Chromatographic Analyses of Isoaccepting tRNAs from Avian Tumor Viruses

M. W. TAYLOR, S. WANG, R. M. KOTHARI, AND P. P. HUNG

Department of Microbiology, Indiana University, Bloomington, Indiana 47401 and Department of Virology, Abbott Laboratories, North Chicago, Illinois 60064

Received for publication 27 June 1974

Low-molecular-weight RNA from transforming viruses (Rous sarcoma virus-Rous-associated virus 1, Schmidt-Ruppin strain of Rous sarcoma virus, and sarcoma-B₇₇), from nontransforming viruses (Rous-associated virus 1 and sarcoma-NTB₇₇), and from chicken liver, chicken embryo fibroblast, and Rous sarcoma virus-Rous-associated virus 1-transformed chicken embryo fibroblast was isolated and purified. To determine if there are modified, qualitatively or quantitatively different isoaccepting species of tRNA in these avian sarcoma viruses as compared with the cell of virus origin, chicken embryo fibroblast or normal chicken liver, methionyl-, arginyl-, and lysyl-tRNA (with high amino acid acceptance activity), and aspartyl- and glutamyl-tRNA from viral-transformed cells (with low viral amino acid acceptance activity) were co-chromatographed on reversed phase-5 chromatography columns, and elution profiles were compared. Although in each case the elution profile between a particular viral and host cell tRNA differed quantitatively, there was no qualitative difference in the profiles of corresponding tRNAs from either transforming or nontransforming viruses examined. Minor quantitative differences in the elution profiles might be a reflection of the metabolic state of the cells, since all evidence points to acceptor activity being of host rather than viral origin. Since, with the exception of selective packaging of methionyl-tRNA (IV) species by both transforming and nontransforming viruses, no selectivity was found for isoacceptor species of other tRNAs, it seems that such preferential packaging of methionyl-tRNA (IV) species has no bearing on the event of viral transformation.

One of the classes of RNAs found in the avian tumor viruses is of low molecular weight (4 to 7S) and has been shown to possess amino acid acceptor activity (2, 3, 5, 7, 11, 22, 25, 26). There has been some discussion as to whether this low-molecular-weight RNA is a contamination of host origin adsorbed externally to the viral envelope (1) or whether it is host RNA packaged inside the virion (3). The bulk of the experimental evidence supports the latter view (5, 7, 22, 23). We have previously reported that the tRNAs of transforming viruses (see below) and the nontransforming virus (see below) constitute a unique population of tRNAs, and a unique species of methionyl-tRNA (met-tRNA_{met}, IV) is preferentially packaged by the virion (25, 26). It was therefore thought worthwhile to examine whether isoacceptor species of other tRNA of host origin were preferentially packaged into the virion of transforming as well as nontransforming viruses. For this purpose, we have compared reversed phase-5 chromatography (RPC-5) profiles of isoacceptor species of

five viral tRNAs from a broad spectrum of avian tumor viruses.

MATERIALS AND METHODS

Preparation of viral low-molecular-weight RNA. Low-molecular-weight RNAs were isolated from the following avian tumor viruses by phenol-sodium dodecyl sulfate extraction of virus banded in sucrose gradients, as previously described (15, 25).

Transforming viruses used were (i) Bryan high-titer strain of Rous sarcoma virus (RSV-Rous-associated virus [RAV1]), (ii) Schmidt-Ruppin strain of RSV (SR-RSV), and (iii) sarcoma virus B₇₇ (RSV-B₇₇, kindly supplied by P. K. Vogt).

Nontransforming viruses used were (i) RAV-1, and (ii) nontransforming virus sarcoma-NTB₇₇ (RSV-NTB₇₇).

Preparation of host cell tRNA. Transfer RNAs were isolated from the following sources by the methods of Rogg et al. (20) and by Taylor et al. (21): (i) chicken liver, (ii) chicken embryo fibroblast (CEF), and (iii) RSV-RAV-1-transformed CEF.

Both host-cell tRNAs and viral low-molecular-weight RNAs were further purified by chromatography through DEAE-cellulose (Whatman DE-52,

microgranular) with a gradient of 0.2 to 2.0 M KCl, buffered at pH 4.5. The material eluting between 0.4 to 0.5 M KCl was deacylated, ethanol precipitated, and dissolved in sterile 0.15 M NaCl.

Aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases were prepared from chicken liver as described by Taylor et al. (21) except that Sephadex G-50 was used to free the synthetases from endogenous tRNA, nucleases, and low-molecular-weight impurities. The synthetase preparation was quite stable at -70 C over a period of 6 months.

Aminoacylation procedure. The following amino acids were obtained from New England Nuclear, Boston, Mass.: [^{14}C]arginine (25.9 mCi/mmol), [^{14}C]aspartic acid (146 mCi/mmol), [^{14}C]glutamic acid (234 mCi/mmol), [^{14}C]lysine (254 mCi/mmol), [^{14}C]methionine (280 mCi/mmol), [^3H]arginine (20.6 Ci/mmol), [^3H]aspartic acid (26 Ci/mmol), [^3H]glutamic acid (16.2 Ci/mmol), [^3H]lysine (55 Ci/mmol), and [^3H]methionine (9 Ci/mmol).

The extent of aminoacylation of chicken liver tRNAs by homologous aminoacyl-tRNA synthetase was optimized for amino acid concentration, pH, amount of synthetase, period of incubation, and concentrations of Mg^{2+} , K^+ , ATP, and CTP (25). In each case, tRNA was the limiting component, and at least a twofold molar excess of amino acid was added to ensure maximal aminoacylation. To confirm that plateau levels of aminoacylation were actually obtained with the viral tRNAs, using the amount of amino acids employed for chicken liver tRNA, the effect of amino acid concentration on the extent of aminoacylation was studied for 4S RNA from sarcoma virus B₇₇ as representative of viral tRNAs. The efficiency of counting was 90% for ^{14}C and 22% for ^3H .

RPC-5. The chromatography was performed as described by Kelmers and Heatherly (16). About 10,000 counts/min of ^{14}C -labeled aminoacyl-host cell tRNAs and 30,000 counts/min of ^3H -labeled aminoacyl-viral tRNAs were co-chromatographed for each chromatographic run. The absorbance was in the range of 0.3 to 0.6 units of tRNA at 260 nm. Aminoacyl-tRNAs were eluted from a column (0.9 by 25 cm) at 27 C using a 200-ml linear salt gradient (0.05 M sodium acetate, 0.01 M magnesium chloride, and 0.001 M β -mercaptoethanol [pH 4.5] containing 0.4 to 0.9 M sodium chloride). Fractions (1.0 ml) were collected and processed as previously described (26). Recovery of counts/min ranged between 70 and 85%.

RESULTS

In the present study, aminoacyl-tRNAs with high levels of aminoacylation from several avian tumor viruses were compared by co-chromatography with chicken liver aminoacyl-tRNAs. Due to limited amounts of viral 4S RNA preparations, a preliminary comparison was made between chicken liver and RSV-RAV-1-transformed CEF tRNA to ensure detection of all isoaccepting species. The viral 4S RNA preparation was always acylated with high-specificity ^3H -labeled amino acid to provide maxi-

mal sensitivity of detection. Since the synthetase used throughout the studies was isolated from chicken liver, differences in elution profiles cannot be attributed to this source. The elution profiles were fairly reproducible with at least two batches of liver synthetases, two or three preparations of viral 4S RNAs, and with RPC-5 preparations, prepared in the laboratory by the procedure of Pearson et al. (18), or with RPC-5 purchased from Miles Laboratories, Kankakee, Ill.

Methionyl-tRNA. An analysis of methionyl-tRNA from sarcoma-B₇₇ and the nontransforming isolate, sarcoma-NTB₇₇, gave elution profiles similar to those of RSV-RAV-1 and RAV-1, respectively (Fig. 1A and B). Our earlier work has shown that chicken liver contained four (I through IV) species of methionyl-tRNA, with approximately the same proportions in normal CEF (25). It was further shown that species I and II were initiating methionyl-tRNA, whereas species III and IV were the internal methionyl-tRNA (27). However, an examination of the isoacceptor species of methionyl-tRNA from RAV-1 and RSV-RAV-1 revealed that species IV predominated, with the first three peaks being present in low amounts, probably representing some endogenous tRNA in the synthetase preparation (26). Similar data have subsequently been reported by Elder and Smith (6) and by Gallagher and Gallo (11) for avian myeloblastosis virus (AMV).

