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Enzyme-Mediated Binding of Tyrosine to Brome-Mosaic-Virus Ribonucleic Acid

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Incubation of tyrosine with the RNA from brome-mosaic-virus under conditions favourable for aminoacylation of tRNA resulted in binding of tyrosine to the viral RNA. Sucrose-density-gradient centrifugation showed that tyrosine binds to three and probably to all four of the viral RNA components. The bound amino acid was readily released in mild alkaline solutions. Of 20 amino acids tested, only tyrosine was found to bind. The maximum extent of binding observed was 0.58mol of tyrosine/mol of brome-mosaic-virus RNA.

The RNA of plant viruses is believed to function as a messenger carrying the genetic information required for the synthesis of viral proteins. However, it has been shown by Pinck *et al.* (1970) that valine can be enzymically bound to turnip-yellow-mosaic-virus RNA in a manner similar to the charging of $tRNA^{Val}$, suggesting that this RNA may play some additional role. Sela (1972) has demonstrated that fragments of tobacco-mosaic-virus RNA can accept serine and methionine.

BMV-RNA* is known to consist of four components, three of which are essential for infectivity (Lane & Kaesberg, 1971). Thus its functions are divided among several RNA species and it seems quite possible that some of the BMV-RNA components may be messengers, whereas others may serve different purposes. In common with all known tRNA species, each of the four BMV-RNA components has a nucleotide sequence terminating in $C\text{-}C\text{-}A_{OH}$ (Leppla, 1969; Glitz & Eichler, 1971). It was therefore decided to test BMV-RNA for amino acid acceptor activity. To approximate conditions found *in vivo* we used the aminoacyl-tRNA-synthesizing system developed from plants (Tao & Hall, 1971*a*,*b*).

We find that tyrosine, but no other amino acid, binds to BMV-RNA. Moreover, all four components appear to serve as acceptors.

Experimental

Methods

Preparation of aminoacyl-tRNA synthetases. Synthetase enzyme fractions were prepared from cotyledons of developing French bean (*Phaseolus vulgaris* L. cv. Tendergreen) seeds. The plants were grown in aerated liquid nutrient culture under artificial illumination (37600lx) with 16h days (26°C day, 17°C night). The cotyledons used were 11–15mm in length and were taken from plants 45–55 days old. The extraction procedure was similar to that described for the preparation of synthetase fractions A and B from bean leaves (Tao & Hall, 1971*b*), except that 100g of cotyledons were ground into 200ml of extraction buffer. Unless otherwise stated enzyme fraction A was used in the aminoacylation reactions. The protein content of the enzyme preparation was determined by the method of Lowry *et al.* (1951) with dry bovine serum albumin as standard. The enzyme preparations were stored in 0.25ml portions at −90°C; each enzyme portion was used immediately after thawing and any remaining enzyme was discarded. No decrease in activity was noted after storage for 2 months.

Preparation of RNA fractions. BMV-RNA was extracted with phenol (Kirby, 1965) from purified virus (Shih *et al.*, 1972) which had been grown on barley (*Hordeum vulgare* L. cv. Moore) plants. The final preparations were stored as ethanolic precipitates, or in solution at a concentration of approx. 6mg/ml (assuming E_{260} is 23 for a solution containing 1mg of RNA/ml) in 5mM-sodium acetate buffer (pH5.0) containing 5mM-magnesium acetate. Tyrosyl-BMV-RNA was also recovered by phenol extraction after its enzymic synthesis. At all stages of extraction the aqueous phase containing the RNA was maintained at an acid pH value (lower than pH5.5, with 0.5M-sodium acetate buffer). A fraction rich in tRNA was prepared from bean-seed cotyledons by method 3 described previously (Tao & Hall, 1971*b*). After charging *in vitro*, the tyrosyl-tRNA was recovered in a similar manner to that used for tyrosyl-BMV-RNA.

* Abbreviation: BMV-RNA, brome-mosaic-virus RNA.

