

Transfer Ribonucleic Acid Synthetase Activity Associated with Avian Myeloblastosis Virus

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Avian myeloblastosis virions purified by conventional techniques were shown to be associated with or to contain transfer ribonucleic acid synthetase activity. Arginine, tryptophan, cystine, and lysine synthetase activities were observed.

A number of enzyme activities have been found to be associated with the virions of ribonucleic acid (RNA) tumor viruses. Adenosine triphosphatase and ribonuclease activities associated with avian myeloblastosis virus (AMV) have been known for some time (9, 10). Since the discovery of deoxyribonucleic acid (DNA) polymerase in the virions (1, 13), the list of virion-associated enzymes has increased (6-8). Some of these enzyme activities are probably related to the transfer of the genetic information in the virus into DNA which can be integrated into the infected cell in a stable form (12).

The virions of RNA tumor viruses contain low-molecular-weight RNA, a portion of which, at least in some cases, appears to be host transfer RNA (tRNA) which is not sensitive to ribonuclease while it is associated with the virion (2, 3, 5, 14, 15). This observation raises questions concerning essential roles for tRNA in the early events of viral infection. At this time, no published evidence is available about the function of virion tRNA. Participation of tRNA in the early events of infection might occur at the level of aminoacylation by an amino acid synthetase brought into the cell by the virion. Consequently, we examined AMV for several tRNA synthetase activities and report the results in this communication.

MATERIALS AND METHODS

Preparation and purification of AMV and preparation of tRNA and chick embryo synthetases have been described previously (4, 5). The assay for aminoacylation of tRNA was carried out as described previously (4).

RESULTS AND DISCUSSION

Test for synthetase activities. In the standard aminoacylation procedure, the synthetase activity present in AMV preparations was compared with that in enzymes partially purified from chick em-

bryos. Chick embryo synthetases were used in these experiments to verify that conditions of charging were adequate, and to determine the presence of a particular acceptor RNA. The tRNA used in these experiments was originally extracted from 10- to 12-day-old chick embryos and had been deacylated and purified by sucrose gradient sedimentation.

The results presented in Table 1 were obtained by use of 150 μ g of viral total protein or 200 μ g of chick embryo enzyme protein and 20 μ g of tRNA per reaction mixture. There was little effect of endogenous viral tRNA in these experiments because the virus contributes less than 1 μ g of tRNA per reaction. The results (Table 1) indicate that several synthetase activities are associated with the virion. Virus that had not been treated with the nonionic detergent Nonidet P-40 yielded no activity in the assays used. Therefore, in this respect, the enzyme activity is presumably protected by a membrane similar to the DNA polymerase activity in the virion (13). The conditions used in the reactions carried out here have not been optimized for the aminoacylation of any particular tRNA. The values shown are relative and give only a qualitative indication of activity.

Analysis of the product of the reaction. To demonstrate that an aminoacyl tRNA had been formed in the reaction, 750 μ g of AMV was used to charge 100 μ g of 32 P-labeled chick embryo tRNA for 20 min with tryptophan as described above. The reaction mixture was then adjusted to pH 4.85 with sodium acetate, and was extracted with phenol three times. The nucleic acid was precipitated twice with ethanol and centrifuged through a sucrose gradient with 16S RNA as a sedimentation rate marker. As shown in Fig. 1, the 3 H-tryptophan and the 32 P-labeled tRNA have sedimentation coefficients of approximately 4S. The 32 P-labeled tRNA shows evidence of degra-

TABLE 1. Amount (picomoles) of amino acid accepted per 20 μg of tRNA^a

Amino acid	Chick embryo enzyme	AMV
Arginine.....	47.3	4.7
Tryptophan.....	1.9	6.9
Cystine.....	4.6	1.6
Lysine.....	40.7	3.4
Phenylalanine.....	15.5	<0.003
Leucine.....	3.9	0.040
Tyrosine.....	6.1	<0.001
Aspartic acid.....	20.9	<0.2