In all of the chromatographic profiles discussed above, aminoacylations were performed with chicken liver methionyl-tRNA synthetase. There are reports (12, 13) that the use of nonhomologous aminoacyl-tRNA synthetase can affect chromatographic profiles. In order to verify such claims, we prepared aminoacyl-tRNA synthetase from RSV-RAV-1-transformed CEF by the usual procedure and used this to acylate the two sources of tRNA. The chromatographic profile was similar (not shown in the figure) to that obtained when using chicken liver synthetase to acylate the tRNAs, confirming earlier observation from this laboratory (21, 25). It should be mentioned that AMV virions have been reported to carry aminoacyl-tRNA synthetases (9, 24) and RSV virions undoubtedly do as well; however, it is very probable that these synthetases are identical to host cell synthetases, and it is unlikely that the RPC-5 profile of viral methionyl-tRNA would be altered if the virion synthetase has been used for acylation.

Lysyl-tRNA. Studies on lysyl-tRNA were of particular interest since it is one of the rela-

FIG. 1. RPC-5 chromatographic elution profiles of methionyl-tRNA from (A) CEF, RSV-RAV-1, and sarcoma-B₇₇, and (B) chicken liver, RAV-1, and sarcoma-NTB₇₇.

tively abundant tRNAs in the several viral 4S RNA preparations studied, and since AMV virions have been shown to contain lysyl-tRNA synthetase activity (9, 24).

When chicken liver and CEF lysyl-tRNA were chromatographed on RPC-5, four peaks were obtained. Peak II and IV were the major peaks, with peak II being greater than peak IV

(Fig. 2A). A qualitatively similar elution pattern, with a minor variation in the proportion of species II and IV, was found for RAV-1 and for RSV-RAV-1-transformed CEF lysyl-tRNAs (Fig. 2B). Lysyl-tRNA from sarcoma-B₇₇ and sarcoma-NTB₇₇ also gave a typical four-peak pattern, where the profiles resembled those of RSV-RAV-1 (not shown) and RAV-1 lysyl-

FIG. 2. RPC-5 chromatographic elution profiles of lysyl-tRNA from (A) chicken liver, CEF, and sarcoma-B₇₇, and (B) RSV-RAV-1-transformed CEF, RAV-1, and sarcoma-NTB₇₇.

tRNA, respectively (Fig. 2A and B). Similar observations were made also by Gallagher and Gallo (11) on AMV lysyl-tRNA profiles.

Arginyl-tRNA. In general, arginyl-tRNA profiles on RPC-5 were not very reproducible. The difficulty of obtaining reproducible profiles was much more pronounced in sarcoma-B₇₇, where the presence of an early elutable additional peak, not observed in chicken liver arginyl-tRNA, was unpredictable. This is in concurrence with earlier observations that profiles for eukaryotic arginyl-tRNA varied; it appears that at least two or three species can be resolved on RPC-2 or RPC-5 though the degree of resolution of the peak(s) after the first peak varied considerably in different reports (10, 12, 16, 17). However, in all of these groups studied there was no qualitative difference in elution patterns of arginyl-tRNAs between the tissues being studied.

The elution patterns of arginyl-tRNA from chicken liver and CEF resembled each other and showed a fairly separated first peak, followed by a broad peak with a leading shoulder, probably

second and third peaks that were not well resolved (Fig. 3A). Sarcoma-NTB₇₇ and RAV-1 arginyl-tRNA furnished similar profiles, but with a more prominent second peak (Fig. 3A). RSV-RAV-1-transformed CEF arginyl-tRNA gave a profile in which the second peak was more prominent (Fig. 3B). Sarcoma-B₇₇ arginyl-tRNA furnished a qualitatively different elution profile when compared to chicken liver; however, since its reproducibility is questionable with different batches of viral tRNA, it cannot be compared directly with the corresponding profiles of tRNA from either CEF or RSV-RAV-1-transformed CEF (Fig. 3B). Gallagher and Gallo have also noted qualitative differences in the profiles of the arginyl-tRNA from AMV (11).

