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Isoaccepting Transfer Ribonucleic Acids in Specialized Mammalian Tissues*

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ABSTRACT: The transfer ribonucleic acids of two specialized mammalian tissues, lens and muscle, were isolated and compared by cochromatography on reversed-phase Freon columns. A comparison of each isoaccepting tRNA species showed several significant differences between these two tissues. Quantitative differences of two- to threefold were seen in the amount of at least one isoaccepting tRNA species with aspartyl-, isoleucyl-, leucyl-, and lysyl-tRNA. Alanyl-tRNA from muscle and methionyl- and tyrosyl-tRNA from lens contained one species that was almost completely absent in the other tissue. Artifacts due to isolation, aminoacylation, chromatographic conditions, and ribonuclease activity were eliminated. These data suggest that specialized mammalian tissues have unique populations of tRNA which may function to promote the rapid synthesis of the proteins peculiar to that tissue.

Several different hypotheses have been advanced which propose that protein synthesis could be controlled at the translational level by tRNA (Sueoka and Kano-Sueoka, 1970). Supportive evidence is usually obtained by comparing the tRNA of two organisms or tissues to determine what significant differences exist. These can be either differences in the amount of one isoaccepting tRNA species or differences in the number of isoaccepting species present. In mammalian tissues differences of these types have been reported when comparisons were made between erythrocytes and reticulocytes (Lee and Ingram, 1967), normal and leukemic lymphocytes (Gallo and Pestka, 1970), normal liver and hepatoma (Baliga *et al.*, 1969), normal and hormone-treated cells (Busby and Hele, 1970; Jackson *et al.*, 1970), normal and regenerating liver (Agarwal *et al.*, 1970), and different lines of cells in tissue culture (Taylor *et al.*, 1968). Comparisons have also been made between various normal tissues and also between tissues from different species (Taylor *et al.*, 1967; Holland *et al.*, 1967). In most instances, little or no differences were seen, however, a recent report by Hatfield and Portugal (1970) has shown that major differences do exist between the seryl-tRNAs of brain and liver tissues. We would like to report here the results of a study of the tRNA populations of two specialized mammalian tissues, lens and muscle. These tissues were selected because they have different specialized protein-synthetic functions and different embryological origins.

Materials and Methods

Isolation of tRNA from Lens Tissue. Frozen bovine lenses (500 g) were homogenized in a high-speed Waring blender with 3 vol of an extraction buffer containing 0.15 M KCl–0.001 M Tris-HCl buffer (pH 7.5)–0.01 M $MgCl_2$–0.001 M Na_2EDTA–0.02% polyvinyl sulfate. This homogenate was stirred with an equal volume of water-saturated phenol at 45° for 1 hr. This mixture was centrifuged at 40,000*g*, for 15 min, and the aqueous layer was removed. The nucleic acids were precipitated from the aqueous layer by the addition of 2.5 vol of 95% ethanol. After storing overnight at −15° the RNA was collected by centrifugation and dissolved in a solution containing 0.05 M NaCl–0.01 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.001 M Na_2EDTA. This sample was applied to a 2.0 × 40 cm DEAE-cellulose column which had been previously equilibrated with the sample buffer. The column was washed with 250 ml of sample buffer, followed by 250 ml of sample buffer containing 0.30 M NaCl. The tRNA was then eluted with sample buffer containing 0.70 M NaCl. The peak of 260-nm-absorbing material was pooled, and 2.5 vol of ethanol was added. The precipitated tRNA was collected by centrifugation, dissolved in water to a concentration of 40 A_{260} units/ml, and stored at −15°.

Isolation of Aminoacyl-tRNA Synthetases from Lens Tissue. Bovine eyes were obtained from the local slaughterhouse soon after the death of the animal and transported to the laboratory in an ice bucket. The lenses were rapidly removed and the nucleus of each lens was removed by punching out the center with a no. 8 cork borer. The remainder of the lens was homogenized with a Teflon in glass homogenizer in a solution containing 0.03 M KCl–0.01 M Tris-HCl buffer (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–0.001 M Na_2EDTA (1.0 ml of buffer/g of lens tissue). This homogenate was centrifuged at 30,000*g* for 20 min followed by another centrifugation at 160,000*g* for 75 min. Glycerol was added to the resulting supernatant to make a 10% solution (v/v). This mixture was applied to a 2.5 × 80 cm reverse-flow Sephadex G-75 column which had been previously equilibrated with 0.01 M KCl–0.05 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–10% glycerol. The column was eluted with equilibrating buffer and the absorbance

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of each tube was measured at 280 nm. The peak which emerged in the void volume was pooled, glycerol was added up to 20%, and the enzyme was stored in small aliquots at −15°.

Isolation of tRNA from Muscle Tissue. Bovine diaphragm muscle ("hanging tenderloin") was obtained fresh from the local slaughterhouse and used immediately. The muscle tissue was trimmed, cut into strips, and put through a meat grinder. The ground meat was extracted with an equal volume of a buffer containing 0.25 M sucrose–0.05 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–0.01 M KCl–0.02% polyvinyl sulfate for 1 hr in ice. This mixture was squeezed through several layers of cheesecloth and the resulting extract was centrifuged at 30,000*g* for 20 min. The supernatant was mixed with an equal volume of water-saturated phenol and stirred vigorously for 1 hr under ice-cold conditions. This mixture was separated by centrifugation, the aqueous layer was taken, and 2.5 vol of ethanol was added. The tRNA was then purified by the DEAE-cellulose method described above.

In one muscle tRNA preparation, the procedure was modified by the use of diethyl pyrocarbonate, a potent nuclease inhibitor. After the first phenol extraction, the alcohol-precipitated nucleic acids were dissolved in 200 ml of the original buffer and diethyl pyrocarbonate was added to make a 2.5% solution. This solution was heated at 37° for 5 min and cooled, and an additional phenol extraction was carried out. This preparation was also purified by DEAE-cellulose chromatography.

