TYMV RNA AS A SUBSTRATE OF THE tRNA NUCLEOTIDYLTRANSFERASE

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1. Introduction

It has been recently shown that in the presence of partly purified enzyme preparations from *Escherichia coli* and ATP, the RNA (M.W. = 2×10^6) of Turnip Yellow Mosaic Virus (TYMV) reacts with valine forming an ester bond analogous to that existing in aminoacyl-tRNA; the valine esterifies the 3′-terminal adenosine of TYMV RNA [1, 2]. The enzyme which catalyses this reaction is the valyl-tRNA synthetase (VRS).

Such esterification as known for tRNAs implies that the 3′-end of the polynucleotide molecule carries a terminal adenosine. Since the partly purified preparations used to bind valine to TYMV RNA contained tRNA nucleotidyltransferase (EC 2. 7. 7. 25) (CCA-enzyme), it appeared interesting to study whether the esterification of adenosine by valine in TYMV RNA was preceded by the incorporation of AMP catalysed by the CCA-enzyme. Using a highly purified preparation of this enzyme and pure VRS we were able to demonstrate that, prior to the esterification by valine, TYMV RNA reacts as an acceptor of AMP from ATP.

Moreover, analysis of the alkaline hydrolysate or pancreatic RNase digestion product of TYMV RNA having incorporated ^{32}P-AMP showed that CMP is the penultimate nucleotide at the 3′-end of the RNA molecule and that the preceding nucleotide is also a pyrimidine. Thus we conclude that the RNA isolated from TYMV bears the 3′-terminal sequence: pCpC.

2. Material

The CCA-enzyme was purified from *E. coli* as described elsewhere [3]. VRS from *E. coli* was a kind gift of Dr. M. Yaniv. TYMV RNA was purified by the phenol method after incubation with pronase [4], and was stored at −20° in 66% ethanol in the presence of 0.1% bentonite. ^{3}H-ATP was purchased from Schwarz BioResearch. α-^{32}P-ATP and ^{14}C-valine were from the Commissariat à l'Énergie Atomique, Saclay.

3. Methods

3.1. *CCA-enzyme assay.*

The reaction mixture contained in 0.2 ml: 10 μmoles tris pH 8.4; 2 μmoles $MgCl_2$; 0.4 μmole β-mercaptoethanol; 1 nmole ^{3}H-ATP (1 mCi/μmole); 50 pmoles of TYMV RNA and 10–15 μg of purified CCA-enzyme. Incubations were carried out at 37° for different times and the reaction was stopped by the addition of 1 mg of 0.1% bovine serum albumin and 0.2 ml of 50% trichloroacetic acid. After centrifugation, the supernatant was discarded and the precipitate rapidly dissolved in 0.2 ml of 0.2 N NaOH and reprecipitated with 10% trichloroacetic acid. This procedure was repeated three times. The final precipitate was filtered, washed extensively with 5% trichloroacetic acid and counted.

3.2 *VRS assay*

When the incorporation of valine into TYMV RNA was tested after preincubation in the presence or absence of the CCA-enzyme, the incubation mixture was identical to that used for the CCA-enzyme assay except that tris at pH 7.5 and unlabeled ATP (0.1 μmole) were used. The preincubation was carried out for 60 min with or without CCA-enzyme; 3 nmoles of ^{14}C-valine (108 mCi/mmole) and 1 μg of VRS were

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then added and the incubation pursued for different times as 30°. After addition of 0.1 mg of bovine serum albumin and 1 ml of 10% trichloroacetic acid, the mixture was filtered; the precipitate washed extensively with 5% trichloroacetic acid and the radioactivity retained on the filters determined.

3.3. *Glycerol gradient centrifugation*

Approximately 100 pmoles of TYMV RNA were submitted to a 5–20% glycerol gradient centrifugation in 0.01 M tris pH 7.5, for 75 min at 420,000 *g*. The fractions were collected and the absorbance measured at 260 nm; the radioactivity was determined after filtration of the 5% trichloroacetic acid precipitate.

