Patterns of codon recognition by isoacceptor aminoacyl-tRNAs from wheat germ

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Received 3 August 1978

ABSTRACT

Isoacceptors of Ala-, Arg-, Glu-, Gln-, Ile-, Leu-, Lys-, Ser-, Thr- and Val-tRNAs from wheat germ have been resolved by reverse phase chromatography. Codon recognition properties have been determined on isolated fractions of each of these aa-tRNAs and codon assignments have been made to a number of isoacceptors. Evolutionary changes which have occurred in patterns of codon recognition by isoacceptor aa-tRNAs in wheat germ and other organisms are discussed.

INTRODUCTION

Patterns of codon recognition of isoacceptor aa-tRNAs have been characterized in *E. coli* (1-3), yeast (1,2) and mammals (3,4). They have not been characterized in higher plants. We, therefore, have examined the patterns of codon recognition for ten aa-tRNAs fractionated from wheat germ. Such studies, in conjunction with coding studies from other species (1-4), provide an evolutionary record of the changes which have occurred in the patterns of codon recognition between different organisms.

MATERIALS AND METHODS

[³H]Amino acids were commercial products and their specific activities are listed in Table 1. Wheat germ was obtained from General Mills Co. and was the gift of Dr. Bruce Paterson. 50 g of wheat germ in 250 ml of buffer (10 mM Tris-Cl, pH 7.4, 100 mM KCl, 1 mM MgAc₂, 2 mM CaCl₂ and 6 mM 2-mercaptoethanol) were blended in a Servall Omni-mix, the heavy particulate matter removed by centrifugation and tRNA prepared from this extract with phenol and chloroform (5,6) followed by centrifugation at 150,000 x g for 2.5 hours. Aminoacyl-tRNA synthetases were prepared from an extract (7) containing 60 ml of 10 mM Hepes buffer, pH 7.5, 10 mM MgCl₂ and 20 mM 2-mercaptoethanol by the procedure of Muench and Berg (8) as previously described (4-6). Transfer RNA was aminoacylated with one labelled (0.05 mM

to 0.1 mM final concentration) and 19 unlabelled (0.5 mM) amino acids in 60 mM Tris-Cl, pH 7.4, 10 mM MgCl₂, 6 mM ATP, and enzyme under limiting tRNA conditions. The resulting [³H]aa-tRNAs were resolved on a reverse phase chromatographic column (designated RPC-5) (9), as previously described (4), fractions pooled from developed columns as shown in the figures by hatched areas, precipitated in ethanol and prepared for codon recognition studies (6). The linear gradients employed for separation of [³H]aa-tRNAs on RPC-5 columns were carried out at pH 4.5 from 0.45 to 0.65 M NaCl in a total volume of 160 ml unless otherwise noted in legends of Figures. Ribosomal binding studies were carried out by the procedure of Nirenberg and Leder (10) as given (4,6). A₂₆₀ units of tRNA, cpm of labelled aa-tRNA and Mg⁺⁺ levels used in the ribosomal binding studies are summarized in Table 1.

RESULTS

I. Fractionation and codon responses of aa-tRNAs

Ten aa-tRNAs from wheat germ were fractionated on a RPC-5 column (Figures 1 & 2). Fractions were pooled from the developed columns and designated with a Roman numeral in the order of elution from the columns. Each fraction was assayed for its codon recognition properties and responses to codons are shown in the Figures. Amounts of aa-tRNAs and Mg⁺⁺ levels used in the ribosomal binding studies are shown in Table 1. Codon assignments were made to most isoacceptor aa-tRNAs and are indicated in the text by the underlined codon(s).

Chromatography and codon recognition properties of isolated fractions of Ala-, Arg-, Gln-, Glu-, Ile-, and Leu-tRNAs are shown in Figure 1. The results are as follows:

A. Ala-tRNA:

- 1) The binding of Fraction I to ribosomes is stimulated in the presence of GCU, GCC and GCA.
- 2) Fraction II responds most strongly to GCG, but also responds to the other codons.
- 3) Fraction III responds to GCG.
- 4) The small initial eluting shoulder of Peak I responds to GCU, GCC and GCA (data not shown).

