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Conformational requirements of tobacco mosaic virus RNA for aminoacylation and adenylation

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ABSTRACT

The RNA conformational requirements for both aminoacylation and adenylation emerging from our studies performed using the valine- and the tyrosine-accepting plant viral RNAs are now strongly supported by the histidine-accepting tobacco mosaic virus RNA: an 'L'-shaped conformation is recognized by the aminoacyl-tRNA synthetase whereas only the aminoacyl RNA domain (equivalent in tRNAs to the continuous helix formed by the acceptor stem and the T stem and loop) interacts with the tRNA nucleotidyltransferase.

INTRODUCTION

The 3' terminus of the genome of several plant RNA viruses can be aminoacylated with a specific amino acid (for a review see ref. 1). Interestingly, investigations bearing on the folding of the 3' tRNA-like region of the valine-accepting turnip yellow mosaic virus (TYMV) RNAs and on those of the tyrosine-accepting bromovirus, cucumber mosaic virus and barley stripe mosaic virus RNAs have revealed two very different and unconventional ways of reaching an 'L'-shaped conformation (fig. 1) recognized by aminoacyl-tRNA synthetases (2-8, for a review see ref. 9). In line with these unusual foldings, the determination of the length of the shortest 3' terminal viral RNA fragment that can be adenylated led us to propose that in the 'L'-shaped structure, only the aminoacyl RNA domain (equivalent in tRNAs to the continuous helix formed by the acceptor stem and the T stem and loop) is required by the tRNA nucleotidyltransferase for adenylation (3,6).

Tobacco mosaic virus (TMV) RNA accepts histidine. We have established that the tRNA-like structure in TMV RNA is composed of the 3' terminal ∿95 nucleotides. In this structure also, as in the case of TYMV RNA (3-5), the aminoacyl RNA domain interacting with the tRNA nucleotidyltransferase must be formed by only the 3' half of the tRNA-like region since viral RNA fragments as short as ∿55 nucleotides can be adenylated. These results are compatible

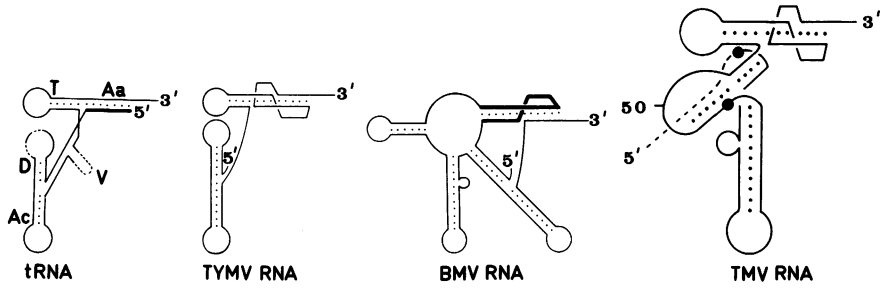

Fig. 1. Schematic representation of the 'L'-shaped conformation of tRNAs and viral tRNA-like regions. The thick line corresponds to the contribution of the 5' region to the formation of the aminoacyl RNA domain. BMV = brome mosaic virus. Aa = acceptor stem; T = T stem and loop; V = variable stem and loop; Ac = anticodon stem and loop; D = D stem and loop. In the case of TMV RNA, the region beyond nucleotide 95 is dashed; the position of nucleotide 50 from the 3' end is indicated; ● = nucleotides 55 and 95. From Joshi et al. (6) and adapted from Rietveld et al. (10).

with the folding of the tRNA-like region of TMV RNA (fig. 1) recently proposed (10). Furthermore, they strongly support the RNA conformational requirements for aminoacylation and adenylation that derive from our previous studies performed using the valine- and the tyrosine-accepting viral RNAs (3,6, for a review see ref. 9).

