The nucleotide sequence of *Scenedesmus obliquus* chloroplast elongator methionine-accepting tRNA

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The chloroplast elongator methionine-accepting tRNA from the green alga *Scenedesmus obliquus* was purified and its nucleotide sequence determined. The sequence does not bear close homology to higher-plant chloroplast tRNA^{Met} species, nor to the chloroplast tRNA^{Met} species from *Euglena gracilis*. This tRNA is unique among all tRNAs in having pseudouridine at position 8, and this has implications with regard to the proposed interaction of aminoacyl-tRNA synthetases with their cognate tRNAs at this position. The primary sequence of the tRNA is: pG-C-C-U-G-C-U-Ψ-A-G-C-U-C-A-G-U-D-Gm-G-C-C-A-G-A-G-C-A-U-C-C-G-Ψ-Ψ-U-C-A-U-m²A-C-G-C-G-G-A-A-m⁷G-D-C-A-C-U-A-G-T-Ψ-C-G-A-A-U-C-U-A-G-U-A-G-C-A-G-G-C-A-C-C-A-OH.

INTRODUCTION

Although the sequences of several different elongator methionine-accepting tRNAs from the bacterial, animal and plant kingdoms have been published [1], the structure of only one chloroplast tRNA^{Met} (that from spinach [2]) has been fully determined. Previously we have published the sequences of the cytoplasmic [3] and chloroplast [4] initiator tRNA^{Met} species from the unicellular green alga *Scenedesmus obliquus* and have reported the partial sequence of the cytoplasmic elongator tRNA^{Met} species [5]. We now report the sequence of a *S. obliquus* chloroplast tRNA^{Met} species, which has enabled us to compare the sequences of chloroplast tRNA^{Met} from the higher-plant and lower-plant kingdoms. Also, by determination of the RNA sequence (rather than deduction of the RNA sequence from the gene structure), we have been able to study the pattern of modification to the nucleosides. Other tRNAs from *S. obliquus* have shown unique features in their modification patterns [6, 7], and here also in the tRNA^{Met} species several unique and interesting modifications have been observed. The possible functional implications of these modifications are discussed.

A preliminary report of this tRNA sequence was presented at the EMBO–FEBS 9th International tRNA Workshop, Strasbourg.

MATERIALS AND METHODS

T4 RNA ligase was obtained from PL Biochemicals, and [5′-³²P]pCp was either prepared according to the method of Silberklang *et al.* [8] or purchased at 2000–3000 Ci/mmol from Amersham International. Otherwise chemicals, enzymes, radiochemicals and chromatographic materials were obtained or prepared as described previously [3]. Total crude tRNA was isolated

from *S. obliquus* by the method of Osterman *et al.* [9]. The chloroplast tRNA^{Met} was purified by successive steps of column chromatography on BD-cellulose, arginine–agarose and two stages of RPC-5 [10], when it had methionine-acceptance activity in the peak fractions (material from which was used for sequential analysis) of approx. 2000 pmol of methionine/A₂₆₀ unit of tRNA with *Escherichia coli* methionyl-tRNA synthetase (see ref. [10]). The tRNA hybridized to the chloroplast rather than to the nuclear fractions of *S. obliquus* DNA fractionated by CsCl-density-gradient centrifugation [10].

The sequence was deduced by using a combination of techniques including the ‘limited-hydrolysis’ method [11], mobility-shift analysis [8] on 5′- and 3′-end-labelled tRNA and direct read-off gels [12]. For this last-mentioned method 5′-end-labelled tRNA was cleaved by a series of partial enzymic digestions [3], whereas for 3′-end-labelled tRNA the tRNA was cleaved either by partial enzymic digestions or by specific chemical reactions [13]. The modified nucleosides were identified by the tritium derivative method [14] and by the results of the ‘limited-hydrolysis’ procedure after two-dimensional t.l.c. by the methods of Silberklang *et al.* [8] and Nishimura [15]. The 5′- and 3′-terminal nucleotides were characterized after total digestion of 5′-³²P-labelled tRNA with nuclease P₁ and 3′-³²P-labelled tRNA with ribonuclease T₂.