B. Arg-tRNA:

- 1) Fraction I responds strongly to CGU, CGC and CGA.
- 2) Fraction II responds strongly to CGG.
- 3) Fraction III responds most strongly to AGG and less well to

Figure 1. RPC-5 chromatography of six $[^3\text{H}]\text{aa-tRNAs}$ from wheat germ and their codon responses. Fractions were pooled from developed chromatographic columns as shown by the hatched areas and the resulting pooled fractions were prepared for coding studies (6). Coding studies were carried out as given in Materials and Methods and in Table 1. The results of the coding studies are shown in the Figure. Codons are given in the first column to the left. The order in which the cpm are listed by columns corresponds to the binding obtained with each fraction in response to codons in the order of fraction elution (designated by Roman numerals) from RPC-5 columns. The cpm were obtained by subtracting the number of cpm bound in the absence of codon from that bound in the presence of codon. Cpm obtained in the absence of codon are given in parentheses and listed as NONE.

AGA, CGG and CGA. Responses to the latter two codons are probably due to incomplete resolution of adjacent peaks which respond strongly to CGG and CGA, respectively. It is not clear if the response to AGA is due to 2 out of 3 base recognition (11), if this response demonstrates that this isoacceptor also recognizes AGA or if a minor isoacceptor which recognizes

Figure 2. RPC-5 chromatography of four [^3H]aa-tRNAs from wheat germ and their codon responses. Fractionation, preparation of pooled fractions for coding studies and coding studies were carried out as given in the legend to Figure 1. The small, initial eluting peak of Val-tRNA was pooled as shown in the Figure and re-chromatographed on the RPC-5 column in a linear 0.475 M to 0.6 M NaCl gradient as shown in the insert. Coding studies on the initial eluting peak were performed with the re-chromatographed fraction.

AGA co-elutes with the isoacceptor which recognizes AGG. However, since none of the other Arg-tRNA fractions from wheat germ manifest a response to AGA and since other wheat germ aa-tRNAs which show a strong response to codewords with G in the 3' position may also show weaker responses to codewords with A (e.g., see Glu-tRNA below), we tentatively assign AGA to this isoacceptor.

4) The terminal eluting peak responds strongly to CGA.

C. Glu-tRNA:

1) All three eluting peaks recognize GAG and respond slightly to GAA.

2) The middle peak appears to respond more strongly to GAA in proportion to GAG than other peaks and is tentatively assigned to GAA.

D. Gln-tRNA:

1) The initial eluting minor shoulder and the major peak (Fractions I and II) recognize CAG.

TABLE I. COMPONENTS OF THE RIBOSOMAL BINDING ASSAY^a

[³ H]AA-tRNA	Specific Activity of Amino Acid	Fract No.	[Mg++] M	CPM Added	A ₂₆₀ Units Added
Ala	37 Ci/mM	I	0.01	13509	0.012
		II	0.01	16130	0.050
		III	0.01	24524	0.068
Arg	28.75 Ci/mM	I	0.02	2763	0.003
		II	0.01	8168	0.009
		III	0.01	5692	0.006
		IV	0.01	6464	0.017
Glu	45 Ci/mM	I	0.01	4465	0.017
		II	0.01	5224	0.007
Gln	21 Ci/mM	I	0.02	3622	0.012
		II	0.02	6538	0.007
		III	0.02	4105	0.150
Ile	80.5 Ci/mM	I	0.02	6650	0.006
		II	0.02	6485	0.007
		III	0.02	5864	0.012
Leu	110 Ci/mM	I	0.02	8802	0.019
		II	0.02	8988	0.016
		III	0.02	6663	0.060
		IV	0.02	6110	0.100
Lys	63.1 Ci/mM	I	0.01	3607	0.002
		II	0.01	2837	0.027
		III	0.01	3839	0.003
		IV	0.01	2807	0.005
		V	0.01	3611	0.016
Ser	8.9 Ci/mM	I	0.02	5086	0.003
		II	0.02	5045	0.009
		III	0.02	4824	0.003
		IV	0.02	5148	0.138
Ser	8.9 Ci/mM	I	0.02	8101	0.019
		II	0.02	6141	0.065
		III	0.02	5548	0.006
Thr	2 Ci/mM	I	0.02	5828	0.058
		II	0.01	6764	0.082
		III	0.01	4092	0.077
Val	19 Ci/mM	I	0.01	10658	0.022
		II	0.01	10417	0.019
		III	0.01	9300	0.064
		IV	0.01	7743	0.038

^aAssays were carried out by the procedure of Nirenberg and Leder (10) as given previously (4,6). Mg++ levels used in assays and total cpm of [³H]aa-tRNA and total A₂₆₀ units added to assays are shown in the table. Fractionation of [³H]aa-tRNAs on RPC-5 columns, pooled fractions from developed columns corresponding to Fraction numbers shown in the table and the results of the ribosomal binding studies are shown in Figures 1 and 2.