Isolation of Aminoacyl-tRNA Synthetases from Muscle Tissue. A concentrated muscle tissue extract was prepared as described above using 200 g of ground muscle tissue and 100 ml of an extraction buffer containing 0.25 M sucrose–0.05 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–0.01 M KCl–10% glycerol. This extract was centrifuged at 30,000*g* for 20 min and at 160,000*g* for 75 min. The crude synthetases were isolated from the 160,000*g* supernatant by Sephadex G-75 gel filtration and stored in the same manner as the lens synthetases. The rat liver synthetases were also prepared in the same manner except that the tissue extract was prepared by homogenizing the liver tissue in 2 vol of a buffer containing 0.25 M sucrose–0.05 M KCl–0.01 M Tris-HCl (pH 7.5)–0.01 M $MgCl_2$–0.01 M MSH–10% glycerol.

Assay Systems. The amino acid accepting activity of each tRNA preparation was assayed by the filter paper disc method of Mans and Novelli (1961) as described by Yang and Novelli (1968a). Reaction mixtures contained 80 μmoles of Tris-HCl (pH 7.5), 10 μmoles of KCl, 1.6 μmoles of $MgCl_2$, 1.6 μmoles of ATP, 0.2 μmole of Na_2EDTA, 10 μmoles of MSH, 1 μCi of [^{14}C]amino acid, 0.5 A_{260} unit (1 nmole) of tRNA, and 0.1 ml of the synthetase preparation in a total volume of 0.5 ml. The protein content of the various synthetase preparations was 8 mg/ml for the muscle synthetase, 12 mg/ml for the liver synthetase, and 40 mg/ml for the lens synthetase. All reactions were run at room temperature and aliquots were taken at various time intervals. Plateau values were considered to be maximum acceptance values. Variations in the assay system to obtain maximum incorporation with certain amino acids will be mentioned later. All [^{14}C]- and [^{3}H]amino acids were uniformly labeled and were purchased at the highest specific activity available.

Postcharging assays were carried out by a procedure similar to that above. Samples of 200 A_{260} units of each tRNA preparation were chromatographed separately on RPC-2 columns. The tRNA in each fraction was precipitated with alcohol, collected on a nitrocellulose filter (0.45 μ pore size), and eluted with 1.0 ml of H_2O. A 0.05-ml sample was removed from every other tube and assayed with 40 μmoles of Tris-HCl (pH 7.5), 5 μmoles of KCl, 0.8 μmole of $MgCl_2$, 0.8 μmole of ATP, 0.1 μmole of Na_2EDTA, 5 μmoles of MSH, 0.25 μCi (0.001–0.002 μmole) of [^{14}C]amino acid, 0.05 μmole each of 19 unlabeled amino acids, and 0.025 ml of rat liver synthetase in a total volume of 0.25 ml. One sample was taken at 30 min.

Preparation of Aminoacyl-tRNAs and Chromatographic Separations. Preparations of [^{14}C]- and [^{3}H]aminoacyl-tRNAs were made by increasing every component of the assay mixture tenfold. The aminoacyl-tRNAs were isolated from this mixture by either phenol extraction at pH 4.5 or by DEAE-cellulose chromatography as described by Yang and Novelli (1968b). RPC-2 columns were prepared by the method of Weiss and Kelmers (1967). Unless otherwise stated, all RPC-2 chromatography was carried out using a 2-l. linear gradient from 0.3 to 0.6 M NaCl in 0.01 M sodium acetate (pH 4.5), 0.01 M $MgCl_2$, 0.001 M Na_2EDTA, and 0.03 M MSH. Samples of [^{14}C]- and [^{3}H]aminoacyl-tRNA ($1–3 \times 10^4$ cpm each) were cochromatographed on a 1×240 cm RPC-2 column at 10°. Fractions of 10 ml were collected, and the RNA in tubes 40–160 was precipitated by the addition of 2.0 ml of 50% trichloroacetic acid. The contents of each tube were filtered on a nitrocellulose filter (0.45 μ pore size) and washed with 10 ml of 70% ethanol. Each filter was dried under a heat lamp and counted in a dual-channel Packard scintillation counter.

Results

Amino Acid Acceptance Activities of Lens and Muscle tRNA. Several preparations of tRNA were isolated from lens tissue. The average yield was 1.5–2.0 A_{260} units of tRNA per lens. When these preparations were assayed with lens synthetases the results shown in Table I were found. The synthetase preparation had sufficient activity to yield plateau values with most amino acids tested using 0.5 A_{260} unit of tRNA, however, no activity was seen with either glutamine, alanine, or asparagine, and low activity was seen with isoleucine and valine. Optimum activity for leucine required a Mg^{2+}:ATP ratio of 0.5 whereas all others were best at 1.0. The lens histidyl synthetase was completely inactive at pH 7.5, but had good activity at pH 8.5. Assays for amino acid acceptance activity with several lens tRNA preparations gave almost identical results.

The isolation procedures used for muscle tRNA and synthetases were adopted in an effort to remove any contamination due to either connective tissue or erythrocytes. Contamination by erythrocytes was estimated by measuring the amount of hemoglobin which eluted from the Sephadex G-75 column during the preparation of the synthetases. The presence of hemoglobin was estimated by measuring the amount of 415-nm-absorbing material which eluted directly after the excluded peak on G-75 Sephadex. The intense myoglobin peak, which eluted considerably later, did not interfere. Of all the preparations of synthetase tested in this manner (6–7), only one showed a hemoglobin peak, however, the absorbance at 415 nm of this peak was less than 0.1% of the myoglobin peak. The hemoglobin content was not measured in any tRNA preparation, however, the method of tissue extraction was the same as that for the synthetases. The yield of tRNA with this method was variable, but usually about 1.0 A_{260} unit was obtained per gram of muscle tissue. The synthetase fraction showed excellent activity for all the amino acids tested except asparagine and glutamine. In all the work involving these two amino acids, rat liver synthetase was used.