Aspartyl- and glutamyl-tRNA. Methionyl- and lysyl-tRNAs were present in comparatively high levels in the 4S RNAs of the three transforming viruses examined (RSV-RAV-1, SR-RSV, and sarcoma-B₇₇). In the case of these tRNAs, there were some quantitative differ-

FIG. 3. RPC-5 chromatographic elution profiles of arginyl-tRNA from (A) chicken liver, RAV-1, and sarcoma-NTB₇₇, and (B) RSV-RAV-1-transformed CEF and sarcoma-B₇₇.

ences in the ratio of isoaccepting species between RSV-RAV-1-transformed CEF and CEF. We wondered whether there was any correlation between the presence of high levels of tRNAs in the transforming viruses and quantitative differences in the corresponding tRNAs in transformed cells as compared to normal cells. Therefore, we co-chromatographed two tRNAs, aspartyl- and glutamyl-tRNAs, from transformed and normal CEF, that were present in low levels in the virions of the transforming viruses.

Aspartyl-tRNA from normal and transformed CEF gave qualitatively identical patterns (Fig. 4A). Briscoe et al. (4) have reported that tRNAs from tumors show a minor third peak on RPC-2 columns, whereas normal cells do not. Gallo and Pestka (12) did not find a third aspartyl-tRNA peak in either normal or leukemic human lymphoblasts on RPC-2. However, we did observe a third peak in normal as well as transformed CEF. Frazer and Yang (10) also found a third peak in normal mouse liver on RPC-2 columns, but not in mouse brain.

Glutamyl-tRNA from normal and transformed CEF gave quantitatively different patterns (Fig. 4B). The first [^{14}C]glutamyl-tRNA peak (Fig. 4B, dotted line) was probably an artifact, since it did not appear if the isotopic labels were reversed. The resolution of the peak or peaks between the two major peaks was not very good. It appears that the transformed fibroblasts have a lesser proportion of the major first peak than do normal fibroblasts. Reversing the isotopic labels did not alter this proportion. Other workers (10, 12) have found only two major species of glutamyl-tRNA, both being present in equal amounts in chicken embryo and chicken liver (19).

DISCUSSION

We have further demonstrated the selective packaging of methionyl-tRNA species IV, namely, internal methionyl-tRNA by other strains of avian sarcoma virus. Thus, all avian tumor viruses examined to date (various strains of Rous sarcoma, avian myeloblastosis, and both the transforming and nontransforming variants of sarcoma B₇₇ virus) selectively incorporate internal methionyl-tRNA (6, 11, 25, 26).

Eukaryotic methionyl-tRNAs fractionated on RPC-2 (10, 12, 17, 27, 28) columns have usually been reported to give three peaks. The largest peak, and the first to elute from a salt gradient, is F-methionyl-tRNA. We found that chicken liver methionyl-tRNA gave four peaks on the RPC-5 column. However, if a large quantity of methionyl-tRNA was loaded, the second peak

FIG. 4. RPC-5 chromatographic elution profiles of (A) aspartyl-tRNA, and (B) glutamyl-tRNA, from CEF and RSV-RAV-1-transformed CEF.

appeared as a shoulder on the trailing edge of the major first peak; the chromatogram was then very similar to previously published chromatograms (10, 12, 17, 27, 28). Gallo and Pestka (12) have found that there is some increase of the last methionyl-tRNA peak in leukemic lymphoblasts as compared to normal human lymphoblasts. Gonano et al. (14) found a large increase in the proportion of the second methionyl-tRNA peak in Morris hepatoma 5123 as compared to normal rat liver. In fact, the change in proportion of the second peak of methionyl-tRNA to the first peak was very similar to the change we observed for transformed CEF as compared to normal CEF (25, 26). Using *in vivo* labeled aminoacyl-tRNAs, Lee and Ingram (17) found a remarkable shift in the proportions of the two major methionyl-tRNA peaks in reticulocytes from adult chickens and blood cells of 4-day-old embryos, on both methylated albumin-kieselguhr and RPC-2 columns. They suggested that the change in the methionyl-tRNA ratio could be involved in translational regulation at the level of initiation.

