Aminoacylation of RNA from Several Viruses: Amino Acid Specificity and Differential Activity of Plant, Yeast and Bacterial Synthetases

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SUMMARY

The RNA of broad bean mottle virus (BBMV), cowpea chlorotic mottle virus (CCMV), and cucumber mosaic virus (CMV) bound tyrosine, as does brome mosaic virus (BMV) RNA. Other amino acids were not bound. Comparison of the efficiency of synthetase enzymes from Escherichia coli, bean, wheat, and Saccharomyces cerevisiae in aminoacylation of RNA from these viruses, and from tobacco mosaic virus (TMV) and turnip yellow mosaic virus (TYMV), showed that the plant enzymes were effective in tyrosylation of RNA from bromoviruses and CMV, whereas the yeast and bacterial enzymes were ineffective. The E. coli enzyme did not esterify TMV RNA with histidine, and the bean enzyme was poor in this ability. All enzymes were able to catalyse valine binding to TYMV RNA. However, 40 mM-KCl inhibited valine binding to TYMV RNA by the bacterial enzyme while its ability to catalyse binding by tRNA was not affected.

INTRODUCTION

The ability to bind specific amino acids at the 3'-terminus of RNA from plant viruses has been reported for turnip yellow mosaic virus (TYMV) and several other members of the tymovirus group (Yot et al. 1970; Pinck et al. 1972), for tobacco mosaic virus (TMV) by Öberg & Philipson (1972) and by Sela (1972), and for brome mosaic virus (BMV) by Hall, Shih & Kaesberg (1972). Although the binding is similar in many respects to aminoacylation of tRNA, it is not yet clear if all, or some, of the charged virus RNAs can function as tRNAs in donating the bound amino acid during protein synthesis (Chen & Hall, 1973; Haenni et al. 1973).

The present experiments were performed to determine the relative activity of synthetase enzymes from several organisms to esterify an amino acid to RNA from viruses known to contain aminoacylatable RNA, and to RNA from broad bean mottle virus (BBMV), cowpea chlorotic mottle virus (CCMV) and cucumber mosaic virus (CMV), which have not previously been reported as having aminoacylatable RNA. Heterologous charging systems have been used in most of the previous reports, and the possibility existed that mischarging had occurred because heterologous systems are prone to this complication (Jacobson, 1971). In addition, we wished to confirm preliminary results which suggested that synthetases from some sources were completely inactive in charging certain of the virus RNAs.
METHODS

Sources of virus RNA. BMV RNA, BBMV RNA and CCMV RNA were extracted by standard phenolization procedures (Bockstahler & Kaesberg, 1965), from viruses respectively grown in barley (Hordeum vulgare), broad bean (Vicia faba) and cowpea (Vigna sinensis) plants. The original inocula were gifts from Dr L. C. Lane; additional BBMV RNA was generously prepared and donated by Dr R. W. Fulton. Dr Louis van Vloten-Doting most kindly supplied samples of CMV RNA and TYMV RNA; TMV RNA was the gift of Dr J. W. Davies.

Preparation of synthetase enzyme fractions. Bean (Phaseolus vulgaris) seed cotyledon and wheat (Triticum aestivum) germ synthetase fractions were extracted, then stripped free from contaminating tRNA by DEAE-Sephadex A-25 treatment as described previously (Chen & Hall, 1973). Yeast (Saccharomyces cerevisiae, strain A224A) and bacterial (Escherichia coli, strains Q13, MRE600 and CA274MM3) enzymes were obtained as supernatant fluids of 100000 g sedimentation (S100) kindly donated by Mr D. Scheindler, Dr J. W. Davies and Mr M. Mauer. tRNA was removed from these enzymes in a similar manner to that used for the plant synthetases, using 0.8 × 20 cm plastic columns (Kontes Co., Vineland, N.J. 08360, U.S.A.). The column eluate was rapidly dialysed against 0.1 M-EDTA in 0.1 M-imidazole-Cl (pH 7.7) by passage through a Bio-Fiber dialyser (Bio-Rad Laboratories, Richmond, CA 94804, U.S.A.). The dialysed enzyme was made to 5 mm-dithiothreitol and 5 mm-glutathione (reduced) before being dispensed into small portions for storage at −90 °C.

Aminoacylation reaction. Amino acid binding to virus RNA was measured using the filter-paper disc technique as described previously (Tao & Hall, 1971; Hall et al. 1972), 0.01 ml portions being taken from the 0.05 ml reaction mixtures detailed in the text. The amino acid substrate mixtures used were designed to minimize the possibility of mischarging, and controls were run to reveal the relative specificity for histidine, tyrosine and valine binding by the different virus RNAs. Thus, when a single amino acid substrate, e.g. [3H]-tyrosine was used, an excess of each of the other 19 amino acids found in protein was also added. The control incubation for virus RNAs which bound tyrosine contained tyrosine plus a mixture of 19 radioactive amino acids, to which relative large amounts of [3H]-histidine and [3H]-valine were added (see legend to Table 1).