- 2) The minor eluting terminal peak (Fraction III) recognizes CAA.

E. Ile-tRNA:

- 1) The large initial and second eluting peaks (Fractions I and II) recognize AUU, AUC and AUA.

- 2) The third eluting peak (Fraction III) recognizes AUA.

- 3) The peak which elutes in fractions 69 to 80 recognizes AUU, AUC and AUA (data not shown). However, on re-chromatography this peak elutes in an earlier position from the column near Peak 1. This peak may, therefore, migrate on the column as a tRNA dimer (12) which disassociates before the coding assay.

F. Leu-tRNA:

- 1) The initial eluting shoulder (Fraction I) responds to UUG.

- 2) The large eluting peak (Fraction II) responds to CUG.

- 3) Fraction III responds to CUG and slightly to CUA.

- 4) A minor peak (Fraction IV) responds most strongly to UUA.

- 5) The terminal eluting peak bound tightly to ribosomes in the absence of codons and did not manifest a positive response to any of the leucine codons (data not shown).

Fractionation and codon responses of Lys-, Ser-, Thr- and Val-tRNAs are shown in Figure 2. The results are as follows:

A. Lys-tRNA:

- 1) The large initial, and the second minor, eluting peaks (Fractions I and II) recognize AAG.

- 2) Fraction III responds to both AAG and AAA, but more strongly to AAG.

- 3) Fractions IV and V also respond to AAG and AAA, but preferentially recognize AAA.

- 4) About 40% of the response obtained in the presence of AAG as template was observed with UAG as template with peaks I-IV (data not shown).

Responses of different lysine isoacceptors from black-eyed peas (13) and from lupin seeds (14) to AAG and to AAA and AAG, respectively, have been reported.

B. Ser-tRNA:

- 1) Fraction I responds to UCU, UCC, UCA, and UCG. Additional fractionation and coding studies on the initial eluting peak are given below.

- 2) Fraction II responds to all six serine codons.

- 3) Fractions III and IV respond to AGU and AGC.

- 4) No response to UGA (5) was observed with any of the wheat germ Ser-tRNA fractions (data not shown).

C. Thr-tRNA:

- 1) Fraction I responds to ACU, ACC and ACA.
- 2) The most pronounced response of Fraction II is to ACG.
- 3) The most pronounced response of Fraction III is to ACA.
- 4) The peak which elutes just before the column flush in high salt responds to ACU, ACC and ACA (data not shown). However, on re-chromatography this peak elutes in an earlier position from the column. Therefore, the peak which elutes just before the high salt wash may migrate on the column as a tRNA dimer (12).
- 5) The small peak which elutes first from the column recognizes ACU, ACC and ACA (data not shown).

D. Val-tRNA:

- 1) Fraction I, which was re-chromatographed before being examined in the coding assay (see insert of Val-tRNA in Figure 2), responds to GUU, GUC, GUA and GUG.
- 2) The large eluting peak (Fraction II) responds to GUU, GUC, GUA and GUG. This isoacceptor may be assigned all four codons by analogy to a mammalian Val-tRNA (15).
- 3) Fractions III and IV respond most strongly to GUA, but also respond to GUU and GUG. Additional chromatography would be required to determine if the responses to GUU and GUG are due to incomplete resolution of these fractions and the major isoacceptor.

The initial eluting peak of Ser-tRNA was divided into two fractions and each fraction re-chromatographed (see Figure 3) to determine if it consists of an isoacceptor which recognizes UCU, UCC and UCA and a different isoacceptor recognizing UCG (3-5). The earlier eluting portion of this peak (Fraction I of insert A) responds strongly to UCU, UCC and UCA. Fraction II responds to UCU, UCA, and weakly to UCC and UCG. This fraction may be assigned the codon UCC by analogy to a mammalian Ser-tRNA (3-5). The later eluting portion (Fraction III of insert B) responds to UCU, UCC, UCA, and UCG. These data suggest that peak I of Ser-tRNA contains an isoacceptor recognizing UCU, UCC and UCA (3-5). Additional fractionation would be required to determine if an isoacceptor is present in this peak which recognizes only UCG (3-5).

II. Distribution of isoacceptors

The distribution of wheat germ isoacceptors resolved by RPC-5 chromatography is shown in Table 2 along with their codon assignments.

