# FURTHER STUDIES ON THE ENZYMATIC AMINOACYLATION OF TMV-RNA BY HISTIDINE

Eloisa CARRIQUIRY and Simon LITVAK
*Department of Chemistry, Faculty of Medicine, University of Chile, Casilla 6679, Santiago, Chile*

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## 1. Introduction

The initial discovery that TYMV*-RNA had a tRNA-like structure able to be recognized by several enzymes that utilize tRNA as substrate [1–4] has been complemented by the recent description of other plant viral RNA having similar properties. RNA from bromegrass mosaic virus incorporates tyrosine [5] while TMV-RNA is aminoacylated by histidine in the presence of yeast histidyl-tRNA synthetase [6, 7]. It has also been reported that TMV after being cleaved by plant endonuclease can accept methionine and serine [8].

The present report deals with some properties of the histidine incorporation into TMV-RNA. RNA from four strains of TMV have been used and important differences of the extent of aminoacylation were found. RNAase $T_1$ treatment has shown that histidine is acylated to a tetranucleotide compatible with the known sequence of TMV-RNA at its 3′ end. The difference between the aminoacylation of the four strains of TMV-RNA was studied in some detail and it was found that this difference may be related to a difference in structure at the 3′ end.

* *Abbreviations:* TMV = tobacco mosaic virus; TYMV = turnip yellow mosaic virus; DMSO = dimethyl sulfoxide; TCA = trichloroacetic acid; PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene.

## 2. Materials

TMV-RNA of the following strains: Vulgare, $U_2$, dahlmensis and HRG were a kind gift of Dr. K.W. Mundry from Tübingen; TMV was a gift of Dr. Vincent Santilli from the Faculty of Sciences, Santiago, Chile; total yeast tRNA was purchased at Nutritional Biochemical Co.; [$^{14}$C]histidine (43 mCi/mmole) was obtained from the Commissariat a l'Energie Atomique, Saclay, France; RNAase $T_1$ was obtained from Worthington and DMSO from Sigma Chemical Co.

## 3. Methods

### 3.1. *Histidine incorporation onto TMV-RNA*

In a total volume of 0.2 ml, the incubation mixture contained 10 μmoles of Tris–HCl pH 7.5, 2 μmoles of $MgCl_2$, 0.4 μmoles of β-mercaptoethanol, 1 μmol of ATP, 2 μCi of [$^{14}$C]histidine, 20–50 μg of HRS and 50 pmoles of TMV-RNA, unless otherwise stated. The incubation was carried out at 37°C for different lengths of time. The reaction was stopped by addition of 2 ml of cold bovine serum albumin (50 mg/ℓ) and 0.1 ml of 50% TCA. The precipitate was filtered through a Whatman glass fiber filter GF/A, washed with 50 ml of cold 5% TCA containing cold histidine, dried and counted in a toluene–PPO–POPOP scintillation mixture.

### 3.2. *Partial purification of yeast HRS*

Fifty grams of yeast (a kind gift of La Trigueña Ltd. Santiago, Chile) were suspended in half a volume

of a buffer containing 0.05 M Tris–HCl, pH 7.5, 0.01 M $MgCl_2$ and 0.001 M $\beta$-mercaptoethanol, to form a viscous slurry. This suspension was passed through a French press homogenizer at 9000 psi. The broken cells were centrifuged at 10 000 *g* for 30′ and the supernatant centrifuged in a Spinco model L ultracentrifuge for 2 hr at 105 000 *g*. The high speed supernatant was adsorbed to a DEAE-cellulose column equilibrated with the buffer described above. Proteins were eluted using a KCl gradient (0–0.4 M, 250 ml of each) prepared in the same buffer. Fractions of 5 ml were collected and the HRS activity was detected by [$^{14}$C]histidine incorporation into total yeast tRNA (4 nmoles per assay). The tubes showing HRS activity were pooled, precipitated to 80% saturation with $(NH_4)_2SO_4$, dissolved and dialyzed against buffer A and kept at −20°C in 30% glycerol.

### 3.3. *DEAE-cellulose chromatography of the RNAase $T_1$ digest of TMV-RNA charged with [$^{14}$C]histidine*

The method was identical to the one described by Yot et al. for RNAase $T_1$ digest of [$^{14}$C]valyl-TYMV-RNA [2].