Assay for amino acid binding to RNA. The conditions established for aminoacylation of bean-leaf RNA *in vitro* (Tao & Hall, 1971*a*) were also optimum for experiments with cotyledon synthetase fractions. Reaction mixtures contained 0.64mM-ATP, 5mM-magnesium acetate and 40mM-KCl in addition to synthetase and RNA. The synthetase fraction was dissolved in 5mM-dithiothreitol and 1mM-GSH. Hence 0.02ml of enzyme provided final concentrations of 0.2mM-GSH and 1.0mM-dithiothreitol in a 0.1ml reaction mixture. The system was buffered with 1M-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)-ethanesulphonic acid] at pH7.6 (30°C) except for experiments where the pH was varied. For the latter experiments the buffer was 1M-Hepes–250mM-Pipes [piperazine-*NN'*-bis(2-ethanesulphonic acid)] and the pH of the solution of cations plus buffer was varied by addition of acetic acid or 10M-NaOH to give a range of pH values between 5 and 10 (at 30°C). The actual pH value of the individual incubation mixtures was found by adding 1ml of water to 0.01ml of the reaction mixture and determining the pH with a Corning model 476050 combination electrode on a Corning model 12 pH-meter.

Most reactions were carried out at 30°C in a final volume of 0.1ml. Samples (0.02ml) were removed at 0, 15, 30 and 45min for assay by the filter-paper-disc method of Rubin *et al.* (1967). When tyrosyl-BMV-RNA was to be recovered, the reaction was scaled up to as much as 5ml. In these cases samples (0.01ml) were removed at the beginning and at the end of the reaction to check the extent of amino acid binding. The reaction was terminated by the addition, with mixing, of 0.1vol. of 1M-sodium acetate buffer, pH5.0, and 0.1vol. of 10% (w/v) sodium dodecyl sulphate and this was followed by phenol extraction (Kirby, 1965). The specific radioactivity of the final preparation was determined directly by measurement of the radioactivity and u.v. absorbance of a diluted sample. Determination of the specific radioactivity of tyrosyl-BMV-RNA by this method and by the disc method differed by less than 10%.

Sucrose-density-gradient centrifugation. Samples of tyrosyl-BMV-RNA were applied to 5–20% (w/v) sucrose gradients and centrifuged for 17h at 25000rev./min at 4°C in the SW 25 rotor on a Spinco model L2-65 ultracentrifuge. Fractions (0.5ml) were collected by using an Isco model D density-gradient fractionator, and radioactivity and u.v. absorbance were determined.

Radioactivity measurements. Filter-paper discs were assayed as described previously (Tao & Hall, 1971*b*). The radioactivity of solutions was counted in a dioxan scintillator solution (Hall & Weiser, 1966) except for samples from sucrose gradients. These were counted in 10ml of 'B-42' scintillator which contained 8g of 2,5-diphenyloxazole, 6mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene, and 50g of naphthalene in 1 litre of solution (900ml of dioxan, 100ml of Triton X-100). Water was added to the scintillator (0.5ml/10ml) immediately before counting. A clear solution was obtained on addition of the sucrose solution; however, the sucrose crystallized out, resulting in uniform counting efficiency for the entire gradient (31% for ^3H counts and 92% for ^{14}C counts, or 50% for ^{14}C counts at a discriminator setting which excluded over 99.5% of the ^3H counts). This scintillator is also useful for radioactivity counting in the presence of high salt (e.g. 1M-NaCl) concentrations.

Chemicals

Scintillation fluors and Triton X-100 were obtained from Research Products Inc., Elk Grove Village, Ill., U.S.A. Naphthalene was the once-recrystallized product of Eastman Chemicals, Rochester, N.Y., U.S.A., and commercial-grade dioxan (North Central Chemical Co., Cottage Grove, Wis., U.S.A.) was used. Other reagent chemicals were those used previously (Tao & Hall, 1971*b*). Radioactive amino acids were obtained from Schwarz/Mann, Orangeburg, N.Y., U.S.A., or from Amersham/Searle, Arlington Heights, Ill., U.S.A.

