CONVERSION OF *ESCHERICHIA COLI* $tRNA^{Trp}$ TO GLUTAMINE-ACCEPTING tRNA BY CHEMICAL MODIFICATION WITH SODIUM BISULFITE

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1. Introduction

Recently Soll et al. [1,2] showed that the amber suppressor su^+_7 tRNA of *E. coli* which inserts glutamine in response to the amber codon, UAG [3], has the same nucleotide sequence as $tRNA^{Trp}$, except for a U instead of a C in the second position of the anticodon. Thus it seemed likely that chemical modification of *E. coli* $tRNA^{Trp}$ in vitro by sodium bisulfite, which is known to convert C residues in a tRNA molecule to U residues [4–8, for example], should give rise to tRNA molecules which mischarge with glutamine.

This paper describes results of studies on this using $tRNA^{Trp}$ purified from *E. coli*: results showed that a) glutamine-chargeable molecules, when assayed with GlnRS*, appeared following treatment of $tRNA^{Trp}$ with bisulfite. b) This glutaminyl-$tRNA^{Trp}_{HSO_3}$ bound to ribosomes in response to the nonsense codon, UAG, and c) TrpRS inhibited the charging of $tRNA^{Trp}_{HSO_3}$ with glutamine the presence of tryptophan.

2. Materials and methods

E. coli $tRNA^{Trp}$ which accepted 1600 pmol of tryptophan per A_{260} unit was purified from strain B by column chromatographies on DEAE-Sephadex A-50 at pH 7.5 and pH 4.0, BD-cellulose, and RPC-5 at pH 7.5 and pH 4.3 (S. Nishimura, unpublished results, see ref. 9). *E. coli* $tRNA^{Gln}_2$ which accepted 500 pmol of glutamine per A_{260} unit was obtained as described previously [10]. Both tRNAs were generously supplied by Dr S. Nishimura.

GlnRS was prepared from *E. coli* B as described by Folk [11] except that the final step of gel filtration on a Sephadex G-200 column was omitted. Partially purified TrpRS was obtained during preparation of this GlnRS at the step of hydroxylapatite column chromatography. Both preparations were stored at −20°C in the presence of 50% glycerol until used.

E. coli Ribosomes were prepared from *E. coli* B as described by Nishimura et al [12].

[^{14}C]Aminoacyl-tRNA was prepared as described previously [13]. The amino acid acceptor activity of tRNA was assayed as described previously [13], except that sodium cacodylate buffer (pH 7.0) was used in place of Tris–HCl (pH 7.5) in the reaction.

^{14}C-labelled glutamine and tryptophan were obtained from the New England Nuclear Corp. and the Radiochemical Centre, respectively. UpApG and UpApA were gifts from the late Professor T. Ukita, Tokyo University. CpApG was prepared by digestion of *E. coli* tRNA with ribonuclease T_1. Poly (U_4, G) was a commercial product from Miles Laboratory.

3. Results and discussion

3.1. *Appearance of glutamine-accepting $tRNA^{Trp}$ following HSO_3-treatment*

Tryptophan tRNA was dissolved in freshly prepared 3 M $NaHSO_3$ (pH 6.0) – 0.01 M magnesium acetate at a concentration of 10 A_{260} units/ml. Small portions

*Aminoacyl-tRNA synthetases are abbreviated as the standard three-letter designation for each amino acid followed by the letters RS, e.g. GlnRS and TrpRS.

of the solution (0.1–0.2 ml each) were placed in capillary tubes which were then sealed at both ends with parafilm. After incubation for various times at 23°C, samples were diluted by adding 10 vol of 0.15 M NaCl – 0.01 M magnesium acetate – 0.05 M Tris–HCl (pH 9.0) and dialysed at 4°C against the same buffer solution to remove bisulfite, then against 0.15 M NaCl – 0.05 M Tris–HCl (pH 9.0) and finally against 0.05 M Tris–HCl (pH 9.0). Then they were incubated overnight at 37°C in 0.05 M Tris–HCl (pH 9.0) to convert the intermediate 5,6-dihydrouracil 6-sulfonate residues to uracil residues. These procedures were performed as described by Goddard and Schulman [7] with a slight modification. Samples were desalted by dialysis against distilled water at 4°C and assayed for glutamine- and tryptophan-acceptor activity. Fig.1 shows that glutamine-chargeable

