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AFFINITY CHROMATOGRAPHY OF TYROSINE TRANSFER RNAs

Carlos M. Garcia and Ram P. Singhal[1]

Department of Chemistry

Wichita State University, Wichita, Kansas 67208

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Summary: *Ricinus communis* agglutinin, a lectin from castor beans has an affinity for β-D-galactose and tyrosine tRNAs of mammalian tissues have galactose in gal-Q base of their anticodons. We have studied interaction between tyrosine tRNAs and this lectin immobilized on solid supports using spacer arms of different lengths. Tyrosine tRNAs are separated from nineteen other tRNAs of bovine liver by affinity chromatography using the lectin immobilized to an agarose matrix. The results indicate that a spacer arm length of 10 Å between the agarose bead and the lectin gives the best separation. Two tyrosine tRNA isoacceptors are separated from each other and from other tRNAs in one step using this affinity column chromatography.

A lectin from castor beans, *Ricinus communis* agglutinin[2] (type 1, 120,000 daltons), binds specifically with β-D-galactopyranose. Tyrosine tRNAs from mammalian tissues contain β-D-galactopyranose linked to the cyclopentene ring of gal-Q base, present in the Wobble position of their anticodons (1,2). Okada, et al. (2) recently examined the interaction between Sepharose-bound RCA and tRNAs from rabbit liver and reported only partial retention of $tRNA^{Tyr}$ on this matrix. In order to achieve practical separation of $tRNA^{Tyr}$ from other tRNAs, we have examined three kinds of affinity matrices where agarose beads were linked directly to RCA or linked to RCA through a spacer arm of 2 or 10 Å length. The results indicate that the spacer arm length is critical for binding of $tRNA^{Tyr}$ to an agarose-RCA matrix.

MATERIALS

Castor bean agglutinin, RCA, was purchased from either Sigma Chemical Co. or E. Y. Laboratories, San Mateo, California. The material supplied in solution as 12.5 mg RCA per ml in 50% glycerol (E. Y. Labs.) was 3 to 4 times

[1] Address all inquiries to this author.

[2] The abbreviations used are: RCA, *Ricinus communis* agglutinin type I; DEAE-cellulose, diethylaminoethyl-cellulose; A_{260} unit, one unit is the amount of substance in 1 ml of solution that gives an absorbance of 1.0 when measured at 260 nm with a path length of 10 mm.

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more active for tRNA binding than the one supplied as a freeze-dried powder (Sigma Chem. Co.). Purified RCA covalently linked to the agarose beads by imido-ester linkages was also obtained from E. Y. Labs. Each one ml of the beads contained about 4 mg of RCA. Agarose beads with 2 Å spacer arms and with 10 Å spacer arms were supplied as Affi Gel 10, control Nos. 87-2 and 87-10, respectively, by Bio-Rad Laboratories, Richmond, California.

METHODS

Affinity Column Matrix. In a typical coupling experiment, 5 ml of Affi Gel beads were washed successively with 2-propanol, water, and 0.1 *M* $NaHCO_3$-Na_2CO_3 buffer, pH 8, using a small buchner funnel. About 15 to 25 ml of each solution, precooled to 4°C, was used for the washings. One ml of RCA solution containing 12.5 mg lectin was diluted to 5 ml with 0.1 *M* $NaHCO_3$ buffer, pH 8. Washed Affi Gel beads were mixed with freshly diluted RCA solution in a 25-ml polyethylene vial. The contents were gently mixed by strapping the vial on a rotatory wheel at 4°C. After 18 hours, the solution was separated from the beads by decantation and unreacted lectin was determined at 280 nm. About 1.5 mg of RCA was immobilized to one ml of the beads. The beads were washed with the $NaHCO_3$ buffer, and the unreacted sites on the Affi Gel were derivatized by reaction with two volumes of 0.1 *M* ethanolamine in 0.1 *M* $NaHCO_3$, pH 8 buffer at 10°C for 1 hr. Finally, the beads were thoroughly washed with column buffer A (50 m*M* sodium acetate, 5 m*M* $MgCl_2$ and 0.15 *M* NaCl, pH 6.0).

tRNAs and Synthetases. Transfer RNAs from bovine liver were isolated as described in detail elsewhere (5). The tissue homogenate in a buffer of pH 4.5 was directly extracted with phenol; the aqueous phase was treated with DEAE-cellulose; RNAs recovered by ethanol precipitation were extracted with 1 *M* NaCl to remove rRNAs and tRNAs were separated by gel-filtration chromatography. Mixed aminoacyl-tRNA synthetases were purified from rat liver using differential centrifugation (4) and gel-filtration and DEAE-Sephadex chromatography (3).