Results and Discussion

Specificity of the reaction

Initial experiments showed that when a mixture of 14 radioactive amino acids was incubated in the bean-leaf aminoacylation system (Tao & Hall, 1971*b*), but with an enzyme fraction obtained from developing seeds, a sixfold stimulation was obtained by the addition of 60μg of BMV-RNA to the 0.1ml reaction mixture. To determine if there was selective binding of a particular amino acid 20 individual amino acids were tested. When the individual amino acids were supplied at equivalent molarities and at similar specific radioactivities (Table 1) it was clear that tyrosine binding was greatly increased by the addition of BMV-RNA to the reaction. Although phenylalanine binding was apparently stimulated to 8.5% of the extent of tyrosine, it was found in a separate experiment that phenylalanine binding could not be detected when 10nmol of radioactive phenylalanine was challenged with 0.5nmol of unlabelled tyrosine. No inhibition of BMV-RNA-stimulated binding of [^3H]tyrosine (0.5nmol) was observed when as much as 100nmol of unlabelled phenylalanine was added to the aminoacylation reaction. Similarly, no decrease in tyrosine binding was obtained when a non-radioactive mixture of the other 19 amino acids was added to the reaction. Additional confirmation that tyrosine was the only amino acid that would aminoacylate BMV-RNA was obtained by using amino acids with specific radioactivities 50–100 times greater than in

Table 1. *Specificity for tyrosine in BMV-RNA-stimulated aminoacylation*

BMV-RNA (33 μg) was added to standard 0.1 ml aminoacylation reaction mixtures which were catalysed by bean-seed synthetase fraction A (613 μg of protein) and contained 500 pmol of the amino acid to be tested. With the exception of glutamate and glutamine each L-amino acid was supplied at a specific radioactivity of 500 mCi of ^{3}H/mmol and the radioactivity bound to the filter-paper disc was counted at 17% efficiency. Glutamate and glutamine were ^{14}C-labelled, and had specific radioactivities of 260 and 100 mCi/mmol respectively, and were counted at 74% efficiency. Reactions were at pH 7.5 and at 30°C.

	Radioactivity bound to filter (c.p.m./0.02 ml)							
	−BMV-RNA			+BMV-RNA			Stimulation of binding	
Amino acid	Zero-time	45 min	Difference (1)	Zero-time	45 min	Difference (2)	Difference (2) − difference (1)	fmol/μg of RNA
Alanine	19.4	31.0	11.6	25.2	39.1	13.9	2.3	1.0
Arginine	66.5	194.0	127.5	103.9	186.4	80.7	−46.8	—
Asparagine	35.0	78.0	43.0	37.0	74.0	37.0	−6.0	—
Aspartic acid	34.0	51.7	17.7	27.3	48.6	21.3	+3.6	1.5
Cystine	212.0	101.0	−111.0	226.0	92.0	−134.0	−23.0	—
Glutamine	40.3	81.1	41.0	34.3	64.3	30.0	−11.0	—
Glutamic acid	409.0	442.0	33.0	419.0	452.0	33.0	0.0	—
Glycine	434.0	435.0	+1.0	407.0	397.0	−10.0	−11.0	—
Histidine	58.6	74.1	15.5	58.5	76.5	18.0	+2.5	1.0
Isoleucine	4.3	48.0	43.7	12.1	61.7	49.6	+5.9	2.9
Leucine	177.0	237.0	60.0	158.0	199.0	41.0	−19.0	—
Lysine	16.4	63.7	47.3	15.1	78.4	63.3	+16.0	7.9
Methionine	82.3	79.9	−2.4	93.2	73.1	−20.1	−17.7	—
Phenylalanine	70.0	108.0	38.0	70.0	141.0	71.0	+33.0	16.3
Proline	22.2	133.0	110.8	21.5	132.9	111.4	+0.6	0.3
Serine	7.5	46.6	39.1	16.1	57.2	41.1	+2.0	1.0
Threonine	46.0	73.7	27.7	53.3	88.2	34.2	+7.2	3.5
Tryptophan	142.6	131.4	−11.2	103.1	82.9	−20.2	−9.0	—
Tyrosine	42.2	91.1	48.9	40.0	472.4	432.4	+383.5	189.5
Valine	51.0	149.6	97.7	56.8	13.4	74.6	−23.1	—

the experiments shown in Table 1. Much higher count rates were recorded for [^{3}H]tyrosine bound to the filter discs after the aminoacylation reaction than for any other amino acid (Table 2).

