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PATTERNS OF CODON RECOGNITION BY ISOACCEPTOR AMINO ACYL-tRNAs FROM HYMENOPTERA

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SUMMARY: Isoacceptors of Ala-, Arg-, Glu-, Gln-, Gly-, Ile-, Lys-, Ser-, Thr-, and Val-tRNAs from Hymenoptera were resolved by reverse phase chromatography, isolated, and examined for codon recognition properties. Codon assignments have been made to most isoacceptors. Evolutionary changes which have occurred in patterns of codon recognition and in the relative abundances of isoacceptor aa-tRNAs in Hymenoptera and other organisms are discussed.

INTRODUCTION: Amino acyl-tRNAs have been fractionated and codon recognition properties of the resulting isoacceptors characterized from the tRNA populations of *E. coli* (1-3), yeast (1,2), mammals (3,4) and wheat germ (5). These studies have shown that isoacceptor aa-tRNAs from different organisms may recognize different codons within a synonymous codon set. Furthermore, such studies have shown that distributions of isoacceptors within an amino acid family may vary in different organisms (1-5). Thus, evolutionary changes have occurred in patterns of codon recognition as well as in the relative abundances of isoacceptor aa-tRNAs which recognize specific codons.

An analysis of the patterns of codon recognition by isoacceptor aa-tRNAs from other organisms may provide further insight into how these changes may have occurred in evolution. Therefore, an examination of the patterns of codon recognition of eleven aa-tRNAs fractionated from a lower animal, Hymenoptera, was undertaken. The results of this study are described in the present report.

MATERIALS AND METHODS: [^{3}H]Amino acids were commercial products with the following specific activities: Alanine, 36 Ci/mM; arginine, 28.7 Ci/mM; glutamic acid, 15 Ci/mM; isoleucine, 99.2 Ci/mM; leucine, 118.4 Ci/mM; lysine, 72.13 Ci/mM serine, 19 Ci/mM; threonine, 2 Ci/mM; and valine, 19 Ci/mM. A shipment of six pounds of Hymenoptera (*Apis mellifera mellifera* Starline Hybrid) without queens

ABBREVIATIONS: GUA, UCG, etc., Guanylyl-3',5'-uridylyl-3',5'-adenosine diphosphate and uridylyl-3',5'-cytidylyl-3',5'-guanosine diphosphate, etc.

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was obtained from J. L. O'Farrell, La Belle, Fla. On arrival the deceased Hymenoptera were discarded and the remaining organisms stored at -80°C until ready for use. Transfer RNA was isolated from Hymenoptera (4-7) and aa-tRNA synthetases prepared from Hymenoptera and from rabbit reticulocytes by the procedure of Muench and Berg (8) as described (4-7). Transfer RNA was aminoacylated with [^{3}H]amino acid under limiting tRNA conditions (4-7). [^{3}H]Arg-, Ile-, Leu-, Lys-, Ser-, Thr- and Val-tRNAs were prepared in the presence of Hymenoptera synthetases and [^{3}H]Ala-, Glu-, Gln-, and Gly-tRNAs in the presence of reticulocyte synthetases. Attempts to obtain preparations from Hymenoptera which were active for all synthetases by the Muench and Berg procedure (8) using either 0.02 M 2-mercaptoethanol or 0.001 M dithiothreitol or by alternative procedures (6,9) were unsuccessful. [^{3}H]AA-tRNAs were resolved on a reverse phase column [designated RPC-5; (10)], as described (4), fractions pooled from developed columns as shown in the figures by hatched areas, and prepared for codon recognition studies (7). Ribosome binding studies were carried out by the procedure of Nirenberg and Leder (11) as given (4,7). 0.01 M Mg++ was used in ribosome binding studies with [^{3}H]-Ala-, Arg-, Glu-, Gly-, Lys-, Thr- and Val-tRNAs and 0.02M with [^{3}H]Gln-, Ile-, Leu- and Ser-tRNAs. 2.0 A_{260} units of E. coli ribosomes were used in binding assays with each fraction of aa-tRNA with the exception of Ile-tRNA Fraction III and IV which used 1.2 A_{260} units and Lys-tRNA Fractions I-VI which used 0.4 A_{260} units.