RESULTS

Most of the sequence information including the nucleoside modifications was obtained from the ‘limited-hydrolysis’ method. From this method it was possible to deduce all of the sequence from residues 4 to 71 except for residue 27, for which no band was obtained on the gel. The modified nucleosides Ψ-8, D-17, Gm-18, Ψ-31,

Abbreviations used: m²A, 2-methyladenosine; m⁶A, N⁶-methyladenosine; t⁶A, N-[(9-β-D-ribofuranosylpurin-6-yl)carbamoyl]threonine; mt⁶A, N-[(9-β-D-ribofuranosylpurin-6-yl)-N-methylcarbamoyl]threonine; D, dihydrouridine; m⁷G, 7-methylguanosine; Gm, 2′-O-methylguanosine; T, 5-methyluridine; Ψ, pseudouridine; s⁴U, 4-thiouridine.

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Fig. 1. Identification of modified nucleosides at positions 8 and 37 of *S. obliquus* chloroplast tRNA_{Met}

5'-³²P-labelled mononucleotides, obtained from the 'limited-hydrolysis' method [11], were subjected to two-dimensional t.l.c. on cellulose. (a) First dimension, isobutyric acid/conc. NH₃/water (66:1:33, by vol.); second dimension, 0.1 M-sodium phosphate buffer (pH 6.8)/solid (NH₄)₂SO₄/propan-1-ol (50:30:1, v/w/v). (b) First dimension, isobutyric acid/conc. NH₃/water (124:3:73, by vol.); second dimension, propan-2-ol/conc. HCl/water (14:3:3, by vol.).

Ψ-32, m³A-37, m⁷G-46, D-47, T-54 and Ψ-55 were identified by using the two-dimensional t.l.c. system described by Silberklang *et al.* [8]. The identities of Ψ-8 and m²A-37 were confirmed by using the two-dimensional t.l.c. system described by Nishimura [15] (see Fig. 1).

Owing to the inefficient 5'-³²P-labelling of *S. obliquus* chloroplast tRNA_{Met} [16], rapid read-off gel sequencing was severely limited. However, sufficient labelled material was obtained to perform one sequencing experiment, and from this it was possible to obtain sequence confirmation from residues 8 to 49. It was not possible to distinguish

between all of the C and U residues, and the modified nucleosides were not identified. 'Mobility-shift' analysis performed on [5'-³²P]tRNA_{Met} gave sequence information on residues 2-9. This provided the sequence of residues 2 and 3 and confirmed the sequence 4-9.

3'-([5'-³²P]pCp)-labelled tRNA_{Met} was used for 'mobility-shift' analysis, and direct read-off gels where the tRNA was digested partially with ribonuclease T₁, ribonuclease U₂, *Physarum polycephalum* nuclease or *Bacillus cereus* nuclease [3] or by chemical cleavage as described by Peattie [13]. Although from these experi-

Fig. 2. Clover-leaf structure of *S. obliquus* chloroplast tRNA_m^{Met}

ments the results were not as clear-cut as the results from the 5'-labelled material, valuable information at the 3'-end of the molecule was obtained. From these methods partial sequence information was given for residues 63–73, 10–71, and 59–75 respectively.

The 5'- and 3'-end residues were shown to be C and A respectively.

From the data the sequence for *S. obliquus* chloroplast tRNA_m^{Met}, which is shown in Fig. 2 in clover-leaf form,

was deduced. The direct read-off gels indicated that residue 27 is most likely to be U, but we do not have evidence to show whether or not it is modified.

DISCUSSION

Comparisons of *S. obliquus* chloroplast tRNA_m^{Met} with tRNA_m^{Met} species from other sources [1] do not reveal many similarities. Only one other chloroplast tRNA_m^{Met} structure, that from spinach, has been fully determined [2], but the sequences of other chloroplast tRNA_m^{Met} species can be deduced (except for nucleoside modifications) from gene structures [17]. *S. obliquus* tRNA_m^{Met} has only 70%, 68%, 66% and 67% homology with tRNA_m^{Met} species from maize, tobacco, spinach and *Euglena gracilis* respectively. The higher-plant chloroplast species, themselves, however, have very close homology (96–99%). Also, *S. obliquus* tRNA_m^{Met} has 77 nucleotides (similarly to *E. coli* tRNA_m^{Met}) whereas the other chloroplast species have 76.