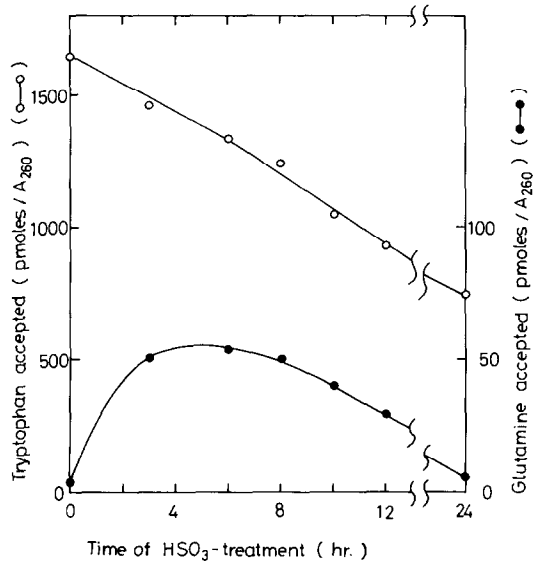

Fig.1. Appearance of glutamine tRNA on treatment of *E. coli* $tRNA^{Trp}$ with bisulfate. The reaction mixtures (100 μl) for assay of tryptophan-acceptor activity (○) contained 0.03 A_{260} unit of samples of $tRNA^{Trp}$ obtained after the various times of bisulfite-treatment indicated, 2 nmol of [^{14}C]tryptophan (specific activity, 52 mCi/mmol), 8 μg of TrpRS and other components as described in the Materials and methods. For measurement of glutamine-acceptor activity (●), 0.5 A_{260} unit of the same tRNA, 2.2 nmol of [^{14}C] glutamine (specific activity, 55 mCi/mmol), and 70 μg of GlnRS were used in place of 0.03 A_{260} unit of tRNA, [^{14}C] tryptophan and TrpRS, respectively, in the above reaction mixture. Mixtures were incubated at 30°C for 10 min for the former reaction and at 30°C for 30 min for the latter

molecules appeared following treatment with HSO_3 and a maximum value of 50 pmol per A_{260} unit was obtained after 3 hr treatment, while about 150 pmol per A_{260} unit of $tRNA^{Trp}$ lost the ability to accept tryptophan. The former value corresponded to about 3% of the initial $tRNA^{Trp}$. Later we found that the value increased to over 5% if more GlnRS (up to 1 mg) and a higher concentration of glutamide (0.1 mM) were used (data not shown). It seemed likely that the C residues in the CCA terminus of the glutamine-chargeable tRNA molecules underwent a C → U transition that destroyed the amino acid-accepting ability of the tRNAs. If this was the case, the glutamide-acceptor activity of these tRNAs should be restored by enzymatic replacement of the CCA terminal sequence [14]. Then, the value would increase still further from the above value of 5%.

3.2. *Ribosome-codon binding of glutaminyl-$tRNA^{Trp}_{HSO_3}$*

Since the glutamine-chargeable tRNA appears to be formed by HSO_3-treatment of $tRNA^{Trp}$, it seemed interesting to examine the site of C→U transition that caused the tRNA to mischarge with glutamine. Studies on the structure of the nonsense suppressor su^+_7 tRNA [2] suggested the possibility of a C→U transition in the second position of the anticodon in glutamine-accepting $tRNA^{Trp}_{HSO_3}$. In the present work this was examined by measuring the codon response of this tRNA.

[^{14}C]Glutaminyl-$tRNA^{Trp}_{HSO_3}$ was prepared from $tRNA^{Trp}$ which had been treated with bisulfite for 4 hr (fig.1) and stimulation of its binding to ribosomes by the amber codon, UAG, or ochre codon, UAA, was tested. Table 1 shows that the binding of [^{14}C] glutaminyl-$tRNA^{Trp}_{HSO_3}$ was specifically stimulated by UpApG, and not by the glutamine codon, CpApG, or poly (U_4, G), which contains the tryptophan codon, UGG. The binding of [^{14}C]tryptophanyl-$tRNA^{Trp}_{HSO_3}$, used as a control, was stimulated by poly (U_4, G) but not by UpApG. Thus it appears that bisulfite treatment caused a C→U transition in the second position of the anticodon of $tRNA^{Trp}$, thus forming glutamine-mischargeable tRNA. The data are quite in agreement with results showing that glutamine-inserting su^+_7 tRNA is a transcription product of the structural gene for $tRNA^{Trp}$ formed by a single mutation which results in a C→U transition in the anticodon of $tRNA^{Trp}$ [1,2]. The possibility that additional C→U transitions