MATERIALS AND METHODS

Viral RNA and enzymes

TMV BL strain, an isolate from Leiden, was kindly supplied by L. Van Vloten-Doting (University of Leiden); the total RNA was extracted from the virus (11) and stored at -70°C. Purified tRNA nucleotidyltransferase from Escherichia coli (12) was provided by D.S. Eusèbe-Carré, and from baker's yeast (13) by H. Sternbach (Max-Planck-Institut für Experimentelle Medizin, Göttingen). Partially purified sheep liver histidyl-tRNA synthetase was a gift from J.P. Waller and M. Mirande (Ecole Polytechnique, Palaiseau); the enzyme was further purified by successive chromatography (14) on DEAE-cellulose (Whatman) and phosphocellulose (Whatman). RNase T1 was from Sankyo, alkaline phosphatase from Sigma, and bovine serum albumin from Bethesda Research Laboratories.

Preparation of viral RNA devoid of its 3' terminal A residue (RNA-CC)

The 3' terminal adenosine was removed from the native viral RNA (RNA-CCA) by sodium periodate and lysine treatments (15). Alkaline phospha-

tase was then used to eliminate the 3' terminal phosphate (6, 16).

Partial RNase T1 and alkali digestion

The conditions were essentially those described previously (6).

For the RNase T1 digestion, RNA-CCA, RNA-CC or RNA-CC-^{3}H-A (1 mg/ml) was incubated in the presence of 7 M urea, 20 mM sodium citrate pH 5, and 5 mM EDTA at 50°C for 10 min after which RNase T1 (1, 3 or 10 U/ml) was added and incubation continued for 20 min. The samples were then boiled for 2 min, chilled, diluted three times with water and phenol extracted; the aqueous phases were extracted three times with ether to remove residual phenol, and the RNAs were ethanol precipitated; the dried pellets were resuspended in water and stored at -70°C.

For the alkali digestion, RNA-CCA, RNA-CC or RNA-CC-^{3}H-A (1 mg/ml) was boiled in 25 mM sodium bicarbonate pH 9 for 1, 2 or 3 min and then chilled. The RNAs were ethanol precipitated and the dried pellets were resuspended in water and stored at -70°C.

Adenylation and aminoacylation

The conditions were essentially those described previously (6).

Adenylation was performed using 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 10 µM ^{3}H-ATP (32 Ci/mmol; Amersham), 500 µg/ml of RNase T1 or alkali fragments of TMV RNA-CC and 150 µg/ml of E. coli tRNA nucleotidyltransferase for 20 min at 37°C; when the tRNA nucleotidyltransferase from yeast (0.6 µg/ml) replaced the E. coli enzyme, the incubation mixture also contained 100 µg/ml of bovine serum albumin.

Aminoacylation was performed using 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 2 mM ATP, 10 µM ^{3}H-histidine (44 Ci/mmol; Amersham), 250 µg/ml of RNase T1 or alkali fragments of TMV RNA-CCA, 100 µg/ml of bovine serum albumin and 50 µg/ml of purified sheep liver histidyl-tRNA synthetase for 15 min at 30°C.

The samples were treated as indicated previously (3) and analyzed by gel electrophoresis.

To obtain molecular weight markers, TMV RNA-CC was first adenylated using ^{3}H-ATP and the resulting viral RNA-CC-^{3}H-A was partially digested by RNase T1 or alkali as indicated above.

Polyacrylamide gel electrophoresis

Polyacrylamide (12%), bis-acrylamide (0.6%), 7 M urea gels were prepared, run and treated as indicated previously (17); the samples layered into each well contained 30,000 to 200,000 cpm; exposition at -70°C was for 1 to 3 weeks.

RESULTS AND DISCUSSION

To determine the length of the shortest 3' terminal TMV RNA fragment that can be aminoacylated or adenylated, the following strategy was used (3,6). Native RNA (RNA-CCA) or the viral RNA devoid of its 3' terminal A residue (RNA-CC) was partially pre-digested with either RNase T1 or alkali. The mixture of resulting fragments was then either aminoacylated with ^{3}H-histidine or adenylated using ^{3}H-ATP. The material was analyzed on polyacrylamide-urea gels: only those fragments that are still capable of being aminoacylated or adenylated appear on the fluorogram. The fragments obtained by partial RNase T1 or alkali digestion of RNA-CC-^{3}H-A served as molecular weight markers based on the sequence of the RNA (18). For aminoacylation and adenylation, the incubation time and enzyme concentrations were reduced to a minimum to limit RNA degradation (fig. 2; lanes 1 and 2).