The binding observed in the absence of added RNA ('−BMV-RNA' columns in Tables 1 and 2) resulted from traces of endogenous tRNA present in the enzyme. Although the bean-seed amino acyl-tRNA synthetase fraction used was partially purified, no specific steps were taken to remove endogenous tRNA, and it is known that complexes between the synthetase and its corresponding tRNA occur (Mitra *et al.*, 1970; Bandyopadhyay & Deutscher, 1971). Although this endogenous tRNA resulted in low extents of aminoacylation being detected in the absence of added RNA, it also provided evidence that binding of the supplied amino acid could occur under the experimental conditions chosen, i.e. that the requisite amino acyl-tRNA synthetase was present in the enzyme fraction used. This was considered important, as bean-leaf enzyme fractions eluted from the calcium phosphate gel differed in regard to synthetase activity for individual amino acids (Tao & Hall, 1971*b*). Differences in synthetase activity have also been noted in enzyme fractions prepared from bean seeds, although activity for binding of individual amino acids to tRNA is not the same for similar fractions from seeds and leaves (T. C. Hall, unpublished work), possibly as a result of different proportions between these tissues of cytoplasm- and chloroplast-derived synthetases (Burkard *et al.*, 1970; Guderian *et al.*, 1972).

Incubation of cystine in the aminoacylation reaction resulted in decreasing amounts of radioactivity bound to the filter over the assay period (Table 1); however, cysteinyl-tRNA was synthesized in the presence of synthetase fraction A and a seed tRNA preparation when the incubation was at pH 6.1. No stimulation of cysteine binding was obtained on the addition of BMV-RNA at acid pH values. The results

Table 2. *Tyrosine-specific binding of amino acids supplied at high specific radioactivity*

Conditions were as given in Table 1, except that the ^{3}H-labelled amino acids were supplied at the specific radioactivities and in the amounts shown.

Amino acid	Specific radioactivity (Ci/mmol)	Amount supplied (pmol/0.02ml)	Radioactivity bound to filter (c.p.m./0.02ml)						Stimulation of binding	
			−BMV-RNA			+BMV-RNA				
			Zero-time	45min	Difference (1)	Zero-time	45min	Difference (2)	Difference (2)− difference (1)	fmol/μg of RNA
Alanine	51	20	705.3	901.8	196.5	607.0	559.1	−47.9	−244.4	—
Histidine	35	29	1330.0	3436.0	2106.0	1535.0	4025.0	2490.0	+384.0	3.3
Isoleucine	34	29	700.0	2765.0	2065.0	686.0	3152.0	2466.0	+401.0	4.7
Leucine	52	19	400.0	5371.0	4971.0	360.0	6696.0	6336.0	+1365.0	10.5
Lysine	41.6	24	571.1	3760.7	3189.6	463.0	3306.9	2843.9	−347.9	—
Phenylalanine	51	20	1020.4	3540.8	2520.4	2347.6	3786.3	1438.7	−1081.7	—
Tyrosine	52	19	998.4	2986.6	1988.2	1276.1	30615.1	29339.0	+27350.8	211.2

in Table 1 also show little or no endogenous charging for glycine, methionine or tryptophan. However, addition of the seed tRNA preparation to aminoacylation reaction mixtures containing these amino acids confirmed that they were charged, hence synthetases for these amino acids were present in the enzyme fraction used.

The demonstration of aminoacylation of tRNA species that were present in the enzyme preparation is not a rigorous test that all tRNA species could be charged, since the conditions for charging some tRNA species and isoaccepting species of tRNA appear to be very dependent upon cation concentration (Burkard *et al.*, 1970; Vanderhoef *et al.*, 1970) and pH (Tao & Hall, 1971*a*). For these reasons our experiments do not entirely eliminate the possibility that different reaction conditions and different enzyme fractions might reveal an ability of BMV-RNA to bind amino acids other than tyrosine.