RESULTS: I. Fractionation and codon responses of aa-tRNAs: Eleven aa-tRNAs from Hymenoptera were fractionated on a RPC-5 column and coding assays were performed with resolved isoacceptors as shown in Figure 1. Codon assignments were made to most isoacceptor aa-tRNAs and are indicated in the text by underlined codon(s). The results are as follows:

A. Ala-tRNA: The peak which elutes first (Fraction I) from the column binds to ribosomes most strongly in the presence of GCG. Fractions II and III bind slightly in presence of all four codons[1]. The binding of fraction IV is stimulated significantly in the presence of GCU, GCC and GCA and Fraction V in the presence of GCU, GCC, GCA and GCG.

B. Arg-tRNA: The minor, initial eluting peak (Fraction I) responds to CGU, CGC, and CGA and the second peak (Fraction II) to CGU, CGC, CGA and slightly to CGG. Fraction III responds strongly to AGG and Fraction IV to AGA.

[1]Compared to the binding of Fractions I, IV and V, Fractions II and III bind less well to ribosomes in the presence of any of the four codons in proportion to the amount of Ala-tRNA added to assays. Since the binding of Fractions II and III is low and since [^{3}H]Ala-tRNA was prepared in the presence of mammalian synthetases, these two fractions may be the result of misrecognition of Hymenoptera tRNA by mammalian synthetases. Attempts to prepare active Ala-tRNA synthetase have thus far been unsuccessful (see Materials and Methods).

