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Amino-Acid Acceptor Activity of the "70S-Associated" 4S RNA from Avian Myeloblastosis Virus

(oncogenic RNA virus/tRNA bound to 70S RNA)

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ABSTRACT **The "70S-associated" 4S RNA from avian myeloblastosis virus contains transfer RNA, as determined by aminoacylation. The various amino acids esterified, plus the great number of minor bases found in this "70S-associated" 4S RNA indicate that a sizeable fraction, but not all, of this RNA is transfer RNA.**

Oncornaviruses have been reported by several laboratories to contain a "70S-associated" 4S RNA that is released from the 60–70S RNA (called for convenience, "70S RNA") under conditions of mild denaturation such as heat or dimethylsulfoxide (Me_2SO) (2–4). Our laboratory has recently reported on the minor base composition of the "70S-associated" 4S RNA from avian myeloblastosis virus (AMV) (1). This composition (with the exception of the value for m^5c) was strikingly similar to that of AMV "free" 4S RNAs previously determined (5). Other work from our laboratory (manuscript submitted for publication) demonstrates an *in vitro* association of myeloblast tRNA with 70S RNA from AMV. These results raise alternative explanations that (*a*) the "70S-associated" 4S RNA binds to the 70S RNA during packaging of virus particles as they bud from the host cell, or (*b*) the association occurs during rupture of the virion and isolation of the total RNA fraction. In further exploration of this problem, we have determined that the "70S-associated" 4S RNA fulfills criteria for transfer RNA, as defined by aminoacylation.

Viral 70S RNA was obtained from purified AMV after sucrose-gradient centrifugation (6). Pooled 70S RNA was obtained after centrifugation in Me_2SO–sucrose gradients. The "70S-associated" 4S RNA was recovered by the addition of solid LiCl to 1 M concentration, followed by 2.5 volumes of cold ethanol. From 500 ml of viremic plasma, about 60 A_{260} units of 70S RNA was obtained by surose gradient centrifugation, and by dissociation of the latter in a 70% Me_2SO sucrose gradient about 3 A_{260} units of "70S-associated" 4S RNA were collected. Thus, the amount of "70S-associated" 4S RNA is severely limited and has permitted only restricted studies. Nevertheless, because of the possible importance of this RNA in the oncogenic viral problem, we report the present results.

The formation of aminoacyl-tRNA was measured as follows: the reaction mixture contained, in 100-μl final volume: 0.05 M Tris·HCl buffer (pH 8), 5 mM ATP, 5 mM KCl, 0.01 M $MgCl_2$, 5 mM 2-mercaptoethanol, 0.3 mg of protein from a crude aminoacyl-tRNA ligase preparation isolated from the myeloblasts of infected chickens, 5–10 μg of the 4S RNA, and 20 μCi of L-[^{3}H]aminoacids. The aminoacyl-tRNAs were charged with either L-[^{3}H]aminoacid mixture (17 Ci/mmol), or a mixture of the following: L-[^{3}H]-tyrosine (29 Ci/mmol), L-[^{3}H]phenylalanine (16 Ci/mmol), L-[^{3}H]proline (30 Ci/mmol), L-[^{3}H]alanine (41 Ci/mmol), [^{3}H]glycine (11 Ci/mmol), and L-[^{3}H]lysine (27 Ci/mmol), all from New England Nuclear Corp. Individual labeled amino acids were also used where indicated. Specific activities of these amino acids were as follows: L-[^{3}H]threonine

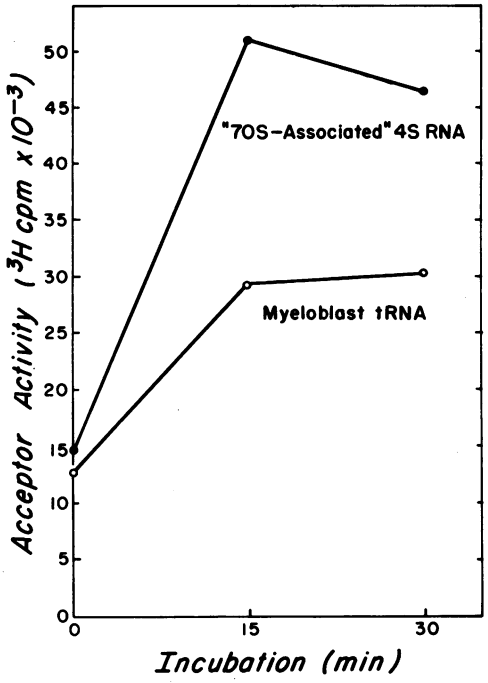

FIG. 1. Time course of aminoacylation of the "70S-associated" 4S RNA and myeloblast tRNA. Total acceptor activity was measured with an L-[^{3}H]aminoacid mixture (17 Ci/mmol). At each time point, reactions were stopped by the addition of 5 ml of cold 5% Cl_3CCOOH. After 15 min at 4°, the precipitated RNA was collected on Whatman 2.4 cm glass-fiber filters and washed vigorously with a total of 75 ml of cold 5% Cl_3CCOOH. Filters were placed in scintillation-counting vials and dried for 20 min. After this, 0.1 ml of water and 0.5 ml of NCS tissue solubilizer (Amersham/Searle) were added to each vial, and the vial and contents were allowed to stand at room temperature (24°) for 1 hr. Counting was done in 10 ml of toluene containing 0.3% 2,5-diphenyloxazole (PPO) and 0.03% 1,4-bis-2-(4-methyl-5-phenoxazoyl-benzene(dimethyl POPOP), as scintillation fluid.