RESULTS

Aminoacylation of RNA from BBMV, CCMV and CMV

The finding that RNA from several viruses in the tymovirus group (Harrison et al. 1971) bound valine (Pinck et al. 1972) suggested to us that a similar group characteristic might exist for the bromoviruses, except that the amino acid bound was expected to be tyrosine, because we had previously shown the binding of this amino acid to BMV RNA (Hall et al. 1972). The data in Table 1 confirm this situation, synthetase enzymes from both wheat embryo and bean seed being capable of tyrosylating the RNA from BBMV and CCMV. The bromoviruses all have four RNA components, having mol. wt. of approx. 1.1, 0.9, 0.7 and 0.3 × 10^6 (Lane & Kaesberg, 1971; Hull, 1972). Because the proportion of each RNA component varies from virus to virus, and also in different strains of one virus, the average mol. wt. of a mixture of RNA components varies. We have assumed average mol. wt. of 7.5 × 10^6 for RNA from BBMV, BMV, CCMV; 1 × 10^6 for CMV; and 2 × 10^6 for TMV and TYMV. Using these mol. wt., fmol of amino acid bound per pmol of RNA was calculated for the highest amount of aminoacylation for each virus RNA. The degree of binding is as follows:
Aminoacylation of virus RNAs

Table I. Activity of synthetase enzymes from several organisms in aminoacylation of virus RNAs

<table>
<thead>
<tr>
<th>Virus RNA</th>
<th>Amino acid</th>
<th>Source of synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phaseolus vulgaris</td>
</tr>
<tr>
<td>Broad bean mottle virus</td>
<td>Tyrosine</td>
<td>Triticum aestivum</td>
</tr>
<tr>
<td></td>
<td>Mixture A</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli Q13</td>
</tr>
<tr>
<td>Brome mosaic virus</td>
<td>Tyrosine</td>
<td>Radioactivity bound (ct/min/0.01 ml)</td>
</tr>
<tr>
<td></td>
<td>Mixture A</td>
<td>22 196</td>
</tr>
<tr>
<td>Cowpea chlorotic mottle virus</td>
<td>Tyrosine</td>
<td>17 694</td>
</tr>
<tr>
<td></td>
<td>Mixture A</td>
<td>13 585</td>
</tr>
<tr>
<td>Cucumber mosaic virus</td>
<td>Tyrosine</td>
<td>12 816</td>
</tr>
<tr>
<td></td>
<td>Mixture A</td>
<td>5 653</td>
</tr>
<tr>
<td>Tobacco mosaic virus</td>
<td>Histidine</td>
<td>2 17</td>
</tr>
<tr>
<td></td>
<td>Mixture B</td>
<td>3 27</td>
</tr>
<tr>
<td>Turnip yellow mosaic virus</td>
<td>Valine</td>
<td>3 874</td>
</tr>
<tr>
<td></td>
<td>Mixture C</td>
<td>12 420</td>
</tr>
<tr>
<td>Cognate tRNA</td>
<td>Tyrosine</td>
<td>18 664</td>
</tr>
<tr>
<td></td>
<td>Histidine</td>
<td>12 874</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>9 307</td>
</tr>
</tbody>
</table>

Each reaction mixture (50 μl) contained: 0.1 M-Hepes-NaOH, pH 7.6; 0.64 mM-ATP; 5 mM-MgOAc₂; 40 mM-KCl; approx. 3 pmol virus RNA (6.8 μg BBMV RNA; 4 μg BMV RNA; 4 μg CCMV RNA; 6 μg CMV RNA; 10 μg TMV RNA; 9 μg TYMV RNA); synthetase enzyme (see Methods), and radioactive amino acid substrate. Amino acid substrates used were: 12 μCi [³H]-tyrosine (40 Ci/mmol) plus 19 non-radioactive amino acids (60 μM each, tyrosine omitted); 12 μCi [³H]-histidine (58 Ci/mmol) plus 19 non-radioactive amino acids (60 μM each, histidine omitted); 12 μCi [³H]-valine (16 Ci/mmol) plus 19 non-radioactive amino acids (60 μM each, valine omitted); mixture A contained 19 radioactive amino acids (0.37 μCi each, except for histidine and valine, which were 1 μCi each) plus non-radioactive tyrosine (1 mM); mixture B contained 19 radioactive amino acids (0.37 μCi each, except for tyrosine and valine, which were 1 μCi each) plus non-radioactive histidine (1 mM); mixture C contained 19 radioactive amino acids (0.37 μCi each, except for tyrosine and histidine, which were 1 μCi each) plus non-radioactive valine (1 mM). Each value is the mean of duplicate 10 μl samples withdrawn after 30 min reaction at 30 °C, and is corrected for both zero time binding to the filter disc, and for a control reaction in which virus RNA was omitted. The tRNA used was the cognate tRNA for each enzyme and was 9 μg for bean tRNA, 8 μg for wheat tRNA, 9 μg for yeast tRNA, and 10 μg for Escherichia coli tRNA. Reactions having less radioactivity bound to the disc after 30 min incubation than at zero time are recorded as zero values. Counting efficiency for [³H] was 17%.