3.4. *Alkaline hydrolysis of ^{32}P-AMP-labeled TYMV RNA*

TYMV RNA (125 pmoles in 0.01 M tris pH 8.4) was incubated for 60 min at 37° in the presence of the CCA-enzyme and ^{32}P-ATP (480 mCi/mmole); the RNA was extracted by the phenol method and precipitated by ethanol in the presence of potassium acetate. After extensive washing of the precipitate with ethanol saturated with unlabeled ATP, the RNA was submitted to alkaline hydrolysis for 14 hr at 37° in 0.2 M KOH. The products of the hydrolysis were analysed both by paper chromatography (Whatman No 1) in 0.1 M sodium phosphate pH 6.8–ammonium sulphate–propanol, (100 : 60 : 2) and by paper electrophoresis. The paper electrophoresis (Whatman No 3) was run at 50 V/cm for 2 hr at 4° in 0.05 M sodium citrate pH 3.6. Either methods separated all four 2′-3′-phosphonucleosides. A Dunnschicht Apparatus was used for radioactivity scanning of paper strips.

4. Results and discussion

As shown in table 1, when 50 pmoles of TYMV RNA were incubated for 60 min in the presence of ^{3}H-ATP and CCA-enzyme, 19 pmoles of the nucleotide were incorporated into RNA. Addition of magnesium ions stimulated the reaction, and pancreatic ribonuclease abolished it. In other experiments not described here it was found that, as in the case of the incorporation of AMP into the 3′-end of tRNA deprived of terminal adenosine [3], β-mercaptoethanol stimulated the reaction. With different preparations of TYMV RNA the incorporations were from 0.3–0.6 mole of nucleotide per mole of RNA. Fig. 1 presents the kinetics of ^{3}H-AMP incorporation into TYMV RNA: the reaction is virtually complete in 10 min at 37°.

Table 1
Requirements for the incorporation of AMP into TYMV RNA.

Conditions	AMP incorporated (pmoles)
Complete system	19
minus $MgCl_2$	2.5
minus RNA	< 1
minus CCA-enzyme	< 1
plus RNase	< 1

The conditions were as given under Methods, and when indicated, 1 μg of bovine pancreatic ribonuclease was added.

Analysis of the incubated mixture by glycerol gradient centrifugation (fig. 2), showed that most of the radioactivity migrated with 25 S RNA. When this material was digested by pancreatic ribonuclease and analysed by paper electrophoresis, all the radioactivity was located in a single spot coinciding with marker adenosine. Thus, as in the case of tRNA, the CCA-enzyme catalyses the transfer of AMP from ATP into the 3′-end of TYMV RNA. Using various conventional methods that allow dissociation of nucleic acids and of nucleic acid-protein complexes [2], it was established that the ^{3}H-AMP-bearing material was not a tRNA contaminant.

In order to examine the valine accepting capacity

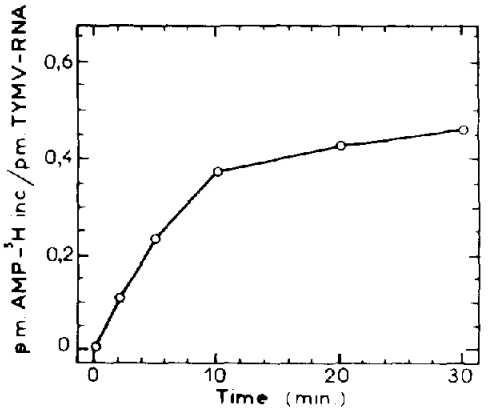

Fig. 1. Kinetics of ^{3}H-AMP incorporation into TYMV RNA by the CCA-enzyme. Conditions were as given under Methods.

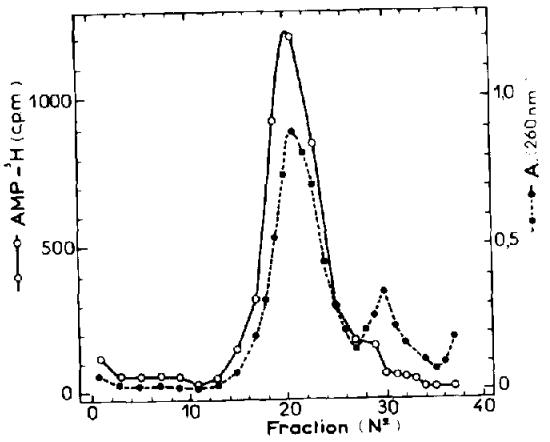

Fig. 2. Glycerol gradient centrifugation of ^{3}H-AMP labeled TYMV RNA. ○—○, cpm; ●- - -●, Absorbance 260 nm. The conditions were as given under Methods.

