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Nucleotide sequence of a spinach chloroplast valine tRNA

H.Mae Sprouse, M.Kashdan, L.Otis and B.Dudock

Department of Biochemistry, State University of New York at Stony Brook, Stony Brook, NY 11794, USA

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ABSTRACT

The nucleotide sequence of a spinach chloroplast valine tRNA (sp. chl. $tRNA^{Val}$) has been determined. This tRNA shows essentially equal homology to prokaryotic valine tRNAs (58-65% homology) and to the mitochondrial valine tRNAs of lower eukaryotes (yeast and N. crassa, 61-62% homology). Sp. chl. $tRNA^{Val}$ shows distinctly lower homology to mouse mitochondrial valine tRNA (53% homology) and to eukaryotic cytoplasmic valine tRNAs (47-53% homology). Sp. chl. $tRNA^{Val}$, like all other chloroplast tRNAs sequenced, contains a methylated GG sequence in the dihydrouridine loop and lacks unusual structural features which have been found in several mitochondrial tRNAs.

INTRODUCTION

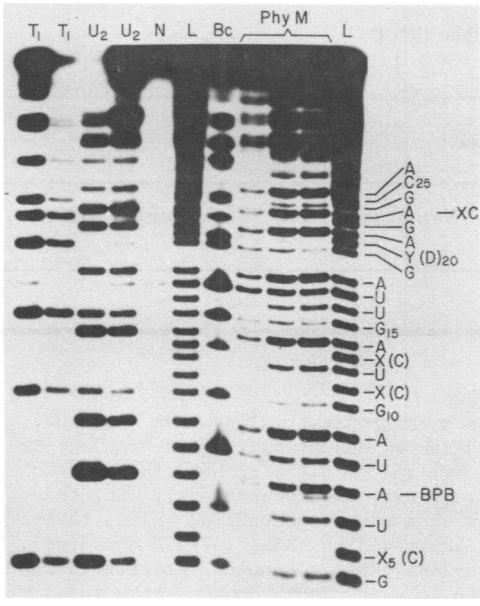
The nucleotide sequences of twelve valine tRNAs have been determined. Five are from prokaryotes (1), six are from eukaryotes (1) and one is from N. crassa mitochondria (2). In addition, two mitochondrial valine tRNA genes, from yeast (3) and mouse L cells (4), have also been reported. We have now determined the nucleotide sequence of a valine tRNA from spinach chloroplasts and compare this sequence to the valine tRNAs of prokaryotes, eukaryotes and mitochondria.

MATERIALS AND METHODS

The isolation of chloroplasts from Spinacia oleracea L. var 424, the isolation of total spinach chloroplast tRNA and the methods used in nucleotide sequence determination and hybridization have been discussed previously (5-7).

RESULTS

Purification of tRNA. The pure valine tRNA used for the sequence analysis and hybridization studies reported here was obtained from the same chromatographic procedure from which spinach chloroplast $tRNA^{Thr}_3$ was obtained (6). Fractions 134-136 of Fig. 1C of reference 6 were found to contain a pure tRNA as judged by a single band on a denaturing 20% polyacrylamide gel, stained



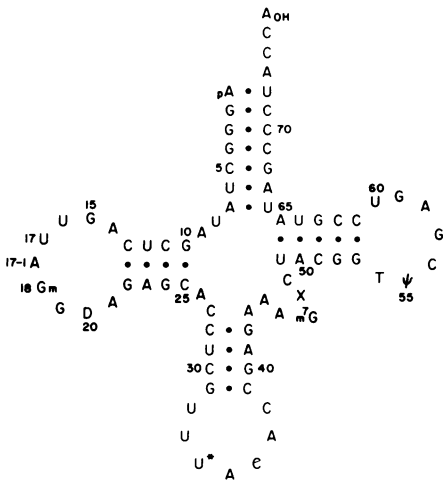


Figure 1. Sequence analysis of sp. chl. $tRNA^{Val}$. Autoradiogram of RNA sequencing gel of oligonucleotides from partial digests of sp. chl. [5'-^{32}P] $tRNA^{Val}$. The base-specific cleavages were obtained using ribonucleases T_1 (G), U_2 (A) and PhyM (U and A) and the pyrimidine-specific ribonuclease from B. cereus (U and C). Limited formamide digests were used to obtain the "ladders" (L). The sp. chl. [5'-^{32}P] $tRNA^{Val}$ control was untreated (N). The locations of the dyes xylene cyanol FF (XC) and bromophenol blue (BPB) are indicated.

Figure 2. Nucleotide sequence of a spinach chloroplast valine tRNA.

with ethidium bromide (data not shown). This tRNA was shown by subsequent sequence analysis and aminoacylation to be a valine tRNA.