As pointed out by Gallagher and Gallo (11), there are broad similarities between the amino-

acylation patterns of AMV and RAV-1 tRNAs. We confirmed further that sarcoma-NTB₇₇ also shows a similar pattern of aminoacylation (R. M. Kothari and M. L. Taylor, manuscript in preparation). No significant differences in the RPC-5 profiles of the corresponding tRNAs from AMV, RAV-1, and sarcoma-NTB₇₇ could be found. We have noted high degrees of resemblance in the RPC-5 profiles of corresponding tRNAs also from avian transforming viruses, RSV-RAV-1, SR-RSV, and sarcoma-B₇₇.

Since all of the avian tumor viruses examined, including those that do not transform in culture, selectively incorporate internal methionyl-tRNA species, we are led to the belief that this tRNA species is not selectively involved in the transformation event, but reflects either a metabolic alteration of the infected cell during viral replication or is essential for some yet unknown viral function. No such selectivity was found for any of the other tRNAs studied (viz, lysyl-, arginyl-, aspartyl-, and glutamyl-tRNA). It is possible that due to the uniqueness of F-methionyl-tRNA, its distribution in the cell differs from that of other tRNA species, and that the incorporation of internal methionyl-tRNA is due to compartmentalization of the virus.

There has been some controversy as to the origin of the viral low-molecular-weight RNA material (1). All evidence points to it being of host origin rather than viral origin. It has been difficult to prove that it is not random contamination of host tRNA, but the selectivity of met-tRNA_{met} and the uniqueness of the tRNA population of several viruses argue against this (25, 26). However, it is possible that a low percentage of the host tRNA may be a contamination in viral 4S RNA preparation. Perhaps more problematic is to distinguish tRNA which is "free" in the virion from the 70S-associated tRNA (8), which may play a functional role in either the structure of the viral genome or in viral replication. Although the 4S RNA used in these experiments was free tRNA from the virion, it is possible that some of this is derived from 70S-associated RNA due to degradation during purification. However, the bulk of it is probably incorporated in the virion during the maturation stage(s) as part of the host-viral envelope complex. As such, the differences in populations of tRNA observed between RSV-RAV-1 and RAV-1 and also between sarcoma-NTB₇₇ and RAV-1 (R. M. Kothari and M. W. Taylor, manuscript in preparation) may reflect site of maturation rather than viral function. •

ACKNOWLEDGMENTS

We thank Peter K. Vogt for kindly providing us with sarcoma virus-B₇₇.

This work was supported by Public Health Service grant CA 10417 from the National Cancer Institute and Public Health Service training grant GM 01046-11 from the National Institute of General Medical Science.

