SEVEN LYSINE ISOACCEPTING tRNA's FROM POLYOMA VIRUS-TRANSFORMED CELLS

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Summary. Chromatography of lysyl-tRNA from polyoma virus-transformed mouse fibroblasts on benzoylated diethylaminoethyl cellulose gives four isoaccepting species. One of the isoacceptors is a major species in transformed cells but not in normal cells. In a reverse-phase chromatographic system, five isoacceptors are clearly resolved, one is poorly resolved, and one is observed only with difficulty. A comparison of the results of the two systems reveals a total of seven identifiable isoacceptors of lysyl-tRNA.

Introduction. Jacobson et al. (1) using BDC[1] chromatography demonstrated four isoaccepting lysyl-tRNA's in polyoma virus-transformed mouse cells. Only three peaks are observed in normal cells. Because Ortwerth and Liu (2), using reverse-phase chromatography (RPC-5), reported five isoaccepting species of lysyl-tRNA in various mammalian cells, we compared RPC-5 and BDC in the separation of isoaccepting lysyl-tRNA's. The results presented here correlate the various peaks observed in the two chromatographic profiles and demonstrate the presence of seven isoaccepting lysine tRNA's in polyoma-transformed mouse cells.

Material and Methods. [^{3}H]lysine (7 Ci/mM) and [^{14}C]lysine (312 mCi/mM) were obtained from Schwarz/Mann. Material for RPC-5 chromatography was prepared according to Pearson, Weiss, and Kelmers (3), and BDC was prepared according to Gillam et al. (4).

Mouse fibroblasts transformed by polyoma virus (Py3T3) were grown in roller bottles at 37° in Eagle's minimum essential medium (5) as described (1) using only the antibiotics penicillin and streptomycin.

tRNA and aminoacyl-tRNA ligases were obtained as described (1), and tRNA was purified further by DEAE-cellulose chromatography (6). tRNA was

[1] BDC: benzoylated diethylaminoethyl cellulose

aminoacylated in a reaction mixture containing in 150 µl: Tris-HCl, 5 µmoles; ATP, 0.2 µmoles; magnesium acetate, 2 µmoles; 2-mercaptoethanol, 6 µmoles; 19 nonradioactive L-amino acids, 1 nmole each; 25 µCi of [^{3}H]lysine or 2.5 µCi of [^{14}C]lysine; 0.5 A_{260} units[2] of tRNA; and 40 µg of protein from the ligase preparation. After incubation for 20 min at 37°, aminoacyl-tRNA was recovered by DEAE-cellulose chromatography (6).

BDC chromatography was done on a 0.9 x 110 cm column. After sample application, the column was washed with 60 ml of acetate buffer (10 mM sodium acetate buffer, pH 5, 10 mM $MgCl_2$ and 0.4% sodium azide) containing 0.65 M NaCl. A linear gradient (200 ml) from 0.65 M NaCl to 1.2 M NaCl with acetate buffer was used for elution. A final wash consisted of 60 ml of acetate buffer containing 1.5 M NaCl and 15% methoxyethanol. Fractions of 3 ml were collected at a flow rate of 1 ml/min.

RPC-5 chromatography was done on a 0.9 x 100 cm column. After sample application, the column was eluted with a linear gradient (500 ml) from 0.5 to 0.65 M NaCl in acetate buffer without sodium azide. Fractions of 4 ml were collected at a flow rate of 1.3 ml/min. Radioactivity in each fraction was determined in 15 ml of a 2:1 mixture of toluene-based scintillation fluid and Triton X-100.

Results. The chromatographic profile of Py3T3 lysyl-tRNA on BDC is shown in Fig. 1 and is similar to previous results (1) with four peaks observed. Peak III is found in appreciable amounts in polyoma virus-transformed cells but not in normal cells. The RPC-5 system reveals six peaks of lysyl-tRNA (Fig. 2A). Although results with the different chromatographic systems are qualitatively similar, it is not possible to identify by inspection the various peaks in the two systems. To identify the RPC-5 peaks, Py3T3 lysyl-tRNA was chromatographed on BDC and the fractions containing the various peaks were individually pooled as indicated in Fig. 1. Each pooled

[2] One A_{260} unit is the amount of tRNA/ml giving an absorbance of one at 260 nm for a 1 cm light path.