TABLE I: Amino Acid Acceptance Activities of Lens and Muscle tRNA.

Amino acid	pmoles of amino acid accepted/nmole of tRNA	
	Lens	Muscle
Alanine	58.4[a] ± 0.9	45.8 ± 3.2[b]
Arginine	60.9 ± 0.2	93.6 ± 5.5
Asparagine	39.1[a] ± 4.8	42.0[a] ± 15.2
Aspartic acid	61.4 ± 0.1	41.4 ± 0.4
Glutamic acid	16.0[a] ± 2.0	19.3 ± 3.4
Glutamine	16.0[a] ± 2.6	24.1[a] ± 2.5
Glycine	84.5 ± 4.1	57.4 ± 3.7
Histidine	18.3 ± 3.2	14.3 ± 2.1
Isoleucine	15.0 ± 5.2	12.0 ± 3.6
Leucine	56.9 ± 2.9	69.5 ± 2.6
Lysine	23.1 ± 0.2	29.3 ± 5.5
Methionine	16.4 ± 1.8	21.6 ± 2.5
Phenylalanine	23.1 ± 0.3	12.2 ± 2.4
Proline	21.0 ± 6.2	36.0 ± 4.0
Serine	91.8 ± 1.2	85.1 ± 3.2
Threonine	41.4 ± 0.5	52.8 ± 1.5
Tyrosine	14.1 ± 2.1	15.3 ± 0.7
Valine	50.2 ± 2.3	30.6 ± 2.0
Total acceptance	707	702

[a] Determined with rat liver synthetase. [b] Deviation shown is the standard deviation.

When the amino acid acceptance activity was measured for the muscle tRNA preparations, variable results were obtained. However, when the data were calculated as the per cent of total acceptance, the results were equivalent. Apparently, the muscle tRNA preparations contained small amounts of ribonuclease. The total acceptance values of three preparations were 702, 660, and 560 pmoles per nmole of tRNA. To eliminate the possibility that the ribonuclease present in these preparations may have affected our results, one preparation of muscle tRNA was made using diethyl pyocarbonate. This reagent reacts with proteins and therefore destroys all nuclease activity (Abadom and Elson, 1970). All RNase activity was apparently removed by this treatment, since an incubation of this tRNA preparation at 23° for 20 hr caused no detectible loss in acceptor activity.

The average amino acid acceptance activities of the tRNA preparations from muscle are also presented in Table I. The values determined for the muscle tRNA preparations were corrected to 702 pmoles total acceptance for each preparation. In almost all cases a standard deviation of less than 10% was seen. When a comparison was made between the amino acid acceptor activity of the lens and muscle preparations, significant differences were seen with arginine, aspartic acid, glycine, leucine, phenylalanine, proline, and valine, even though both preparations had a similar total acceptance activity.

The values shown in Table I are most important as a basis for plotting the data obtained from RPC-2 chromatography. The results of RPC-2 chromatography indicate only the number of isoaccepting tRNAs present and the relative amounts of each. In order to accurately reflect the amount of each tRNA species which is actually present in the tissue, the acceptance activity for each amino acid in each preparation must be considered. If assays indicate that there is more of a particular type of tRNA in lens than in muscle, then the area under the lens chromatographic profile should be correspondingly greater than the area under the muscle profile. The ratio of the total counts plotted for the lens preparation over the total counts plotted for the muscle preparation always equals the acceptance activity of the lens preparation over the acceptance activity of the muscle preparation. In this manner the exact amount of each isoaccepting tRNA species in each tissue can be determined.

Reversed-Phase Chromatographic Comparisons of Lens and Muscle tRNA. Figure 1 shows the chromatograms obtained with asparagyl-, glutamyl, glycyl-, histidyl-, prolyl-, seryl-, threonyl-, and valyl-tRNA. In most cases these represented the tRNAs which were poorly separated by RPC-2 chromatography. The quantitative differences seen in Figure 1 usually reflected an overall increase or decrease in all the isoaccepting species, and was therefore not considered significant. In general the separations were similar to those seen by other investigators using RPC-2 chromatography, however, the results obtained with seryl-tRNA were not. Using other tissues, seryl-tRNA separates into at least 3 well-defined peaks, however, this was not seen with either lens or muscle seryl-tRNA. Chromatograms were run with different tRNA preparations and different packing preparations, both with and without MSH and EDTA in the gradient and using different synthetases and different methods of aminoacyl-tRNA isolation. In every case seryl-tRNA profiles similar to those in Figure 1 were obtained.

Differences in the amounts of one specific isoaccepting species were seen with arginyl-, phenylalanyl-, aspartyl-, isoleucyl-, and lysyl-tRNAs. These chromatograms are shown in Figure 2. Arginyl-tRNA was present in much greater amount in muscle than in lens. Peak I arginyl-tRNA was threefold higher in muscle, however, since all of the peaks were increased in muscle, it cannot be certain that this is a specific effect on peak I. The chromatogram for phenylalanyl-tRNA showed only one peak for muscle, but possibly two peaks for lens. When both preparations were aminoacylated with the rat liver synthetase and the labels were reversed, identical patterns were seen. To eliminate the possibility that the additional peak seen with lens tRNA was due to either a conformational change or a ribonuclease-nicked phenylalanyl-tRNA, both preparations were heated to 85° and cooled according to the procedure of Nishimura and Novelli (1965). When these preparations were chromatographed, both phenylalanyl-tRNA preparations gave one symmetrical peak, suggesting that the shoulder seen previously with the lens phenylalanyl-tRNA was an artifact. However, when the heated samples of lens and muscle tRNA were chromatographed on a RPC-5 column (Pearson *et al.*, 1971), lens Phe-tRNA showed an additional peak which eluted at a lower salt concentration. This confirmed the difference shown in Figure 2B.

