NUCLEOTIDE SEQUENCE OF $tRNA_2^{Gly}$ FROM THE POSTERIOR SILK GLANDS OF *BOMBYX MORI*

Makoto KAWAKAMI, Kohji NISHIO and Shosuke TAKEMURA

Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusaku, Nagoya, Japan

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1. Introduction

The posterior silk glands of *Bombyx mori* exclusively produce fibroin from day 5–8 of the 5th instar. In proportion to the amino acid content of fibroin, this organ synthesizes much larger amounts of tRNAs specific for glycine, alanine and serine than those specific for the other amino acids [1,2]. Because of the characteristic synthesis and accumulation of the particular tRNA species, the silk glands should be promising as a system to study on the correlation of tRNA and mRNA syntheses. In spite of an interest to the function of the posterior silk glands, sequencing of tRNA from this organ was scarcely carried out so far. Glycine tRNA from the posterior silk glands is composed of two major species, i.e., $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ [3]*. We present here the primary structure of the $tRNA_2^{Gly}$.

* The two isoaccepting $tRNAs^{Gly}$ from the posterior silk glands of *B. mori* are described [3] as $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ in the order of elution from a DEAE-Sephadex A-50 column. Here, the previous naming is changed to another one, in which they are expressed in the order of their amounts. Thus $tRNA_1^{Gly}$ and $tRNA_2^{Gly}$ in this paper respectively correspond to $tRNA_2^{Gly}$ and $tRNA_1^{Gly}$ designated [3].

Abbreviations are used according to the 1969 recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature for abbreviations and symbols for nucleic acids, polynucleotides and their constituents.

2. Materials and methods

Unfractionated tRNA was prepared from the posterior silk glands of *B. mori*, hybrid from Japanese and Chinese strains, on day 5 and 6 of the 5th instar as in [3]. Glycine $tRNA_2$ was isolated by chromatographic procedures on columns of DEAE–Sephadex A-50 and benzoylated DEAE-cellulose after naphtoxyacetylation of glycyl-$tRNA_2^{Gly}$ as in [4]. The purity was estimated to 90% from the glycine accepting activity.

The purified $tRNA_2^{Gly}$ was completely digested with pancreatic RNAase and with RNAase T_1. The products were separated by chromatography on a DEAE–Sephadex A-25 column and then by rechromatography on the same column in 7 M urea in acidic conditions or an AG 1 × 2 column in acidic conditions. The fragments thus obtained were identified by further enzymatic digestion, i.e., pancreatic RNAase, RNAase T_1, RNAase T_2, RNAase U_2, RNAase P_1, snake venom phosphodiesterase, silkworm endonuclease, and bacterial alkaline phosphatase were used with slight modifications of conditions in [5,6]. In order to overlap the fragments, some larger oligonucleotides were isolated from a partial RNAase T_1 digest of the tRNA. From the results a unique sequence was deduced.

3. Results and discussion

Figure 1 shows the nucleotide sequence of *B. mori* $tRNA_2^{Gly}$ arranged in a clover leaf model. The chain

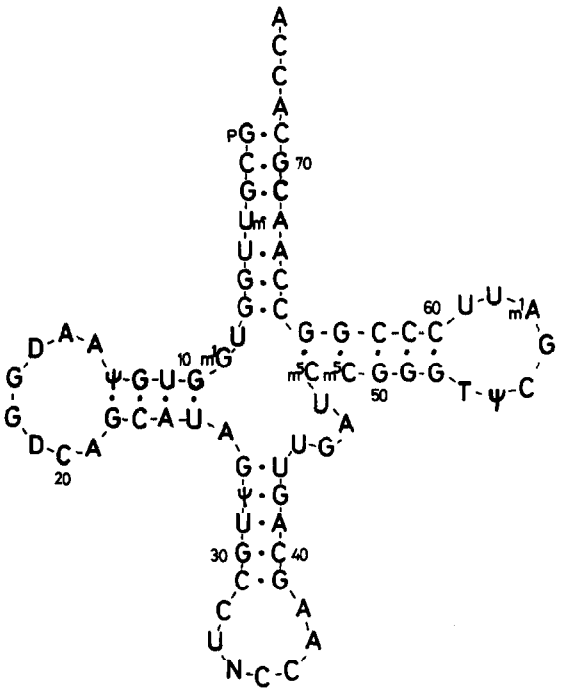

Fig. 1. Nucleotide sequence of $tRNA_2^{Gly}$ from the posterior silk glands of *Bombyx mori*. The $tRNA_2^{Gly}$ contains the N–C–C anticodon, in which N contains 2 unknown modified nucleosides, *N and **N. They seem to be the derivative of uridine.