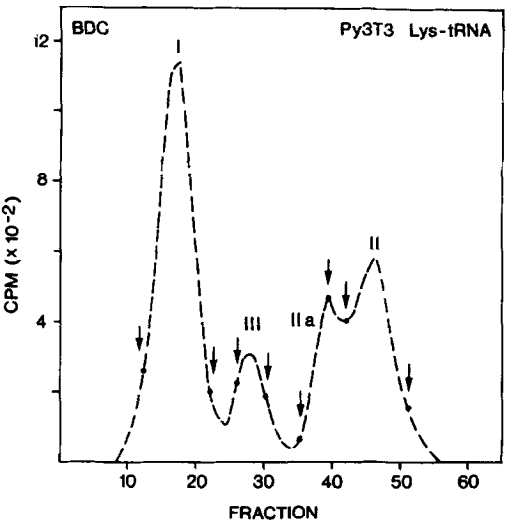

Fig. 1. BDC chromatography of Py3T3 lysyl-tRNA. Five A_{260} units of Py3T3 tRNA, aminoacylated with [^{3}H]lysine (6 x 10^5 cpm), was used for BDC chromatography; 100 µl of each fraction was spotted on glass fiber discs and radioactivity was determined by liquid scintillation counting. The fractions between the arrows, corresponding to each of the peaks, were pooled, and tRNA was precipitated with 2.5 volumes of ethanol after the addition of 2 mg of E. coli B tRNA as carrier. tRNA was recovered by centrifugation, dried in vacuo, and dissolved in 0.5 ml of RPC-5 starting buffer.

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peak was chromatographed on RPC-5 with control Py3T3 lysyl-tRNA. BDC peak I chromatographs as RPC peaks 1, 2, and 4 (Fig. 2A). BDC peak II is identified as RPC peak 5 (Fig. 2B). Although RPC peak 5a was not resolved in that chromatographic analysis, it is clear that the leading edge of the control RPC peak 5 is not identical with BDC peak II. BDC peak IIa appears on the leading edge of RPC peak 5 (Fig. 2C) and is, therefore, RPC peak 5a. The extra peak found in polyoma-transformed cells, BDC peak III, splits into two peaks in the RPC system with a portion of the peak appearing as RPC peak 3 and a portion appearing as RPC peak 6 (Fig. 2D). Variations in the relative amounts of peaks in the control Py3T3 tRNA derive from using two different preparations of Py3T3 tRNA. The correspondence of the peaks is summarized in Table I.

Five peaks of lysyl-tRNA have been obtained by Ortwerth and Liu (2) in the RPC-5 system with tRNA from various mammalian tissues. To compare our