Table 1
Codon response of $tRNA^{Trp}_{HSO_3}$ which mischarges with glutamine

tRNA	tRNA bound to ribosomes (Δ cpm) Template				
	None	UpApG	UpApA	Poly (U_4, G)	CpApG
[^{14}C]Gln-$tRNA^{Trp}_{HSO_3}$	(70)	200	2	60	10
[^{14}C]Trp-$tRNA^{Trp}_{HSO_3}$	(100)	0	0	320	–
[^{14}C]Trp-$tRNA^{Trp}$	(75)	50	5	410	–
[^{14}C]Gln-$tRNA^{Gln}_2$	(75)	10	0	–	510
[^{14}C]Gln-$tRNA^{Gln}_1$	(50)	10	–	–	50

Ribosome binding was assayed by the procedure of Nirenberg and Leder [15] in a volume of 50 μl containing 0.02 M magnesium acetate, 0.05 M KCl, 0.1 M Tris–HCl (pH 7.5), 1 A_{260} unit of ribosomes and 0.1 A_{260} unit of trinucleotides or polynucleotides as specified. The specific activities of [^{14}C]glutamine and [^{14}C]tryptophan were 178 mCi/mmol and 52 mCi/mmol, respectively. Approx. 4000 cpm of [^{14}C]aminoacyl-tRNA were assayed. Values show the binding of aminoacyl-tRNA to ribosomes in the presence of a template minus the binding in the absence of the template. Values are averages of results of three separate experiments.

occurred in other regions of the glutamine-accepting $tRNA^{Trp}_{HSO_3}$ was not examined in the present study. It should be noted that the binding of glutaminyl-$tRNA^{Trp}_{HSO_3}$ to ribosomes was not stimulated by the ochre codon, UAA (table 1), while a mutant of su^+_7 tRNA which suppresses the ochre codon is reported to have C → U transitions in both the first and second positions of the anticodon [2]. However, it is uncertain whether the mutant su^+_7 tRNA inserts glutamine into the ochre codon.

3.3. *Amount of GlnRS required for the charging reaction of HSO_3-treated $tRNA^{Trp}$*

Fig.2 shows that much more GlnRS was required for maximum charging of $tRNA^{Trp}_{HSO_3}$ with glutamine than for charging of $tRNA^{Gln}_2$. Glutamine-charging of $tRNA^{Gln}_2$ was not influenced by the presence of about 20-fold excess of $tRNA^{Trp}$ in the reaction. Hence, the requirement of a large amount of GlnRS for charging of glutamide-accepting $tRNA^{Trp}_{HSO_3}$ is probably not due to some sort of interference by $tRNA^{Trp}$ that predominates over glutamine-accepting $tRNA^{Trp}_{HSO_3}$. The data in fig.2 agree with results showing that su^+_7 tRNA required 30 times more GlnRS than $tRNA^{Gln}$ for charging glutamine [2].

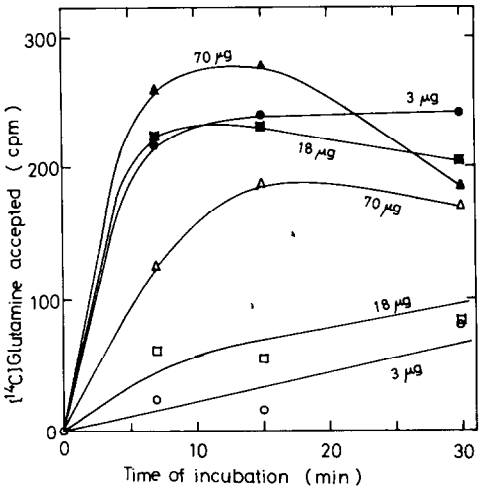

Fig.2. Glutamine-charging of bisulfite-treated $tRNA^{Trp}$ with various amounts of GlnRS. Reaction mixtures (100 μl) contained 12 nmol of [^{14}C]glutamine (specific activity, 41 mCi/mmol), the various amounts of GlnRS specified, 0.2 A_{260} unit of $tRNA^{Trp}$ treated with bisulfite for 8 hr (open symbols) or 0.035 A_{260} unit of $tRNA^{Gln}_2$ (closed symbols), and other components as described in the Materials and methods.