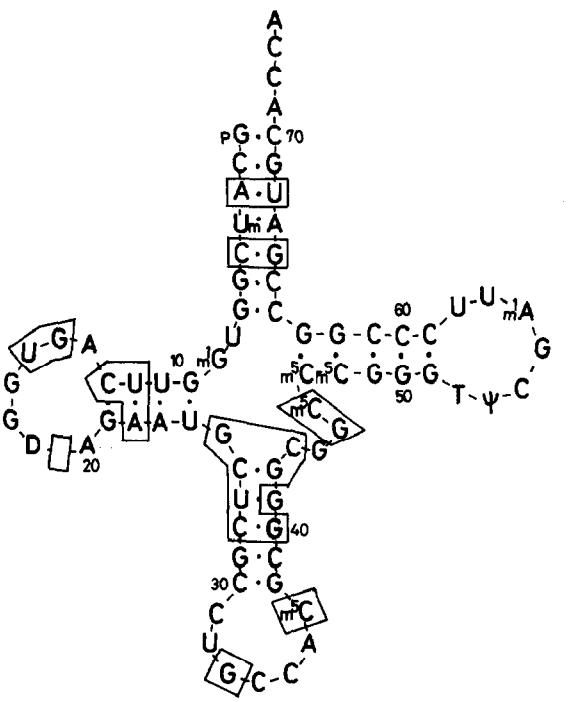

Fig.2. Nucleotide sequence of $tRNA_1^{Gly}$ from the posterior silk glands of *B. mori* [7]. The boxes show the sequences different from *B. mori* $tRNA_2^{Gly}$.

length of this tRNA is 75 including 15 nucleosides invariable in almost all tRNAs; i.e., the nucleosides and their positions from the 5′-terminus are U8, A14, G17, G18, A21, U33, G52, T53, Ψ54, C55, m^1A57, C60, C73, C74, and A75. Besides the modified nucleosides included in the invariable positions, this tRNA contains the following modified nucleosides; Um4, m^1G9, Ψ13, D16, D19, Ψ28, N34 (unknown modified nucleosides), m^5C48, and m^5C49. The nucleoside Um is found also in position 4 in *B. mori* $tRNA_1^{Gly}$ [7]. In $tRNAs^{Gly}$ from yeast [8] and wheat germ [9], Um of position 4 is replaced to Cm. In prokaryotic $tRNAs^{Gly}$ from *Escherichia coli* [10–12], *Salmonella typhimurium* [12] and *Staphylococcus epidermidis* [13], however, no modified nucleoside is found in this position. The occurrence of 2′-*O*-methyl group in this position seems to be characteristic of eukaryotic $tRNA^{Gly}$. Prokaryotic $tRNAs^{Gly}$ contain U in the 4th nucleoside from the 3′-terminus, whereas eukaryotic $tRNAs^{Gly}$ from *B. mori*, wheat germ and yeast contain A.

Figure 2 shows the sequence of *B. mori* $tRNA_1^{Gly}$ reported [7]. It contains a G–C–C anticodon which is able to decode GGU found in fibroin mRNA [14]. The sequences of *B. mori* $tRNA_2^{Gly}$ and $tRNA_1^{Gly}$ differ in 21 residues. In a dihydrouridine loop the $tRNA_1^{Gly}$ lacks C which is found in position 20 of the $tRNA_2^{Gly}$. Hence the chain length of the $tRNA_1^{Gly}$ is 74, 1 residue shorter than the $tRNA_2^{Gly}$. The $tRNA_2^{Gly}$ contains 2 contiguous residues of m^5C in the T–Ψ–C stem as does the $tRNA_1^{Gly}$. The $tRNA_1^{Gly}$ contains 2 more residues of m^5C at the anticodon and the extra loops. The $tRNA_1^{Gly}$ contains only 1 residue of Ψ located at the T–Ψ–C loop. In the $tRNA_2^{Gly}$, 3 Ψ residues are found in the dihydrouridine stem, the anticodon stem and the T–Ψ–C loop.

In position 34 of the $tRNA_2^{Gly}$, we found equimolar amounts of 2 unknown nucleosides, *N and **N, as the first letter of the anticodon. In fig.1 these are shown by N. The ultraviolet spectra of *N and **N resemble those of T, 5-carboxymethyl U

(cm^5U) and the methylester of cm^5U, but *N and **N could not be identified as any of these known nucleosides because the unknowns showed different mobility from the knowns in several solvents of thin layer chromatography. Each of the unknowns is possibly the derivatives of uridine. The nucleotide *Np was eluted together with mononucleotides from a DEAE–Sephadex A-25 column with a NaCl concentration gradient in 0.02 M Tris–HCl buffer (pH 7.5) containing 7 M urea; however, **Np was eluted together with dinucleotides. Accordingly, **Np should contain a negatively-charged group, e.g., carboxyl group, in addition to phosphate. These facts show that the purified $tRNA_2^{Gly}$ is composed of $tRNA_{2a}^{Gly}$ including *N and $tRNA_{2b}^{Gly}$ including **N. However, we cannot still separate them from each other.

From the experimental results of glycine codon-dependent glycyl-tRNA binding to ribosomes, we know that the $tRNA_2^{Gly}$ can decode GGA well and GGG weakly (unpublished data). Since both of *N and **N were found in the anticodon in *B. mori* $tRNA_2^{Gly}$, it remains unsolved whether or not both of the 2 anticodons, *N–C–C and **N–C–C, can base-pair with a glycine codon GGA which is present predominantly in fibroin mRNA together with GGU [14].

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