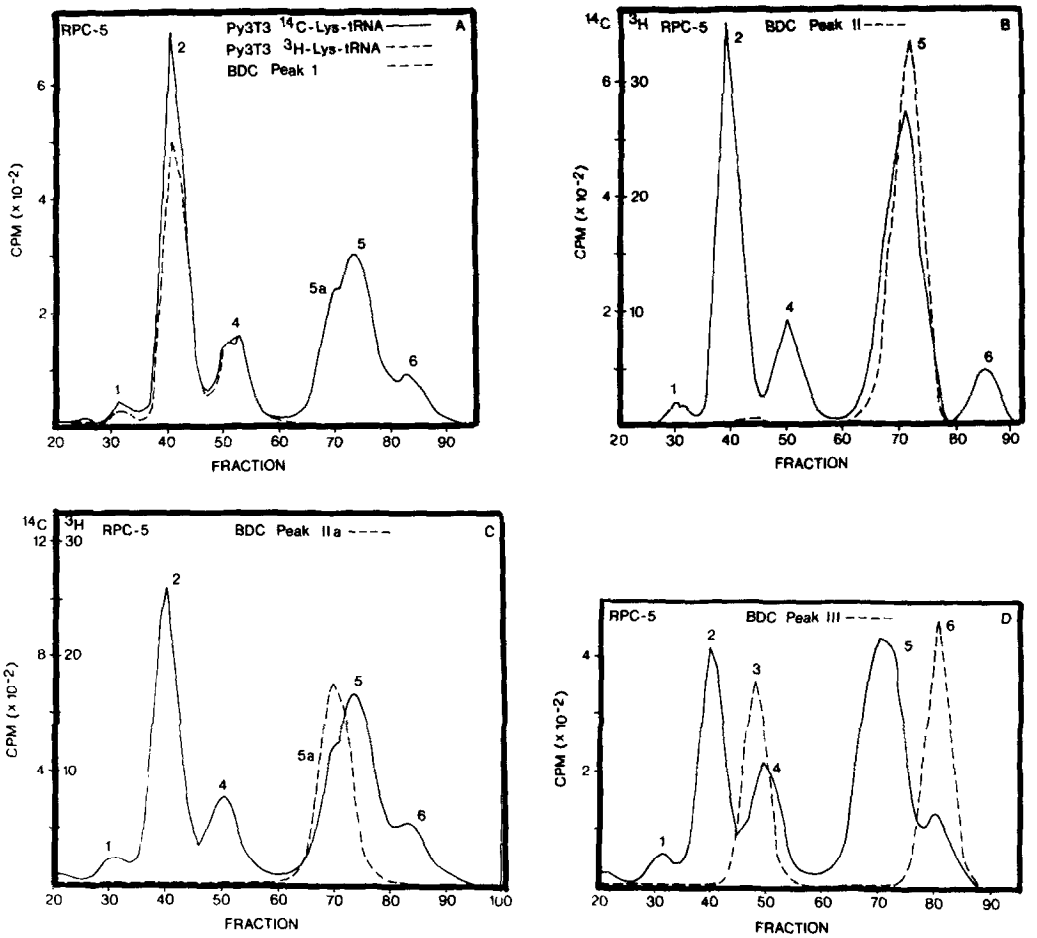

Fig. 2. Correlation between BDC and RPC-5 chromatography of Py3T3 lysyl-tRNA. The peaks obtained from BDC chromatography as indicated in Fig. 1 were cochromatographed in the RPC-5 system with Py3T3 [^{14}C]lysyl-tRNA.

observations with those reported (2) we obtained from Dr. B. J. Ortwerth mouse lymphoma tRNA charged with lysine and chromatographed it with Py3T3 lysyl-tRNA in the RPC-5 system. Both profiles resembled that of the control of Fig. 2B except that RPC peak 6 is not present in lymphomic tRNA (data not shown); peak 5a was not resolved from peak 5. RPC peak 3 is usually masked in the region between peaks 2 and 4 and is difficult to determine (2); it may be present in small amounts in some tissues, especially rapidly dividing tissues (2).

Because BDC peak III split into two peaks (3 and 6) on RPC-5, Py3T3

tRNA was subjected to the denaturing and renaturing conditions of Lindahl et al. (7) and after charging with lysine was cochromatographed with native Py3T3 lysyl-tRNA on RPC-5 to determine whether peak 3 or peak 6 might be an artifact. The treatment did not alter any of the chromatographic peaks.

An additional check on the possibility that RPC peak 3 is an artifact of chromatography, arising perhaps from RPC peak 2 or BDC peak I, involved isolating RPC peak 4 from a chromatographic run of Py3T3 lysyl-tRNA on RPC-5 and rechromatographing it against total Py3T3 lysyl-tRNA on BDC. Peak 4 split to correspond with BDC peaks I and III with most of peak 4 chromatographing with peak I. The data do not indicate that artifacts of chromatography account for the peaks.

Discussion. Five isoaccepting lysyl-tRNA's from RPC-5 chromatography have been reported for various mammalian tissues including mouse tissues (2,8). Other workers using RPC-5 have reported four isoacceptors in rat liver (9), Zajdela ascitic hepatoma cells (9), avian myeloblastosis virus (10), chicken liver (10), and chick embryos (11). Six isoacceptors of lysyl-tRNA from Drosophila were separated by RPC-5 (12). Neither lysyl-tRNA peak III from BDC nor the part of that peak chromatographing as RPC peak 6 has been reported in mammalian cells by other workers. RPC peak 6 seems to be associated with polyoma virus-transformed cells; it has not been observed in

Table I. Comparison of Isoaccepting Lysyl-tRNA's by Benzoylated DEAE-Cellulose and RPC-5 Chromatography

BDC Peak	Corresponding Peaks on RPC-5
I	1,2,4
IIa	5a
II	5
III	3,6

normal cells (Juarez and Hedgcoth, unpublished observations). The part of BDC peak III chromatographing as RPC peak 3 may appear as a very minor peak in some cells (2,12).

By correlating the various peaks from BDC and RPC, we find that there are at least seven isoacceptors for lysine in the tRNA of polyoma-transformed cells. We find BDC peak IIa (RPC peak 5a) in both normal and transformed cells (Juarez and Hedgcoth, unpublished observations). That peak has not been reported by others in either BDC or RPC systems, possibly because of lack of resolution with BDC peak IIa eluting almost under peak II and a similar problem occurring for the two peaks on RPC. Alternatively, the isoacceptor may not be present in all cells or under all growth conditions.

This work illustrates a case of the complementary use of two superior chromatographic systems for satisfactory resolution of a high multiplicity of tRNA isoacceptors: peak 5a is separated on BDC but not on RPC-5 and peaks 1,2, and 4 are resolved on RPC-5 but not on BDC.

Sequential fractionations of lysyl-tRNA on BDC and RPC-5 avoid the ambiguities arising from the use of either system alone when preparing lysyl-tRNA for codon assignments and functional studies. Our finding of two species of lysyl-tRNA usually chromatographing on RPC-5 as peak 5, which is clearly separated on BDC into peaks IIa and II, probably explains the report by Ortwerth et al. (8) that RPC peak 5 is probably composed of two species with different chemical properties and codon responses.

The codon response and the ability to participate in protein synthesis of the seven lysyl-tRNA isoacceptors separated using the two chromatographic systems should be determined.

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