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Nucleotide sequence of a cucumber chloroplast proline tRNA

SHAILAJA HANDE and C JAYABASKARAN*
Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

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Abstract. The nucleotide sequence of a proline tRNA (anticodon UGG) from cucumber chloroplasts has been determined. The sequence is: pAAGGAUGUAGCGCAGCUUCA-DAGCGCAΨUUGUUUUGGNΨFACAAAAUm^{7}GUCACGGGTΨCAAAUCCUG-UCAUCCUUACCA$_{OH}$. It shows 93% homology with spinach chloroplast tRNA^{Pro} (UGG) and 72% homology with bean mitochondrial tRNA ^{Pro} (UGG), the other two known plant organellar tRNAs^{Pro}.

Keywords. Nucleotide sequence; cucumber chloroplast; proline tRNA.

1. Introduction

Chloroplasts contain their own protein-synthesizing apparatus. All tRNAspecies involved in chloroplast protein synthesis are believed to be encoded by chloroplast DNA. Although detailed studies on sequence analyses of the completely sequenced chloroplast genomes of four land plants, namely tobacco (Shinozaki *et al* 1986), rice (Hiratsuka *et al* 1989), liverwort (Ohyama *et al* 1986) and maize (Maier *et al* 1995) have revealed the structures of approximately 30 to 32 different tRNA genes in each of these chloroplast genomes, knowledge of their RNA sequences including modified nucleosides is still poor. The information about occurrence and position of modified nucleotides in the primary structures have been of particular interest because several modified nucleotides have been shown to be associated with a range of biological functions, such as maintenance of translational fidelityand efficiency, codon usage and tRNA-protein interactions (Persson 1993). Only a few plant chloroplast tRNAs have been sequenced so far. They are tRNA^{Glu} (UUC) and tRNA^{Gln} (UUG) from *Hordeum vulgäre,* tRNA^{Ile} (GAU) from maize, tRNA^{Leu} (CAA), tRNA^{Leu}(UAA), tRNA^{Leu}(UAG) from soybean, tRNA ^{Phe} (GAA), tRNA^{Leu} (CAA), tRNA^{Leu} (UAA), tRNA^{Leu} (UAG) and tRNA^{Trp} (CCA) from bean and tRNA^{phe} (GAA), tRNA^{Ile} (GAU), tRNA^{Ile} (NAU), tRNA^{Leu} (UAG), tRNA^{Met} (CAU), tRNA^{Pro} (UGG), tRNA^{Thr} (GGU), tRNA^{Trp} (CCA) and tRNA^{Val} (UAC) from spinach (Sprinzl *et al* 1989). In order to understand primary and secondary structures of plant chloroplast tRNAs, a detailed study of cucumber chloroplast tRNAs has been undertaken. Here we present the nucleotide sequence of a cucumber chloroplast proline tRNA.

2. Materials and methods

2.1 *Materials*

Cucumber seeds were obtained from National Seed Corporation, Bangalore, Acryla mide, DEAE-cellulose and nuclease PI were purchased from Sigma Chemicals Co. USA.

*Corresponding author (Fax, 91-80-3341814, 3341683; Email, cjb@biochem.iisc.ernet.in).

Cellulose thin layer plates were obtained from Mechery-Nagel, Germany. T4 polynuc leotide kinase was purchased from New England BioLabs. [γ^{-32} P] ATP was obtained from Board of Radiation and Isotope Technology, Mumbai.All other chemicals used were of analytical grade.

2.2 *Purification of cucumber chloroplast tRNA^{Pro}*

Total cucumber chloroplast tRNA was isolated from cucumber cotyledons as de scribed previously (Jayabaskaran and Hande 1995). Cucumber chloroplast tRNA^{Pro} was purified by the combined use of RPC-5 column chromatography and two-dimensional Polyacrylamide gel electrophoresis (PAGE) procedure (Jayabaskaran and Puttaraju 1993).

2.3 *Sequencing of tRNA*

To determine the primary structure of the tRNA^{Pro}, we used single hit hydrolysis in deionized formamide followed by T4 polynucleotide kinase [^{32}P]-labelling, PAGE separation of labelled fragments and enzymatic analysis of the end nucleotides by one-dimensional thin-layer chromatography (TLC) as previously described (Jayabas karan and Puttaraju 1993). Two-dimensional TLC was employed for the identification of modified nucleotides using the solvents isobutyric acid: 25% NH_4 OH: H_2O (66:1:33 v/v/v) in the first dimension and isopropanol: Cone. HCl: H_2O (70:15:15 v/v/v) in the second dimension (Keith 1995).

2.4 *Computer analysis*

Sequence comparisons were carried out using the GCG software package (Genetics Computer Group) on Micro VAXII VMS version 4·6 operating system.