BBMV, 687 fmol tyr/pmol RNA; BMV, 715 fmol tyr/pmol RNA; CCMV, 931 fmol tyr/pmol RNA; CMV, 598 fmol tyr/pmol RNA; TMV, 622 fmol his/pmol RNA; and TYMV, 898 fmol val/pmol RNA.

CMV typically contains four RNA components, three of which are necessary for infectivity (Peden & Symons, 1973). This situation is similar to that of the bromoviruses; in addition, the CMV RNA components are similar in size to those of the bromovirus group (Lot et al., 1974). The results shown in Table I augment these similarities of CMV with bromoviruses in that CMV RNA is specifically aminoacylated with tyrosine.

Specificity for synthetases from different sources

In previous studies (Hall et al., 1972; Chen & Hall, 1973) synthetase preparations from bean seed were used to tyrosylate BMV RNA. While the enzyme from this source is very active, and stable for long periods, the charging reaction was heterologous in the sense that BMV does not infect bean plants. However, BMV does infect wheat, and BMV RNA has
Table 2. Comparison of bean and bacterial enzymes in charging turnip yellow mosaic virus RNA with valine in presence and absence of KCl

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Cognate tRNA</th>
<th>TYMV RNA +40 mM-KCl (ct/min/0.01 ml sample)</th>
<th>TYMV RNA -KCl (ct/min/0.01 ml sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaseolus vulgaris</td>
<td>4270</td>
<td>2495</td>
<td>3725</td>
</tr>
<tr>
<td>Escherichia coli MRE600</td>
<td>2478</td>
<td>0</td>
<td>514</td>
</tr>
<tr>
<td>E. coli CA274MM3</td>
<td>2967</td>
<td>0</td>
<td>311</td>
</tr>
</tbody>
</table>

Reaction conditions were identical to those given in Table 1 except that the [3H]-valine used had a specific activity of 7 Ci/mmol.

been shown to be capable of directing the synthesis of authentic virus proteins in a cell-free system from wheat embryo (Shih & Kaesberg, 1973) and from wheat germ (Davies & Kaesberg, 1973). Table 1 shows that synthetase from wheat embryo showed the same specificity for tyrosine charging that was obtained for RNA from the bromoviruses and CMV by bean synthetase. The bean enzyme can be considered homologous for charging BMMV RNA and CCMV RNA because both of these viruses infect *Phaseolus vulgaris*.

The bean and wheat enzymes showed differential activity in their ability to esterify TMV RNA with histidine, the wheat enzyme being more efficient (Table 1). Synthetase from yeast is even more effective in catalysing this reaction, and has been used for this purpose by other investigators (Öberg & Philipson, 1972; Litvak et al. 1973; Carriquiry & Litvak, 1974). However, yeast synthetase is inactive, or very inefficient, in catalysing tyrosylation of RNA from CMV and the bromoviruses.

Under our experimental conditions, the bacterial enzyme only catalysed aminoacylation in the absence of KCl (Table 2). Synthetases from two different strains of *Escherichia coli* were used and compared with bean synthetase. The presence of KCl inhibited the aminoacylation of valine to TYMV RNA when bacterial enzyme was used but had little effect on the aminoacylation catalysed by the bean enzyme. Bacterial enzyme was unable to aminoacylate any other virus RNAs in the absence of KCl.

**DISCUSSION**

Aminoacylation of virus RNA has now been established for all members of the bromovirus group, and CMV (this paper); for several members of the tymovirus group (Pinck et al. 1972) and for TMV RNA (Öberg & Philipson, 1972). Association of tRNA with Rous sarcoma virus (Wang et al. 1973) and avian myoblastosis virus (Rosenthal & Zamecnik, 1973) has been established. The widespread occurrence of tRNA association with RNA viruses suggests that the association has a physiological importance. The present observations indicate that eukaryotic (cytoplasmic) synthetases from the plant sources tested are efficient at aminoacylating RNA from viruses such as BMV which probably multiply in the host cell cytoplasm, while the prokaryotic synthetase from *Escherichia coli* is poor at catalysing such reactions. Conversely, the bacterial enzyme was capable of aminoacylating RNA from TYMV which is a virus thought to be associated with plastids (Bové et al. 1967), which have prokaryotic protein synthesizing systems. It will be interesting to see if there is a general relationship between the site of virus multiplication and the source of enzymes capable of aminoacylating the virus RNA. A recent report (Salmon & Littauer, 1974) of aminoacylation of mengovirus RNA with histidine showed a requirement for an animal synthetase, enzymes
from *E. coli* being inactive. These results suggest that aminoacylation of the virus RNA can occur within the host cell; however, no intrinsic biological role is as yet evident.

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**REFERENCES**


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