SELECTIVE UTILIZATION OF VALYL-tRNA HAVING A PARTICULAR CODING SPECIFICITY IN A RABBIT HEMOGLOBIN SYNTHESIZING SYSTEM

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SUMMARY

Transfer of valine into globin chains from various valyl-tRNA species isolated from Escherichia coli and yeast, which differ in the specificity for codon recognition has been studied using a rabbit reticulocyte cell-free system The evidence was obtained which suggests that the transfer of valine into both α- and β-chains occurs preferentially in response to codon GUG and possibly to GUA, but scarcely to codons GUU and GUC.

The highly degenerate nature of the genetic code was clearly established by the studies in which synthetic templates were used. In order to determine whether or not the amino acid codons are degenerate in natural messengers, a rabbit hemoglobin-synthesizing system was employed by Weisblum et al. and it was revealed that there are at least two distinct codons for leucine in a hemoglobin messenger (1). Thereafter, the similar coding degeneracy was demonstrated for several amino acids in the hemoglobin synthesis (2-5).

This time we attempted to assign a codon for valine in a particular position of globin chain, based on the results of valine incorporation into hemoglobin from several valyl-tRNA fractions, of which coding specificity has been definitely determined by the trinucleotide-stimulated ribosomal binding technique. In this case it was assumed that valyl-tRNAs applied to the reaction incorporate valine into globin chain with a high fidelity to their corresponding coding specificity observed in the ribosomal binding.

In this paper we report the transfer of valine into α- and β-globin chains preferentially in response to codon GUG and possibly to GUA in a rabbit reticulocyte cell-free system.

MATERIALS AND METHODS

Uniformly labeled L-[^{14}C] glutamic acid and L-[^{3}H] valine were purchased from the New England Nuclear Co.

E. coli B $tRNA^{Val}_{1}$ (more than 95 % pure) was prepared by the procedures as described in the previous paper (6, 7), the primary structure of which has been established (8-10). E. coli $tRNA^{Val}_{2}$ (approx. 50 % pure), the nucleotide sequence of which was recently determined (11), was partially purified by the combined use of DEAE-Sephadex A-50 column (6) and benzoylated DEAE-cellulose column (12). These two tRNA preparations were free from cross-contamination.

A $tRNA^{Val}$ preparation (at least 80 % pure), tentatively designated $tRNA^{Val}_{I}$, was prepared from baker's yeast (Saccharomyces cerevisiae) by successive use of DEAE-Sephadex A-50 column (13) and benzoylated DEAE-cellulose column (12). A partially purified $tRNA^{Val}$, designated $tRNA^{Val}_{Fr.3}$ for convenience, was obtained from the later elutable part of $tRNA^{Val}$ fractions separated when yeast mixed tRNA was applied onto DEAE-Sephadex A-50 column. These two fractions of yeast $tRNA^{Val}$ are not necessarily a single species.

Each $tRNA^{Val}$ was charged with [^{3}H] valine (specific activity, 2.88 Ci/mmole) using the homologous aminoacyl-tRNA synthetase.

The preparation of a hemoglobin-synthesizing system from rabbit reticulocytes, the incorporation of radioactive amino acids into globin and the separation of α- and β-chains were carried out by the essentially same procedures as detailed in our previous paper (5).

RESULTS AND DISCUSSION

The coding specificity of valyl-tRNA preparations used for valine transfer experiment as described below was determined by their binding ability to E. coli ribosomes in the presence of the trinucleotides GpUpU, GpUpC, GpUpA and GpUpG. The results were summarized in Table I. The present test confirmed the previous observation that E. coli $tRNA^{Val}_{1}$ responded to the triplet codons GUA and GUG, and to a smaller extent, to GUU, and that E. coli $tRNA^{Val}_{2}$ responded to the triplets GUU and GUC (7, 14). Yeast

Table I

Specificity of various valyl-tRNA preparations in codon recognition determined by ribosomal binding

Template	[^{14}C] Valyl-tRNA bound (cpm)			
	E. coli		Yeast	
	$tRNA^{Val}_{1}$	$tRNA^{Val}_{2}$	$tRNA^{Val}_{I}$	$tRNA^{Val}_{Fr.3}$
GUU	111	596	408	130
GUC	8	676	126	1
GUA	550	33	83	116
GUG	540	1	14	86
None	(46)	(61)	(266)	(138)

The assay of valyl-tRNA binding to E. coli ribosomes was carried out by the procedure of Nirenberg and Leder (18). The reaction conditions used for E. coli tRNA binding (7) and for yeast tRNA binding (5) were the same as described previously, except for those as specified below. Each reaction mixture contained 0.05 $A_{260\ nm}$ unit of trinucleoside diphosphate with the exception of 0.083 $A_{260\ nm}$ unit of the nucleotide in the case of the binding of yeast $tRNA^{Val}_{I}$. 30 mM magnesium acetate was used in the assay for yeast tRNA binding.

