Chromatographic Analyses of Isoaccepting tRNAs from Avian Myeloblastosis Virus

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Avian myeloblastosis virus (AMV) 4S RNA was tested for amino acid acceptor activity for 18 of the 20 amino acids. A nonrandom distribution of viral tRNAs was found compared with tRNA from normal liver or from AMV-infected leukemic myeloblasts, confirming previous reports. Methionine and proline tRNAs were considerably enriched, whereas glutamic acid, glutamine, serine, tyrosine, and valine tRNAs were markedly depleted in AMV relative to homologous cellular tRNAs. The seven AMV tRNAs with the greatest amino acid acceptance capacities, which were in order methionine, proline, lysine, arginine, histidine, isoleucine, and threonine tRNAs, were compared with homologous tRNAs from leukemic myeloblasts and liver by reversed-phase 5 chromatography. Of the 25 isoaccepting chromatographic fractions identified, no tRNA species unique to AMV was detected. Only methionyl-tRNA showed a substantial quantitative variation in isoaccepting species compared with the host cell. Thus, viral selectivity for amino acid-specific tRNAs is not, generally, paralleled by selectivity for individual isoaccepting tRNA species. Qualitative differences in arginyl- and histidyl-tRNA isoaccepting species were discovered in virus and leukemic myeloblasts compared with liver. This indicates the existence of structural differences in these tRNA species which could be related to virus replication or expression.

RNA tumor viruses have been demonstrated to contain substantial amounts of a heterogeneous population of 4S RNA molecules in addition to the single-stranded, genomic 60-70S RNA molecular complex (2, 17). Many of the 4S RNA species have the properties of tRNA, because they contain characteristic methylated nucleic acid bases (16) and because they can be aminoacylated (2, 6, 7, 22, 27). Recently, the evidence has been summarized which indicates that these viral tRNAs are predominately derived from host cells, apparently being incorporated within the viral envelope during the process of budding from the cell membrane (27).

Some investigators have found 4S RNA species associated with the 60-70S RNA complex of RNA tumor viruses (4, 8), and a recent study of modified nucleic acid bases suggests that associated 4S RNA is very similar in composition to free 4S RNA in avian myeloblastosis virus (AMV) (18). However, this is not resolved, since other investigators do not find 4S RNA molecules associated with the 60-70S RNA complex of closely related avian oncornaviruses (1, 19).

Reported aminoacylation data indicate that

viral tRNAs do not represent a random assortment of cellular tRNA species (7, 22, 27). Leukosis viruses and sarcoma viruses derived from the same cells are reported to contain a different complement of tRNA molecules (27). On the other hand, there does appear to be some consistency in tRNA species which are associated with or excluded from avian leukosis viruses (*vide infra*). This selectivity could indicate some special function for these molecules. This might be related to the presence in virions of other translational elements, including ribosomes and aminoacyl-tRNA synthetases (9, 24). Alternatively, selective tRNA species might be involved in the reported stimulation of viral RNA-dependent DNA synthesis by 70S RNA-associated 4S RNA primers (4, 25). At present, however, there is no direct evidence that selectivity is related to specific function, and it is possible that viral tRNA species reflect fortuitous inclusion or inactivation of cellular tRNA species.

In this study, we screened the 4S species from AMV for aminoacylation with 18 of the 20 amino acids. The seven viral tRNAs with the greatest amino acid acceptance capacity were

studied by reversed-phase 5 chromatography (RPC-5) to determine whether there are unique, modified, or quantitatively different isoaccepting tRNA species in AMV as compared with the cell of virus origin, the leukemic myeloblast, or normal chicken liver. These tRNA species might then be purified and tested for specific function in viral transcription or translation.