LITERATURE CITED

1. Bader, J. P., T. L. Steck, and T. Kakefuda. 1970. The structure of the RNA of RNA-containing tumor viruses. *Curr. Top. Microbiol. Immunol.* **51**:105-113.
2. Bishop, J. M., W. E. Levinson, N. Quintrell, D. Sullivan, L. Fanshier, and J. Jackson. 1970. The low molecular weight RNAs of Rous sarcoma virus. I. The 4S RNA. *Virology* **42**:182-195.
3. Bonar, R. A., L. Sverak, D. P. Bolognesi, A. J. Langlois, D. Beard, and J. W. Beard. 1967. Ribonucleic acid components of BAI strain A (myeloblastosis) avian tumor virus. *Cancer Res.* **27**:1138-1157.
4. Briscoe, W. T., W. Taylor, A. C. Griffin, R. Duff, and F. Rapp. 1972. Aspartyl-transfer RNA profiles in normal and cancer cells. *Cancer Res.* **32**:1753-1755.
5. Carnegie, J. W., A. O'C. Deeney, K. C. Olson, and G. S. Beaudreau. 1969. An RNA fraction from myeloblastosis virus having properties similar to transfer RNA. *Biochim. Biophys. Acta* **190**:274-284.
6. Elder, K. T., and A. E. Smith. 1973. Methionine transfer ribonucleic acids of avian myeloblastosis virus. *Proc. Nat. Acad. Sci. U.S.A.* **70**:2823-2826.
7. Erikson, E., and R. L. Erikson. 1970. Isolation of amino acceptor RNA from purified avian myeloblastosis virus. *J. Mol. Biol.* **52**:387-390.
8. Erikson, E., and R. L. Erikson. 1971. Association of 4S ribonucleic acid with oncornavirus ribonucleic acids. *J. Virol.* **8**:254-256.
9. Erikson, E., and R. L. Erikson. 1972. Transfer ribonucleic acid synthetase activity associated with avian myeloblastosis virus. *J. Virol.* **9**:231-233.
10. Frazer, J. M., and W. K. Yang. 1972. Isoaccepting transfer ribonucleic acids in liver and brain of young and old BC₃F₁ mice. *Arch. Biochem. Biophys.* **153**:610-618.
11. Gallagher, R. E., and R. C. Gallo. 1973. Chromatographic analyses of isoaccepting tRNAs from avian myeloblastosis virus. *J. Virol.* **12**:449-457.
12. Gallo, R. C., and S. Pestka. 1970. Transfer RNA species in normal and leukemic lymphoblasts. *J. Mol. Biol.* **52**:195-219.
13. Goldman, M., W. M. Johnston, and A. C. Griffin. 1969. Comparison of transfer ribonucleic acids and aminoacyl synthetases of liver and ascites tumor cells. *Cancer Res.* **29**:1051-1055.
14. Gonano, F., V. P. Chiarugi, G. Pirro, and M. Marini. 1971. Transfer ribonucleic acids in rat liver and Morris 5123 minimal deviation hepatoma. *Biochemistry* **10**:900-908.
15. Hung, P. P. 1973. Ribonucleases of Rous sarcoma virus. *Virology* **51**:287-296.
16. Kelmers, A. D., and D. E. Heatherly. 1971. Columns for rapid chromatographic separation of small amounts of tracer-labeled transfer ribonucleic acids. *Anal. Biochem.* **44**:486-495.
17. Lee, J. C., and V. M. Ingram. 1967. Erythrocyte transfer RNA change during development. *Science* **158**:1330-1332.
18. Pearson, R. L., J. F. Weiss, and A. D. Kelmers. 1971. Improved separation of transfer RNA's on polychlorotrifluoro-ethylene-supported reversed-phase chromatography. *Biochim. Biophys. Acta* **228**:770-774.
19. Portugal, F. H. 1972. Elution profiles of lysine and tyrosine transfer RNA during avian development. *Mech. Ageing Develop.* **1**:3-21.
20. Rogg, H., W. Wehrli, and M. Staehelin. 1969. Isolation of mammalian transfer RNA. *Biochim. Biophys. Acta* **195**:13-15.

21. Taylor, M. W., C. A. Buck, G. A. Granger, and J. J. Holland. 1968. Chromatographic alterations in transfer RNAs accompanying speciation, differentiation and tumor formation. *J. Mol. Biol.* **33**:809-828.
22. Travnicek, M. 1969. Some properties of amino acid acceptor RNA isolated from avian tumor virus BAI strain A (avian myeloblastosis). *Biochim. Biophys. Acta* **182**:427-439.
23. Travnicek, M., and J. Riman. 1970. Chromatographic differences between lysyl-tRNA's from avian tumor virus BAI strain A and virus-transformed cells. *Biochim. Biophys. Acta* **199**:283-285.
24. Travnicek, M., and J. Riman. 1973. Occurrence of aminogcyl-tRNA synthetase in RNA oncogenic virus. *Nature N. Biol.* **241**:60-62.
25. Wang, S., R. M. Kothari, M. W. Taylor, and P. P. Hung. 1973. Transfer RNA activities of Rous sarcoma and Rous associated viruses. *Nature N. Biol.* **242**:133-135.
26. Wang, S., R. M. Kothari, M. W. Taylor, and P. P. Hung. 1974. Selective incorporation of host cell methionyl-transfer RNA by RNA tumor viruses. *Biochim. Biophys. Acta* **340**:52-63.
27. Yang, W.-K., A. Hellman, D. H. Martin, K. B. Hellman, and G. D. Novelli. 1969. Isoaccepting transfer RNA's of LM cells in culture and after tumor induction in C₃H mice. *Proc. Nat. Acad. Sci. U.S.A.* **64**:1411-1418.
28. Yang, W.-K., and G. D. Novelli. 1968. Multiple isoaccepting transfer RNA's in a mouse plasma cell tumor. *Proc. Nat. Acad. Sci. U.S.A.* **59**:208-215.