In fig. 2, a comparison of the bands obtained when the RNase T1 or alkali fragments of TMV RNA-CCA were aminoacylated (lanes 4 and 6) with those obtained by digestion under identical conditions of TMV RNA-CC-^{3}H-A (molecular weight markers; lanes 3 and 5) indicates that all the 3' terminal fragments longer than ∿95 nucleotides can be aminoacylated. Identical results were obtained with fragments from three different RNase T1 concentrations and three alkali digestion times. In the folding of the tRNA-like region of TMV RNA (fig. 1), an 'L'-shaped conformation recognized by the aminoacyl-tRNA synthetase is indeed formed within the first ∿95 nucleotides from the 3' terminus; the stem involving the RNA region beyond these ∿95 nucleotides (dashed line in fig. 1) is not essential.

On the other hand, in fig. 3A, a comparison of the bands obtained when the RNase T1 or alkali fragments of TMV RNA-CC were adenylated with the E. coli tRNA nucleotidyltransferase (lanes 2 and 4) with those of the molecular weight markers (lanes 1 and 3) indicates that the minimum length requirement for adenylation is ∿55 nucleotides. Similarly, the yeast tRNA nucleotidyltransferase also requires only ∿55 nucleotides from the 3' terminus of the RNA-CC for adenylation as determined using either the RNase T1 or the alkali fragments (fig. 3B; lanes 6 and 8). Thus the structural requirements of the RNA for adenylation by the E. coli or yeast tRNA nucleotidyltransferase are identical as might have been expected. It is noteworthy that in the folding of the tRNA-like region of TMV RNA (fig. 1), ∿55 nucleotides are sufficient for the formation of the aminoacyl RNA domain which is required by the tRNA nucleotidyltransferase.

All the adenylation experiments were performed using three RNase T1 con-

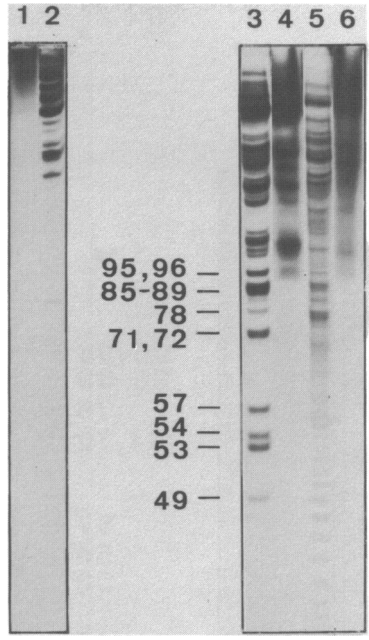

Fig. 2. Gel electrophoretic analysis of the TMV RNA-CCA fragments aminoacylated by the histidyl-tRNA synthetase using ^{3}H-histidine. Lanes 4 and 6: RNase T1 and alkali fragments (70 µg) aminoacylated respectively. Lanes 3 and 5: molecular weight markers obtained by the RNase T1 and alkali digestion of RNA-CC-^{3}H-A (30 µg) respectively. In lanes 3 and 4, RNase T1 was 3 U/ml, and in lanes 5 and 6, alkali digestion was for 1 min. Lane 1: ^{3}H-His-RNA (25 µg). Lane 2: RNA-CC-^{3}H-A (15 µg) obtained using the E. coli tRNA nucleotidyltransferase. The length of the RNase T1 digests is indicated. All lanes are from the same gel.

