{Tyr}$ have not been identified. Yeast $tRNA^{Trp}$ contains an unmodified adenosine at this position (14) and $tRNA^{Tyr}$ from rat liver and silk worm has been reported to contain N-[N-(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine (4), which presumably occurs adjacent to the anticodon in the $tRNA^{Tyr}$ molecules. The major wheat germ $tRNA^{Leu}$ species that were inactive in the tobacco bioassay may represent tRNA species that respond to codons beginning with C.

The restriction of cytokinin modifications to a relatively few tRNA species within the U-group may be characteristic of tRNA preparations from higher eukaryotes. The pattern of cytokinin distribution in *Drosophila* tRNA (23) appears to be similar to that of wheat germ, and the scattered information available from mammalian systems is consistent with this interpretation. The distribution of label in tobacco callus tRNA prepared from tissue grown on [^{14}C]mevalonate (16) suggests a somewhat more complex pattern of cytokinin distribution than that observed in wheat germ tRNA, and it is possible that the distribution of cytokinin moieties in tRNA from the dormant wheat embryos may not be identical to that in tRNA from growing plant tissues or in other plant species. A more complex pattern of cytokinin distribution may also occur in plant tissues in which chloroplast tRNA species represent a significant proportion of the total tRNA population. The extent to which the results reported here for wheat germ tRNA may be typical of plant tRNA preparations is not certain, but it appears likely that the distribution of cytokinin moieties in plant tRNA species will be considerably more restricted than in microbial systems.

TABLE I. Cytokinin Activity of Partially Purified Wheat Germ tRNA Species

Wheat germ tRNA fractions corresponding to peaks of isoacceptor activity were compared to unfractionated tRNA with respect to amino acid acceptor activity and cytokinin activity. Cytokinin activity is expressed as Microgram Kinetin Equivalents (μg KE) as defined in Materials and Methods.

		Amino Acid Acceptor Activity		Cytokinin Activity	
tRNA Species	Fraction Tested	Specific Activity (nmoles/A_{260} unit)	Enrichment (Crude tRNA =1)	Specific Activity (μg KE/A_{260} unit)	Enrichment (Crude tRNA =1)
Cys	III-4	0.31	7.5	0.00	0.0
Leu[1]	V	0.37	4.2	0.00	0.0
	VI-32	0.29	3.4	0.05	4.4
Phe	VI-31	1.29	19.2	0.00	0.0
Ser[2]	V-23	0.61	8.7	0.15	10.2
	V-24	0.63	9.4	0.13	8.7
	VI-21	0.48	7.1	0.04	3.2
	VI-22	0.55	8.2	0.01	1.1
Trp	III-1	0.23	5.6	0.00	0.0
Tyr	IV-2	0.82	27.3	0.00	0.0
	IV-3	1.32	44.0	0.00	0.0

[1]The cytokinin-active $tRNA^{Leu}$ fraction VI-32 represented ca. 10% of the total leucine aceptor activity present in crude wheat germ tRNA.

[2]Fractions V-23 and V-24 represented ca. 80% of the total serine acceptor activity present in crude wheat germ tRNA. The remainder of the serine acceptor activity was present in fractions VI-21 and VI-22.

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TABLE II. Analysis of the Cytokinin Composition of Wheat Germ tRNA Fractions

Wheat germ tRNA fractions from the BD-cellulose fractionation shown in Figure 1 were analyzed for cytokinin-active ribonucleosides as described in Materials and Methods. Cytokinin activity is expressed as Microgram Kinetin Equivalents (μg KE) as defined in Materials and Methods.

BD-Cellulose tRNA Fraction[1]	Cytokinin Activity of Ribonucleosides (μg KE per 100 A_{260} units tRNA)			
	io^6A	i^6A	ms^2io^6A	ms^2i^6A
V (Salt Gradient)	3.60	0.14	0.16	0.17
VI (Ethanol Purge)	2.30	0.71	0.14	0.21

[1]Fraction V contained ca. 79% of the total cytokinin activity recovered from the BD-cellulose column. The remainder of the activity was recovered in Fraction VI.

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