COMPARISON BETWEEN THE 3'OH END RNA SEQUENCE OF TWO STRAINS OF TOBACCO MOSAIC VIRUS (TMV) WHICH MAY BE AMINOACYLATED

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1. Introduction

It is well known that the 3' ends of a great number of plant viral RNAs can serve as substrates for aminoacyl-tRNA synthetases [1–3]. For this reason, a tRNA-like structure has been assigned to the 3' extremities of these RNAs. Recently, this laboratory has reported the sequence of the first 71 nucleotides from the 3' end of tobacco mosaic virus RNA, which binds histidine [4]. No abnormal bases are found and the secondary structure of this 3' end could not be folded into the clover-leaf structure characteristic of tRNAs. In order to see if such a structure is unique to TMV wild type or is to be found in other strains as well, we have sequenced the 3' terminal region of the RNA of GTAMV [5], a strain of TMV, which also binds histidine [6]. The present paper describes the sequence of 74 nucleotides at the 3' end of GTAMV RNA. A secondary structure, which looks like that of TMV RNA is proposed. Some resemblances with $tRNA^{His}$ are also found and are discussed.

2. Methods

^{32}P-labeled GTAMV RNA was prepared according to the methods described elsewhere [4].

After partial digestion of ^{32}P-labeled RNA with T1 RNase (1 unit per 50 μg RNA) in the presence of 0.06 M sodium pyrophosphate, pH 7.25, for 30 min, at 0°C, the mixture of RNA fragments was fractionated by electrophoresis in a 10% polyacrylamide gel. Identification of the fragment arising from the 3' end of the GTAMV RNA was performed as described by Guilley et al. [4], i.e. total T1 RNase digestion of each fragment followed by electrophoresis at pH 2.5. The sequence of the 3' terminal T1 oligonucleotide is such that only the fragment which originated from the 3' end of the molecule gives a product which migrates toward the cathode at pH 2.5. Once identified, the 3' terminal fragment was purified on a 15% polyacrylamide gel and its sequence was determined according to the methods of Sanger et al. [7].

3. Results

After partial T1 RNase hydrolysis and separation of the fragments on a 10% polyacrylamide gel, only one fragment was found to give rise to a product which migrates toward the cathode at pH 2.5 after total T1 RNase digestion. The sequence of this material is $CCCA_{OH}$ as deduced from pancreatic, alkaline and venom phosphodiesterase (VPDE) hydrolysis.

The total T1 RNase and pancreatic RNase digestion products of the 3' terminal fragment were separated by two-dimensional electrophoresis and each oligonucleotide was characterised by further digestion with the complementary RNase, VPDE, and U2 RNase.

Partial digestion products of the fragment containing the 3' end oligonucleotide $CCCA_{OH}$ were obtained by T1 RNase digestion (0.3 U per 50 μg of RNA) or pancreatic RNase digestion (0.002 μg per 50 μg of RNA). Each purified fragment was then sequenced and the sequence of the whole 3' end fragment shown in fig.1 was deduced by overlapping of these small fragments. It is worth noting that, in contrast with the wild type RNA, the sequence of oligonucleotide UUCG, which is characteristic of the TψC loop of tRNA, was firmly determined. As previously shown

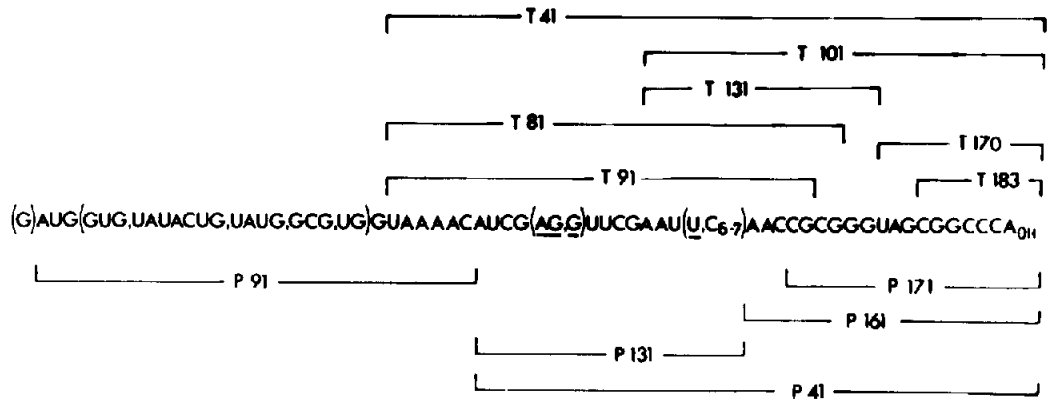

Fig.1. Nucleotide sequence of the fragment T3'OH. The numbered lines refer to the various products obtained by partial digestion of the fragment with T1 or pancreatic ribonuclease. If a product is underlined, it was present in two molar yield.

in the case of TMV RNA, no abnormal bases were found in GTAMV RNA after total hydrolysis and thin-layer chromatography [4].

