Search for tRNA-like properties in tomato aspermy virus RNA

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The 3′-terminal sequence of tomato aspermy virus (TAV, a cucumovirus) RNA can be arranged in an 'L'-shaped conformation similar to that of the 3′-tRNA-like regions of the tyrosine-accepting bromo-, cucumo- and hordeivirus RNAs examined to date. We report here that TAV RNA is recognized by the tRNA nucleotidyltransferase and can be adenylated nearly as efficiently as brome mosaic virus (BMV, a bromovirus) RNA. However, using tyrosyl-tRNA synthetase from rat liver or from yeast, TAV RNA cannot be tyrosylated under conditions that allow aminoacylation of BMV RNA. Structural features in the 3′-region could be responsible for the recognitory discrimination between the BMV and TAV RNAs by these eukaryotic tyrosyl-tRNA synthetases.

plant viral RNA *Tomato aspermy virus* *tRNA-like structure* *Aminoacylation* *Adenylation*

1. INTRODUCTION

The genome of several plant RNA viruses is said to possess a tRNA-like structure at its 3′-end since it is efficiently recognized in vitro by many tRNA-specific enzymes (for reviews, see [1–3]). Bromoviruses such as brome mosaic virus (BMV), broad bean mottle virus (BBMV) and cowpea chlorotic mottle virus (CCMV), cucumber mosaic virus (CMV, a cucumovirus) and barley stripe mosaic virus (BSMV, a hordeivirus) have a tripartite genome and the three RNAs (designated RNA 1, 2 and 3) that make up the genome of a given virus are all aminoacylatable at their 3′-terminus with tyrosine. A comparison of the nucleotide sequence of the 3′-region of these viral RNAs has revealed striking intraviral and interviral homologies between these untranslated regions, even though the coding regions which precede them are totally different. This is particularly apparent when comparing the tRNA-like regions of the bromovirus RNAs and of the CMV RNAs [4]. The folding of the 3′-region of the BMV RNAs in their tRNA-like configuration, recently established [4–6], is presented in fig.1A; this folding can be extended to the other tyrosine-accepting viral RNAs [5,7].

It has been proposed that the 3′-terminal nucleotide sequence of tomato aspermy virus (TAV, a cucumovirus with a tripartite RNA genome) RNA [8] can also be arranged [5] in a similar 'L'-shaped conformation (fig.1B). However, it is not known whether TAV RNA possesses tRNA-like properties. We have therefore examined whether the tRNA nucleotidyl transferase and the tyrosyl-tRNA synthetase can adenylate and aminoacylate TAV RNA as efficiently as BMV RNA, used as control.

It is a pleasure to dedicate this paper to Prakash Datta as an expression of our admiration for his devotion to FEBS Letters and for the international exchange he has constantly promoted among Biochemists

2. MATERIALS AND METHODS

Total TAV RNA was generously supplied by R.H. Symons (University of Adelaide) and total BMV RNA by P.A. Verduin (University of Wageningen). Purified *Escherichia coli* tRNA

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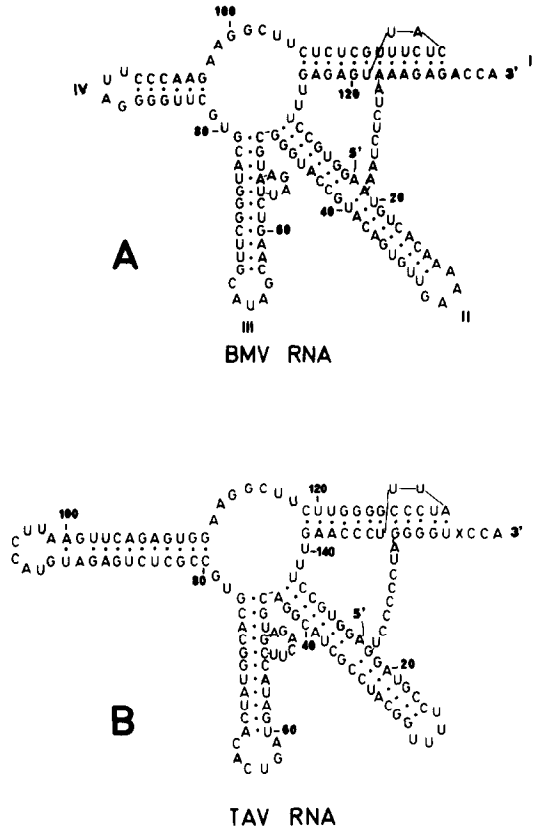

Fig.1. The tRNA-like structure of BMV RNA (A) and possible folding of the 3′-region of TAV RNA (B). The sequences shown are those of RNA 3. Every 20th nucleotide is numbered starting from the 3′-end. I–IV in the BMV RNA structure correspond to stems and loops, and also refer to the corresponding stems and loops in the TAV RNA structure. Adapted from [5].

nucleotidyltransferase was a kind gift from D.S. Eusèbe-Carré. Highly purified tyrosyl-tRNA synthetase from rat liver was kindly provided by F. Deák (Institute of Biochemistry, Szeged), and from yeast by H. Faulhammer (University of Bayreuth). [^{3}H]ATP (31.7 Ci/mmol) was from Amersham (England) and [^{3}H]tyrosine (60 Ci/mmol) from the Centre de l'Energie Atomique (France).

2.1. *Preparation of viral RNA-CC$_{OH}$*

The 3′-terminal A residue in TAV and BMV RNAs was removed by sodium periodate, aniline and alkaline phosphatase treatments as described [5].