MATERIALS AND METHODS

Preparation of virus and cells. Avian myeloblasts and AMV (BAI strain A virus) were obtained from heparinized blood of leukemic chickens (kindly provided by J. W. and D. Beard, Duke Univ., Durham, N.C.). Myeloblasts were separated from erythrocytes by sedimentation at 37°C for 30 min. The myeloblasts were washed twice with phosphate-buffered saline, pH 7.3, and stored at -70°C until used. Virus was prepared from clarified plasma by centrifugation onto a 100% glycerol pad (SW-27 rotor at $96,000 \times g$ for 1 h). The viral pellet was resuspended in TNE buffer (0.01 M Tris, pH 8.5, 0.01 M NaCl, and 0.001 M EDTA), and the centrifugation onto a 100% glycerol pad was repeated twice. Finally, the virus was banded on a 20 to 80% glycerol gradient in an SW27 rotor by centrifugation at $53,000 \times g$ for 14 h. Normal livers were obtained from white male Leghorn chickens (Truslow Farms, Chestertown, Md.) and used fresh or stored at -70°C.

Preparation of viral and cellular RNA. The banded virus was diluted in TNE buffer, pH 7.5; bentonite was added to a final concentration of 2 mg/ml and sodium dodecyl sulfate was added to 1%. After shaking gently for 30 s on ice, 0.5 vol of phenol-cresol saturated with TNE buffer was added and gentle shaking was continued for 5 min. After centrifugation at $2,000 \times g$ to separate the aqueous and phenol phases, the aqueous phase was extracted twice with phenol-cresol and once with chloroform. The aqueous phase was precipitated with 0.1 vol of potassium acetate and 2.2 vol of ethanol overnight at -20°C. The precipitate was collected by centrifugation at $15,000 \times g$ for 15 min. The pellet was suspended in 0.5 ml of TNE buffer, pH 7.5, and centrifuged through a 10 to 30% glycerol gradient in an SW-41 rotor at $196,000 \times g$ for 4 h. Fractions were collected from the bottom of the tubes, and determinations of absorbancy at 260 nm (A_{260}) were taken on samples across the gradient. The peaks of 70S, 35S, and 4S RNAs were separately pooled and precipitated with 0.1 vol of 20% potassium acetate and 2.2 vol of ethanol at -20°C. For use in these studies, the 4S RNA was resuspended in a small volume of TNE and A_{260} determinations were recorded. Cellular tRNAs were prepared by the method of Yang and Novelli (29). The ratio of absorbance of light at A_{260} to A_{280} was 2.0 ± 0.05 for all RNA preparations.

Preparation of aminoacyl-tRNA synthetase. Fresh normal chicken livers were finely minced, freeze-thawed three times, suspended in 2 vol of buffer A (0.01 M KPO_4 , pH 7.5, 0.005 M KCl, 0.001 M $MgCl_2$, 0.02 M 2-mercaptoethanol, and 15% glycerol) and homogenized for 2 min at 4°C in a microblender (Eberbach Corp., Ann Arbor, Mich.). The homogenate was clarified by centrifugation at $4,000 \times g$ in a

Sorvall centrifuge, and the supernatant fluid was centrifuged at $101,000 \times g$ in a type 40 rotor for 1 h. The supernatant fluid was directly applied to a DEAE-cellulose column, as described elsewhere (11), and washed with buffer A until all protein had been eluted, as determined by A_{260} monitoring. Then, the tRNA was eluted from DEAE-cellulose with buffer B (0.01 M KPO_4 , pH 6.5, 0.25 M KCl, 0.005 M $MgCl_2$, 0.002 M 2-mercaptoethanol and 15% glycerol) and stored at -20°C. This procedure was found to remove more than 90% of the endogenous aminoacyl-tRNA synthetase activity for all amino acids except tryptophan and cystine.

Aminoacylation procedures. The following amino acids were obtained from New England Nuclear Corp., Boston, Mass.: [^{14}C]arginine (313 mCi/mmol), [^{14}C]asparagine (179 mCi/mmol), [^{14}C]aspartic acid (167 mCi/mmol), [^{14}C]cystine (255 mCi/mmol), [^{14}C]glutamic acid (269 mCi/mmol), [^{14}C]glycine (104 mCi/mmol), [^{14}C]histidine (282 mCi/mmol), [^{14}C]leucine (311 mCi/mmol), [^{14}C]lysine (312 mCi/mmol), [^{14}C]proline (255 mCi/mmol), [^{14}C]methionine (212 mCi/mmol), [^{14}C]phenylalanine (466 mCi/mmol), [^{14}C]serine (128 mCi/mmol), [^{14}C]threonine (208 mCi/mmol), [3H]threonine (2.38 Ci/mmol), [^{14}C]tryptophan (592 mCi/mmol), [^{14}C]tyrosine (513 mCi/mmol), and [^{14}C]valine (210 mCi/mmol). The following amino acids were purchased from Schwarz Bio-research, Orangeburg, N.J.: [^{14}C]glutamine (187 mCi/mmol), [3H]histidine (3.5 Ci/mmol), [^{14}C]isoleucine (312 mCi/mmol), [3H]isoleucine (7 Ci/mmol), [3H]lysine (8 Ci/mmol), [3H]methionine (2.6 Ci/mmol), and [3H]proline (23 Ci/mmol).