The results seen with muscle and lens aspartyl-tRNA are shown in Figure 2C. These profiles were obtained using the rat liver synthetase to aminoacylate both preparations. When the homologous synthetases were used identical results were obtained, except that peak I was almost completely absent from the lens profile. Generally, muscle tissue appears to have at least twice as much of peak I aspartyl-tRNA, but only half as much of peak II when compared to lens tissue. Results similar to those in Figure 2C, were obtained when the labels were reversed, when the tRNA preparations were heated to

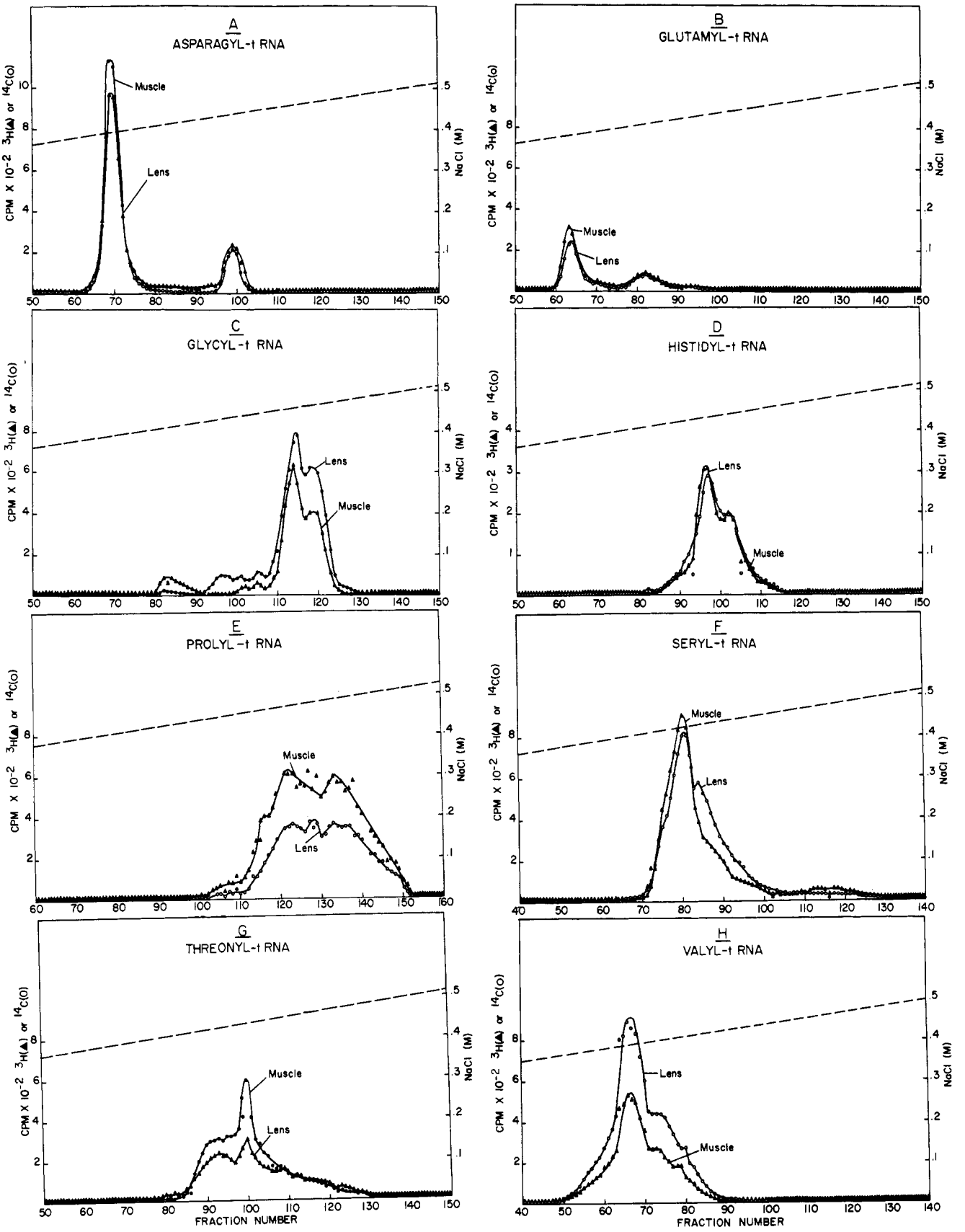

FIGURE 1: RPC-2 profiles of (A) asparagyl-tRNA, (B) glutamyl-tRNA, (C) glycyl-tRNA, (D) histidyl-tRNA, (E) prolyl-tRNA, (F) seryl-tRNA, (G) threonyl-tRNA, and (H) valyl-tRNA from lens and muscle tissues. Samples of [^{14}C]- (○) and [^{3}H]- (▲) aminoacyl-tRNAs were cochromatographed using a linear gradient of 0.3–0.6 M NaCl.

