BINDING OF HISTIDINE TO TOBACCO MOSAIC VIRUS RNA

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SUMMARY

Tobacco mosaic virus (TMV) can be aminoacylated with the amino acid histidine in the presence of ATP, magnesium ions and a crude ligase preparation from yeast or mammalian cells.

The RNA from turnip yellow mosaic virus (TYMV) has been enzymatically esterified with valine in a manner similar to the esterification of tRNA (1, 2, 3). No other amino acid was accepted and extracts from E. coli, yeast or rabbit liver could be used as charging enzymes (3). The function of the valine aminoacylation of TYMV RNA is not known and it is possible that TYMV-RNA by mere chance has a $tRNA^{val}$ like structure at its 3′ end (3). If, however, other viral RNAs also could accept amino acids, it would be of interest to study the functional aspects in great detail.

In the course of studies on aminoacylation of polio RNA, other viral RNAs were tested as substrates for amino acid charging enzymes from yeast or KB-cells. Only tobacco mosaic virus RNA (TMV-RNA) was found to accept an amino acid which was identified as histidine.

MATERIALS AND METHODS

RNA. Poliovirus RNA was prepared as described previously (4), Qβ RNA was obtained by phenol extraction of phage Qβ purified by isopycnic banding in CsCl (5), satellite tobacco necrosis virus (STNV) purified by electrophoresis (6) was kindly provided by Dr. B. Strandberg, purified TMV (7) was kindly supplied by Dr. P. Oxelfeldt and RNA from STNV and TMV was obtained by phenol extraction as for poliovirus RNA.

All RNA preparations were precipitated with ethanol and dissolved in 0.05 M Tris-HCl pH 7.2. Yeast tRNA was from Schwarz Bioresearch (Orangeburg, N. Y.).

Enzymes. Yeast aminoacyl transferase was kindly supplied by Dr. T. Lindahl as a crude extract with a protein content of 38 mg/ml. A crude preparation of aminoacyl transferase from KB cells was prepared as described by Raska et al. (8).

Isotopes. L-amino acid-^{3}H-(G) mixture (1 mC/ml) was from New England Nuclear (Boston, Mass.). The histidine content was 15 μC/mC mixture and had a specific activity of 2.44 C/mmole. Other materials and methods have been described earlier (4). The amino acid analyses were performed as previously described (9). Eluted fractions were collected and radioactivity determined.

RESULTS AND DISCUSSION

Charging of RNA from Qβ, polio, STNV and TMV with KB cell aminoacyl transferase and ^{3}H-amino acids did only in the case of TMV-RNA significantly increase the radioactivity in acid precipitable material as is shown in Table 1. The conditions used for incubation were the same as described for binding of valine to TYMV-RNA (3). The KB cell aminoacyl transferase could be substituted with yeast aminoacyl transferase with the same result. Incubating TMV-, Qβ-, STNV and polio-RNA at pH 8.5 in 0.05 M Tris-HCl for 15 min at 23°C prior to charging with ^{3}H-amino acids did not result in RNA with changed ability to be aminoacylated. This makes it unlikely that the native RNA had an amino acid attached, which would prevent charging.

To determine whether the radioactivity bound to TMV-RNA was due to contamination of cellular tRNA, amino acylated TMV-RNA prepared by phenol extraction of the incubation mixture was analyzed on a Sephadex^{R} G 75 column as shown in Fig. 1A. The radioactivity

TABLE 1 Charging of viral RNA with ^{3}H-amino acids

RNA	mole RNA 10^{-11}	Counts/min Exp. 1	Exp. 2
-	0	1044.6	1012.8
Qβ	1.9	1160.3	1175.3
STNV	6.7	1144.0	997.3
Polio	0.38	1083.8	882.0
TMV	2.0	3012.0	3140.0

The incubation mixture contained the indicated amount of RNA, 90 mM Tris-HCl, pH 7.2, 5 mM $MgCl_2$, 1 mM dithiothreitol, 10 μC ^{3}H-amino acid mixture, 10 mM KCl, 1 mM ATP, 0.1 mM CTP and 20 μl of KB-cell enzyme. The total volume was 200 μl and the incubation 30 min at 35° C. The mixture was precipitated with 2 ml of cold 5 % trichloric acetic acid (TCA) and washed with 5 % TCA on millipore filter, and the filters were counted.

