GLUTAMINE COGNATE CODONS IN RABBIT HAEMOGLOBIN mRNAs

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1. Introduction

Comparisons of isoaccepting tRNA species from different cell types have led to the suggestion that tRNA patterns are adapted to an optimal translation efficiency of a unique mRNA population [1]. Thus, it has been shown that concentrations and codon recognitions of isoaccepting tRNA species from cells with a limited number of mRNAs, such as rabbit reticulocytes, are significantly different from those which have a broad variety of different mRNAs, such as rabbit liver cells [2]. Moreover, it has been demonstrated that the production of α- and β-chains of rabbit haemoglobin can be selectively influenced by the addition of certain tRNA fractions to a cell free system which is dependent on tRNA [3].

A more direct way of testing this hypothesis is the use of aminoacylated isoaccepting tRNAs with different codon recognition as substrates for in vitro translation of a defined mRNA. We chose to undertake such an experiment with haemoglobin mRNA. In this case a comparison of the CAA and CAG recognizing $tRNA^{Gln}$ species seemed most suitable. The CAA-recognizing $tRNA^{Gln}$ is present in reticulocytes to only 2% of total isoaccepting $tRNA^{Gln}$, whereas in rabbit liver the fraction of $tRNA^{Gln}$ recognizing exclusively CAA is 25% [2]. It was established that the reading capacity of the aminoacylated $tRNA^{Gln}_{CAA}$ is dramatically suppressed indicating a complete absence of the glutamine CAA codon in the α- and β-globin mRNAs.

2. Materials and methods

2.1. *Sources of materials*

L-[^{14}C] glutamine (252 Ci/mol) was obtained from New England Nuclear, Boston, Mass. USA). Plaskon CTFE and Adogen 464 were purchased from Allied Chemical Corporation and Oleochin, Brussels, respectively. Na_3ADP, Na_3GDP and CpA were obtained from Pharma-Waldhof. Polynucleotide phosphorylase (*M. luteus;* 30 units/mg) was delivered by Boehringer, Mannheim and cellulose nitrate filters (pore size 0.45 μm) were obtained from Sartorius, Göttingen.

2.2. *Preparation of isoaccepting aminoacylated $tRNA^{Gln}$*

Total uncharged tRNA was isolated from rabbit liver and separated into isoaccepting tRNAs via RPC-5 [4] chromatography as reported recently [2]. The rechromatography of CAA recognizing $tRNA^{Gln}$ was performed under identical conditions after the pooled fractions were concentrated by vacuum dialysis and dialysed against the starting buffer of the column chromatography. Isoaccepting $tRNA^{Gln}$ containing fractions were concentrated to a volume of 1 ml each and aminoacylated with [^{14}C]glutamine in a total volume of 4 ml as published [2].

2.3. *Ribosomal binding assay and rabbit haemoglobin synthesis*

The trinucleotide CAA was prepared according to Thach [5] and CAG according to the modification

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of Pongs [6]. Ribosomes of *E. coli* MRE 600 were isolated following the method of Matthaei and Nirenberg [7] with only one washing in 0.5 M NH_4Cl. The ribosomal binding assay of Nirenberg and Leder [8] was carried out in total volumes of 50 μl and a Mg^{++} concentration of 15 mM. The samples contained approx. 4 pmol of radioactive Gln-tRNA (2000 c.p.m.). Unseparated tRNA and the two isoacceptors charged with [^{14}C]glutamine were used separately for amino acid incorporation into the globin in a reticulocyte lysate system [9,10]. In addition to the radioactive Gln-tRNA as a substrate a 100-fold molar excess of nonradioactive free glutamine was included to the incubation mixture. The experimental conditions are outlined in the legend of table 1.