The aminoacylation reactions were run as previously detailed (11) except that, for each reaction, samples were taken with and without added tRNA at 1, 5, 15, and 30 min to ensure that the maximum capacity of acylation had been achieved for a limited amount of tRNA.

RPC-5. The column materials were obtained and prepared exactly as described by Pearson et al. (15). Aminoacyl-tRNAs were eluted from a column (0.9 by 25 cm) using a 500-ml linear salt gradient (0.05 M sodium acetate, pH 4.5, and 0.01 M $MgCl_2$ containing 0.3 to 0.8 or 1.0 M sodium chloride). Samples were collected and processed as previously described (11). Ten thousand to 20,000 counts/min of ^{14}C -labeled aminoacyl-tRNAs and 20,000 to 30,000 counts/min of 3H -labeled aminoacyl-tRNAs were applied for each chromatographic run. Recovery of counts per minute exceeded 70% of those applied, and the recovery of ^{14}C - and 3H -counts per minute were always comparable for a given chromatographic run. Data are reported as the percentage of the total counts per minute recovered in each eluate fraction in order to normalize the ^{14}C - and 3H -counts per minute and to facilitate the accurate comparison of the relative amounts of isoaccepting species for the two aminoacyl-tRNAs being simultaneously analyzed (12).

RESULTS

Aminoacylation of viral and cellular tRNAs. The extents of aminoacylation achieved with 18 amino acids for tRNA from AMV, leukemic myeloblasts, and normal

chicken liver are presented in Table 1. The reported values are the results of individual kinetic enzyme reactions in which tRNA was shown to be limiting. Because the optimal reaction conditions (e.g., pH, salt concentration, etc.) were not determined for each aminoacyl-tRNA, the acceptance capacity values represent minimal estimates. Although results from individual experiments are reported, similar values were achieved with tRNA from different virus preparations. Furthermore, these values are quite similar to those previously reported for 15 amino acids by Travnicek (22; Table 2).

Cystinyl- and tryptophanyl-tRNAs would be of particular interest to study since the respective tRNA synthetases have been detected in AMV (9). Unfortunately, cystine and tryptophan were the only two amino acids not included in this report because we were unable to remove the endogenous tRNAs for these amino acids from the liver aminoacyl-tRNA synthetase preparation. Endogenous tRNA synthetase activity was determined to be absent or present in only trace amounts for all other amino acids with the ^{14}C -labeled amino acids used for experiments summarized in Table 1 and with high-specific-activity ^3H -labeled amino acids for all aminoacyl-tRNAs studied by chromatography.

RPC-5 of aminoacyl-tRNAs from AMV, leukemic myeloblasts, and liver. RPC-5 has been demonstrated to produce high resolution

TABLE 1. Amino acid acceptance capacities of tRNA from avian myeloblastosis virus (AMV), leukemic myeloblasts, and liver

Amino acid	Acceptance capacities ^a		
	Liver tRNA	Myeloblast tRNA	AMV tRNA
Alanine	38.7	23.5	6.8
Arginine	41.8	14.1	19.3
Asparagine	29.9	16.9	4.0
Aspartic acid	22.3	10.7	5.0
Glutamic acid	17.3	22.5	<1.0
Glutamine	21.7	15.1	<1.0
Glycine	39.7	60.4	8.5
Histidine	17.3	14.9	16.4
Isoleucine	32.2	22.3	13.4
Leucine	56.9	41.8	6.9
Lysine	37.6	45.1	23.2
Methionine	17.1	22.9	47.8
Phenylalanine	22.8	24.8	3.6
Proline	28.7	23.1	36.0
Serine	55.1	39.7	<1.0
Threonine	32.5	29.4	10.8
Tyrosine	10.6	11.0	<1.0
Valine	51.0	17.4	1.4

^a Expressed as picomoles of amino acid accepted per A_{260} unit of tRNA.