Fig.2a shows the secondary structure which may be proposed for the region starting from the 3' end. The two hydrogen-bonded loops have free energies of formation of −9.2 Kcal/mole and −15.8 Kcal/mole respectively, as estimated by the rules of Tinoco et al. [8].

4. Discussion

By using the unusual property of the 3' terminal oligonucleotide $CCCA_{OH}$, the sequence of an RNA fragment of 74 nucleotides originating from the 3' end of the GTAMV RNA molecule has been established. If we compare its sequence and secondary structure to those of wild type RNA, several homologies are apparent (fig.2a and 2b). The GTAMV RNA fragment can be folded into a secondary structure similar to that of TMV. Furthermore, the primary structure of the unpaired parts of both loops is homologous, except for an extra guanine in the loop proximal to the 3' end of the molecule. Although GTAMV is considered as a strain of TMV, the large number of aminoacid changes in the coat protein (27%) [9], and the fact that the 5' extremity of GTAMV RNA cannot be recognized by TMV protein (M. C. Morel, personal communication) suggest that the strain relationship is not a close one. Moreover, if we notice the great variability in the sequence

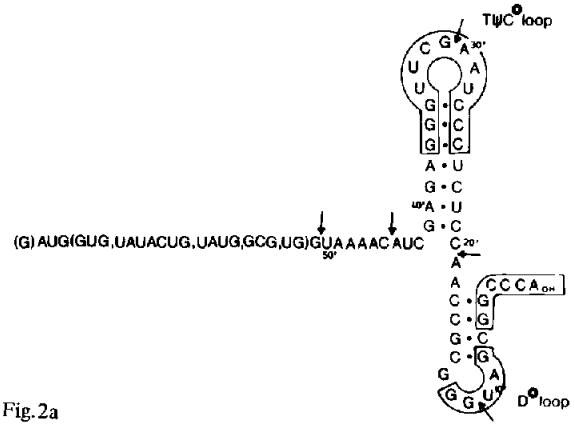

Fig.2a

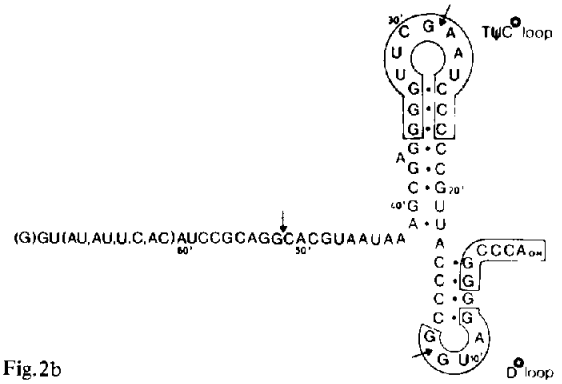

Fig.2b

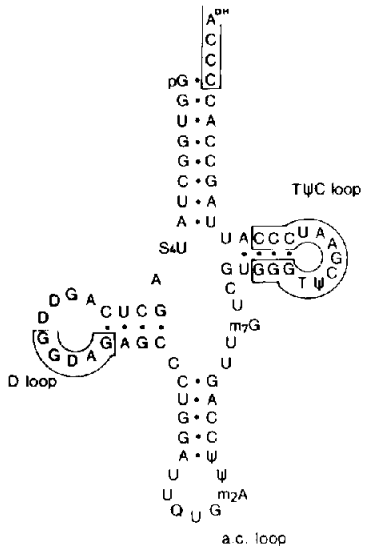

Fig.2c

Fig.2. (a) A possible model for the secondary structure of the 3′ end fragment of GTAMV RNA. (b) Model proposed by Guilley et al. [4] for the secondary structure of TMV RNA. (c) $tRNA^{His}$ of *S. typhimurium* [10]. Boxed areas of $tRNA^{His}$ indicate sequence homologies with TMV and GTAMV RNAs. Boxed areas of TMV and GTAMV RNAs indicate constant regions. Stars indicate parts of the secondary structure which could be assigned to the TψC or D loop of the generalized tRNA cloverleaf structure. Arrows indicate preferential breaks which occur during partial T1 or pancreatic hydrolysis.

at the 5′ ends of the 3′ terminal fragments, it seems likely that the conserved portion of the sequences at the 3′ end is involved in an important function common to both viruses.

It should be noted that the conserved hairpin structures are themselves homologous with portions of the *S. typhimurium* $tRNA^{His}$ molecule [10], i.e. parts of the TψC and D loops and the 3′ terminal tetranucleotide (fig.2c). Up until now, none of these regions has been definitely implicated in the recognition interaction with the aminoacyl-tRNA synthetase. Nevertheless, Klug and co-workers [11] have shown that the tertiary structure of yeast $tRNA^{Phe}$ is mainly determined by interchain liaisons between invariant bases within the D and TψC arms. It therefore seems likely that the 3′ terminal secondary structure described above is involved in the mechanism of its recognition by the histidyl-tRNA synthetase.

It will be of great interest to determine if the fragment carrying this portion of the sequence is in itself structurally sufficient to participate in the aminoacylation reaction. If so, studies on the reaction between such fragments and their specific aminoacyl-tRNA synthetase should be useful in ascribing functions to various parts of the tRNA molecule.

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