Characteristics of tyrosyl-BMV-RNA formation

Tyrosylation of the BMV-RNA had very similar properties to tyrosyl-tRNA synthesis by cell-free systems from plants. The reaction was enzyme- and energy-dependent and was not stimulated by the addition of CTP to the system (Table 3). The kinetics of the reaction are shown in Fig. 1. A lag frequently occurred over the first 5min of incubation, which may give the impression of an acceleration over the 45-min reaction period (Fig. 1). Usually amino acid binding was linear between 15 and 45min of incubation. Rather rapid deacylation occurred with incubations over 45min. Binding of tyrosine to BMV-RNA occurred over a fairly wide range of pH values, but activity rapidly decreased above pH7.8 (Fig. 2). The rapid decrease in binding at high pH values is in part due to rapid hydrolysis of the ester bond. The stability of tyrosyl-BMV-RNA on storage was markedly dependent on pH, as shown in Table 4. The presence of tris catalysed hydrolysis, as has been observed with alkaline hydrolysis of valyl-turnip-yellow-mosaic-virus RNA (Pinck & Schuber, 1971); the half-life of tyrosyl-BMV-RNA in the presence of 0.01M-tris (pH8.5) was approx. 15min.

The K_m for tyrosyl-BMV-RNA synthesis with the bean-seed synthetase fraction at pH7.5 was 9.1×10^{-6}M-tyrosine in the presence of 66μg of BMV-RNA/0.1ml (Fig. 3). When 1.6nmol of [^{14}C]tyrosine (450mCi/mmol) was supplied to the standard aminoacylation reaction mixture and the BMV-RNA concentration varied, the maximum amount of tyrosine bound was 16.76pmol/0.02ml sample (Fig. 4). Since 1μg of the BMV-RNA used contained 1.49pmol of RNA (0.14pmol of component 1, 0.23pmol of component 2, 0.65pmol of component 3 and 0.47pmol of component 4) the apparent K_m was

Table 3. *Requirements for the binding of tyrosine to BMV-RNA*

The complete system contained: ATP (64nmol), magnesium acetate (500nmol), KCl (4μmol), dithiothreitol (100nmol), GSH (20nmol), Hepes (100μmol, adjusted to pH7.6 with NaOH), synthetase fraction A (613μg of protein), L-[3,5-^{3}H]tyrosine (2μCi, specific radioactivity 58Ci/mmol), and BMV-RNA (31μg) in a final volume of 0.1ml. Incubation was at 30°C.

Experimental conditions	Radioactivity bound (c.p.m./0.02ml)		
	Zero-time	45min	Difference
Complete	1921	22815	20894
CTP (20nmol) added	455	20850	20395
ATP omitted	1518	1457	−61
Enzyme omitted	400	399	−1
Enzyme heated (100°C for 2min)	365	407	+42

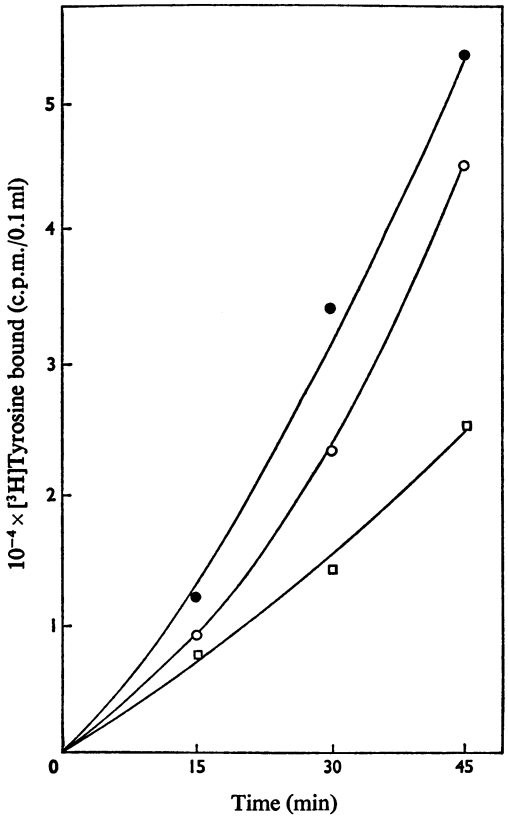

Fig. 1. *Time-course of tyrosine binding at several concentrations of BMV-RNA*

The reaction conditions were as given in Table 3, except that 4μCi of [^{3}H]tyrosine (specific radioactivity 43Ci/mmol), 360μg of enzyme, and different amounts of BMV-RNA (●, 36μg; ○, 24μg; □, 12μg) were included in the 0.1ml reaction mixture. The results were corrected for tyrosyl-tRNA synthesis by subtracting values obtained from reaction mixtures from which BMV-RNA was omitted.