^a Conditions of aminoacylation were as follows. Each reaction was carried out in 0.3 ml of buffer [0.1 M tris(hydroxymethyl)aminomethane, pH 8.0, 0.01 M MgCl₂, 0.01 M KCl, 0.006 M mercaptoethanol] containing 2 μmoles of adenosine triphosphate, 1 μmole of cytidine triphosphate, 1 μCi of ³H-amino acid, and, in the reactions with AMV, 0.05% Nonidet P-40; 150 μg of viral protein or 200 μg of chick embryo protein was used. Reactions were carried out at 37 C for 20 min, and were stopped by the addition of 1 ml of cold 10% trichloroacetic acid; acid-precipitable radioactivity was determined after filtration through membrane filters (Millipore Corp.). The ³H-amino acids were purchased from Schwarz/Mann (tryptophan, cystine), New England Nuclear Corp. (arginine), and Amersham (leucine, aspartic acid, lysine, phenylalanine, tyrosine).

dition in that it was distributed to a size somewhat less than 4S. This is not surprising in view of the ribonuclease activity observed in the virion (10). The ³H-tryptophan could be removed from the tryptophanyl-tRNA, by a 1-hr incubation in tris (hydroxymethyl)aminomethane at pH 8.1, which is additional evidence on the nature of the reaction product (11).

Kinetics of tryptophanyl-tRNA formation. Equal concentrations of tRNA were aminoacylated with two different concentrations of AMV (Fig. 2). Although the reaction was complete in both cases by 10 min, the yield of charged tRNA was less when less virus was used. Upon addition of more tRNA (arrow, Fig. 2), enzyme activity was still observed. This suggested that perhaps ribonuclease degradation of added tRNA was taking place during the reaction, rendering it unable to accept an amino acid. This argument is strengthened by the observation that preincubation of the reaction mixture for 10 min without tryptophan resulted in no detectable synthetase activity when tryptophan was added. The AMV preparations used in these experiments had approximately 10 times more ribonuclease activity than the chick embryo enzyme preparation, as determined by the extent of degradation of 29S ribosomal RNA produced by these preparations for 20 min at 37 C. With a constant amount of AMV (150 μg), incorporation of tryptophan was

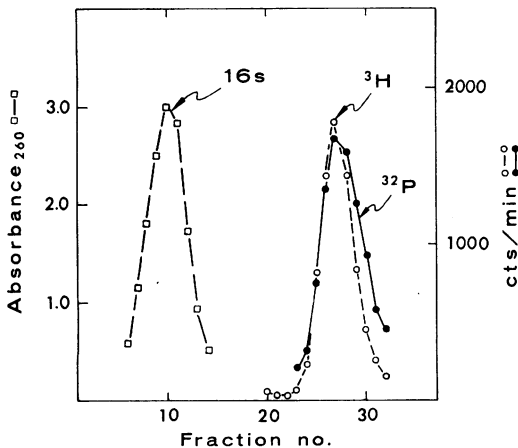


FIG. 1. Sedimentation of the reaction product. The nucleic acid prepared from the reaction mixture as described in the text was dissolved in 0.2 ml of sodium acetate (0.14 M, pH 4.85). A 100- μg amount of chick cell 16S ribosomal RNA was added, and this mixture was sedimented through a 5 to 20% sucrose gradient prepared with 0.15 M sodium acetate, pH 4.85. Sedimentation was in an SW 50.1 rotor (45,000 rev/min for 4 hr). Fractions of 0.15 ml were collected, absorbance was determined, and 0.1 ml of each fraction was used for determination of radioactivity. Recovery of radioactivity was 100% for ³²P and 75% for ³H.