$tRNA^{Val}_{I}$ preparation was found to respond to GUU and, to a smaller extent, to GUC and GUA, but not to GUG. This specificity for codon recognition is similar to that of $tRNA^{Val}_{1}$ or $tRNA^{Val}_{3}$ as reported by Mirzabekov et al. (15) It was found that yeast $tRNA^{Val}_{Fr.3}$ fraction bound to ribosomes in response to the triplets GUU, GUA and GUG.

Four kinds of valyl-tRNA as mentioned above were separately added to a hemoglobin-synthesizing cell-free system. Free[^{14}C] glutamic acid was simultaneously added as a reference to each reaction mixture, in order to compare the amounts of hemoglobin synthesized. After the mixture was incubated, the doubly labeled globin was isolated and separated into α- and β-globin chains by carboxymethylcellulose column chromatography. The results are shown in Fig. 1. The ordinate for [^{3}H] valine incorporated was

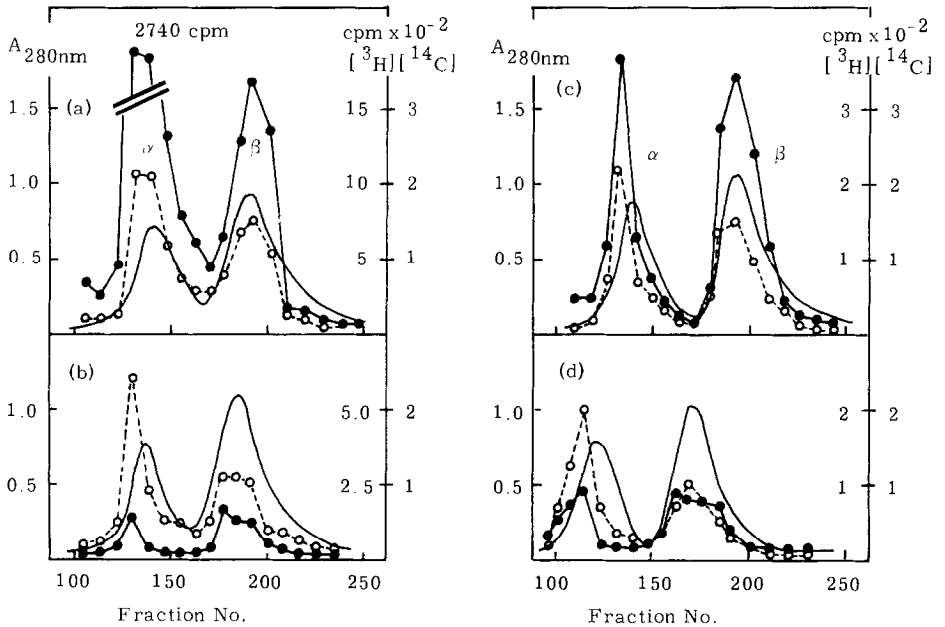

Fig. 1. Separation of α- and β-chains of double labeled globin by carboxymethylcellulose column chromatography. The reaction mixture for amino acid transfer into rabbit globin contained the following components in a final volume of 5 ml : 500 μmoles of Tris-HCl (pH 7.6), 125 μmoles of KCl, 20 μmoles of $MgCl_2$, 40 μmoles of β-mercaptoethanol, 2.5 μmoles of GTP, 5 μmoles of ATP, 50 μmoles of phosphoenolpyruvate, 250 μg of phosphoenolpyruvate kinase, 1 μmole each of the eighteen kinds of unlabeled amino acid (minus valine and glutamic acid), 10 μmoles of unlabeled valine, 5 μCi of [^{14}C] glutamic acid (specific activity : 206 mCi/mmole), [^{3}H] valyl-tRNA specified below and 450 $A_{260\ nm}$ units of the "pH 5" pellet prepared from reticulocytes. [^{3}H] valyl-tRNAs added to each reaction mixture were as follows : (a) E. coli [^{3}H] valyl-$tRNA^{Val}_1$, 2.8×10^6 cpm; (b) E. coli [^{3}H] valyl-$tRNA^{Val}_2$, 1.3×10^6 cpm; (c) yeast [^{3}H] valyl-$tRNA^{Val}_{Fr.3}$, 7.2×10^5 cpm; and (d) yeast [^{3}H] valyl-$tRNA^{Val}_I$, 6.7×10^5 cpm. After incubation at 37°C for 45 min and subsequent addition of unlabeled globin as a carrier, the double labeled globin was isolated and applied to carboxymethylcellulose column (2.8 cm x 36 cm) for chain separation. Elution was performed using a linear gradient made of 800 ml of 0.2 M formic acid and 0.02 M pyridine in the mixing chamber and 800 ml of 2 M formic acid and 0.2 M pyridine in the reservoir. Fractions of 5 ml were collected and radioactivity in 3 ml samples was assayed. ——, absorbance at 280 nm: ●——●, [^{3}H] valine incorporated; o---o, [^{14}C] glutamic acid incorporated.