3.4. *Inhibition by TrpRS of glutamine-charging of HSO_3-treated $tRNA^{Trp}$*

The possibility that glutamine-chargeable tRNAs in the HSO_3-treated $tRNA^{Trp}$ still maintain the specificity to interact with TrpRS was tested by adding TrpRS in the glutamine-charging reaction of $tRNA^{Trp}_{HSO_3}$. The glutamine-charging tRNA was not separated from the remainder of the $tRNA^{Trp}_{HSO_3}$, so its tryptophan-acceptor activity was not measured directly. As shown in table 2, glutamine-charging was inhibited by the presence of TrpRS and unlabeled tryptophan. No significant inhibition was observed on addition of either TrpRS or tryptophan alone. Moreover no inhibition was observed in a control, using a mixture of $tRNA^{Gln}_2$ and a large excess (20 fold) of $tRNA^{Trp}$ in place of $tRNA^{Trp}_{HSO_3}$. Hence, it seems that the inhibition was specific for glutamine-accepting tRNA obtained by treatment of $tRNA^{Trp}$ with bisulfite. The results could be explained as follows; i) the glutamine-accepting $tRNA^{Trp}_{HSO_3}$ accepted tryptophan in the reaction of TrpRS in competition with glutamine-charging in the reaction of GlnRS, and consequently radioactivity in [^{14}C]glutaminyl-$tRNA^{Trp}_{HSO_3}$ decreased, or ii) although TrpRS could not charge glutamine-accepting $tRNA^{Trp}_{HSO_3}$ with tryptophan, the enzyme together with tryptophan could compete with GlnRS in the step of binding to tRNA in the process of aminoacylation reaction. If the latter is the case, TrpRS may undergo some conformational change when tryptophan binds to it, enabling it to interact with glutamine-accepting $tRNA^{Trp}_{HSO_3}$. Experiments should be performed with glutamine-accepting tRNA isolated from $tRNA^{Trp}_{HSO_3}$ to decide between two alternatives. It should be noted that su^+_7 tRNA is reported to be unable to accept tryptophan with TrpRS (cited in [2]).

Thus the present results support on the genetical origin of glutamine-inserting su^+_7 tRNA of *E. coli*, indicating that it is a transcription product of the structural gene for $tRNA^{Trp}$ in which a single mutation has resulted in a C→U transition in the anticodon [1,2].

Work is in progress on isolation of glutamine-chargeable tRNA from HSO_3-treated $tRNA^{Trp}$ to determine the sites of C→U transition in it by nucleotide sequence analysis and also to examine its amino acid specificity.

Acknowledgements

I am greatly indebted to Dr S. Nishimura for

Table 2
Inhibition by TrpRS of glutamine-charging of $tRNA^{Trp}_{HSO_3}$

	μg TrpRS with 70 μg GlnRS	Tryptophan (0.1 mM)	[^{14}C]Gln charged (cpm)	Inhibition (%)
$tRNA^{Trp}_{HSO_3}$	0	–	810	0
	80	–	730	10
	230	–	650	20
	0	+	720	11
	80	+	470	42
	230	+	180	78
$tRNA^{Gln}_2$ + $tRNA^{Trp}$	0	–	430	0
	230	–	470	−10
	0	+	420	2
	230	+	370	14

Reaction mixtures (100 μl) contained 0.17 A_{260} unit of $tRNA^{Trp}$ treated with bisulfite for 3 hr or 0.015 A_{260} unit of $tRNA^{Gln}_2$ mixed with 0.3 A_{260} unit of normal $tRNA^{Trp}$, 10 nmol of [^{14}C]glutamine (specific activity, 49 mCi/mmol) and other components as indicated in the table. Mixtures were incubated at 30°C for 15 min. For other reaction conditions, see Materials and methods.

generous supply of tRNAs and for stimulating discussions. I would also like to thank Dr D. Söll of Yale University who encouraged me to start this work during my visit to his laboratory in 1972. I am also grateful to Dr H. Hayatsu of Tokyo University for information on bisulfite treatment of tRNA, to the late Professor T. Ukita of Tokyo for kind gifts of trinucleotides, and to Ms A. Shirai for participation in the earlier part of this work.

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