3. Results and discussion

While purification of isoleucine isoacceptor tRNAs from cucumber total chloroplast tRNA by RPC-5 column chromatography followed by identification of individual fractions for isoleucine-accepting activity and two-dimensional PAGE of the tRNA^{Ile} enriched fractions (Hande 1992), one of the 19 major spots well separated on the 2D-gel was identified by amino acylation to be a tRNA^{Pro} (data not shown) and its two-dimensional PAGE mobility corresponds to tRNA^{Pro} reported earlier (Jayabaskaran and Hande 1995). The tRNA was further purified by denaturing 15% PAGE and subjected to the sequence analysis. The sequence of this tRNA in the clover leaf form is shown in figure 1. Its length is 76 nucleotides including 7 modified nucleotides. These modified nucleotides are: one each of D, m^7G, T and N (unknown modified nucleoside) and three Ψ.

We have compared all 11 known tRNAs^{Pro} (UGG) sequences with the cucumber tRNA^{Pro} (UGG). As shown in table 1 it is very similar to spinach chloroplast tRNA^{Pro} (UGG) (93% homology). It shows 72% homology to bean mitochondrial tRNA^{Pro} (UGG) but is considerably less homologous with *S. cerevisiae* mitochondrial tRNA^{Pro}

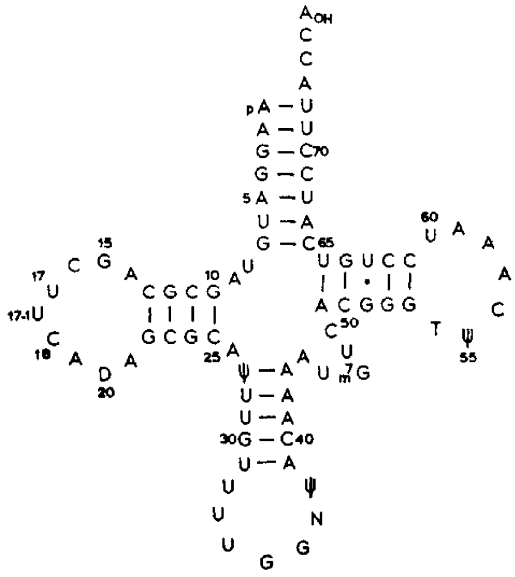

Figure 1. Secondary structure of cucumber chloroplast tRNA^{Pro}(UGG). The numbering system of nucleotides is according to Sprinzl *et al* (1989). N (37): unidentified modified nucleotide.

Table 1. Similarity comparison between the cucumber chloroplast tRNA^{Pro} (UGG) and its homologues in other species and organelles. All these tRNA sequences can be found in Sprinzl *et al* (1989).

Species	Percentage
Spinacia oleracea (chloro)	93
Phaseolus vulgaris (mito)	72
Bacillus subtilis	69
Mycoplasma mycoid	68
Salmonella typhimurium	64
Phage T4	59
Saccharomyces cerevisiae	49
Saccharomyces cerevisiae (mito)	44
Halobacterium volcanii	44
Phage T5	43
Torulopsis utilis	43

(UGG) (44% homology). This higher homology with plant organelle tRNAs^{Pro} sug gests that tRNAs^{Pro} from these organelles in plants may be similar with respect to their structure-function relationship. It should be noted that the cucumber tRNA^{Pro} (UGG) exhibits greater homology with eubacterial (64-69% homology) than with its archaebacterial counterparts.

It has been shown previously upon fractionation of cucumber chloroplast tRNA population by two-dimensional PAGE (Jayabaskaran and Hande 1995) that cucumber chloroplast contained only one $tRNA^{Pro}$. Fractionation and identification of chloro plast tRNAs of bean, spinach maize and *Euglena* on two-dimensional gels has also revealed one proline tRNA (Mubumbila *et al* 1980). Moreover, it is also known from four completely sequenced chloroplast genomes of land plants namely tobacco, liverwort, rice and maize (Shinozati *et al* 1986; Ohyama *et al* 1986; Hiratsuka *et al* 1989; Maier *et al* 1995) that only a single gene for $tRNA^{Pro}$ is present in these genomes which has a UGG anticodon. The anticodon of this $tRNA^{Pro}$, namely UGG, should be able to read only two proline codons CCA and CCG, but not CCU or CCC. Marechal Drouard *et al* (1993) have proposed that the single plant chloroplast $tRNA^{Pro}$ (UGG) may be able to read all the four proline CCN codons in chloroplasts and suggested that a "two out of three mechanism" (only the first two bases of the codon which pair with the anticodon) (Lagerkvist 1978) involving two strong G:C base pairs can operate in chloroplasts allowing $tRNA^{Pro}$ (UGG) to read all four proline codons. It may therefore, be concluded that this cucumber chloroplast $tRNA^{Pro}$ (UGG) probably reads all the four proline codons in the chloroplasts.

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Corresponding editor: K DHARMALINGAM