TABLE 2. Relative amino acid acceptance capacities of avian leukosis virus tRNAs and cellular tRNAs^a

Amino acid	Myeloblast tRNA			Liver tRNA	
	I ^b	II ^c	III ^d	IV ^e	V ^f
Alanine	0.28	0.48	NR ^g	0.18	0.16
Arginine	0.46	0.48	0.34	0.46	0.44
Asparagine	0.24	NR	NR	0.13	0.52
Aspartic acid	0.47	0.57	0.19	0.22	0.86
Glutamic acid	<0.05	<0.05	NR	<0.05	0.82
Glutamine	<0.05	NR	NR	<0.05	0.34
Glycine	0.14	NR	0.52	0.21	0.52
Histidine	1.10	0.84	NR	0.95	0.25
Isoleucine	0.60	0.29	<0.05	0.42	0.09
Leucine	0.17	0.16	NR	0.12	0.45
Lysine	0.51	1.09	NR	0.62	0.50
Methionine	2.09	1.76	0.81	2.80	0.68
Phenylalanine	0.15	0.09	<0.05	0.16	0.15
Proline	1.56	1.97	0.81	1.25	0.57
Serine	<0.05	<0.05	<0.05	<0.05	0.07
Threonine	0.37	0.28	<0.05	0.33	0.10
Tyrosine	<0.05	0.14	<0.05	<0.05	<0.05
Valine	<0.05	<0.05	1.82	<0.05	0.21

^a Determined by dividing the reported amino acid acceptance capacities of AMV (column I-IV) or RAV-1 (column V) tRNAs by the amino acid acceptance capacities of the respective homologous cellular tRNAs.

^b Calculated from values for AMV and myeloblasts tRNA in Table 1.

^c Calculated from values reported by Travnicek (22).

^d Calculated from values reported by Erikson and Erikson (7).

^e Calculated from values for AMV and liver tRNA in Table 2.

^f Calculated from values reported by Wang et al. (27).

^g Not reported.

of isoaccepting species of tRNA (15). For comparative purposes, tRNAs from two sources are charged with ^3H - or ^{14}C -labeled amino acids, and the oppositely labeled aminoacyl-tRNAs are coeluted in the same chromatographic run to permit precise comparison and to minimize artifacts.

In the present study, seven aminoacyl-tRNAs derived from AMV 4S RNA with the highest levels of aminoacylation were compared by cochromatography with homologous cellular aminoacyl-tRNAs. Due to the limited amounts of AMV 4S RNA available, each aminoacyl-tRNA was initially screened by elution across a broad chromatographic salt gradient (0.3–1.0 M NaCl), with liver and myeloblast tRNA, to ensure detection of all isoaccepting species. Then, acylated viral tRNA was cochromatographed with liver tRNA, with a narrower salt gradient (0.3–0.8 M NaCl) to increase resolution. The viral tRNA preparation was always

acylated with high-specific-activity ^3H -labeled amino acids to provide maximal sensitivity of detection, and in different chromatographic runs liver tRNA was oppositely charged with ^{14}C - or ^3H -labeled amino acids to detect possible artifacts due to radioisotope impurities. Because the same source of enzyme was used for all acylation procedures, differences in elution profiles cannot be attributed to this source. As previously mentioned, endogenous tRNA synthetase activity, i.e., amino acid acceptor activity due to the presence of tRNA in the liver enzyme preparation, which would skew the chromatographic profiles in favor of the liver tRNA pattern, was first determined to be very low or absent for each amino acid used.

Aminoacyl-tRNAs with little or no chromatographic differences. Three aminoacyl-tRNAs, lysyl-, prolyl-, and isoleucyl-tRNA, showed no significant quantitative or qualitative differences in chromatographic elution profile between AMV and normal liver (Fig. 1). Although not depicted, the elution profiles for these aminoacyl-tRNAs from avian myeloblasts were identical to those of AMV and liver.

