ISOACCEPTING tRNA's IN MOUSE PLASMA CELL TUMORS THAT SYNTHESIZE DIFFERENT MYELOMA PROTEIN*

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Different mouse plasma cell tumors have been shown to produce different homogenous species of myeloma immunoglobulin in large amounts and are currently thought to consist of monoclonal cells (Potter et al., 1965; Lennox and Cohn, 1967). We have previously observed multiple isoaccepting tRNA's in a tumor of this type, MOPC 31C (Yang and Novelli, 1968a). In the present work, comparative studies of isoaccepting tRNA's by co-chromatography on a reversed-phase chromatographic column have been performed on cell lines of transplantable mouse plasma cell tumors that produce different kinds of myeloma immunoglobulin. Similar chromatographic patterns were observed for most of the aminoacyl-tRNA's examined in these tumors. A marked difference, however, was found between the seryl-tRNA's of an immunoglobulin-A producer (MPC 62) and those of immunoglobulin-G producers (MPC 47 and MOPC 31C).

MATERIALS AND METHODS

MOPC 31B and 31C were originally supplied by Dr. M. Potter of the National Cancer Institute, NIH (Potter and Kuff, 1964). MPC 47 and MPC 62, kindly provided by Drs. D. C. Hooper and J. F. Albright of the Biology Division, ORNL, were obtained originally from the laboratory of Dr. R. Mervin of NIH. The tumors were maintained by serial 2-week transplantations in 8-week-old female BALB/c Cum mice.

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Except for modifications described below, methods of preparing tRNA, aminoacyl-tRNA synthetases, and labeled aminoacyl-tRNA's have been reported elsewhere (Yang and Novelli, 1968a). To prepare labeled aminoacyl-tRNA, 10 A_{260} units of tRNA and 1.5 mg of synthetase protein were usually used in a 5-ml reaction mixture that included an excess amount of 19 other nonlabeled amino acids in addition to the labeled one. After incubation at 30°C for 45 min, the reaction mixture was poured onto a DEAE-cellulose column (1.0 X 3.0 cm) that had been equilibrated with a solution containing 0.25 M NaCl, 0.01 M $MgCl_2$, and 0.001 M EDTA (pH 4.5-5.0). After the column was washed with 15-20 column volumes of the solution, the labeled aminoacyl-tRNA's were eluted from the column in a small volume of 0.7 M NaCl, 0.01 M $MgCl_2$, and 0.001 M EDTA. This method, devised at the Biology Division by Dr. L. C. Waters, is simple and gives excellent recovery of tRNA from small sample operations. The eluted aminoacyl-tRNA solution was diluted with an equal volume of 0.01 M Na acetate (pH 4.5) before application to the reversed-phase column.

The procedures for making the reversed-phase column RPC-II (formerly called the reversed-phase Freon column) and for its operation have been described previously (Weiss and Kelmers, 1967; Yang and Novelli, 1968a). Analyses were made by using 2 to 10 A_{260} units of [^{3}H] and [^{14}C] aminoacyl-tRNA mixture. A 2-liter linear gradient of 0.35-0.65 M NaCl at pH 4.5 was usually employed. Inclusion of 0.001 M ß-mercapto-ethanol in the solutions gave better resolution of seryl-tRNA's. The columns were operated at 23°C with a 1.5 ml/min flow rate. Aminoacyl-tRNA's eluted from the column were precipitated by mixing the 10-ml fractions with 4 A_{260} units of calf thymus DNA (0.5 ml) and 2 ml of 50% cold trichloroacetic acid solution. The precipitates were collected on Millipore filters, washed twice with 70% ethanol, dried under infrared light, and counted in a Nuclear Chicago liquid scintillation counter with settings for ^{3}H-^{14}C discrimination. The addition of DNA carrier equalized the quenching of ^{3}H radioactivity throughout the chromatogram.

RESULTS AND DISCUSSION

Co-chromatography of [^{3}H] aminoacyl-tRNA of an immunoglobulin-A producer (MPC 62) and of [^{14}C] aminoacyl-tRNA of an immunoglobulin-G producer (MPC 47 or MOPC 31C), each aminoacylated by its homologous synthetase, were carried out for serine, glycine, tyrosine, methionine, leucine, threonine, and arginine. Several of such co-chromatograms are shown in Figure 1. Apart from seryl-tRNA's, other aminoacyl-