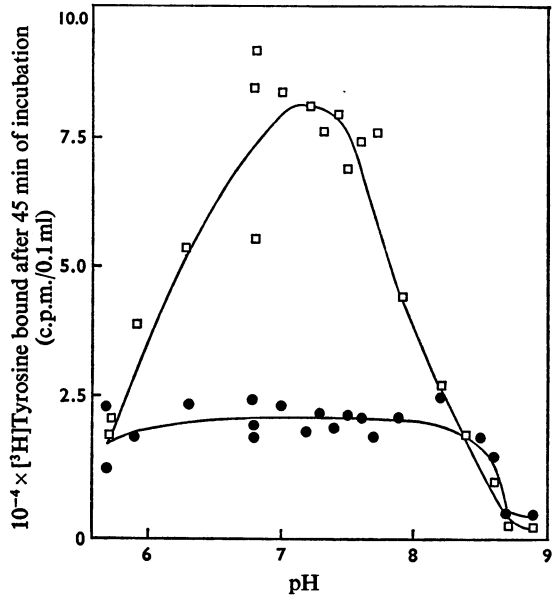

Fig. 2. *Effect of pH on tyrosyl-BMV-RNA synthesis*

The reaction system was that given in Table 3, except that the pH was varied as described in the Experimental section. Values for tyrosyl-BMV-RNA synthesis (□) are corrected for tyrosyl-tRNA formation (●), which resulted from endogenous tRNA present in the enzyme fraction.

7.5×10^{-7} M with respect to the BMV-RNA substrate. The values for molar proportions of the individual RNA components are based on the molecular-weight determinations of Lane & Kaesberg (1971). The maximum extent to which the BMV-RNA was tyrosylated was 12.75pmol of tyrosine/22.5pmol of BMV-RNA (Fig. 4), i.e. 58% of the BMV-RNA molecules were charged with tyrosine, assuming equal tyrosylation of each component.

Table 4. *Stability of tyrosyl-BMV-RNA*

Tyrosyl-BMV-RNA was prepared as described in the Experimental section and 50 μl samples (50000 c.p.m.) were added to a series of tubes containing 0.2 ml of 0.01 M-sodium acetate solutions of different pH values (adjusted with NaOH). At various time-intervals 25 μl samples were withdrawn and the extent of deacylation with time was determined by the filter-paper-disc assay. Results are for 30°C, except that after 24h at 30°C the pH 5 solution was frozen at −90°C.

pH of tyrosyl-BMV-RNA solution	Half-life
5.0	Stable for over 6 weeks
6.1	18 h
7.2	3 h
7.5	2 h
9.0	1 h

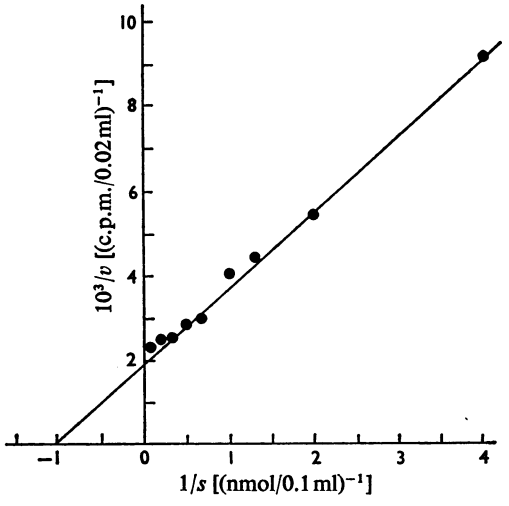

Fig. 3. *Michaelis constant for tyrosyl-BMV-RNA synthesis (tyrosine rate-limiting)*

The reaction conditions were as given in Table 1 except that the stimulation of tyrosine binding obtained in the presence of 66 μg of BMV-RNA was plotted against various concentrations of [^{3}H]-tyrosine (500 mCi/mmol) supplied to the 0.1 ml incubation mixtures. Results are corrected for tyrosyl-tRNA synthesis.

