PROPERTIES OF ISOACCEPTING tRNA^{Val} FROM RABBIT RETICULOCYTES; FRACTIONATION AND CODON RECOGNITION

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1. Introduction

The possibility that changes in the structure and abundance of individual tRNAs may be an important aspect of cell differentiation has been suggested by several authors [1–5]. As reticulocytes are highly specialized for the synthesis of the two polypeptide chains of hemoglobin, a comparison of reticulocyte tRNA with tRNA from other tissues may be relevant to the molecular level of this differentiated state.

In this paper we wish to report on the fractionation of tRNA^{Val} from rabbit reticulocytes into four isoaccepting subspecies and their properties in codon recognition. It was established that the ribosomal binding of two of the three tRNA^{Val} subspecies investigated is stimulated exclusively by the trinucleotide GpUpG. The use of tRNA subspecies of this kind in a hemoglobin synthesizing system could be of value for determining the number of GpUpG among the valine codons of reticulocyte mRNAs.

2. Materials and methods

The following materials were obtained from commercial sources: L-[^{14}C]valine (280 mCi/mmole); L-[^{3}H]valine (31.4 Ci/mmole) (Amersham); Na_3ADP, Na_3GDP, Na_3CDP, Na_3UDP and GpU (Papierwerke Waldhof-Aschaffenburg); polynucleotide phosphorylase from *Micrococcus luteus* (30 units/mg) (Boehringer, Mannheim); cellulose nitrate filters (pore size 0.45 μm)(Sartorius, Göttingen); Plaskon CTFE (Allied Chemical Corporation); Adogen 464 (Oleochim, Brüssel).

The isolation of packed reticulocytes and the preparation of tRNA, of aminoacyl-tRNA-synthetase fraction from rabbit reticulocytes as well as the charging reaction of tRNA were carried out as described in a previous paper [6].

The trinucleotides were prepared enzymatically according to the procedure of Thach [7]. The ribosomes of *Escherichia coli* MRE 600 were isolated following the method of Matthaei and Nirenberg [8]. The fractionation of Val-tRNA was carried out by the reversed-phase chromatography system described by Pearson et al. [9]. The size of the column and the elution conditions were modified as specified in the legend to fig. 1. The codon recognition was tested by the ribosomal binding assay according to Nirenberg and Leder [10].

3. Results

The isoaccepting Val-tRNAs from rabbit reticulocytes were fractionated either on BD-cellulose according to Fittler et al. [11] or on RPC-5 as described by Pearson et al. [9]. Although BD-cellulose chromatography allows the isolation of two well-separated fractions (not shown) reversed-phase chromatography seems to be more efficient; as shown in fig. 1 we were able to obtain four subspecies. Several repetitions of the column run showed exact reproducibility of the elution profile. The peak fractions were collected as indicated by the shaded areas. The samples from peak II contained too little material to be examined. A co-chromatography of fraction three and four confirmed that the tRNA fractions collected are not artifacts but