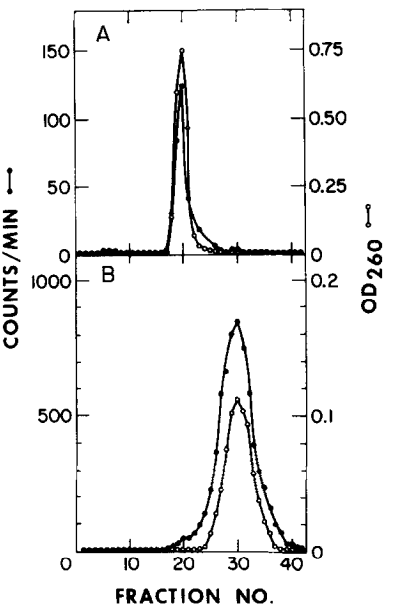

Figure 1 Gelfiltration of amino acylated TMV-RNA and yeast tRNA on Sephadex G 75.

A. 100 μg of TMV-RNA was incubated as described in Table 1. 20 μl of yeast enzyme was used and after incubation 0.5 ml of 0.05 M sodium acetate pH 5.5 was added. The mixture was extracted with an equal volume of phenol, twice with equal volumes of ether and precipitated with 2 volumes of ethanol. LiCl was added to 0.1 M. The precipitate was dissolved in 1 ml 0.05 M sodiumacetate, pH 5.5 and reprecipitated twice. Finally, the precipitate was dissolved in 0.5 ml 2 mM sodium phosphate buffer pH 6.0, 0.1 M NaCl, 1 mM EDTA and 0.5 % butanol, and gelfiltered on Sephadex G 75 in the same buffer. The size of the column was 1.5 x 25 cm and fractions of 0.75 ml were collected.

B. 50 μg of yeast tRNA was charged as described in A, and gelfiltered on the same column.

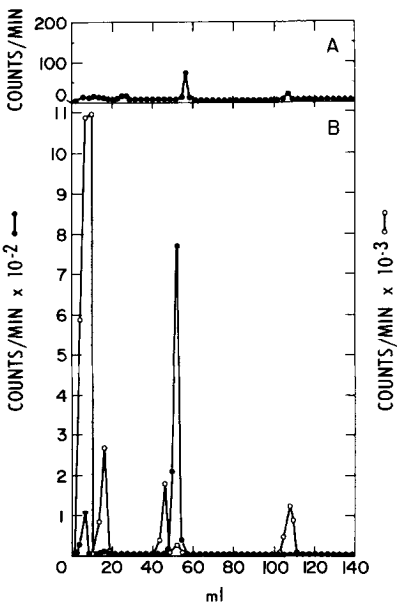

Figure 2 Separation of amino acids charged to TMV-RNA.

50 µg of TMV-RNA was charged with KB cell enzyme and extracted as described in Fig. 1. The RNA preparation was made pH 10 with NaOH and incubated 15 min at 23°C. HCl was added to pH 7 and the mixture was analyzed in a Beckman Amino Acid Analyzer. The ninhydrin step was bypassed and the entire fractions were counted. The ^{3}H amino acid mixture used for charging of the RNA was separated on the anion exchange resin as a control.
A. Acid and neutral amino acids.
B. Basic amino acids.
Hydrolyzate from charged TMV-RNA ●——●
^{3}H-amino acid mixture O——O

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elutes in the void volume together with TMV-RNA. As a comparison yeast tRNA charged and phenol extracted in the same way as the amino acylated TMV-RNA was separated on the same column. As shown in Fig. 1B tRNA is retarded and elutes at about 65 % of the bed volume. This seems to exclude that the amino acylation of TMV-RNA preparation was due to a contamination of cellular tRNA.

Identification of the amino acid bound to TMV-RNA was performed by amino acid analysis. TMV-RNA was first charged with the ^{3}H-amino acid mixture, then phenol extracted and precipitated. The amino acid

was hydrolyzed from the charged TMV-RNA, and the solution was analyzed on a Beckman amino acid analyzer. The elution pattern of acid and neutral amino acids is shown in Fig. 2A. Less than 10 per cent of radioactivity applied to the column, appeared as acid or neutral amino acids. In Fig. 2B is shown the separation of basic amino acids. About 90% of the radioactivity appears as a single peak at the position of histidine. Fig. 2 also shows the separation of the basic amino acids in the ^{3}H amino acid mixture used for charging TMV-RNA.

The number of histidine groups per TMV RNA molecule can be calculated from the specific activity of histidine. In Table 1 the charging of TMV-RNA is 0.06 moles histidine per mole TMV-RNA. It is possible that a higher number could be obtained with a purified amino acyl transferase devoid of any nucleases.

The ability of RNA from two plant viruses, TYMV (1, 2, 3) and TMV, to be charged specifically with valine and histidine respectively, may suggest that esterified RNA has a functional role in translation of the viral mRNA.

Note added at completion of this study: Charging of TMV-RNA with histidine has also been observed by S. Litvak et al. (personal communication).

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