### 3.4. *Preparation of TMV protein*

Four ml of glacial acetic acid were added to 2 ml of ice cold purified TMV at 10 mg/ml in 0.05 M phosphate buffer, pH 7.0. After 1 hr in ice with occasional stirring, the viral suspension was centrifuged for 15 min at 15 000 *g*. The supernatant is dialyzed extensively against distilled water until the solution becomes very milky. This suspension is centrifuged at 105 000 *g* for 1 hr in the cold. The precipitate is suspended in 1 ml of water. The protein is dissolved by adjusting the solution at pH 8 with KOH under constant stirring. We have assumed that 1 mg of TMV protein gives an absorbance at 280 nm of 0.8.

### 3.5. *Assay for the deacylation of histidyl-TMV-RNA*

In a final volume of 0.2 ml the incubation mixture contained 5 $\mu$moles of sodium cacodylate buffer pH 7.0, 1 $\mu$mol of AMP, 1 $\mu$mol of sodium pyrophosphate, 1 $\mu$mol histidine, 10 $\mu$moles of $MgCl_2$, 1 $\mu$mol of $\beta$-mercaptoethanol, 50–100 $\mu$g of HRS, 100–150 $\mu$g of $^{14}$C-labeled TMV-RNA or 20–30 $\mu$g tRNA sterified with [$^{14}$C]histidine.

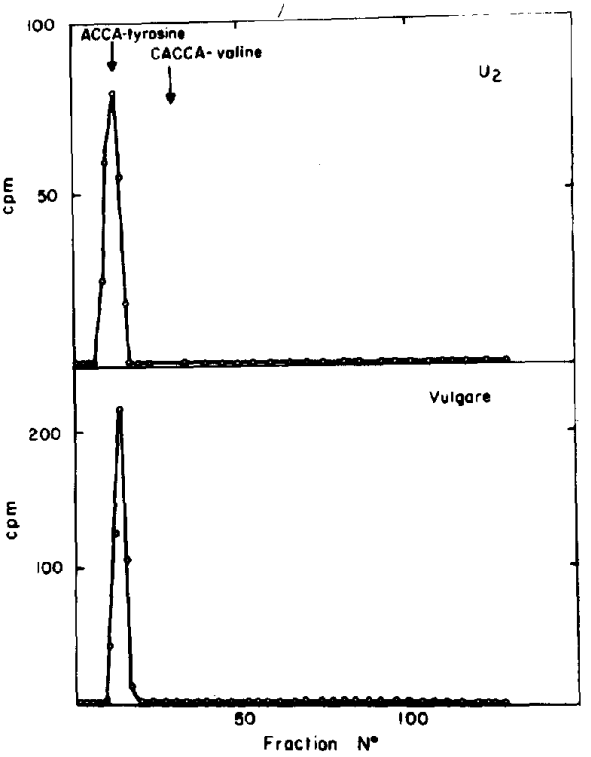


Fig. 1. DEAE-cellulose chromatography of an RNAase $T_1$ digest of histidyl-RNA TMV. TMV-RNA acylated with [$^{14}$C]histidine (1 mg, 25 000 cpm) was digested with RNAase $T_1$ as described previously [2]. The digestion product was put in a DEAE-cellulose column (0.8 × 14 cm) equilibrated with 7 M urea in 0.05 M ammonium acetate, pH 5.5, and then eluted with an ammonium acetate gradient between 0.05 and 0.6 M. One ml fractions were collected and then radioactivity counted.

Incubation was carried out for different lengths of time, at 37°C. The reaction was stopped and the radioactive material washed and counted as described for the HRS assay.

## 4. Results and discussion

### 4.1. *TMV-RNA aminoacylation studies*

As shown previously, TMV-RNA is specifically aminoacylated with histidine but not with any other of the naturally occurring amino acids [7]. The 3′ terminal sequence of TMV-RNA has been determined to be GCCCA-OH [11]. This implies that aminoacylation followed by RNAase $T_1$ digestion should yield an

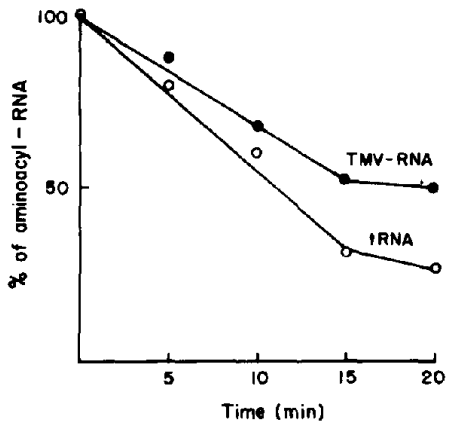


Fig. 2. Enzymatic deacylation of histidyl TMV-RNA. Incubation was carried out as described under Methods.