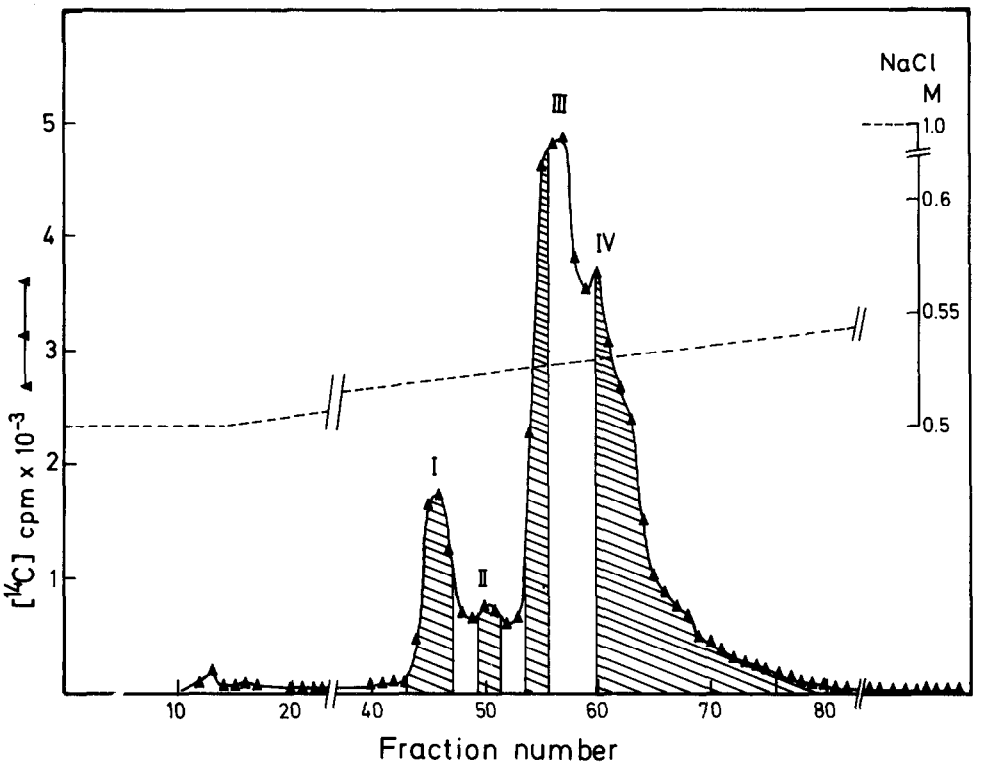

Fig. 1. Fractionation of [^{14}C]Val-tRNA into the isoaccepting subspecies by reversed-phase column chromatography. A sample of aminoacylated [^{14}C]Val-tRNA (1.6. $\times 10^6$ cpm) cleared of protein and dialysed against starting buffer (see below) was applied to an equilibrated RPC-5 column 1×100 cm in size. The elution was carried out at 30°C by a linear gradient of 0.5 litresec starting buffer and 0.5 litres reservoir buffer. Both buffers are composed of 0.01 M sodium acetate pH 4.5, 0.01 M $MgCl_2$, 0.001 M EDTA and 0.003 M 2-mercaptoethanol. The starting and equilibrating buffer in addition contains 0.5 M NaCl and the reservoir buffer 0.65 NaCl. Fractions of 5 ml were collected at a flow rate of about 60 ml/hr. For estimation of the labeled tRNA subspecies 200 µl of each fraction were counted in 5 ml of a scintillation mixture according to Anderson [12]. The shaded areas represent the fractions collected for further examinations.

physically different tRNA subspecies containing only limited cross contaminations (fig. 2). Contamination from subspecies I is not detectable by co-chromatography. A rough estimate yields the following proportions of the four isoaccepting tRNAs: $tRNA_1^{Val}$ 8–12%; $tRNA_2^{Val}$ 3–5%; $tRNA_3^{Val}$ 45–55%; $tRNA_4^{Val}$ 30–40%.

The codon responses in the ribosomal binding test obtained with $tRNA_1^{Val}$, $tRNA_3^{Val}$, and $tRNA_4^{Val}$ as well as with unseparated Val-tRNA are shown in fig. 3 A–D. Unfractionated $tRNA^{Val}$ from rabbit reticulocytes charged with [^{3}H] valine shows a high preference for the recognition of GpUpG (fig. 3A). The trinucleotide GpUpU stimulates the binding to ribosomes only weakly. In the presence of GpUpC and GpUpA no significant stimulation of binding is observed.

This result is confirmed with separated Val-tRNA subspecies (fig. 3 B–D). Val-$tRNA_1$ (fig. 3 C) is bound to ribosomes exclusively in the presence of GpUpG. Isoaccepting Val-$tRNA_3$ (fig. 3 D) responds efficiently to the codon GpUpG and minimally or not at all to the other three valine specific codons. The weak response to GpUpU might be due to a small contamination by Val-$tRNA_4$ as already seen in the co-chromatography (fig. 2). The ribosomal binding of Val-$tRNA_4$ (fig. 3 B) is stimulated by the codon GpUpG and less efficiently by GpUpU; the trinucleotide GpUpA still seems to catalyse some binding to ribosomes, while in the presence of