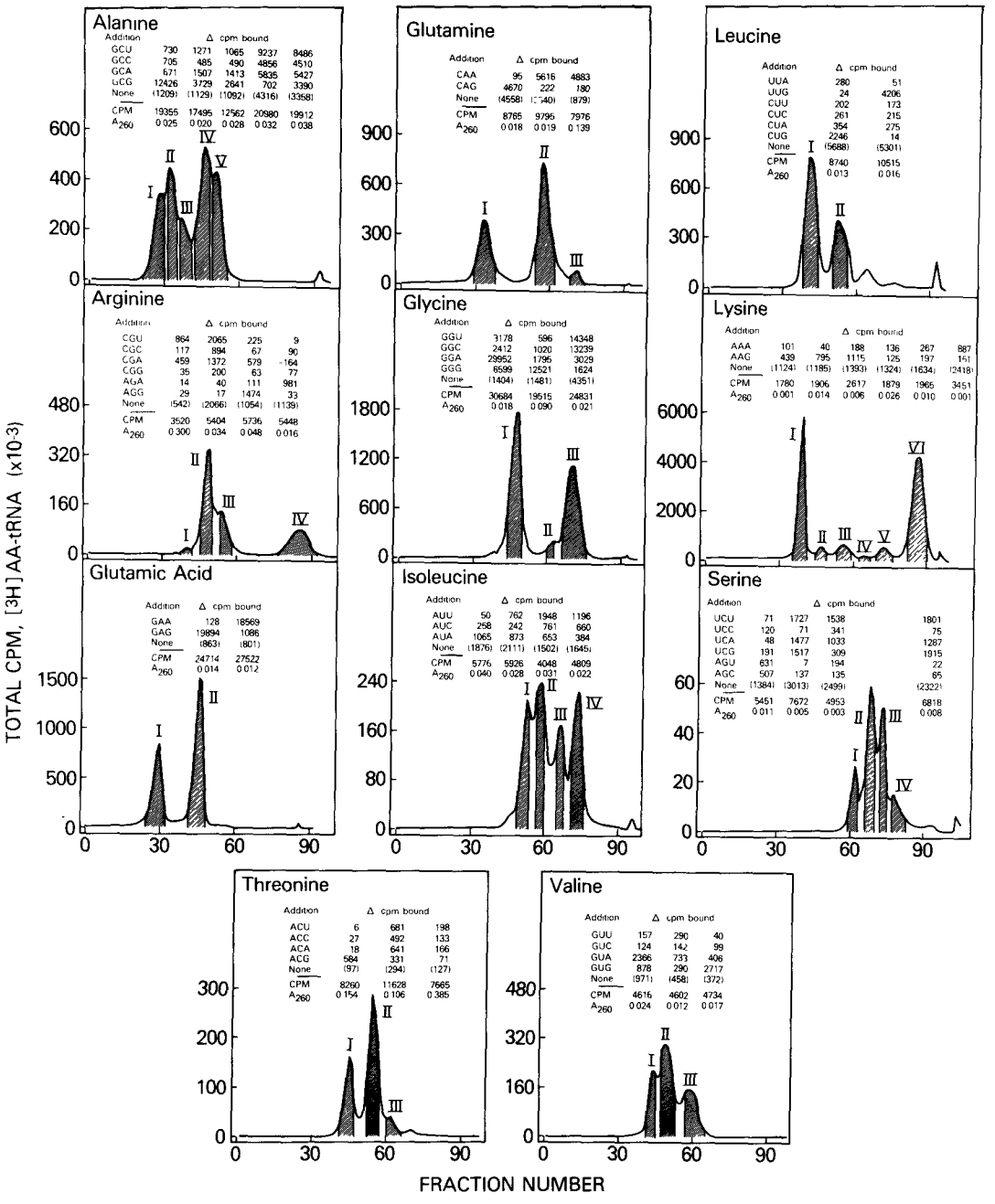

Figure 1. RPC-5 chromatography of [^{3}H]aa-tRNAs from Hymenoptera and their codon responses. Columns were developed, the resulting [^{3}H]aa-tRNA fractions pooled and prepared for coding, and coding studies carried out as given in Materials and Methods. The results of the coding studies are shown in 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

C. Glu-tRNA: The initial eluting peak (Fraction I) recognizes GAG and the second peak (Fraction II) GAA.

D. Gln-tRNA: The initial eluting peak (Fraction I) recognizes CAG and the second and third peaks (Fractions II and III) CAA.

E. Gly-tRNA: The large, initial eluting peak (Fraction I) responds most strongly to GGA, the second minor eluting peak (Fraction II) to GGG, and the third peak (Fraction III) to GGU and GGC.

F. Ile-tRNA: Fraction I responds to AUA and Fractions II, III and IV to AUU, AUC and AUA.

G. Leu-tRNA: The initial eluting peak (Fraction I) recognizes CUG and the second peak (Fraction II) UUG. The later eluting peaks did not manifest a significant response in presence of any of the leucine codons and therefore codon assignments were not made to these isoacceptors (data not shown).

H. Lys-tRNA: The large peak which eluted first from the column and the second and third eluting peaks (Fractions I-III) recognize AAG. The fourth and fifth eluting peaks (Fractions IV and V) respond to AAA and AAG and the large, late eluting peak (Fraction VI) primarily to AAA.

I. Ser-tRNA: The initial eluting peak (Fraction I) responds to AGU, AGC and slightly to UCU, UCA and UCG. The response to the latter codons most certainly is due to overlap of this peak with the second eluting peak (Fraction II) which responds strongly to UCU, UCA and UCG. The second eluting peak also responds weakly to UCC. This peak may be assigned UCC by analogy to a mammalian serine isoacceptor (3,6). Fraction III responds to UCU, UCA, and weakly to UCC and Fraction IV to UCU, UCA, UCG and weakly to UCC. Additional fractionation of the second and fourth eluting peaks would be required to determine if these peaks contain isoacceptors recognizing four serine codons or contain isoacceptors recognizing UCU, UCC and UCA and recognizing UCG (3,6).