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scaled taking the input radioactivity into consideration so that the profiles are directly comparable with each other. As clearly shown in Fig. 1, [^{3}H]-valine charged to E. coli $tRNA^{Val}_1$ (Fig. 1-a) and yeast $tRNA^{Val}_{Fr.3}$ (Fig. 1-c)

was efficiently transferred into both α- and β-globin chains. On the other hand, [^{3}H]valine charged to E. coli tRNA$^{Val}_{2}$ (Fig. 1-b) and yeast tRNA$^{Val}_{I}$ (Fig. 1-d) was scarcely transferred into both chains, whereas [^{14}C]glutamic acid was incorporated to the almost same extent as that in the case of E. coli tRNA$^{Val}_{1}$. These observations were confirmed by an additional experiment, that is, a double-labeling experiment in which the simultaneous transfer of [^{3}H] and [^{14}C]valines charged to appropriately combined two kinds of tRNA^{Val} species among E. coli tRNA$^{Val}_{1}$, E. coli tRNA$^{Val}_{2}$ and yeast tRNA$^{Val}_{I}$ to α- and β-globin chains were estimated (unpublished work).

Taking it into consideration that yeast tRNA$^{Val}_{I}$ responded to GUA only to a smaller extent, the above described findings seem to indicate that valine was transferred into hemoglobin preferentially in response to GUG and possibly to GUA, but scarcely to codons GUU and GUC. The findings thus suggested that hemoglobin mRNA is rich in GUG and possibly GUA among the four synonym valine codons. Other possibilities, however, should be taken in mind in the explanation of the observations obtained this time. Thus (a) the results may be due to the ability of individual valyl-tRNA species to interact with the components, such as binding factors, which are concerned in the translation process in the reticulocyte cell-free system, regardless of its coding specificity. This possibility, however, can be excluded because Gupta et al. reported that in the rabbit reticulocyte cell-free system E. coli tRNA$^{Val}_{2}$ undoubtedly transferred valine at least in response to a synthetic messenger, poly r(G-U-U) (16). (b) The results may be due to the relative amounts of isoaccepting species of endogenous tRNA^{Val} present in the reticulocyte cell-free system. If the amount of endogenous valyl-tRNA species which is able to recognize GUG is much smaller than that of valyl-tRNA species which recognize the other valine codons, [^{3}H]valyl-tRNA added to the incubation mixture which recognize GUG would be utilized more efficiently. In fact any partially separated reticulocyte tRNA^{Val} fractions showed little difference in the stimulation of its binding to reticulocyte ribo-

somes by four valine codons, so that this possibility may be ruled out. (c) The results may be due to the labeling of the limited valine residues near the C-terminus of globins which are coded by GUG, because of few reinitiation in this protein synthesizing system (4). At least in the case of α-chain, however, this possibility can be excluded from the fact that 9 among 11 valine residues found in α-chain are contained in the tryptic peptides, αT9 to αT15, and that αT9 was sufficiently labeled with [^{14}C]glutamic acid which was used as a reference. (d) The results may be due to the incorporation of valine into non-globin peptides which contain many valine residues coded by GUG, because it was reported that E. coli valine tRNA transfers valine mainly into globin peptides, but some valine is transferred into non-globin peptides (17). However, column chromatographic analysis of tryptic digests labeled with the radioactive valine revealed that this possibility could be neglected.

From the above-mentioned considerations, experimental grounds of which will be published elsewhere, it is strongly suggested that mRNA for hemoglobin, at least for α-globin chain, is very rich in GUG and possibly GUA as valine codon.

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