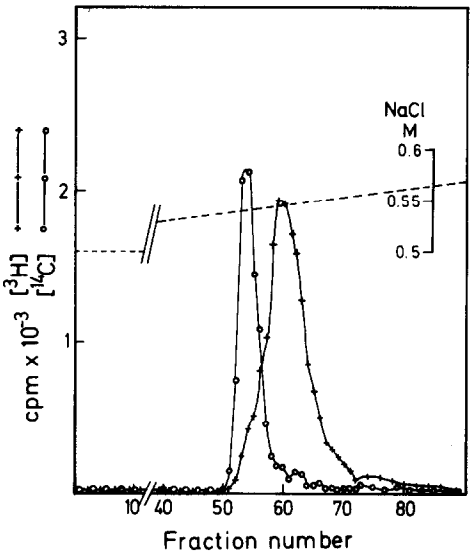

Fig. 2. Co-chromatography of [^{14}C] Val-tRNA$_3$ and [^{3}H] Val-tRNA$_1$ on a reversed–phase column RPC-5. After concentrating the two samples separated by chromatography (see fig. 1), 20000 cpm from each were combined and fractionated as reported in fig. 1. The eluted fractions were precipitated with 10% trichloroacetic acid on glass filters and the radioactivity was discriminately counted in 5 ml of a 0.5% diphenyloxazole–toluene solution.

GpUpC no significant stimulation is observed.

4. Discussion

Valyl-tRNA from rabbit reticulocytes shows a remarkable specificity for the recognition of the codon GpUpG in the ribosomal binding test. All three subspecies investigated respond markedly to GpUpG and only on reduced scale or not at all to the other valine codons. Contradictory results have recently been reported. Takemoto et al. [13] found almost no difference in stimulation by the four valine codons using reticulocyte ribosomes and unseparated as well as partially fractionated Val-tRNA from rabbit reticulocytes in the ribosomal binding test. An explanation for this apparent contradiction might be found in the very high Mg^{2+} concentration (50 mM) used by the authors. Contrary to this, we preferred a relatively low Mg^{2+} concentration (16 mM) and the application of *E. coli* ribosomes. Ribosomal binding of Val-tRNA from rabbit liver is strongly stimulated by all four valine codons under the same conditions (unpublished observations). Therefore, the lack of response to GpUpA and GpUpC obtained with reticulocyte Val-tRNA must be due to a real specificity of the subspecies and not the conditions used.

It is interesting to note that these recognition properties of tRNA^{Val} from rabbit reticulocytes differ distinctly from those observed with tRNA^{Val} from *E. coli*, yeast and rat liver [14–16], as the latter do not show such a pronounced specificity for only one codon.

It has been concluded from rabbit hemoglobin synthesis in vitro with isoaccepting tRNA^{Val} of *E. coli* and yeast [13, 17] that the most frequent codon specific for valine in the mRNAs for hemoglobin is GpUpG and possibly GpUpA. (The results, however, did not allow estimation of the frequency of GpUpA present in the mRNAs of the reticulocyte). The results presented here support this conclusion [13, 17] in that the trinucleotide GpUpG is most often recognized by unseparated as well as by separated tRNA^{Val} from rabbit reticulocytes. However, GpUpA as well as GpUpC, which stimulate ribosomal binding of tRNA^{Val} only weakly, might appear rarely of not at all in valine specific positions or mRNAs or hemoglobin. The fourth valine codon GpUpU, on the other hand, might be the only valine codon in addition to GpUpG present in the mRNAs of hemoglobin. Studies on the incorporation properties of fractionated tRNA^{Val} subspecies in a homologous cell-free system are now in progress; preliminary results seem to support the above mentioned conclusions.