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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. Total cpm and A_{260} units added to codon assays are also shown.

J. Thr-tRNA: Fraction I responds most strongly to ACG. Fraction II responds to ACU, ACC, ACA and ACG, and Fraction III to ACU, ACC, and ACA.

K. Val-tRNA: Fraction I responds strongly to GUA and less well to GUG. Fraction II responds to GUU, GUC, GUA and GUG, and the strongest response is to GUA. Fraction III responds most strongly to GUG.

II. Distribution of Isoacceptors: The distribution of Hymenoptera isoacceptors resolved by RPC-5 chromatography is shown in Table 1 along with their codon assignments. The most abundant isoacceptor of Ala-tRNA recognizes GCU, GCC, and GCA. Approximately equal amounts of the remaining Ala-tRNA respond to GCG and to GCU, GCC, GCA and GCG, respectively. Approximately one-half of the Arg-tRNA recognizes CGU, CGC and CGA while approximately equal quantities of two other arginine isoacceptors respond to AGG and to AGA. More than 60% of the Glu- and Gln-tRNAs respond to GAA and to CAA, respectively. About one-half of the Gly-tRNA responds to GGA, about 40% to GGU and GGC and less than 7% to GGG. Most of the Ile-tRNA responds to AUU, AUC, and AUA while less than 25% responds to AUA. About one-half of Leu-tRNA recognizes CUG while about one-third recognizes UUG. The most abundant isoacceptor of Lys-tRNA responds to AAA and the second most abundant isoacceptor responds to AAG. Most of the Ser-tRNA responds to UCU, UCC, UCA and UCG. About one-third of the Thr-tRNA responds to ACG, and about 60% responds to all four threonine codons. Approximately one-half of the Val-tRNA responds to GUU, GUC, GUA and GUG and about 20% and 29% respond to GUA and GUG and to GUG, respectively.

DISCUSSION: The patterns of codon recognition of eleven aa-tRNAs from Hymenoptera have been characterized. The patterns of recognizing codons by these isoacceptor aa-tRNAs are similar in most cases to those observed in mammals (3,4). Possible exceptions are minor Lys-tRNA isoacceptors which recognize AAA and AAG and serine isoacceptors which may recognize UCU, UCC, UCA and UCG in Hymenoptera, whereas different isoacceptors respond to AAA and to AAG and to UCU, UCC and UCA and to UCG in mammals. Additional differences occur between Hymenoptera, wheat germ and lower organisms. For example, an isoacceptor of Arg-

Table 1. DISTRIBUTION OF HYMENOPTERA ISOACCEPTORS RESOLVED BY RPC-5 CHROMATOGRAPHY

Isoacceptor aa-tRNA[a]	Fractions of Elution[b]	Codon Assignment[c]	Distribution[d]
Ala-tRNA	20-30	GCG	18.8
	31-35	?	18.9
	36-40	?	10.5
	41-49	GCU,GCC,GCA	33.8
	50-60	GCU,GCC,GCA,GCG	17.8
Arg-tRNA	37-42	CGU,CGC,CGA	2.5
	43-51	CGU,CGC,CGA,CGG	48.6
	52-64	AGG	23.3
	76-92	AGA	25.6
Glu-tRNA	20-36	GAG	37.3
	37-51	GAA	61.5
	52-59	?	1.2
Gln-tRNA	24-47	CAG	35.6
	48-67	CAA	59.8
	68-74	CAA	4.6
Gly-tRNA	35-40	?	1.2
	41-55	GGA	52.2
	56-63	GGG	6.2
	64-85	GGU,GGC	40.4
Ile-tRNA	42-47	?	1.5
	48-55	AUA	22.2
	56-62	AUU,AUC,AUA	29.3
	63-70	AUU,AUC,AUA	21.5
	71-86	AUU,AUC,AUA	25.5
Leu-tRNA	33-46	CUG	52.4
	47-58	UUG	33.2
	59-70	?	8.2
	71-80	?	2.4
	91-95	?	3.8
Lys-tRNA	34-43	AAG	34.1
	44-50	AAG	4.5
	51-61	AAG	7.0
	62-67	AAA,AAG	1.7
	68-77	AAA,AAG	5.9
	78-93	AAA	46.8
Ser-tRNA	56-64	AGU,AGC	16.0
	65-71	UCU,UCC,UCA,UCG	42.4
	72-76	UCU,UCC,UCA	26.5
	77-88	UCU,UCC,UCA,UCG	13.0
	89-95	?	2.1
Thr-tRNA	39-49	ACG	30.5
	50-60	ACU,ACC,ACA,ACG	60.6
	61-66	ACU,ACC,ACA	5.9
	67-76	?	3.0
Val-tRNA	35-45	GUA,GUG	20.2
	46-54	GUU,GUC,GUA,GUG	50.6
	55-67	GUG	29.2