Aminoacylation of tRNA. Transfer RNA was aminoacylated with tritium-labelled amino acids in a 0.2 ml reaction mixture containing 100 m*M* 4-(2-hydroxyethyl)-1-piperazinepropane sulfonic acid (Hepps) buffer, pH 8.0, 6.25 m*M* magnesium acetate, 3 m*M* Na_2ATP, 2.5 μ*M* labelled amino acid (about 0.5 nmol), 2 m*M* dithioerytheotol, 1.5 m*M* bovine serum albumin, a saturating amount of aminoacyl-tRNA synthetase, and 0.1 A_{260} unit of tRNA. The mixture was incubated at 37°C for 5 min, and then cooled in ice. These reaction conditions were the best for achieving maximal aminoacylation of tyrosine tRNA with tyrosine (results not shown).

For aminoacylation of 5-10 A_{260} units of tRNAs needed for column chromatography, the reaction was scaled up and the mixture was applied to a small DEAE-cellulose column (4 x 1 cm). The column was first washed with 0.25 *M* NaCl buffer, pH 4.5, to remove unreacted amino acids and proteins, and then with 0.75 *M* NaCl buffer to elute tRNAs and aminoacyl-tRNAs. Transfer RNAs from the eluate were recovered by precipitation with ethanol and were centrifuged to a pallet. The pallet was dissolved in a buffer and applied to the affinity column. For the assay of specific activity of tRNAs in different peaks, aminoacylation was carried out as above and 60 μl aliquots from the 0.2 ml reaction mixture were applied to three filter paper discs (Whatman, code 3MM). The discs were successively washed with 5% trichloroacetic acid, with ethanol-diethyl ether mixture (1:1), and with anhydrous diethyl ether (5). For acid-insoluble radioactivity, a 0.2 ml aliquot of each fraction from chromatography on the affinity column was mixed with two volumes of 50 m*M* sodium acetate buffer, pH 4.5, containing 2 A_{260} units of DNA per ml. The mixture, after cooling in ice for 5 min, was filtered through a glass fiber filter (Whatman, GF/C) presoaked in 10% trichloroacetic acid. The discs were