Figure 3. RPC-5 chromatography of $[^3\text{H}]\text{Ser-tRNA}$ from wheat germ. $[^3\text{H}]\text{Ser-tRNA}$ was chromatographed on a RPC-5 column, the initial eluting peak pooled in two fractions as shown in the Figure and each fraction re-chromatographed on a RPC-5 column in a linear 0.525 M to 0.65 M NaCl gradient as shown in inserts A and B. Coding studies were performed on the pooled fractions I through III.

Most of the Ala-, Ile- and Thr-tRNAs recognize U, C and A in the 3' positions of their corresponding codewords, and presumably each of these isoacceptors has inosine in the wobble position of the anticodon (16,17). Approximately one-third of the Ala-tRNA responds to GCG while 16% of Ile-tRNA recognizes AUA. About one-half of the Arg-tRNA recognizes CGU, CGC and CGA. The second most abundant isoacceptor of Arg-tRNA responds to AGG and possibly to AGA. Smaller amounts of Arg-tRNA respond to CGG and to CGA, respectively. Greater than 90% of the Gln-tRNA and 65% of the Lys-tRNA respond exclusively to G in the 3' position of the corresponding codeword. Only about 7% of the Gln-tRNA recognizes CAA, and the remaining Lys-tRNA responds to both AAA and AAG. All of the Glu-tRNA isoacceptors manifest strong responses to GAG, and the most abundant Glu-tRNA isoacceptor may also recognize GAA. The majority of the Leu-tRNA recognizes CUG, a lesser amount recognizes UUG and less than 5% recognizes UUA. More than 60% of the Ser-tRNA elutes as a single peak and responds to UCU, UCC, UCA and UCG. However, at least a portion of this peak contains an isoacceptor which recognizes UCU, UCC and UCA (see Figure 3). Most of the Val-tRNA recognizes GUU, GUC, GUA and GUG.

DISCUSSION

Codon responses of isolated fractions of ten aa-tRNAs from wheat germ which were resolved by RPC-5 chromatography have been examined. Some of the aa-tRNAs contain isoacceptors which recognize adenosine or guanosine in the 3' position of the codon in different ways. Ile-tRNA has isoacceptors which

TABLE 2. DISTRIBUTION OF WHEAT GERM ISOACCEPTORS RESOLVED BY RPC-5 CHROMATOGRAPHY

ISOACCEPTOR AA-tRNA ^a	FRACTIONS OF ELUTION ^b	CODON ASSIGNMENT ^c	DISTRIBUTION (%) ^d
Ala-tRNA	22-33	GCU,GCC,GCA	6.5
	34-42	GCU,GCC,GCA	53.2
	43-47	?	11.5
	48-59	GCG	28.8
Arg-tRNA	24-39	CGU,CGC,CGA	49.8
	40-45	CGG	10.2
	46-60	AGG [AGA]	33.7
	61-72	CGA	6.3
Glu-tRNA	20-31	GAG	7.6
	32-47	GAG [GAA]	86.1
	55-64	GAG	6.3
Gln-tRNA	14-18	CAG	4.7
	19-32	CAG	88.6
	40-49	CAA	6.7
Ile-tRNA	11-32	AUU,AUC,AUA	48.9
	33-42	AUU,AUC,AUA	20.6
	43-55	AUA	16.6
	69-80	AUU,AUC,AUA*	9.1
	81-91	?	2.3
Leu-tRNA	22-28	UUG	15.2
	29-42	CUG	71.2
	43-51	UUA	4.6
	52-65	?	9.0
Lys-tRNA	11-30	AAG	61.0
	31-41	AAG	4.6
	42-57	AAA,AAG	18.6
	58-70	AAA,AAG	5.2
	89-93	AAA,AAG	10.6
Ser-tRNA	18-40	UCU,UCC,UCA,UCG	62.6
	41-48	?	5.3
	49-63	AGU,AGC	25.3
	90-94	AGU,AGC	6.8
Thr-tRNA	29-37	ACU,ACC,ACA	2.4
	38-46	ACU,ACC,ACA	33.1
	47-50	ACG	21.5
	51-62	ACA (ACU,ACC,ACA)	31.9
	70-78	ACU,ACC,ACA*	6.1
	79-81	?*	4.9
Val-tRNA	43-50	GUU,GUC,GUA,GUG	7.7
	51-62	GUU,GUC,GUA,GUG	77.9
	63-75	GUA**	9.6
	76-90	GUA**	4.8

^aFractionation of Ala-, Arg-, Glu-, Gln-, Ile- and Leu-tRNAs are shown in Figure 1 and Lys-, Ser-, Thr- and Val-tRNAs in Figure 2.