[a] Fractionation of aa-tRNAs are shown in Figure 1.
[b] Fractions in which isoacceptors eluted from the RPC-5 column (see Figure 1).
[c] Codon assignments are given in text and are taken from the data given in Figure 1. A question mark indicates that codon assignments were not made.
[d] Distribution of isoacceptor aa-tRNAs were determined from the elution profiles shown in Figure 1.

tRNA in wheat germ responds to AGG and slightly to AGA and an isoacceptor of Glu-tRNA responds to GAG and slightly to GAA (5), whereas different isoacceptors of Arg- and Glu-tRNAs respond to these codons in Hymenoptera. The major isoleucine isoacceptor in E. coli responds to AUU and AUC (1-3) and serine isoacceptors respond to UCU and UCC and to UCU, UCA and UCG (1-3,12). E. coli and yeast contain valine isoacceptors which recognize GUU, GUA and GUG (13-16). Patterns of codon recognition of the other valine isoacceptors in E. coli (13-15) are different from those in Hymenoptera.

Differences in the relative abundances of specific isoacceptors also exist in the tRNA populations of different organisms. For example, isoacceptors of alanine and threonine which recognize GCG and ACG, respectively, are minor isoacceptors in mammals (3,4), but comprise a significant proportion of the corresponding aa-tRNA families in Hymenoptera. More than 60% of the Gln-tRNA recognizes CAA in Hymenoptera while less than 10% recognizes CAA in wheat germ (5). About 50% of the Arg-tRNA isoacceptors in Hymenoptera responds to AGG and to AGA and the isoacceptors which recognize these codons are present in minor levels in E. coli (17).

Recent studies which report the sequence of mRNAs from procaryotic and eucaryotic sources show that the frequency of usage of certain codons has also undergone evolutionary change (18-24). Thus, evolutionary changes have occurred in patterns of codon recognition, in relative abundances of isoacceptors and in frequency of usage of codons in mRNA. Although the means by which these changes have occurred in evolution are not understood, it seems likely that a tRNA population must maintain an appropriate level of isoacceptors for efficient translation of the corresponding codewords. The initial evolutionary events may involve, therefore, an expansion in the tRNA (anticodon) population. Such an expansion could result either in an increase in the 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 isoacceptor distribution may then occur. Aminoacylation of tRNAs from a variety of organisms under homologous and heterologous conditions are being examined in this laboratory to understand better the evolutionary changes which have occurred in tRNA:synthetase interactions. The fact that evolutionary changes have occurred in patterns of codon recognition, in frequency of codon usage and in tRNA:synthetase interactions raises an important question: "How can genetic information which is carried in one region of a genome (e.g., in a tRNA gene) and codes for interaction with genetic information carried in another region (e.g., in an aa-tRNA synthetase gene) undergo genetic variation?" Studies are in progress to elucidate further the means by which these changes have occurred in evolution.

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