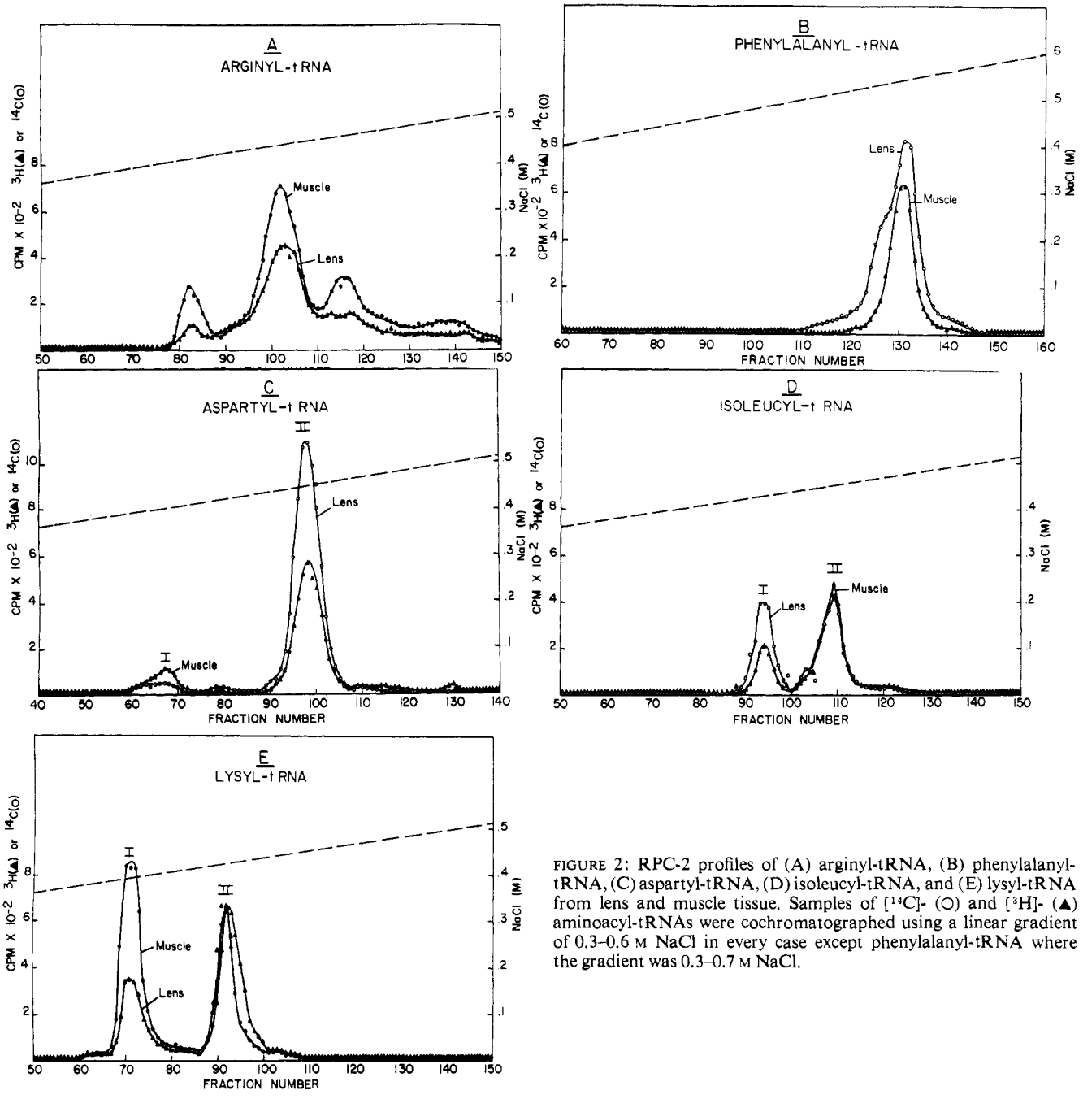

FIGURE 2: RPC-2 profiles of (A) arginyl-tRNA, (B) phenylalanyl-tRNA, (C) aspartyl-tRNA, (D) isoleucyl-tRNA, and (E) lysyl-tRNA from lens and muscle tissue. Samples of [^{14}C]- (○) and [^{3}H]- (▲) aminoacyl-tRNAs were cochromatographed using a linear gradient of 0.3–0.6 M NaCl in every case except phenylalanyl-tRNA where the gradient was 0.3–0.7 M NaCl.

85° before aminoacylation and when the peaks were detected by postcharging. Whether peak I represents a functionally distinct aspartyl-tRNA is uncertain, however, a similar peak was also present in rat liver tRNA. In this tissue peak I represented almost 20% of the total aspartyl-tRNA. We have also shown that peak I could not be eliminated from the muscle profile by adding an excess of 19 other unlabeled amino acids to the reaction mixture. Finally when preparations of [^{14}C]aspartyl-tRNA were hydrolyzed under basic conditions and paper chromatography was carried out on the released amino acids, only one radioactive spot could be detected, which was shown to be aspartic acid.

Figure 2D shows the best chromatographic separation obtained with isoleucyl-tRNA. These tRNAs are difficult to aminoacylate completely and difficult to separate. In two other separations with different preparations of RPC-2 packing, only one peak was seen. When the comparisons were made with reversed labels using the rat liver synthetase, similar results were obtained, however, the total counts eluted were low. It appears certain, though, that muscle tRNA contains a greater amount of peak I isoleucyl-tRNA compared to lens-tRNA under conditions where no difference was seen in peak II.

As can be seen in Figure 2E, lysyl-tRNA also separates into two well-defined peaks. While lens contains slightly more of peak II lysyl-tRNA, it contains considerably less of peak I. Reversal of the labeled amino acids, aminoacylation with rat liver synthetases and postcharging all gave results identical with those in Figure 2E. Heating the tRNA preparations to 85° also had no effect on the lysyl-tRNA profiles. Further experiments in our laboratory have indicated that peak I translates the codon AAG, whereas peak II translates the