2.2. *Adenylation and aminoacylation of viral RNAs*

Adenylation (20 μl) was performed using 50 mM Tris-HCl, pH 9, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 10 μCi [^{3}H]ATP, 10 μg TAV or BMV RNA-CC$_{OH}$, 6 μg tRNA nucleotidyltransferase, and where indicated 50 mM KCl, for 15 min at 37°C.

Aminoacylation (20 μl) was performed using 50 mM Tris-HCl, pH 7.5 or pH 9, 10 mM $MgCl_2$, 10 mM DTT, 2 mM ATP, 10 μCi [^{3}H]tyrosine, 10 μg TAV or BMV RNA, tyrosyl-tRNA synthetase from rat liver (0.2 μg) or from yeast (0.02 μg), and where indicated 50 mM KCl, for 15 min at 37°C.

The reactions were stopped by cold 10% trichloroacetic acid, the acid-precipitable radioactivity was determined and background levels obtained in the absence of added RNA were subtracted. It was verified that both adenylation and aminoacylation had reached the plateau level under these conditions.

3. RESULTS

3.1. *Tyrosylation*

Contrary to what is observed with BMV RNA, TAV RNA is virtually not aminoacylated by the tyrosyl-tRNA synthetase from either rat liver or yeast (table 1) or sheep liver (not shown). The level of aminoacylation of BMV RNA by the rat liver enzyme in the absence of KCl was considerably higher at pH 7.5 than at pH 9; the reverse was true with the yeast enzyme. This level of aminoacylation could be increased by the addition of 50 mM KCl in most assay conditions. However, virtually no tyrosylation of TAV was observed under any of these conditions. Since the proportion of the different viral RNAs extracted from different virus preparations is subject to variations, and since total unfractionated viral RNAs were used here, quantitation of the yield of aminoacylation (and adenylation, see below) is subject to considerable error. Nevertheless, if one assumes that the average M_r of the mixture of RNA components in BMV and TAV is comparable, aminoacylation of TAV RNA was at best ~5% that of BMV RNA. The observation that BMV RNA is efficiently aminoacylated by the yeast enzyme is at variance with previous results [9] and could be due to the

Table 1

Tyrosylation of TAV and BMV RNAs[a]

Source of RNA	KCl (50 mM)	[^{3}H]Tyrosine bound (cpm)			
		pH 7.5		pH 9	
		Enzyme source		Enzyme source	
		Rat liver	Yeast	Rat liver	Yeast
TAV	–	1250	670	1400	400
	+	1460	1160	1780	1650
BMV	–	68190	47170	34300	72740
	+	85960	121380	37340	138340

[a] 1 pmol Tyr-RNA corresponds to ~20000 cpm

high specific activity of the yeast tyrosyl-tRNA synthetase used here.

3.2. *Adenylation*

Since BMV RNA is also recognized by the tRNA nucleotidyltransferase [5], an enzyme with less stringent requirements than aminoacyl-tRNA synthetases, we have examined whether TAV RNA could be a substrate for this tRNA-specific enzyme. TAV and BMV RNAs as extracted from the virus terminate at their 3'-end by the sequence -CCA. Upon removal of the 3'-terminal A residue, the tRNA nucleotidyltransferase can indeed adenylate TAV RNA-CC_{OH} nearly as efficiently as BMV RNA-CC_{OH}, as seen in table 2. The adenylation experiments were performed at pH 9, i.e. close to the optimal pH value reported for this enzyme [10]. Indeed, when performed at pH 7.5 (50 mM Tris-HCl) adenylation was about 35 and 25% of the value obtained at pH 9 for the TAV and BMV RNAs, respectively (not shown). As expected [11], the presence of KCl reduced the level of adenylation of both viral RNAs. Our positive results concerning the recognition of TAV RNA by the tRNA nucleotidyltransferase suggest that TAV RNA has at least retained the tRNA-like features required for adenylation, and supports folding of this region of the viral RNA as proposed previously [5], and as presented in fig.1B.

Table 2

Adenylation of TAV and BMV RNA-CC_{OH}[a]

Source of RNA-CC_{OH}	KCl (50 mM)	[^{3}H]AMP incorporated (cpm)
TAV	–	37120
	+	24120
BMV	–	44360
	+	31310

[a] 1 pmol AMP incorporated corresponds to ~10000 cpm

4. DISCUSSION

One immediate apparent difference between the TAV and BMV RNAs resides in the stem and loop IV which in TAV RNA are about twice as long as in BMV RNA (and in the CCMV and CM [4,5] RNAs). This could be an element of hindrance for recognition of TAV RNA by the rat liver or yeast tyrosyl-tRNA synthetase. It is interesting to point out that stem and loop IV are absent from the tRNA-like regions of the BBMV [4,5] and BSMV [7] RNAs, and thus are not mandatory for tyrosylation. However, other features, such as nucleotide sequence and/or local conformations could be responsible for the lack of recognition of TAV RNA by the tyrosyl-tRNA synthetases used here. Furthermore, aminoacylation of TAV RNA by tyrosyl-tRNA synthetases from other origins cannot be ruled out.

The biological function of the tRNA-like structures in plant viral RNAs still remains enigmatic. It has been reported that the BMV and BSMV

RNAs are aminoacylated in vivo in infected barley protoplasts [12]. Were TAV not to be aminoacylated in vivo, this would be an important fact that would have to be taken into account when considering a general role of the tRNA-like structures in these viral RNAs.

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