Lysyl-tRNA is of particular interest because it is among the most, if not the most (22), abundant tRNA in AMV and because the virion has been demonstrated to contain lysyl-tRNA synthetase (9, 24). Travnick and Riman previously studied lysyl-tRNA in AMV by methylated albumin kieselguhr (MAK) chromatography (23), and they reported that lysyl-tRNA from liver and leukemic myeloblasts eluted as two broad peaks, whereas lysyl-tRNA from AMV showed none (or a markedly reduced amount) of the second, smaller cellular peak.

This description markedly contrasts with our findings by RPC-5 in which four isoaccepting fractions were clearly separated and no large differences were detected in the profile from AMV and liver (Fig. 1A) or myeloblasts. We have also studied lysyl-tRNA by RPC-5 in control and murine sarcoma virus (Kirsten)-transformed mouse cells and, similarly, we find four isoaccepting species (Gallagher, unpublished data), indicating that this lysyl-tRNA complement is not peculiar to chicken cells. In this regard, it is noteworthy that earlier reversed-phase chromatographic systems (RPC-2) resolved only two or three species of mammalian lysyl-tRNA (12, 28). Therefore, we feel that the difference described by Travnick and Riman in AMV (23) is probably due to artifactual differences which may attend chromatographic runs with inadequate resolution (21), although we cannot absolutely exclude the existence of a chemical difference which is resolved by MAK chromatography and not by RPC-5.

Isoleucyl-tRNA shows two clearly resolved isoaccepting species (Fig. 1B), and prolyl-tRNA (Fig. 1C) shows two incompletely resolved isoaccepting species. In addition, there was a consistently demonstrable shoulder on the trailing edge of the major prolyl-tRNA species. These profiles are remarkably similar to those reported in mammalian cells (12, 28).

Aminoacyl-tRNAs with substantial quantitative chromatographic differences. Two aminoacyl-tRNAs, methionyl- and threonyl-tRNA, showed prominent quantitative differences in chromatographic elution profiles between the viral and liver preparations (Fig. 2B

FIG. 1. Reversed-phase 5 cochromatographic elution profiles of three aminoacyl-tRNAs, showing no or minimal profile differences, from AMV and chicken liver. A, lysyl-tRNA; B, isoleucyl-tRNA; C, prolyl-tRNA. Identified isoaccepting aminoacyl tRNA species have been numbered in this figure and in subsequent figures.

FIG. 2. Reversed-phase 5 cochromatographic elution profiles of methionyl-tRNA.

and 3B). The AMV methionyl-tRNA consistently showed a more prominent peak IV and, conversely, a much smaller peak I, as compared with either liver or myeloblast methionyl-tRNAs, which were virtually identical (Fig. 2A). A similar pattern of methionyl-tRNA from Rous-associated leukosis virus (RAV-1) growing in chicken fibroblasts was recently reported by Wang et al. (27). Since previously published chromatographic studies of met-tRNA have demonstrated only three isoaccepting species in mammalian cells (12, 28), it will be of interest to determine whether the fourth met-tRNA species noted in these studies is specific for avian cells or, more likely, is related to improved chromatographic resolution.

Although the presence of five isoaccepting fractions of threonyl-tRNA has been postulated

from an evaluation of shoulders on chromatographic elution profiles (12, 29), the data shown in Fig. 3B demonstrate a considerable improvement in resolution of threonyl-tRNA over previously published results from mammalian cells. Although small, the first isoaccepting fraction was consistently demonstrable with all tRNA sources and also was always less prominent in the tRNA from liver in comparison with either AMV or myeloblast threonyl-tRNA. Conversely, minor isoaccepting fraction V was consistently more pronounced in liver tRNA than in either AMV or myeloblast tRNA. In contrast to the quantitative differences noted with methionyl-tRNA, the viral threonyl-tRNA pattern did not markedly differ from that of leukemic myeloblasts.

FIG. 3. Reversed-phase 5 cochromatographic elution profiles of threonyl-tRNA.