To determine which components of BMV-RNA bind tyrosine we subjected the charged BMV-RNA to sedimentation in sucrose density gradients. Because the tyrosine was readily deacylated it was necessary to use an acidic pH value rather than the alkaline conditions which give the most favourable resolution. The u.v. peaks in Fig. 5 correspond (left to right) to BMV-RNA components 4, 3, and 2+1 (Bockstahler & Kaesberg, 1965; Lane & Kaesberg, 1971). Peaks of radioactivity, from tyrosine, coincided with the extinction peaks of the RNA components. Thus component 1 and/or component 2 and components 3 and 4 were charged. The shape of the patterns coincided in the region of the single peak of components 1 and 2, suggesting that both were charged. An additional peak of radioactivity was observed at the light end of the gradient. This was confirmed to be tyrosyl-tRNA resulting from the endogenous tRNA^{Tyr} present in the synthetase fraction used in the aminoacylation reaction, by sucrose-density-gradient centrifugation of a mixture of bean-seed [^{14}C]tyrosyl-tRNA and [^{3}H]tyrosyl-BMV-RNA, when the ^{14}C label sedimented with the peak at the light end of the gradient (Fig. 5).

Whereas the amount of radioactive tyrosine bound to component 4 appeared much greater than that bound to the other components on a u.v.-extinction

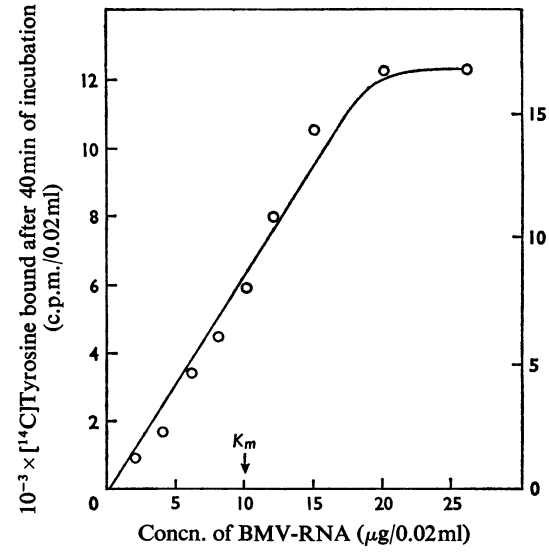

Fig. 4. *Michaelis constant for tyrosyl-BMV-RNA synthesis (BMV-RNA rate-limiting)*

Various amounts of BMV-RNA were charged with 1.6 nmol of [^{14}C]tyrosine (450 mCi/mmol) in the standard aminoacylation reaction (0.1 ml). Incubation was at pH 7.5 (30°C) for 40 min. The results are corrected for tyrosyl-tRNA synthesis. The slope of the graph corresponds to 12.75 pmol of tyrosine charged/22.5 pmol of BMV-RNA (58%).