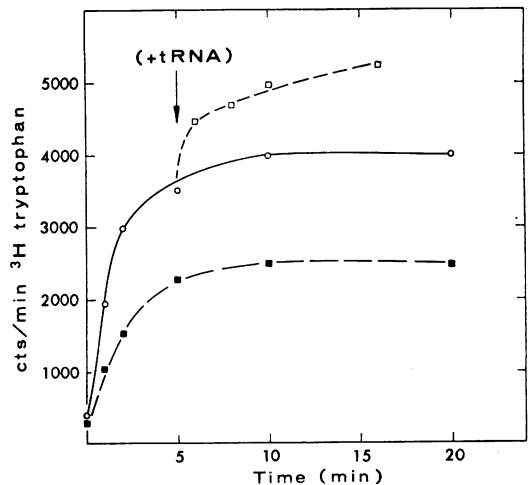


FIG. 2. Kinetics of aminoacylation of tRNA. Aminoacylation was measured at two virus concentrations. At each time point, 0.25 ml of the reaction mixture was added to 1 ml of 10% cold trichloroacetic acid, and acid-precipitable radioactivity was determined. Reaction mixtures contained: tRNA, 120 μg , and AMV protein, 450 μg in 1.8 ml (■); tRNA, 200 μg , and AMV protein, 1.52 mg in 3.0 ml (○). At 5 min, 1.2 ml was removed from the latter tube and reincubated after the addition of 80 μg of tRNA (□).

proportional to the amount of tRNA between 2 and 40 μg .

Sedimentation rate of enzyme activity. The virus used in these experiments was purified by equilibrium sedimentation in sucrose density gradients. To establish further the association of the virion and the enzyme activity, it was important to determine whether the enzyme activity sediments at the same rate as the virus. AMV labeled with ^{32}P and purified by the usual procedures was sedimented through a 15 to 30% sucrose gradient for 30 min at 26,000 rev/min in an SW50 rotor. The distribution of the virus and enzyme activity was measured after sedimentation. The ^{32}P -labeled AMV was shown to sediment with ^3H -uridine-labeled virus in a separate experiment. As the results presented in Fig. 3 show, the virus and the enzyme activity have the same sedimentation rate. The incorporation observed without the addition of tRNA may be the result of virion tRNA.

In conclusion, we have demonstrated tRNA synthetase activities closely associated with the purified virion of AMV. The activity requires activation with nonionic detergent and therefore

is presumably membrane-bound or contained within membranes. Unfortunately, unlike the DNA polymerase activity which has strong supporting evidence for its role in the replication of RNA tumor viruses (12), no supporting biological evidence can be brought forth to suggest an essential role for aminoacylation of specific tRNA preparations in the replication cycle of AMV.

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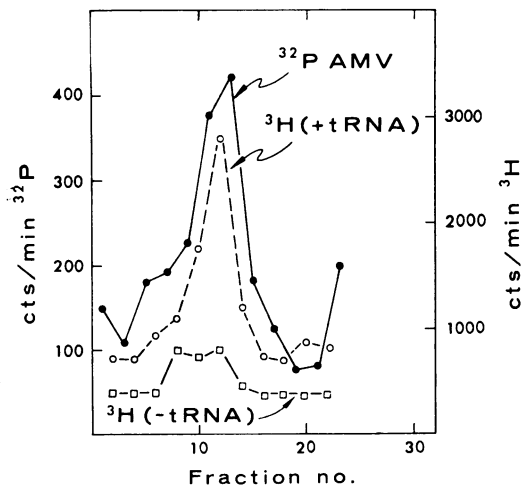


FIG. 3. Sedimentation rate of enzyme activity. A 1.1-mg amount of AMV (^{32}P -labeled) was sedimented through a sucrose gradient as described in the text. Fractions of 0.2 ml were collected. From the odd-numbered fractions, 0.1 ml was used for ^{32}P radioactivity determination (\bullet). The even-numbered fractions were adjusted to 10^{-2} M Mg, and 0.095 ml of each fraction was used to determine enzyme activity. The sample of each fraction was added to a 0.2-ml reaction mixture containing 1 μCi of ^3H -tryptophan, 0.05% Nonidet P-40, and 20 μg of tRNA (\circ) or no tRNA (\square). After 20 min at 37 C, the tubes were chilled in ice, 1 ml of cold trichloroacetic acid was added, and the acid-precipitable radioactivity was determined.