Aminoacyl-tRNA with qualitative chromatographic differences. Arginyl- and histidyl-tRNAs from AMV show a qualitative difference in chromatographic elution profile as compared with liver tRNA (Fig. 4B and 5B). In both cases, this involves a shift in position of a single isoaccepting species (arginyl-tRNA peak II and histidyl-tRNA major peak II) with the viral species eluting after the cellular species. This shift indicates a difference in the chemical composition of these viral and cellular aminoacyl-tRNA species. Also, for both aminoacyl-tRNAs, the viral elution profile is mimicked by tRNA from the cell of origin, the avian myeloblast (Fig. 4A and 5A), indicating that the alterations are not peculiar to free virus particles.

The arginyl-tRNA profiles from chicken liver, which demonstrate three well-resolved isoaccepting fractions and a fourth poorly resolved isoaccepting fraction (Fig. 4A and B) are quite similar to those reported by Yang et al. for mouse LM cells and tumors (28). This similarity in arginyl-tRNA from phylogenetically disparate avian and murine cells enhances the possibility that the shift in position of the second isoaccepting species of arg-tRNA in AMV and myeloblasts may be viral-related, although further studies are required to investigate other possibilities, e.g., that the tRNA complement may be organ specific.

From the profiles presented in Fig. 5, we are unable to accurately determine the number of histidyl-tRNAs. Three major isoaccepting fractions of histidyl-tRNA were consistently demonstrated with liver tRNA by using either ^{14}C - or ^3H -labeled histidine and with different lots of histidine. Similarly, three isoaccepting species of histidyl-tRNA have also been reported in mammalian cells (29). The irregularities noted in the chromatographic profile before the first major isoaccepting fraction were inconsistent in appearance and may have been due to radioisotope impurities, which have been noted in a previous study (12). The first histidyl-tRNA isoaccepting species was always lower in both AMV and myeloblasts than in the liver. Most importantly, when the linear salt gradient was run from 0.3 to 0.8 M NaCl, the second isoaccepting fraction of histidyl-tRNA consistently eluted one tube ahead and was quantitatively reduced in liver histidyl-tRNA compared with either AMV or myeloblast histidyl-tRNA (Fig. 5).

DISCUSSION

The aminoacylation data presented in Table 1 confirm previous reports that tRNA in AMV does not represent a random assortment of

FIG. 4. Reversed-phase 5 cochromatographic elution profiles of arginyl-tRNA.

incorporated cellular tRNAs (7, 22, 27). The relative amounts of various tRNAs which we found in AMV compared to myeloblasts (Table 2, column I) are quite similar to those reported by Travnick (Table 2, column II; 4) and somewhat less similar to those reported by Erikson (Table 2, column III; 5), particularly regarding valine tRNA. In agreement with us, Carnegie et al. also reported a low amount of valine tRNA in AMV (6). Also presented in Table 2 are our values for AMV tRNA relative to liver tRNA (column IV) and calculated ratios from recently reported data on RAV-1 relative to liver tRNA (column V; 8). In comparison to AMV, RAV-1 apparently contains relatively greater amounts of tRNAs for asparagine, as-

partic acid, glutamic acid, glutamine, and possibly valine. tRNAs for methionine, proline, and lysine, and to a lesser degree arginine are relatively enriched compared with other viral tRNAs in both AMV and RAV-1. On the other hand, AMV appears more enriched for histidine tRNA than is RAV-1. Conversely, there is a marked depletion of serine, tyrosine, and phenylalanine tRNAs in both AMV and RAV-1, suggesting that this is a general property of avian leukosis viruses. In this regard, it is notable that these three tRNAs elute late from reversed-phase chromatography (29), and they respond predominantly to mRNA codons beginning with uridine (26) (two of six serine codons begin with adenine instead of uridine). This codon specificity is at least partially related to the presence of modified nucleotides, which

have cytokinin activity in plants, adjacent to the anticodon of these tRNAs (26). These correlations suggest that the chemical properties of tRNA may be important factors in determining whether it is incorporated in virions.