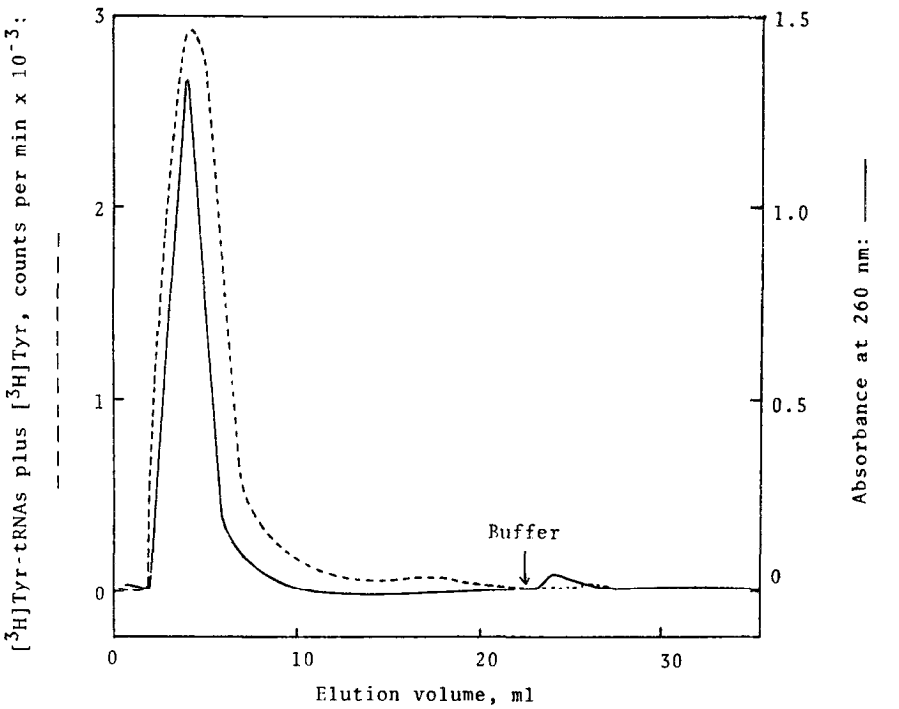

Figure 1. Separation of tyrosine tRNA from 19 other species by affinity chromatography on an agarose-RCA matrix containing no spacer arm (E. Y. Labs). Nine A_{260} units of bovine liver tRNAs and [^{3}H]Tyr-tRNA (26,000 cpm) were applied to the column (5 x 1 cm). The column was first eluted with buffer A (50 m*M* sodium acetate, 5 m*M* $MgCl_2$, 0.15 *M* NaCl, pH 6.0), then with buffer A containing 1 m*M* D-galactose and 0.7 *M* NaCl (indicated by an arrow) at a flow rate of 3 ml per hr. One-ml fractions were collected at 4°C.

washed as described above, and dried discs in scintillation vials were shaken with 10 ml of the scintillator and assayed for radioactivity as described elsewhere (5). For total radioactivity (labelled amino acid + [^{3}H]aminoacyl-tRNA), a 0.4 ml aliquot from each chromatography fraction was mixed with 2.8 ml of Handiflour (Mallinckrodt) in minivials and counted for radioactivity (4,5).

RESULTS

Affinity matrix without spacer arm. When tRNAs and [^{3}H]tyrosyl-tRNAs were applied to an affinity matrix in which RCA was linked to the agarose beads by an imido-ester bond, both [^{3}H]tyrosyl-tRNA and other tRNAs were eluted at the same time. Little radioactivity was eluted from the column upon washing with a buffer containing galactose (see Figure 1). Further washing of the column with a buffer containing 0.5 *M* NaCl eluted very small amounts of strongly bound tRNA, but no radioactivity ($tRNA^{Tyr}$).