Abbreviations: Me_2SO, dimethylsulfoxide; AMV, avian myeloblastosis virus.

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TABLE 1. *Comparison of aminoacid-acceptor activities of viral "70S-associated" 4S RNA and myeloblast 4S RNA*

Amino acid	"70S-Associated" 4S RNA			Myeloblast 4S RNA		
	Blank*	Sample	Sample/blank	Blank	Sample	Sample/blank
1. Lys	4,400; 4,550	61,700; 62,600	14.0; 13.8	5,040; 4,030	62,700; 58,500	12.4; 14.5
2. Phe	4,000	11,700	2.92	2,770	6,680	2.41
3. Pro	3,260; 3,790	7,410; 5,700	2.27, 1.50	1,790	5,300	2.96
4. Thr	3,840	7,100	1.85	2,200	4,880	2.21
5. His	7,050	10,500	1.49	4,030	5,670	1.41
6. Ser	2,500	3,660	1.46	413	2,430	5.89
7. Gly	2,550	3,150	1.24	1,680	2,630	1.56
8. Tyr	11,700	13,500	1.16	7,320	24,600	3.36
9. Ala	3,780	3,470	0.92	1,740	3,930	2.27
10. Leu	6,890	6,180	0.89	2,690	2,300	0.85

* The blank represents the complete incubation mixture minus RNA.

† Sufficient counts were collected for the blank and the sample to yield a standard error for the [cpm sample–cpm blank] $\leq 1\%$ for values of the ratio ≥ 1.46. With the exception of lysine and proline, the values reported are from one determination, due to the limitation in RNA. Incubation was for 20 min and values are cpm throughout.

(2.4 Ci/mmol); L-[^{3}H]serine (1.2 Ci/mmol); L-[4,5-^{3}H]leucine (36 Ci/mmol); L-[^{3}H]histidine (3.1 Ci/mmol). In the case of lysine the radioactive amino acid was diluted with unlabeled lysine to 0.1 mM. In the other incubation mixtures, unlabeled amino acids were not added. Reaction mixtures were incubated at 37° for 20 min, unless otherwise indicated. Formation of [^{3}H]aminoacyl tRNAs was assayed by the Millipore filter procedure (7). In all assays, parallel experiments using myeloblast tRNA were performed to determine the extent of aminoacylation.

To estimate aminoacylation, we tested two preparations of "70S-associated" 4S RNA and a myeloblast tRNA sample, using an L-[^{3}H]aminoacid mixture. Incubations were for 15 and 30 min. The results presented in Fig. 1 show the extent of aminoacylation for "70S-associated" and for myeloblast 4S RNA. Since the amount of incorporation had plateaued by 20 min, this time was chosen for further experiments with the individual labeled amino acids.

Results presented in Table 1 show that the "70S-associated" 4S RNA contains several amino-acid accepting species of transfer RNA; the most abundant acceptor activity is for lysine. In addition, acceptor activity is found for phenylalanine, proline, threonine, histidine, and serine. Because of limitations of RNA, a definitive statement on other amino-acid acceptor activities of the tRNA cannot be made. For the same reason it has not been possible to perform more detailed time-course studies on amino-acid acceptor activities. The results are closely comparable to data of Travnicek (8, 9) on the amino-acid acceptor activities of "free" 4S RNA from AMV.

Quantitation of lysyl-acceptor activity has been performed with the "70S-associated" 4S RNA, "free" viral tRNA, and myeloblast tRNA. The specific activity (nmol/mg of RNA) for "70S-associated" 4S RNA was found to be 1.58, for free viral tRNA 0.68, and for myeloblast tRNA 0.37. Data for the lysyl-acceptor activity of "free" viral tRNA and myeloblast tRNA are in agreement with previously reported work from our laboratory (5). The high lysyl activity for the "70S-associated" 4S RNA is particularly intriguing. Inasmuch as the percentage of minor bases (7%) is considerably lower in the "70S-associated" 4S RNA than in "free" 4S RNA (17%), it appears that a considerable part of this "70S-associated" 4S RNA (as we have isolated it) is not tRNA. This part may represent contamination by degradation products of high molecular weight RNA. It thus would seem that the lysyl-tRNA acceptor activity is much enriched in that tRNA which is in the "70S-associated" 4S RNA fraction.

In summary, the aminoacylation data, the qualitative similarities in minor base composition of "70S-associated" and "free" 4S RNA, plus our evidence for *in vitro* association of myeloblast tRNA to 70S RNA, all indicate that the "70S-associated" 4S RNA contains transfer RNA, and that various amino-acid acceptor species are present.

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