Sequence Analysis. The nucleotide sequence of sp. chl. $tRNA^{Val}$ was determined using formamide fragment analysis, RNA sequence gels and mobility shifts. The formamide fragment analysis method (8,9) provided the most sequence information of the three methods employed and allowed an unambiguous assignment of residues 3 to 69. The dinucleotide GmG in the dihydrouridine loop and all other modified nucleotides obtained were identified as described previously (6). RNA sequence gels (5, 10-12) such as shown in Fig. 1, were used to analyze both [5'-^{32}P] and [3'-^{32}P] labeled sp. chl. $tRNA^{Val}$. These gels confirmed the assignments of residues 2-76. The 5' and 3' ends of $tRNA^{Val}$ were determined by mobility shift procedures (13) which gave residues A_1 to G_{10} at the 5' end and residues G_{63} to A_{76} at the 3' end.

Hybridization of Spinach Chloroplast $tRNA^{Val}$. The sp. chl. [5'-^{32}P] $tRNA^{Val}$ was hybridized to restriction fragments of sp. chl. DNA which were generated by the enzymes SalI, KpnI, and BamHI respectively, using methods previously described (6,7, 14-16). The sp. chl. [5'-^{32}P] $tRNA^{Val}$ hybridized to a 17.0 kilobase SalI fragment, a 5.0 kilobase KpnI fragment and a 5.4 kilobase BamHI fragment. These fragments were derived from a region of the spinach chloroplast genome to which sp. chl. $tRNA^{Val}_1$ has been mapped (17,18).

DISCUSSION

The nucleotide sequence of sp. chl. $tRNA^{Val}$ (Fig. 2) shows essentially equal homology to prokaryotic valine tRNAs (58-65% homology) and to the mitochondrial valine tRNAs of lower eukaryotes (yeast and N. crassa, 61-62% homology). Sp. chl. $tRNA^{Val}$ shows distinctly less homology to mouse mitochondrial valine tRNA (53% homology) and to the eukaryotic cytoplasmic valine tRNAs (47-53% homology). This pattern of sp. chl. $tRNA^{Val}$ sequence homology is distinctly different from that of several other recently sequenced chloroplast tRNAs. For example, the sp. chl. $tRNA^{Thr}_3$ (6) and the chloroplast methionine initiator tRNAs from spinach (7), bean (19) and Scenedesmus obliquus (20) all show considerably greater homology to their prokaryotic compared to their mitochondrial counterparts. This difference underscores the diversity of sequence homologies to be expected upon more detailed studies of organelle tRNAs.

Residue 34 (the wobble position) of sp. chl. $tRNA^{Val}$ contains an unknown modified uridine denoted as U^*. The same residue is also found in the same site in sp. chl. $tRNA^{Pro}$ (H.M.S. and B.D., in preparation). Uridine 5-oxyacetic acid (o^5U) occupies this site in E. coli $tRNA^{Val}_1$ (1) but U^* is not o^5U as judged by differences in chromatographic mobilities. Thus, in the solvents of Gupta and Randerath (9), authentic po^5Up (obtained from E. coli $tRNA^{Val}_1$ or E. coli $tRNA^{Ser}_1$) migrates as follows: R_{pAp}=1.03 (ammonium sulfate), R_{pGp}=0.45 (ammonium formate), while pU^*p migrates as follows: R_{pUp}=0.97 (ammonium sulfate), R_{pUp}=1.05 (ammonium formate). The other unidentified residue in sp. chl. $tRNA^{Val}$ is residue 47 which is denoted as X. The residue pXp migrates as follows: R_{pUp}=1.14 (ammonium sulfate), and R_{pAp}=1.02 (ammonium formate). Residue X is not cleaved by RNases T_1, U_2, PhyM or the pyrimidine specific nuclease from B. cereus.

Fifteen different valine tRNAs or tRNA genes are now sequenced and a detailed comparison of them reveals a variety of features as follows (all residues refer to the numbering system of Fig. 2); A) all eleven non-organelle tRNAs have G as the 5' residue whereas three of the four organelle valine

tRNAs do not indicating that the 5' terminal residue is more highly conserved in non-organelle than in organelle valine tRNAs; B) all fifteen valine tRNAs have a G_{10}-C_{25} base pair and an A at position 73; C) all eukaryotic valine tRNAs have a U at position 11 while all prokaryotic and organelle valine tRNAs have a C at that site; D) all prokaryotic and organelle valine tRNAs have U_{12} and A_{23} while no eukaryotic valine tRNA has a U_{12} or A_{23} residue; E) all non-organelle valine tRNAs have C_{31} and G_{39} while no organelle valine tRNAs have these residues; F) all eukaryotic and organelle valine tRNAs have C_{38} while no prokaryotic valine tRNAs have this residue. These correlations appear significant since they are based upon the sequences of fifteen different valine tRNAs or tRNA genes from a wide variety of sources. It will be most interesting to see how additional valine tRNAs, especially from organelles, fit these correlations.

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