3. Results and discussions

The purity of isoaccepting tRNAs was tested by

Table 1
Incorporation of radioactive glutamine into rabbit globin

Exp.	Substrate applied	Quantity applied (c.p.m.)	(pmol)	Amount of [^{14}C]Gln incorp. into globin (pmol)	(%)	Ratio of radioactivity incorp. β-chain/α-chain
1	2	3	4	5	6	7
1	[^{14}C]Gln-tRNA$_{CAG}$	87 000	182	28.6	15.7	3.7
1	[^{14}C]Gln-tRNA$_{CAA}$	45 000	94	3.8	4.0	3.1
2	[^{14}C]Gln-tRNA$_{unseparated}$	26 770	56	18.5	33	3.6
2	[^{14}C]Gln-tRNA$_{CAG}$	26 770	56	17.9	32	3.6
2	[^{14}C]Gln-tRNA$_{CAG}$	26 770	56	15.7	28	3.6
2	[^{14}C]Gln-tRNA$_{CAA}$ rechromatographed	29 400	62	2.7	4.4	3.4
2	[^{14}C]Gln-tRNA$_{CAA}$ rechromatographed	29 400	62	3.8	6.1	3.4
2	[^{14}C]Gln-tRNA$_{CAA}$ rechromatographed	29 400	62	4.1	6.6	3.1
3	[^{14}C]Gln-tRNA$_{unseparated}$	43 400	91	12.5	13.7	3.8
3	[^{14}C]Gln-tRNA$_{CAG}$	43 400	91	11.9	13.0	3.9
3	[^{14}C]Gln-tRNA$_{CAA}$ rechromatographed	17 200	36	0.35	1	not measurable
	+ Gln-tRNA$_{CAG}$		24			

Red blood cells of rabbits with a reticulocytosis of around 90% were lysed in an equal vol of 3 mM glutathione for 10 min. After centrifugation at 20 000 *g* for 10 min the lysate supernatant was directly used. 775 μl of each incubation sample contained the following: 75 μl of 38 mM haemin in 50 mM Tris–Cl pH 7.5 and 50 mM KCl; 450 μl of lysate supernatant; 200 μl of [^{14}C]Gln-tRNA, whose amounts varied as listed in the table; 50 μl of master mix (100 μl contained: the unlabeled amino acids (composition as described by Hunt et al. [10]. Their concentrations were calculated corresponding to the altered volume of the master mix) 3.5 mg creatinphosphate; 1.5 mg creatinkinase; 15 mM ATP; 3 mM GTP; 100 mM KCl; 30 mM $MgCl_2$; 150 mM Tris–Cl pH 7.3). After incubation at 37°C for 45 min the ribosomes of each sample were centrifuged down at 50 000 rev/min for 2.5 h in a Spinco Ti 50 rotor. Then supernatant was freed of RNA by phenolization and of haemin by acid acetone extraction (0.6 ml of 2 M HCl per 200 ml of acetone). The globin was chromatographed on a CM-cellulose column in order to separate α- and β-chain [15]. Finally, the fractions collected were precipitated with 5% trichloric acetic acid, filtered on glass fiber, and washed with 5% trichloric acetic acid. The radioactivity of the filters was estimated in a liquid scintillation counter.

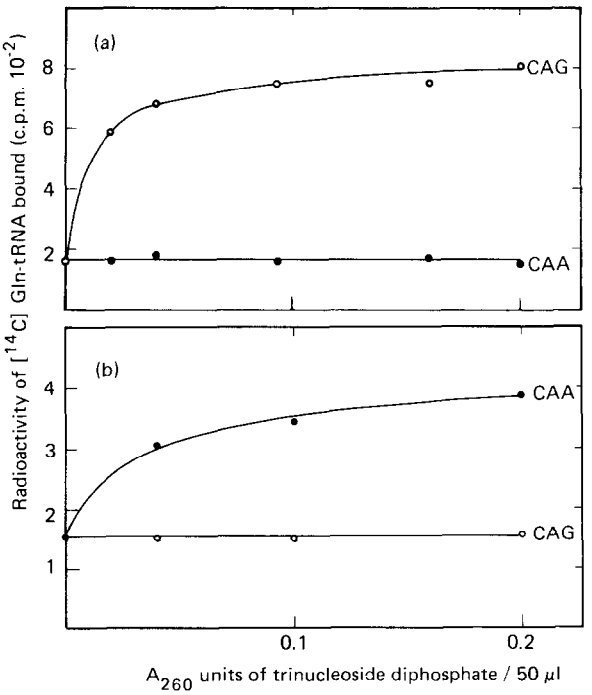

Fig.1. Ribosomal binding assay of isoaccepting [^{14}C]Gln-tRNAs. (A) shows the codon recognition of $tRNA^{Gln}_{CAG}$. (B) shows the codon recognition of rechromatographed $tRNA^{Gln}_{CAA}$.