centrations and three alkali digestion times and similar results were obtained in all cases although those using RNase T1 were not as clear-cut as those using alkali. Our results are at variance with those of Rietveld et al. (10) who found that only fragments of TMV RNA-CC longer than ∿100 nucleotides could be adenylated by the yeast tRNA nucleotidyltransferase. However, only one experimental condition and only one method of generating viral RNA fragments (i.e. RNase T1) is reported. Thus the experimental conditions were probably such that smaller fragments either could not be adenylated or were not generated. Our positive results for adenylation of TMV RNA-CC fragments as short as ∿55 nucleotides are further strengthened by the recent data of Wang et al. (19) who have established that the chemically and enzymatically synthesized aminoacyl RNA domain of yeast tRNA^{Phe} can be efficiently adenylated by the yeast

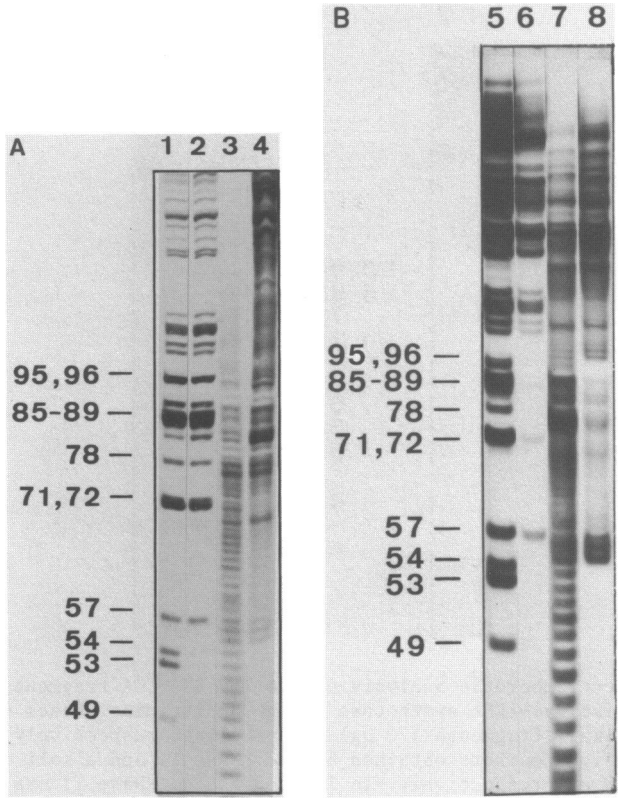

Fig. 3. Gel electrophoretic analysis of the TMV RNA-CC fragments adenylated by the tRNA nucleotidyltransferase from E. coli (A) and from baker's yeast (B) using ^{3}H-ATP. In (A), lanes 2 and 4: RNase T1 and alkali fragments adenylated respectively; lanes 1 and 3: molecular weight markers obtained by the RNase T1 and alkali digestion of RNA-CC-^{3}H-A. Similarly in (B), lanes 6 and 8: RNase T1 and alkali fragments adenylated respectively; lanes 5 and 7: molecular weight markers obtained as in (A). RNase T1 was 3 U/ml in lanes 1 and 2, and 10 U/ml in lanes 5 and 6. Alkali digestion was for 2 min in lanes 3, 4, 7 and 8. For the RNase T1 and alkali digestions, 30 and 50 µg of RNA were used respectively. The length of the RNase T1 digests is indicated.

tRNA nucleotidyltransferase.

Thus, the minimum length requirements of TMV RNA for aminoacylation and adenylation together with the folding of the tRNA-like region of TMV RNA (fig. 1) strongly support our conclusion that an 'L'-shaped conformation is recognized by the aminoacyl-tRNA synthetases and that only the aminoacyl RNA domain interacts with the tRNA nucleotidyltransferase.

Concerning the structure of RNA molecules in general, it is noteworthy

that the folding of viral tRNA-like regions involves base-pairings between loops and distantly located regions leading to short bridges (fig. 1). To our knowledge, none of the presently available computer programs takes into account such interactions, yet these interactions may be of crucial importance in determining the active conformations of nucleic acids (6).

A striking conclusion that emerges when comparing the foldings of tRNAs and of the viral tRNA-like regions (fig. 1) is that the conformation of the RNA is the most important element in recognition by specific proteins and that similar RNA conformations can result from very different secondary structures.

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