aminoacyl-tetranucleotide with a CCCA composition. Fig. 1 gives the results obtained in such an experiment. DEAE-cellulose chromatography of the digest yields a radioactive peak that co-elutes with ACCA-tyrosyl obtained by a similar treatment of yeast tyrosyl-tRNA. The results obtained with the $U_2$ and vulgare strains were identical. As shown previously [7], DMSO and formaldehyde treatment of the acylated TMV-RNA showed that the amino acid was still bound to the 24 S viral RNA, thus eliminating the possibility that the acceptor capacity was due to contaminating tRNA.

Enzymatic deacylation of histidyl-TMV RNA was studied as shown in fig. 2. Yeast histidyl-tRNA synthetase causes deacylation of the viral RNA and requires the presence of pyrophosphate and AMP. The reaction, under comparable conditions, seems to go significantly more slowly than the deacylation of yeast histidyl-

Table 1
Effect of TMV coat protein on the histidine acylation of TMV-RNA ($U_2$) and yeast tRNA.

| Additions | pmoles of Histidine incorporated |
|---|---|
| None (TMV RNA) | 17 |
| Coat protein (40 μg) | 16 |
| Coat protein (80 μg) | 18 |
| None (yeast tRNA) | 89 |
| Coat protein (80 μg) | 91 |
| None (TMV) | <1 |

Table 2
Histidine incorporation into different strains of TMV-RNA.

| TMV strain | pmoles Histidine bound per mol of TMV-RNA |
|---|---|
| $U_2$ | 0.40 |
| Vulgare | 0.37 |
| Dahlmensis | 0.09 |
| HRG | 0.13 |

The incubation was performed for 15 min in the conditions described in the text.

tRNA. Similar experiments performed with valyl-TYMV RNA gave an even lower rate of enzymatic deacylation as compared to valyl-tRNA [10].

The effect of the coat protein on the aminoacylation of TMV RNA was tested as shown in table 1. As can be observed, the whole virus particle cannot be aminoacylated. However, the addition of coat protein does not inhibit the acceptor capacity of TMV RNA or of yeast tRNA. The reconstitution of viral particles by addition of RNA and coat protein can be attained in vitro, but it requires prolonged incubation under special conditions [12]. It has been shown that assembly starts by addition of coat protein to regions of RNA.

### 4.2. *Difference in histidine acceptor capacity of RNA of TMV strains*

Differences in the histidine acceptor capacities of RNA obtained from different strains of TMV have been examined. In table 2 it can be observed that the 4 strains studied can be classified into two groups, RNA's from vulgare and $U_2$ have an acceptor capacity of approximately 0.4 moles of amino acid per mole of RNA. The other two strains, dahlmensis and HRG are poorer histidine acceptors with only a 0.1 value.

This difference is apparently not due to a slower reaction occurring with the RNA's showing low acceptor capacities. This is inferred from the results shown in fig. 3, in which the time course of aminoacylation of $U_2$ and HRG was followed. It is clear that the initial velocities of histidine incorporation were quite similar while the plateaux of labelling differed considerably.

The experiment shown in fig. 4, however, indicates that the lower capacity of the RNA's from some strains is probably due to differences in the folding of

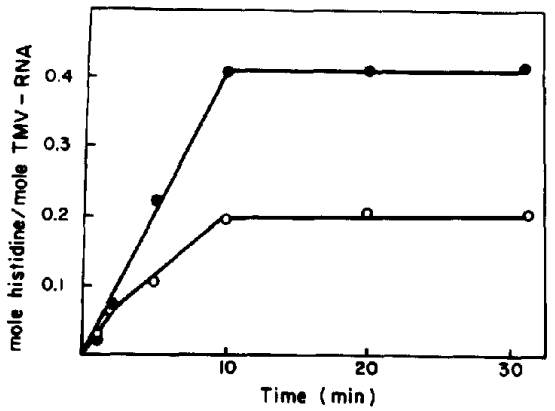


Fig. 3. Kinetics of histidine incorporation into two strains of TMV-RNA. The incubation was performed as described under Methods: TMV-RNA $U_2$ (•—•—•); TMV-RNA HRG (o—o—o).