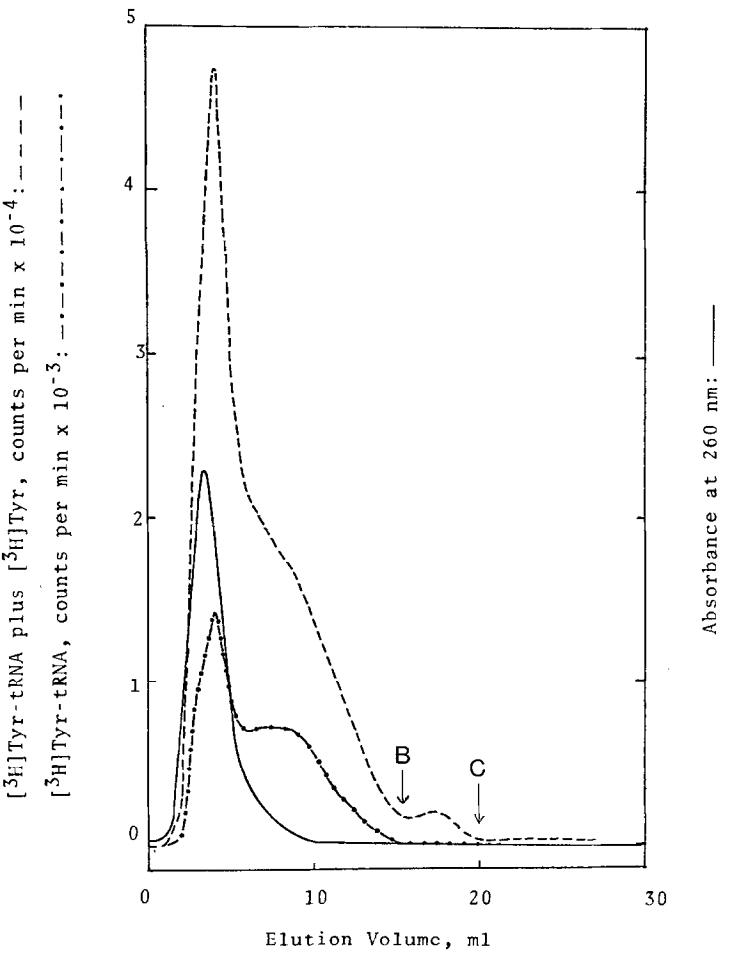

Figure 2. Separation of tyrosine tRNA by affinity chromatography on an Affi Gel-RCA matrix containing 2 Å-long spacer arm (agarose-O-CH_2-CO-RCA). Ten A_{260} units of bovine liver tRNAs and [^{3}H]Tyr-tRNA (171,300 cpm) were applied to the column (4 x 1 cm). The column was eluted with buffer A, then with buffer A containing 1 m*M* D-galactose (buffer B) and, finally, with buffer A containing 0.5 *M* NaCl. For composition of buffer A, flow rate and fraction size, see legends to Figure 1.

Affinity matrix with 2 Å-long spacer arm. The results shown in Figure 2 for this affinity column indicate that although 40-50% tyrosyl-tRNA and other tRNAs eluted in the front peak, the remaining tyrosyl-tRNA was partially retained by the column as indicated by radioactive peaks. Further elution of this column with a buffer containing galactose produced only a small amount of tyrosyl-tRNA. Analogous results were obtained when the amount of tRNA ap-

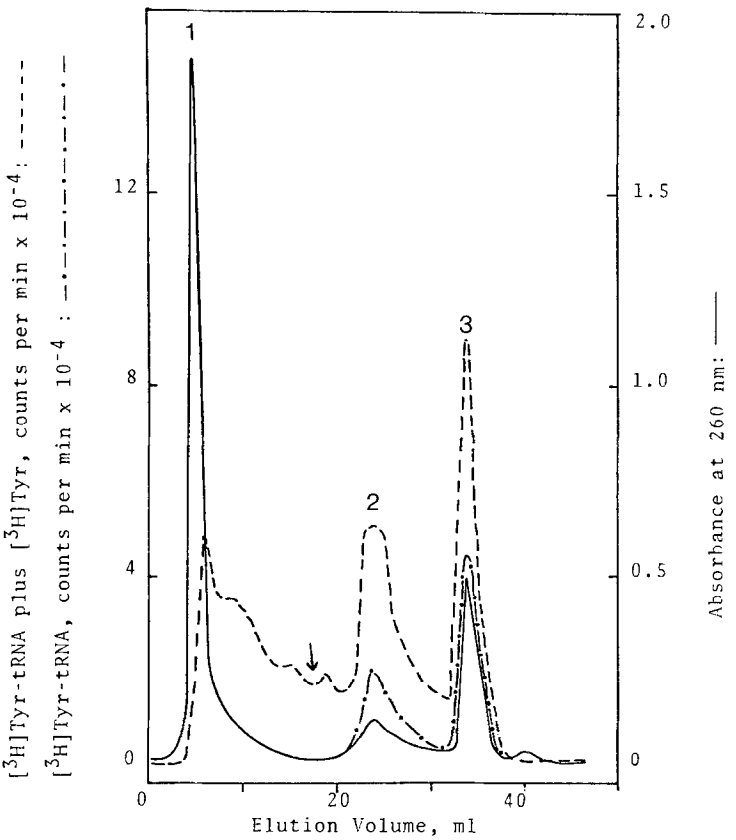

Figure 3. Separation of tyrosine tRNA by affinity chromatography on an Affi Gel-RCA matrix containing 10 Å spacer arm (agarose-O-CH_2-CO-NH-CH_2-CH_2-NH-CO-CH_2-CH_2-CO-RCA). Eight A_{260} units of bovine liver tRNAs and [^{3}H]Tyr-tRNA (1.4 x 10^6 cpm) were applied to the column (7.5 x 1 cm). For column elution, buffers, flow rate, and fraction size, see legends to Figure 1.

plied to this column was reduced to 4-5 A_{260} units, thus indicating that the elution of tyrosyl-tRNA with other tRNAs in the front peak was not caused by a limiting column capacity.