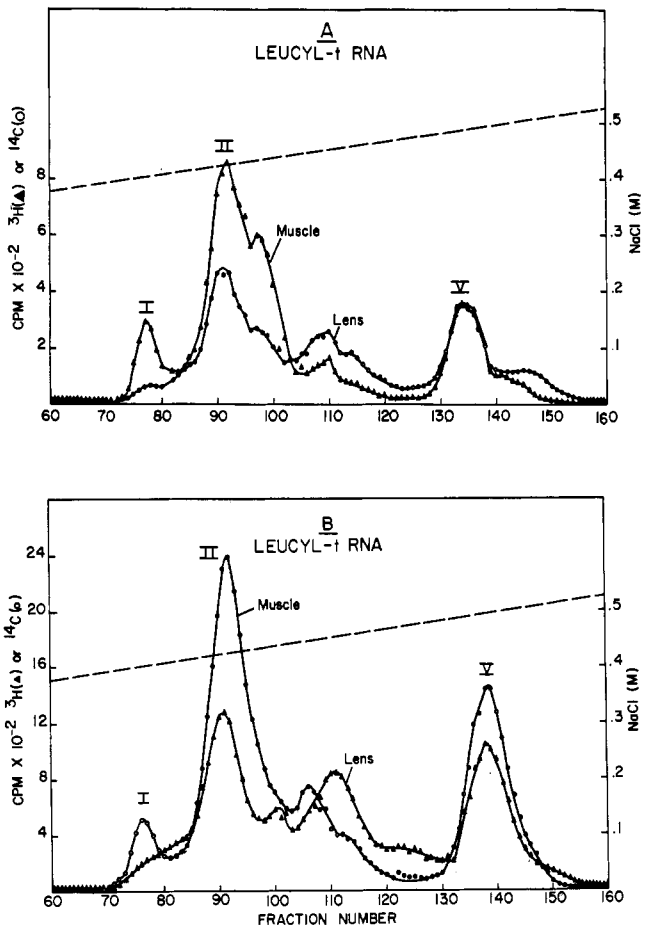

FIGURE 3: RPC-2 profiles of luecyl-tRNA from lens and muscle tissues. Preparations of [^{14}C]- (○) and [^{3}H]- (▲) leucyl-tRNAs were made using either (A) homologous or (B) rat liver synthetases and cochromatographed with a linear gradient of 0.3–0.6 M NaCl.

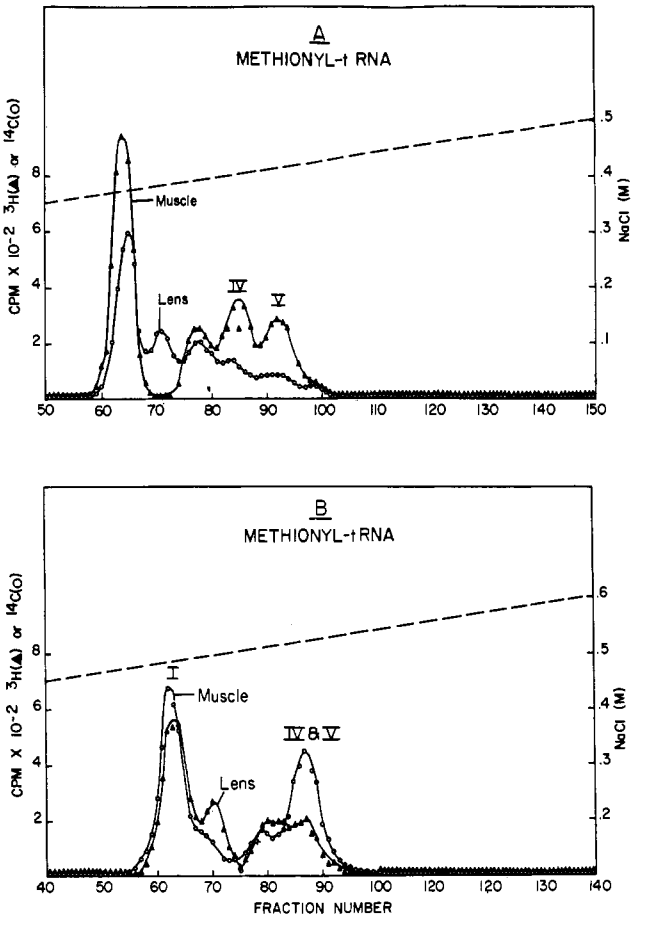

FIGURE 4: RPC-2 profiles of methionyl-tRNA from lens and muscle tissues. Preparations of [^{14}C]- (○) and [^{3}H]- (▲) methionyl-tRNAs were made using either (A) homologous or (B) rat liver synthetases and cochromatographed with a linear gradient of 0.3–0.6 M NaCl.

codon AAA (Liu and Ortwerth, 1971). Therefore, muscle tissue contains almost 3 times as much AAG-specific lysyl-tRNA than does lens, whereas there are almost equivalent amounts of the AAA-specific lysyl-tRNA in the two preparations.

In addition to the quantitative differences already presented, several comparisons showed isoaccepting peaks in one tissue which were almost completely absent in the other tissue. Figure 3 shows two profiles obtained with lens and muscle leucyl-tRNAs. Homologous synthetases were used in Figure 3A, while rat liver synthetases were used in Figure 3B. It is apparent that the first peak represents a significant amount of the leucyl-tRNA in muscle, whereas it is reduced considerably in lens. Since there may be two small peaks which elute before peak II in lens, it is difficult to determine the exact amount of peak I in the lens chromatogram. Peak II leucyl-tRNA is twice as great in muscle than in lens, and differences also exist in the peaks which elute between peaks II and V, however, quantitation is difficult due to the poor separation. Preparations which were heated and cooled gave the same results, however, postcharging could not definitively resolve any differences other than those seen in peaks I and II.

The comparison of the methionyl-tRNA of lens and muscle are shown in Figure 4. Figure 4A represents the results with homologous synthetases, whereas, Figure 4B represents the data obtained with reversed labels using rat liver synthetases. From these results it is apparent that there is a methionyl-tRNA present in lens which is almost completely absent in muscle (peak II). Muscle, however, has more of the species which elute later (peaks IV and V). The last peak in Figure 4B may represent peaks IV and V eluting together. Preparations which were heated to 85° gave similar results to those in Figure 4B, but it was not possible to clearly demonstrate the presence of peak II in lens by postcharging. This is not surprising, however, because the separation of unaminoacylated tRNA with RPC-2 has been shown to be poorer than it is with aminoacylated samples (Yang and Novelli, 1968a).