Despite the selectivity of AMV for certain amino acid-specific tRNAs, the chromatographic data in this report indicate that, with the exception of methionine tRNA, there is very little viral selection for individual isoaccepting tRNA species. In combination with reports from Zamecnik's laboratory on the selective distribution of methylated nucleic acid bases in AMV tRNAs (16), these data argue against the possibility that selective inactivation, i.e., loss of amino acid acceptor activity, accounts for the observed nonrandom distribution of tRNA species. Also, the lack of isoacceptor selectivity virtually rules out the possibility that viral genes code for the vast majority of virion-associated tRNA species. Another proposal has been that viral selection of tRNA species may be due to the membrane association of select tRNAs (16) in a way analogous to the association with and function of certain isoaccepting tRNA species in bacterial cell wall synthesis (20). Although we are unaware of any report of specific tRNAs in eukaryotic cells being associated with the cell membrane, certain tRNAs are specifically associated with other cell organelles (10). Therefore, our chromatographic findings that the same 25 isoaccepting species of seven aminoacyl-tRNAs are present in quantitatively similar amounts (with the exception of two methionyl-tRNA species) in AMV and in whole cell extracts of leukemic myeloblasts argues against selection due to membrane binding of some tRNA species. Perhaps selection is due to some chemical affinity of the incorporated tRNA species for viral components encountered during the process of intracellular virus synthesis and assembly.

Regarding the RPC-5 chromatographic elution profiles several points are emphasized. First, no qualitative chromatographic differences in the elution profiles of the three most abundant viral aminoacyl-tRNAs, methionyl-, prolyl-, and lysyl-tRNA, were noted compared with homologous tRNAs from liver or myeloblast (Fig. 1 and 2). Travnick and Riman previously reported a chromatographic difference in lysyl-tRNA from chicken liver and myeloblasts compared to lysyl-tRNA from AMV by chromatography (23). Although superior resolution of lysine tRNA isoaccepting species was achieved in our studies, some chemical variation recognized by MAK chromatography may not be discriminated by RPC-5 (see Results). Second, methionyl-tRNA is the only

FIG. 5. Reversed-phase 5 cochromatographic elution profiles of histidyl-tRNA.

tRNA in which a significant quantitative alteration was detected in the viral isoaccepting species compared with the myeloblast (Figure 2B). The fourth viral isoaccepting species is increased approximately twofold with a concomitant reduction in the first species relative to the cellular tRNAs. Further, since the aminoacylation capacity of viral methionine tRNA is approximately twofold higher than that of cellular methionine tRNA (Table 2), the net enrichment of this virus isoaccepting species is fourfold. Wang et al. reported a similar increase in the fourth peak of methionyl-tRNA in RAV-1 (27), suggesting that this could be a general feature of avian leukosis viruses. Third, the qualitative differences in the chromatographic elution of single isoaccepting species of arginine and histidine tRNAs indicate that a chemical difference exists between these tRNA species in AMV and in myeloblasts as opposed to liver (Fig. 4 and 5). Such differences might be due to the expression of alternative tRNA genes, which code for entirely different primary nucleotide chains, or, more simply, they could be due to a change in the amino acid moiety after acylation. However, such shifts in isoacceptor position on reversed-phase chromatograms are particularly suggestive of differences in nucleic acid base modification by hydrophobic residues, e.g., methyl groups (5, 14). The later elution of the histidyl- and arginyl-tRNA species in AMV and myeloblasts could, therefore, signify the presence of additional methylated bases. In this regard, it is notable that AMV contains a guanine methylase (13) and has an increased proportion of methylated guanine bases relative to liver tRNA (16). However, since the myeloblast has the same arginyl- and histidyl-tRNA profiles as does AMV and the same methylated base composition as does liver (16), it seems more likely that altered methylated base pattern of AMV tRNA is due to the presence of selected amino acid-specific tRNAs with varying degrees of base methylation rather than to the detection of differences in methylation between a few isoaccepting species. Studies are in progress to determine the effect of methylase enzyme preparations from avian myeloblasts on the chromatographic elution of liver arginyl- and histidyl-tRNAs.

To determine whether the qualitative differences in AMV and myeloblast arginyl- and histidyl-tRNAs are specifically related to AMV, it will be of interest to determine whether avian leukocytes which are normal or transformed by other agents have similar alterations in these tRNA chromatographic profiles. Regardless of origin, the observed alterations in arginyl-, histidyl-, and methionyl-tRNAs may be related

to the production of virus-specific products and possibly to cell regulatory function. In the oncornaviruses, there is the additional possibility that a specific tRNA plays an important role in the transcription of DNA product from the genomic RNA by serving a primer function (4, 25) or possibly by serving other adaptor functions in viral replication (3).

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