Affinity matrix with 10 Å spacer arm. The tRNAs applied to this column were first eluted with a buffer until all material absorbing at 260 nm was removed, and then another buffer containing galactose was employed. The results presented in Figure 3 indicate that the material eluted in the front peak contained tRNAs but no tyrosyl-tRNA. Radioactivity in this peak was not associated with the tyrosyl-tRNA (acid-insoluble counts), but with the free

tyrosine produced by de-esterification of the tyrosyl-tRNA bond during storage and column chromatography. Two isoacceptors of $tRNA^{Tyr}$ were eluted in peaks 2 and 3 of this affinity column. The amount of other Q-base containing tRNAs in peaks 2 and 3 was shown to be less than 1% of the $tRNA^{Tyr}$ by aminoacylations with labelled asparagine, histidine, and aspartate. The specific activity of the $tRNA_1^{Tyr}$ in peak 2 was 1.74 nmol per A_{260} unit, and that of the $tRNA_2^{Tyr}$ in peak 3 was 1.55 nmol per A_{260} unit.

DISCUSSION

The extent of interaction between the affinity matrix (agarose-RCA) and the affinity molecule ($tRNA^{Tyr}$) is highly dependent upon the length of the spacer arm. A spacer arm between the agarose matrix and the lectin molecule of 2 Å or less in length is insufficient in promoting binding of gal-Q base of $tRNA^{Tyr}$ to RCA to yield an adequate separation of this tRNA from other tRNAs.

The results also demonstrate that mammalian $tRNA^{Tyr}$ can be isolated from nineteen other tRNAs in only one column application using the affinity chromatography principle. Tyrosine tRNA prepared by this method contains less than 1% of the tRNA impurities. The low specific activity of the $tRNA^{Tyr}$ (1.55 to 1.74 nmol of tyrosine per A_{260} unit of the tRNA compared to 1.6 to 1.9 nmol of amino acids per A_{260} unit of *E. coli* tRNAs (6) can be explained by the absence of the -CCA end in 15% of bovine liver tRNAs (3), and by the fact that mammalian synthetases exhibit high k_M values.

Differences among $tRNA^{Tyr}$ isoacceptors have been shown to occur in several cases, for example, between normal and cancer cells (7). Another Q-base containing tRNA, $tRNA^{Asp}$, has been found to exhibit similar differences between normal and tumor cells (8). Lack of "mature" Q-base in tumor $tRNA^{Asp}$ was reported to be the basis of tumor-specific $tRNA^{Asp}$ species (9). Using affinity chromatography for purification, $tRNA^{Tyr}$ isoacceptors containing the full complement of the gal-Q base can be separated from other isoacceptors that lack this base. Thus, cancer specific $tRNA^{Tyr}$ can be resolved from normal $tRNA^{Tyr}$ species.

REFERENCES

1. Kasai, H., Nakanishi, K., Macfarlane, R. D., Torgerson, D. F., Ohashi, Z., McClosky, J. A., Gross, H. J. and Nishimura, S. (1976) J. Amer. Chem. Soc. 98, 5044-5046.
2. Okada, N., Shindo-Okada, N., and Nishimura, S. (1977) Nucleic Acids Res. 4, 415-423.
3. Vakharia, V. N., and Singhal, R. P. (1978) (manuscript submitted to Anal. Biochem.).
4. Deutscher, M. P. (1974) Methods Enzymol. 29, 577-583.
5. Kopper, R. A., and Singhal, R. P. (1978) (manuscript stumitted to Anal. Biochem.).
6. Singhal, R. P., and Best, A. M. (1973) Biochim. Biophys. Acta 331, 357-368.
7. Singhal, R. P., Griffin, G. and Novelli, G. D. (1976) Biochemistry 15, 5083-5087.
8. Briscoe, W. T., Griffin, A. C., McBride, C. and Bowen, J. M. (1975) Cancer Res. 35, 2586-2593.
9. Okada, N., Shindo-Okada, N., Sato, S., Itoh, Y. H., Oda, K-I. and Nishimura, S. (1978) Proc. Natl. Acad. Sci. USA 75, 4247-4251.