The results in Figure 5 are those obtained for tyrosyl-tRNA (Figure 5A) and alanyl-tRNA (Figure 5B and 5C). Although the separation is not complete, lens tRNA appears to have two main tyrosyl-tRNA peaks while muscle has only one. This separation was repeated using rat liver synthetases instead of homologous synthetases and again with tRNA preparations which had been heated to 85°. In both cases the lens profile was identical with that shown in Figure 5A, however, the muscle profile contained multiple peaks, all of which eluted at a higher salt concentration. Further investigation showed that these altered tyrosyl-tRNA peaks resulted from the use of diethyl pyrocarbonate in the muscle tRNA isolation procedure. This reagent apparently reacted directly with the tRNA to cause these changes (Ortwerth, 1971). An additional muscle tRNA preparation was therefore isolated without the use of diethyl pyrocarbonate, but with

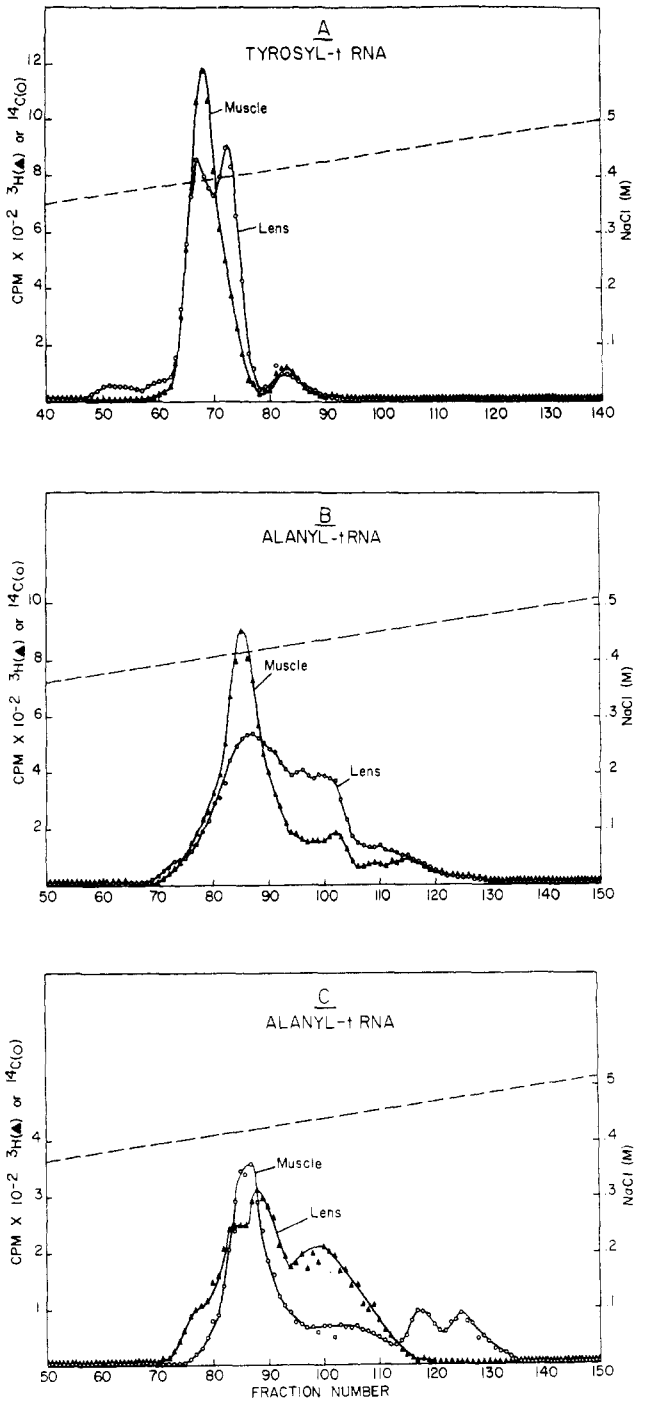

FIGURE 5: RPC-2 profiles of (A) tyrosyl-tRNA and (B and C) alanyl-tRNA from lens and muscle tissues. Preparations of [^{14}C]- (○) and [^{3}H]- (Δ) aminoacyl-tRNAs were made using either (A and B) homologous or (C) rat liver synthetases and cochromatographed with a linear gradient of 0.3–0.6 M NaCl.

the addition of bentonite in the isolation medium to inhibit ribonuclease. This preparation had excellent acceptor activity and gave the same chromatogram as shown in Figure 5A for tyrosyl-tRNA when rat liver synthetases were used.

The comparison of lens and muscle alanyl-tRNAs is shown in Figure 5B and 5C. The profiles appear very different, with lens showing little or no separation, while muscle has one large symmetrical peak and possibly 3 smaller peaks (Figure 5B). When the rat liver synthetase was used and the labels were reversed, lens alanyl-tRNA gave a sharper pattern, but again was considerably different from muscle alanyl-tRNA (Figure 5C). Since the muscle preparation shown in Figure 5C was isolated with diethyl pyrocarbonate, peaks III and IV may be artifacts, however, these peaks appear to be present in the chromatogram in Figure 5B where no diethyl pyrocarbonate was used. Peaks III and IV also readily accept alanine as was demonstrated by postcharging. Heating at 85° also had no effect on the chromatographic profiles. In no case, though, was the lens tRNA aminoacylated with lens synthetase, since the lens preparation had no alanine tRNA synthetase activity. Both preparations were aminoacylated with the muscle synthetase in Figure 5B and with the rat liver synthetase in Figure 5C.