the ribosomal binding assay [8]. As is evident from fig.1 glutaminyl-tRNA from one peak of the chromatogram is bound specifically in the presence of the triplet CAG (fig.1A), whereas material from a second peak after rechromatography is recognized by the CAA codon exclusively (fig.1B). The incorporation of [^{14}C]-glutamine into globin by unseparated [^{14}C]Gln-tRNA varied from one lysate to another as shown in table 1, exp. 2 and 3. Analogous results were published by others [11,12] using amino acids as substrates. However, the relative proportion of glutamine incorporated into the chains is nearly constant. The observed ratios of 3.6 and 3.8 agrees well with the theoretical value of 4 [13]. When the translational properties of charged $tRNA^{Gln}_{CAG}$ were investigated, an incorporation similar to the one with unseparated glutaminyl tRNA species was observed within each set of experiments. Furthermore, the relative proportions of radioactive glutamine incorporated into β-chain and α-chain remain the same (table 1, column 7). From this it seems likely that only CAG glutamine codons, and no CAA codons, are present in both the mRNAs coding for the haemoglobin chains.

Experiments with [^{14}C]Gln-$tRNA^{Gln}_{CAA}$ seem to contradict this interpretation. Contrary to an expected zero incorporation of glutamine from this isoacceptor into the haemoglobin chains, we still observe one quarter (table 1, exp. 1) or one fifth (table 1, exp. 2) of the amount of glutamine inserted, as compared with incorporation from CAG-specific $tRNA^{Gln}$. It seems, however, that this incorporation is due to an aberrant interaction of $tRNA^{Gln}_{CAA}$ with CAG codons of haemoglobin mRNAs in the absence of $tRNA^{Gln}_{CAG}$. This interpretation is based on the result of a competition experiment (table 1, exp. 3). In the presence of nonradioactive CAG-recognizing Gln-tRNA the incorporation of radioactive glutamine from CAA-recognizing [^{14}C]Gln-tRNA is almost completely suppressed. Thus, exclusive recognition of CAA codons by isoaccepting $tRNA^{Gln}_{CAA}$ seems to be guaranteed only in the presence of isoaccepting $tRNA^{Gln}_{CAG}$.

The drastic reduction of the reading capacity of $tRNA^{Gln}_{CAA}$ for CAG codons is not only evident from the poor incorporation (table 1, column 6), but also from a constantly observed lower ratio of glutamine incorporated into the two chains (table 1, column 7). As the β-chain contains four glutamine residues and the α-chain only one [13], incorporation of glutamine into the β-chain should be relatively more hampered. Furthermore, the low content of only 2% of isoaccepting $tRNA^{Gln}_{CAA}$ within rabbit reticulocytes is consistent with our findings of its poor translational efficiency for rabbit haemoglobin synthesis. Thus, the supply of isoaccepting $tRNAs^{Gln}$ in reticulocytes appears to be correlated to the demand of their functional properties in mRNA translation. The small portion of isoaccepting $tRNA^{Gln}_{CAA}$ presumably is involved in glutamine transfer into nonglobin proteins. The experiments described may also lead to the conclusion, that the main function of the modified first base at the 5′ end of the anticodon of $tRNA^{Gln}_{CAA}$ [2,14] is to avoid misreading rather than to guarantee specific recognition of synonymous codons.

The apparent exclusive presence of the glutamine codon CAG in the five codon positions of α- and β-globin messengers is in good agreement with analogous findings. The codon distribution of the amino acids

glutamic acid [16], lysine [9], and valine [17,18] with the two messengers seems to be uneven: isoaccepting tRNAs which interact only to codons ending with guanine at the 5′ end in the ribosomal binding assay transfer their amino acids into a larger number of amino acid positions as compared to those isoacceptors recognizing the codons with a different base than guanine at the 5′ end.

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