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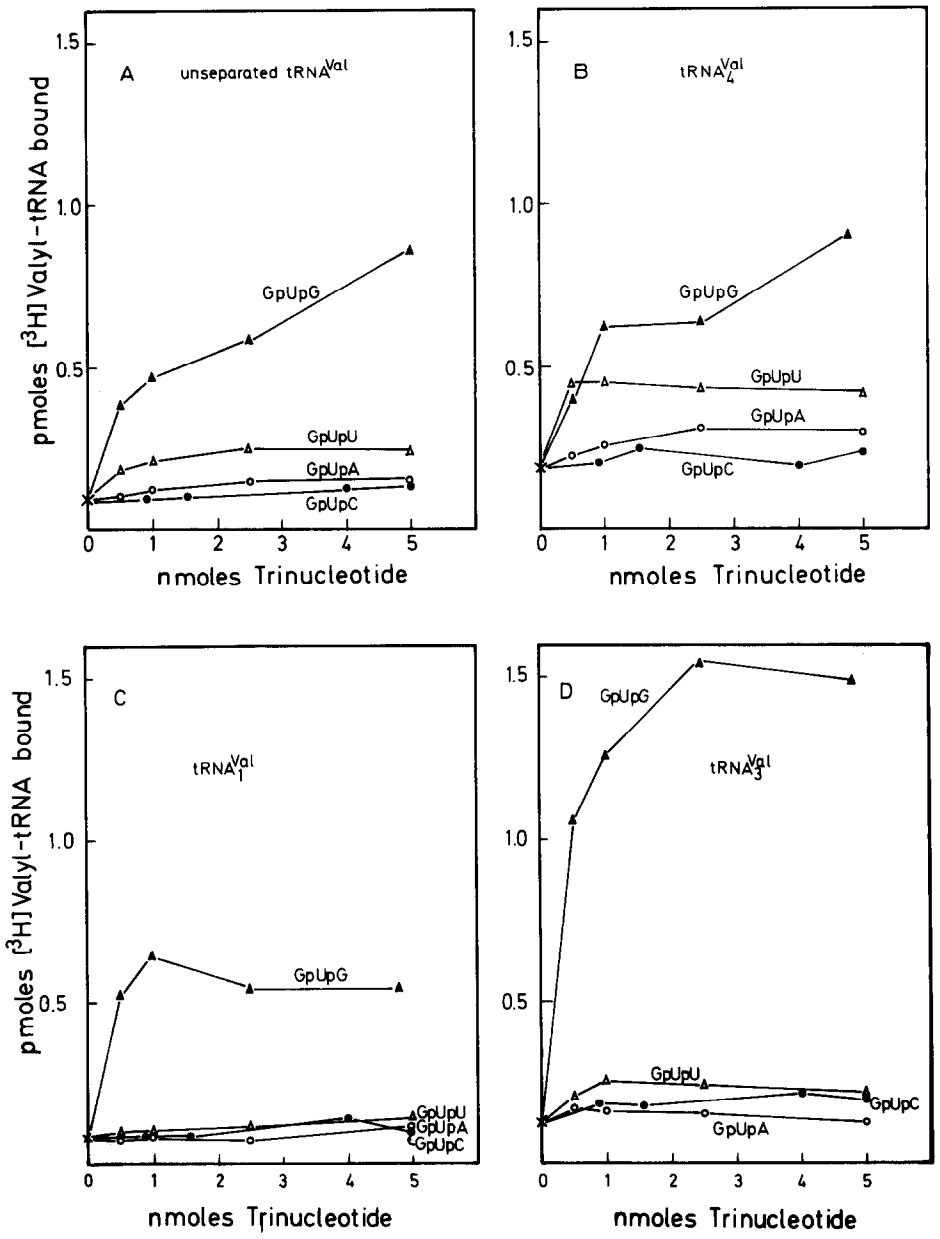

Fig. 3. Properties of isoaccepting Val-tRNAs in codon recognition. The stimulation of ribosomal binding of labeled Val-tRNAs by codons specific for valine was measured according to the technique of Nirenberg and Leder [10]. Each sample of 50 μl total volume contained 0.016 M $MgCl_2$, 0.05 M KCl, 0.02 M Na-cacodylate pH 7.2, 60 pmoles ribosomes, 7.5 pmoles tritiated Val-tRNA subspecies as well as increasing concentrations of trinucleotides. Incubation time was 20 min at 25°C. The samples were immediately cooled and processed as described [10]. The radioactivity on the cellulose nitrate filters was counted in 5 ml of a 0.5% diphenyloxazole–toluene solution.

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