The suggestion that organelle tRNA_m^{Met} species may have certain characteristic common residues [2] (e.g. A-1, A-10, C-16, Ψ-25, U-32, C-38, G-39, A-45, A-57, A-63 and U-72) is given little support by the sequence of *S. obliquus* tRNA_m^{Met}, which has only G-39, A-45, A-57 and A-63 of these residues in common.

Two unusual modifications occur in *S. obliquus* chloroplast tRNA_m^{Met}. These are Ψ-8 and m²A-37. This is the only tRNA having Ψ at position 8. Position 8 is universally occupied by a U residue, but in other tRNAs this is either an unmodified uridine or s⁴U. However, the modification of U-8 to Ψ-8 could still allow this residue to hydrogen-bond by reverse Hoogsteen pairing to A-14 of the D arm, an interaction important in the maintenance of the tertiary structure of tRNA [18,19].

The uridine at position 8 has been implicated as a site for the interaction between tRNAs and aminoacyl-tRNA synthetases [20,21,22]. It has been shown that when *E. coli* isoleucyl-tRNA synthetase and tRNA^{Ile} are incubated together in tritiated water there is an exchange of

Scheme 1. Mechanisms by which an aminoacyl-tRNA synthetase-bound nucleophilic group (X) may bind reversibly to carbon atom 6 of the residue at position 8 of a tRNA

(a) Uridine at position 8 [23]. (b) Pseudouridine at position 8.

hydrogen for tritium at carbon atom 5 of the base in U-8 of tRNA^{11e} [20]. The mechanism proposed for this is shown in Scheme 1(a). The scheme is an example of a Michael addition. The carbon atom at position 6 of the uridine is in an $\alpha\beta$ -unsaturated carbonyl system and is a site for nucleophilic attack by an enzyme-bound group. This enables tritium present in the solution to compete at position 5. As the reaction is envisaged as being reversible, a gradual exchange of hydrogen for tritium occurs at this position. The scheme involving the carbon atom at position 6 of U-8 as a site for synthetase binding is not unique to *E. coli* isoleucyl-tRNA synthetase. Similar schemes have been proposed for *E. coli* valyl-, alanyl- and tyrosyl-tRNA synthetases and also for mammalian tryptophyl-tRNA synthetase [21]. The replacement of U by Ψ at position 8 would require some modification to the mechanism proposed. Scheme 1(b) shows a possible scheme for nucleophilic attack by an enzyme-bound group on carbon atom 6 of Ψ . It will be seen that Ψ also contains an $\alpha\beta$ -unsaturated carbonyl group with carbon atom 6 at one end. However, its orientation in the molecule is different, since carbon atom 5 in this case is used for the glycosidic linkage to ribose. Nevertheless, binding of an enzyme-bound nucleophile to carbon atom 6 of Ψ -8 is still theoretically possible.

According to Nishimura [15] codons beginning with A nearly always result in their complementary tRNAs having t⁶A or mt⁶A at position 37. *E. coli*, yeast and mammalian tRNA^{Met} species have t⁶A-37, whereas plant cytoplasmic tRNA^{Met} species have mt⁶A-37 [1]. *S. obliquus* chloroplast tRNA^{Met} has m⁶A, *Bacillus subtilis* tRNA^{Met} has m⁶A [24] and spinach chloroplast tRNA^{Met} has an unmodified A at position 37 [2]. It is noteworthy that these latter three tRNA species all have C at position 38 whereas all of those tRNAs having t⁶A-37 or mt⁶A-37 have A at position 38. It may be that an A at position 38 is required for modification of A-37 to t⁶A-37. Alternatively there may be a functional reason for the anomalous modification of A-37. The conformation of the codon-anticodon interaction when A-38 is absent may exclude t⁶A as a possible modification of residue 37.

One other modification, that of Ψ -32, is of note. No other tRNA^{Met} species has this modification, but it is found in other amino acid-accepting tRNA species.

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