of TYMV RNA as catalysed by VRS, the incubations were carried out in the presence or in the absence of CCA-enzyme. Fig. 3 shows that only after preincubation with CCA-enzyme and ATP could VRS esterify valine to TYMV RNA. These results show that incorporation of AMP into TYMV RNA is a prerequisite for valine binding to occur. It is not known whether the absence of this nucleotide in the isolated RNA is due to its loss during the isolation of the virus or during the extraction of RNA, or if it is due to the lack of a terminal adenosine in the native RNA.

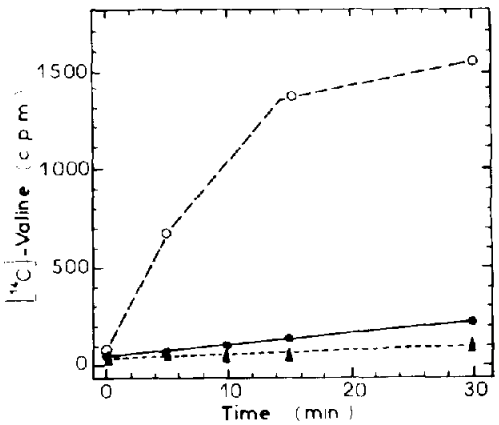

Fig. 3. Effect of preincubation with the CCA-enzyme on the valine binding to TYMV RNA by VRS. The conditions were as given under Methods. ○—○, CCA-enzyme and VRS; ●—●, VRS; ▲- - -▲ CCA-enzyme.

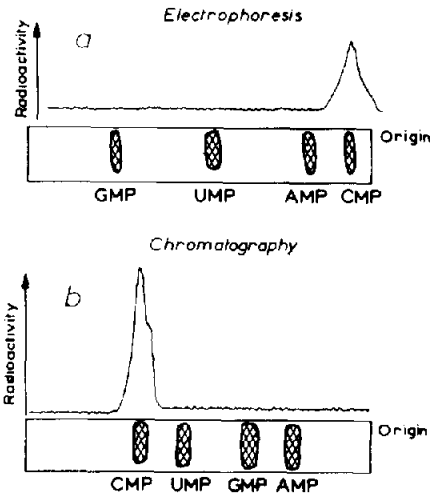

Fig. 4. Analysis of the alkaline hydrolysate products of TYMV RNA having incorporated ^{32}P-AMP. A) Paper electrophoresis; B) Paper chromatography. Other conditions as described under Methods.

The fact that the same enzyme catalyses the transfer of AMP from ATP into tRNA and into TYMV RNA suggests that the sequence of at least two other nucleotides which precede the 3′-terminal adenosine in tRNA, namely pCpC; is also present in TYMV RNA. To examine this point ^{32}P-labeled AMP was incorporated into TYMV RNA, the resulting radioactive RNA was treated with alkali and the products analysed. Fig. 4 shows that all the radioactivity incorporated as AMP migrated with marker 2′, 3′-CMP. When the same material was digested by pancreatic ribonuclease [5] identical results were found. This indicates that, as in tRNA, the nucleotide adjacent to the 3′-terminal adenosine is a cytidilate and that it is itself preceded by a second pyrimidine, probably also a cytidilate. Consequently we find that the RNA extracted from TYMV bears the sequence pPypC at its 3′-terminus.

The presence of this pPypC sequence in TYMV RNA is not surprising since most virus and phage RNAs bear the 3′-terminus: pCpC or pCpCpA; however the fact the CCA-enzyme which is very specific for tRNAs can use TYMV RNA as a substrate is remarkable since it is likely that in tRNA this enzyme recognizes not only the terminal sequence pCpC, but also other part(s) of the molecule [6]. The corresponding struc-

ture should therefore be involved in this recognition in the TYMV RNA molecule. Further analytical work is necessary to determine whether this recognition really reflects an important similarity between the structure of tRNA and the structure close to the 3′-end of TYMV RNA, or if it corresponds only to a coincidence without profound biological significance.

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