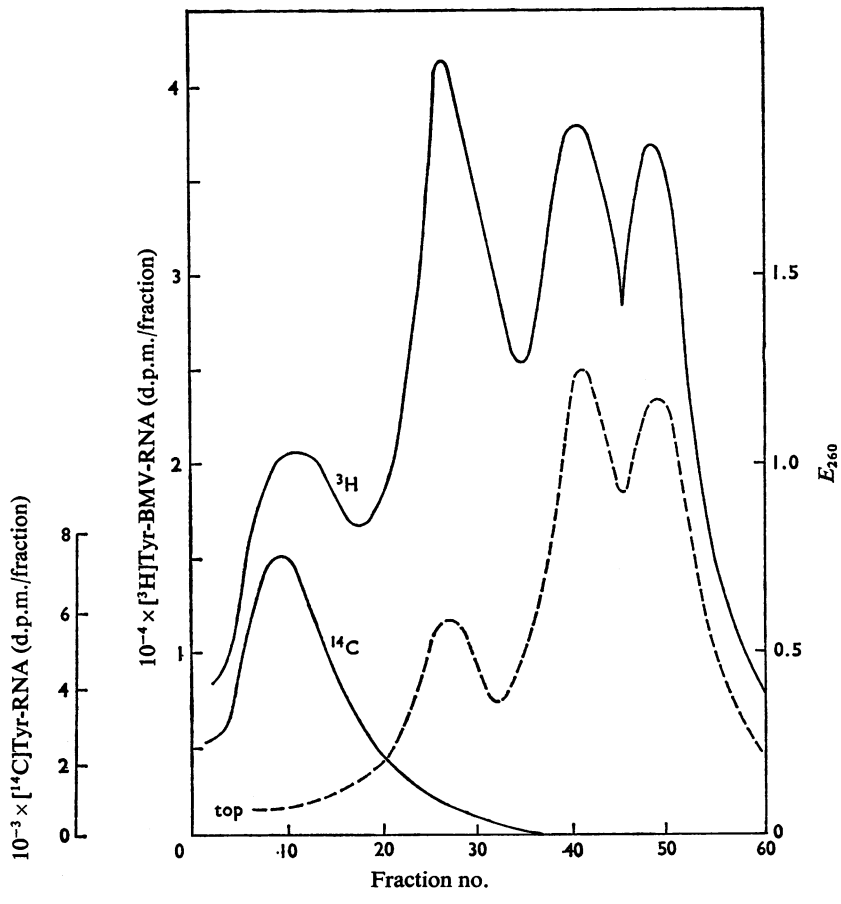

Fig. 5. *Density-gradient centrifugation of a mixture of* [^{14}*C*]*tyrosyl-tRNA and* [^{3}*H*]*tyrosyl-BMV-RNA*

[^{14}C]Tyrosyl-tRNA (125μg; 66000d.p.m.) was mixed with 125μg of [^{3}H]tyrosyl-BMV-RNA (1.9×10^{6}d.p.m.). The mixture was layered on to a 5–20% (w/v) sucrose gradient buffered at pH5.0 with 5mM-sodium acetate and centrifuged under the conditions given in the Experimental section. The radioactivity of tyrosyl-BMV-RNA and that of tyrosyl-tRNA were calculated from scintillation-counting results on the double-labelled fractions collected from the gradient (——). In a separate experiment 700μg of authentic BMV-RNA was centrifuged under identical conditions and the absorption of the fractions measured (----).

basis (Fig. 5), allowance for the smaller size of component 4 suggests a similar specific radioactivity for each component on a molar basis. Better techniques for resolution of the individual components than sucrose-density-gradient centrifugation are required to determine whether significant differences in the extent of aminoacylation of the components occur.

Significance of amino acid binding to plant viral RNA

The observations reported here extend the finding that valine will bind to turnip-yellow-mosaic-virus RNA (Beljanski, 1965; Pinck *et al.*, 1970; Yot *et al.*, 1970) and raise the question as to whether other plant viral RNA species can be charged with amino acids under appropriate conditions. As in the case of valyl-turnip-yellow-mosaic-virus RNA synthesis the molar amount of amino acid bound per mol of RNA approaches, but has not been observed to exceed, unity. The marked specificity for the extensive binding of a single amino acid exhibited by these viral RNA species argues against the binding being fortuitous or caused by plant tRNA bound to the BMV-RNA components.

The biological function for the acceptor activity of these viral RNA species is not known. Possibly

the charging of BMV-RNA has some regulatory function involving protein synthesis, viral RNA replication or the prevention of interference of these processes with each other.

Since the BMV-RNA behaves as a highly purified $tRNA^{Tyr}$ in the aminoacylation reaction it can provide a very sensitive assay system for tyrosyl-tRNA synthetase. Such a system may prove to be of considerable value in devising methods of purification of this synthetase. Should different plant-viral RNA species prove to accept a range of individual amino acids, similar methods could be adapted for their corresponding synthetases.

The question remains as to whether BMV-RNA will function as a tRNA in transferring radioactive tyrosine to growing polypeptide chains in cell-free incorporation systems. The very high specific radioactivity of the amino acid bound to the BMV-RNA should facilitate studying the mechanism of peptide-chain elongation, if direct transfer of the amino acid from the viral RNA to the growing peptide can be shown to occur.

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