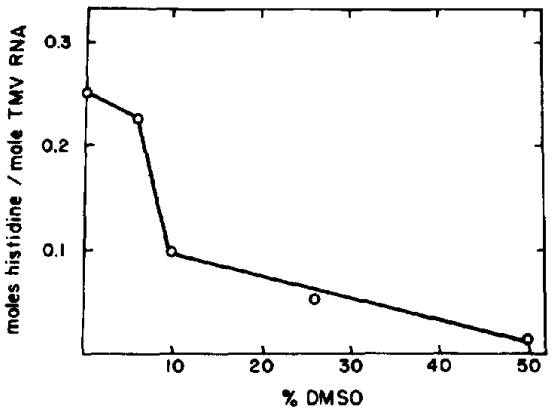


Fig. 5. Effect of DMSO on the histidine incorporation into TMV-RNA. TMV-RNA of the dahlmensis strain was incubated with different amounts of DMSO as described under Methods.

the polynucleotide structure. Pre-heating the HRG strain RNA to 80°C for 10 min apparently increases the amount of RNA molecules capable of accepting histidine to a degree comparable or higher to that of the $U_2$ and vulgare strains. An attempt to show the same effect by using a denaturing agent such as DMSO failed to increase the acceptor capacity. In fact, fig. 5 shows that this compound causes significant inhibition of TMV acylation. DMSO has been shown to increase the aminoacylation of tRNA when heterologous ligases are used [9].

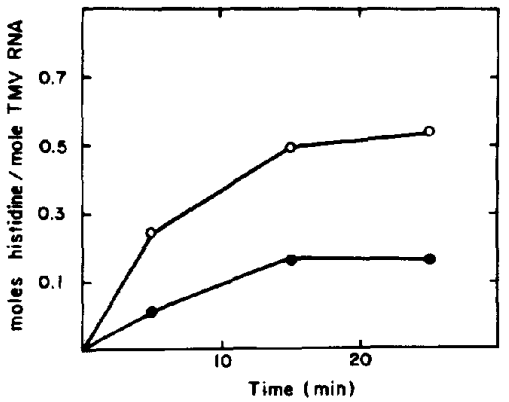


Fig. 4. Effect of temperature on the histidine incorporation into TMV-RNA. TMV-RNA of the HRG strain was preincubated at 80°C for 10 min in the presence of the incubation mixture described in the text with the exception of the aminoacyl-tRNA ligases. The mixture was brought to 37°C, the ligases added and the incubation carried out for different lengths of time (•—•— •) Control; (o—o—o) heated.

## 5. Conclusions

The 3′ end of TMV RNA possesses a tRNA-like structure that can be specifically acylated with histidine by the yeast histidyl-tRNA synthetase.

This enzyme can also catalyze the reaction of deacylation of histidyl-RNA(TMV) in the presence of AMP and $PP_i$. In this reaction the kinetics of the enzyme seem to be slower than for histidyl-tRNA (yeast).

Various strains of TMV RNA differ in the initial acceptor capacity but apparently this is due to the folding of these RNA molecules because pre-heating at 80°C makes the unreactive RNA species accept 0.6 moles of histidine/mole of RNA.

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## References

[1] Pinck, M., Yot, P., Chapeville, F. and Duranton, H. (1970) Nature 226, 954.
[2] Yot, P., Pinck, M., Haenni, A.L., Duranton, H.M. and Chapeville, F. (1970) Proc. Natl. Acad. Sci. U.S. 67, 1345.
[3] Litvak, S., Carré, D.S. and Chapeville, F. (1970) FEBS Letters 1, 316.
[4] Litvak, S., Tarragó-Litvak, L. and Chapeville, F. (1973) J. Virol. 11, 238.
[5] Hall, T.C., Shih, D.S. and Kaesberg, P., J. Virol., in press.
[6] Oberg, B. and Philipson, L. (1972) Biochem. Biophys. Res. Commun. 48, 927.
[7] Litvak, S., Tarragó, A., Tarragó-Litvak, L. and Allende, J.E. (1973) Nature New Biol. 241, 88.
[8] Sela, M., Virology (in press).
[9] Jacobson, K.B. (1971) Progress in Nucleic Acid Research 11, 461.
[10] Yot, P. (1970) Thesis for the degree of Doctor of Sciences. University of Paris VII.
[11] Suyigama, T. and Fraenkel-Conrat, H. (1963) Biochemistry 2, 332.
[12] Fraenkel-Conrat, H. (1957) Virology 4, 1.