Discussion

The results presented here show that bovine lens and muscle tissue have significantly different tRNA populations. Quantitative differences of twofold or greater have been seen for aspartyl-, isoleucyl-, and lysyl-tRNA, while even larger differences were seen with leucyl-, methionyl-, tyrosyl-, and alanyl-tRNA. Since some other surveys of mammalian tissues have shown little or no differences in tRNA populations several procedures were carried out to eliminate artifacts. The importance of these procedures has been emphasized in a recent review article (Sueoka and Kano-Sueoka, 1970). These procedures were carried out on only those profiles which were considered to show significant differences. These were aspartyl-, isoleucyl-, lysyl-, leucyl-, methionyl-, tyrosyl-, and alanyl-tRNAs. We have eliminated the possibility that the differences seen were due to impurities in our radioactive amino acids by switching the labeled amino acids and repeating the separation. This had no effect on the elution profiles.

The possibility that the differences were the result of differences in the aminoacyl-tRNA synthetases was eliminated by aminoacylating both preparations with rat liver synthetases. In every case similar patterns were obtained. To avoid the formation of inactive conformations due to the aminoacylation reaction or chromatographic conditions, both samples of tRNA were separated on RPC-2 and the profiles were determined by measuring the amino acid acceptance activity of each fraction. These results agreed with the precharged profiles in every case except isoleucyl-, methionyl-, and tyrosyl-tRNA, where the separation was not sharp enough to distinguish every peak.

Artifacts due to ribonuclease were eliminated in several ways. The possibility that tRNAs without an intact CCA terminus were confusing our results was not considered, because only tRNAs which had labeled amino acids attached were detected by our procedure. Ribonuclease could, however, hydrolyze some of the single-stranded regions of the tRNA during isolation and produces nicked molecules which would accept amino acids, but chromatograph differently. To avoid this each chromatogram was repeated using preparations which were heated to 85° and slow cooled. This procedure eliminates nicked molecules since they will not renature. Some activity was lost when both preparations were heated, so the profiles were repeated with the active tRNAs remaining. In every case the results were the same as those obtained previously. This suggests that the loss of activity during the heating step may be due to the absence of Mg^{2+} during the procedure rather than the presence of nicked tRNAs. There was also a possibility that ribonuclease could alter the tRNA during the aminoacylation reaction, or that

ribonuclease was not completely eliminated during the phenol extraction of the reaction mixture. This appeared doubtful since an aliquot of all three synthetase preparations had the ability to inhibit added pancreatic ribonuclease due to the presence of a ribonuclease inhibitor protein. To be absolutely sure, however, the heated preparations above were isolated from the aminoacylation reaction mixture by separation on a small DEAE-cellulose column as described by Yang and Novelli (1968b). As stated previously, no differences were seen. In spite of these observations it was known that the muscle tRNA preparation often contained slight amounts of ribonuclease. In an effort to remove this ribonuclease contaminant, a preparation of muscle tRNA was made using diethyl pyrocarbonate. The method used was a modification of the method of Abadom and Elson (1970). Using this tRNA preparation the chromatograms obtained for aspartyl-, leucyl-, lysyl-, and methionyl-tRNA were identical with those previously run, however, the profiles of tyrosyl- and histidyl-tRNA were altered. These modifications, however, were due to the action of diethyl pyrocarbonate on the tRNA rather than the elimination of the ribonuclease.

It is now clear that the levels of several isoaccepting tRNAs can vary greatly in different mammalian tissues. If these isoaccepting species each have a unique coding function, as in the case with the lysyl-tRNAs, then the ability to translate those codons can vary greatly from tissue to tissue. The quantitative differences reported here may not be true for all tissues, but may be a property of specialized tissues only. These tissues, such as lens, muscle, reticulocytes, and hormone-producing cells, may depend upon tRNA to exert a controlling influence on protein synthesis. This may be especially true for lens tissue since much of the synthesis of protein is carried out at a time when the mRNA templates are stable and the cells are experiencing a loss of nuclear control (Stewart and Papaconstantinou, 1967). Since specialized tissues synthesize large amounts of only two or three proteins, the tRNA population may be regulated so that the synthesis of these proteins can proceed in a rapid fashion. As can be seen from the data presented here, certain isoaccepting species of tRNA are present in twofold or greater concentration in one tissue compared to the other, even though other species of the same tRNA type are present in equivalent concentrations. Therefore, it would appear that the tRNA populations are also specialized in these tissues. Recent results by Smith and McNamara (1971) have suggested that the tRNA population may also be specialized in reticulocytes since the amino acid acceptance activities of certain reticulocyte tRNAs appear to correspond to the amounts of these amino acids in hemoglobin.

Lens and muscle tissues were chosen not only because they are specialized tissues, but also because they have different embryological origins. Therefore, it is possible that lens tissue may have a tRNA population which is the same for all tissues derived from ectoderm and muscle may reflect tissues of mesodermal origin. We are currently investigating this possibility in our laboratory, however, preliminary evidence suggests that this is not the case since comparisons of lens and brain tRNA do not give identical patterns, even though both tissues have an ectodermal origin. Differences in tRNA profiles have been repeatedly demonstrated between mammalian tissues and similar cells grown in tissue culture (Taylor *et al.*, 1968; Taylor *et al.*, 1967; Yang *et al.*, 1969). It has been reported that the differences could be decreased if serum were added to the media (Yang *et al.*, 1969). These observations could explain our results, since the lens is unique in that it has no blood supply. Therefore, lens may have a different tRNA population because it resembles cells cultured in a serum-free media. This could have an effect on both tRNA synthesis and the activity of the various methylases and other tRNA-modifying enzymes in the lens. It must be pointed out, however, that the differences reported here do not resemble the differences seen in tissue-cultured cells, nor are the same types of tRNAs involved. It appears more probable, therefore, that the tRNA populations of lens and muscle tissue are different because each tRNA population has become specialized for the rapid synthesis of their particular proteins.

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