^bFractions in which isoacceptors eluted from the RPC-5 column (see Figures 1 and 2).

^cCodon assignments are given in text and are taken from the data given in Figures 1 and 2. A question mark indicates that codon assignments were not made. Brackets around codons indicate tentative assignments. Parenthesis around codons indicate responses to a second isoacceptor which elutes with the major isoacceptor.

^dDistribution of isoacceptor aa-tRNAs were determined from the elution profiles shown in Figures 1 and 2. Distribution of the valine isoacceptors were determined before re-chromatography of the initial eluting peak (see Figure 2).

*These fractions may migrate on the column as tRNA dimers (see text).

**These fractions also respond to other valine codons (see text).

recognize AUU, AUC, and AUA and an isoacceptor which recognizes AUA. Arg-, Thr- and Val-tRNAs also have isoacceptors responding to adenosine in the 3' position of the corresponding codons in different ways. Lys-tRNA has isoacceptors which recognize only AAG and isoacceptors which recognize AAA and AAG.

The studies on fractionation and codon responses of wheat germ aa-tRNAs provide a further example that evolutionary changes have occurred in patterns of codon recognition of isoacceptor aa-tRNAs (see 1-4 for other examples). Patterns of codon recognition of the major isoleucine and valine isoacceptors in wheat germ are similar to those in mammals (3,4,15), but differ from those in *E. coli* (1-3,18-20). The major isoleucine isoacceptor in *E. coli* recognizes AUU and AUC (1-3) and the valine isoacceptors respond to GUU and GUC and to GUU, GUA and GUG, respectively (18-20). A valine isoacceptor in yeast has the same codon recognition properties as an isoacceptor in *E. coli* (20,21), while other yeast valine isoacceptors respond only to GUU, GUC and GUA in the ribosomal binding assay (19-21). Patterns of codon recognition of glutamic acid isoacceptors in wheat germ are different from those in yeast (22), Hymenoptera (23) and mammals (4). Patterns of codon recognition of alanine and serine isoacceptors in wheat germ are different from those in *E. coli* (1-3,18,24).

Evolutionary changes have also occurred in relative abundances of specific isoacceptors (see also 1-4). For example, the alanine isoacceptor which recognizes GUG is a major isoacceptor in wheat germ and Hymenoptera (23) but a minor isoacceptor in mammals (4). The arginine isoacceptor which recognizes AGG is a major isoacceptor in wheat germ and mammals (3,4,6), but a minor isoacceptor in *E. coli* (2,3,25). The Gln-tRNA which recognizes CAA is a minor isoacceptor in wheat germ but comprises more than 50% of the Gln-tRNA in Hymenoptera (23).

It is also of interest to note that the frequency of usage of certain codons is different in the mRNAs which have been sequenced from procaryotic and eucaryotic sources (26-32). Such studies demonstrate that the frequency of usage of codons has also undergone evolutionary changes.

Evolutionary changes have occurred, therefore, in patterns of codon recognition, in relative abundances of specific isoacceptors and in frequency of usage of specific codons in mRNA. The means by which these changes have occurred in evolution are not clearly understood. However, an appropriate amount of aa-tRNA (anticodons) must always be maintained in a tRNA population for efficient translation of the corresponding codewords. Therefore, the

initial evolutionary events may involve an expansion in the tRNA (anticodon) population, either in relative abundance of an isoacceptor or in generation of an isoacceptor with a different pattern of codon recognition. Subsequent changes in codon frequency followed by loss or reduction in expression of the more primitive pattern of codon recognition or in isoacceptor distribution may then occur. Heterologous aminoacylations of tRNA from a variety of organisms are presently being carried out in this laboratory to provide a better understanding of evolutionary changes which have occurred in tRNA: synthetase interactions. The results of these studies, along with a more detailed examination of the evolutionary changes which have occurred in patterns of codon recognition by isoacceptor aa-tRNAs from a number of organisms, including Hymenoptera (23), will be presented elsewhere.

ACKNOWLEDGEMENTS

The authors express their appreciation to Dr. M. W. Nirenberg for the codons, to Ellen Hoffman and Victor Ferrans for their technical assistance and to Ms. Wanda Batts and Ms. Carol Dowling for typing the manuscript.

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