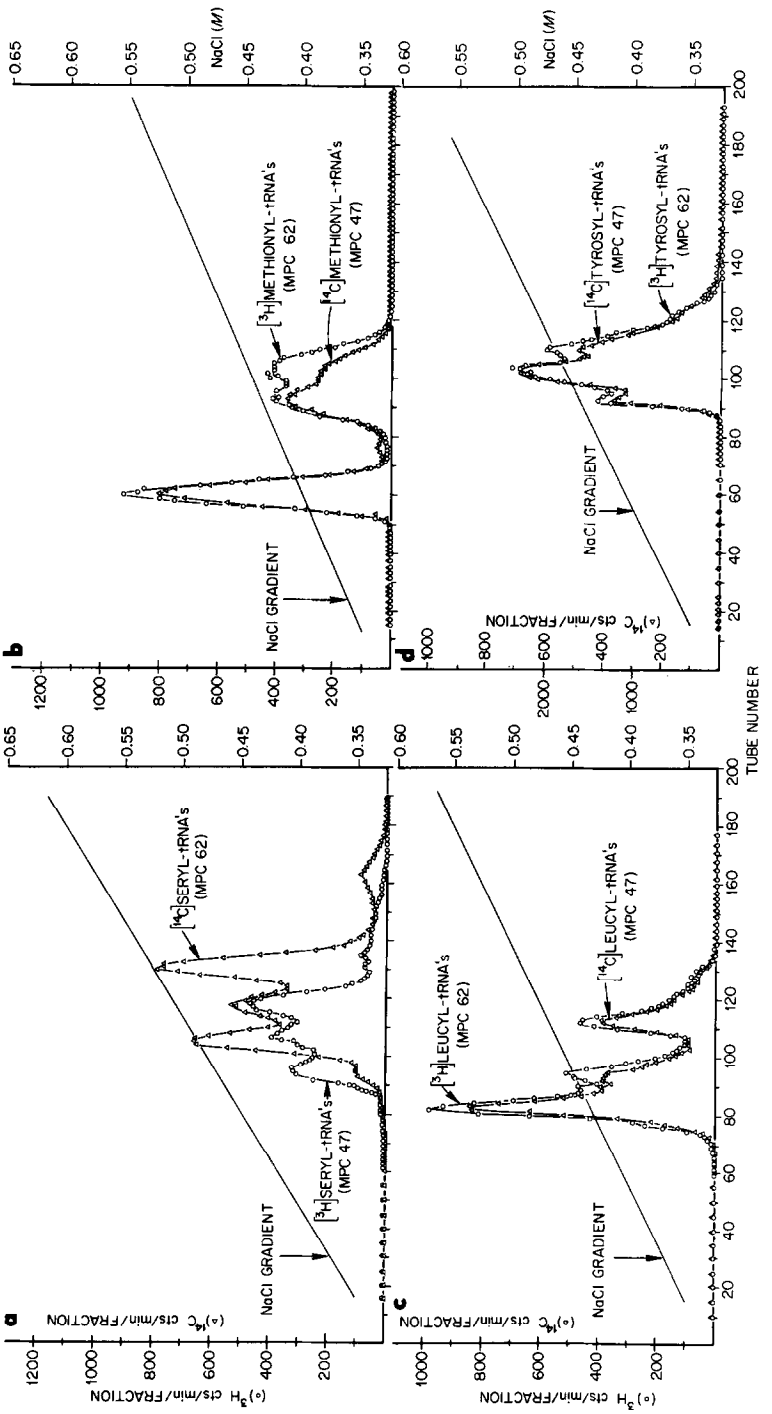

Figure 1. Co-chromatographs of labeled aminoacyl-tRNA's from MPC 62 (IgA) and MPC 47 (IgG) tumors on a reversed phase column (PRC-II). (a) Seryl-tRNA's (MPC 47 = 7.5 A_{260}; MPC 62 = 3.0 A_{260}). (b) Methionyl-tRNA's (MPC 47 = 2.5 A_{260}; MPC 62 = 2.2 A_{260}). (c) Leucyl-tRNA's (MPC 47 = 1.0 A_{260}; MPC 62 = 0.7 A_{260}). (d) Tyrosyl-tRNA's (MPC 47 = 10 A_{260}; MPC 62 = 7.0 A_{260}).

tRNA's derived from these two kinds of tumor tissue were similar in the number and positions of the chromatographic peaks, but were somewhat different in the relative quantities of certain peaks. Reversal of the labeled amino acids gave consistent results. The number of isoaccepting aminoacyl-tRNA peaks detected by the column used in these studies were as follows: 2-3 glycyl-, 3 tyrosyl-, 3-4 leucyl-, 3 methionyl-, 3-threonyl-, and 4 arginyl-tRNA's. A prominent peak of seryl-tRNA (seryl-tRNA$_1$), determined by a Dupont curve resolver, was 27% in MPC 47 and 31% in MOPC 31C, and was nearly absent in MPC 62 (3.5%); whereas a seryl-tRNA peak (seryl-tRNA$_4$) was most marked (35%) in MPC 62 but was hardly detectable in MPC 47 (2%) and MOPC 31C (0%).

To test the authenticity of the observation on seryl-tRNA's, two experiments were performed. First, seryl-tRNA$_1$ and seryl-tRNA$_4$ were isolated from the chromatographic fractions by 70% ethanol precipitation, filtration on Millipore filters, and subsequent elution. Rechromatography demonstrated that both isoaccepting tRNA's were reproduced in single peaks at the same positions on the chromatogram. Secondly, one part of MPC 62 and 2 parts of MPC 47 tumor tissue were mixed. The mixture and two individual samples of tumor tissues were subjected to tRNA preparation in a similar parallel manner. tRNA isolated from this mixture was aminoacylated with [^{3}H] serine, either by MPC 47 or MPC 62 synthetases, and co-chromatographed with [^{14}C] seryl-tRNA prepared from the individual tissue samples. As shown in Figure 2, an intermediate pattern of seryl-tRNA's was observed in the mixture of the two tissues. Analyses of these chromatograms with a Dupont 310 curve resolver clearly demonstrated that the plot of seryl-tRNA's from the mixture was a pure summation of plots of seryl-tRNA's from the two individual portions of MPC 47 and MPC 62 tissues.

Comparative studies were also performed on MOPC 31C, which synthesizes immunoglobulin-G, and on MOPC 31B, which produces the same protein molecule but excretes large amounts of Bence Jones protein (L-chain) and presumably synthesizes unbalanced amounts of subunits L- and H-chain of the protein. Based on the data obtained from aminoacylation (Yang and Novelli, 1968b), leucine, histidine, aspartate, lysine, and methionine were selected for co-chromatographic study. Very similar or identical quantitative patterns were observed for most of the above aminoacyl-tRNA's in these two tissues, except that a slight difference was found in the relative quantities of the three isoaccepting methionyl-tRNA's.

Our current interest has been concentrated on the meaning of the difference in seryl-tRNA's in these IgA and IgG plasma cell tumors. The unique difference persists (a) in tRNA preparations from 34,000 X g supernatants as well as in those from the 34,000 X g

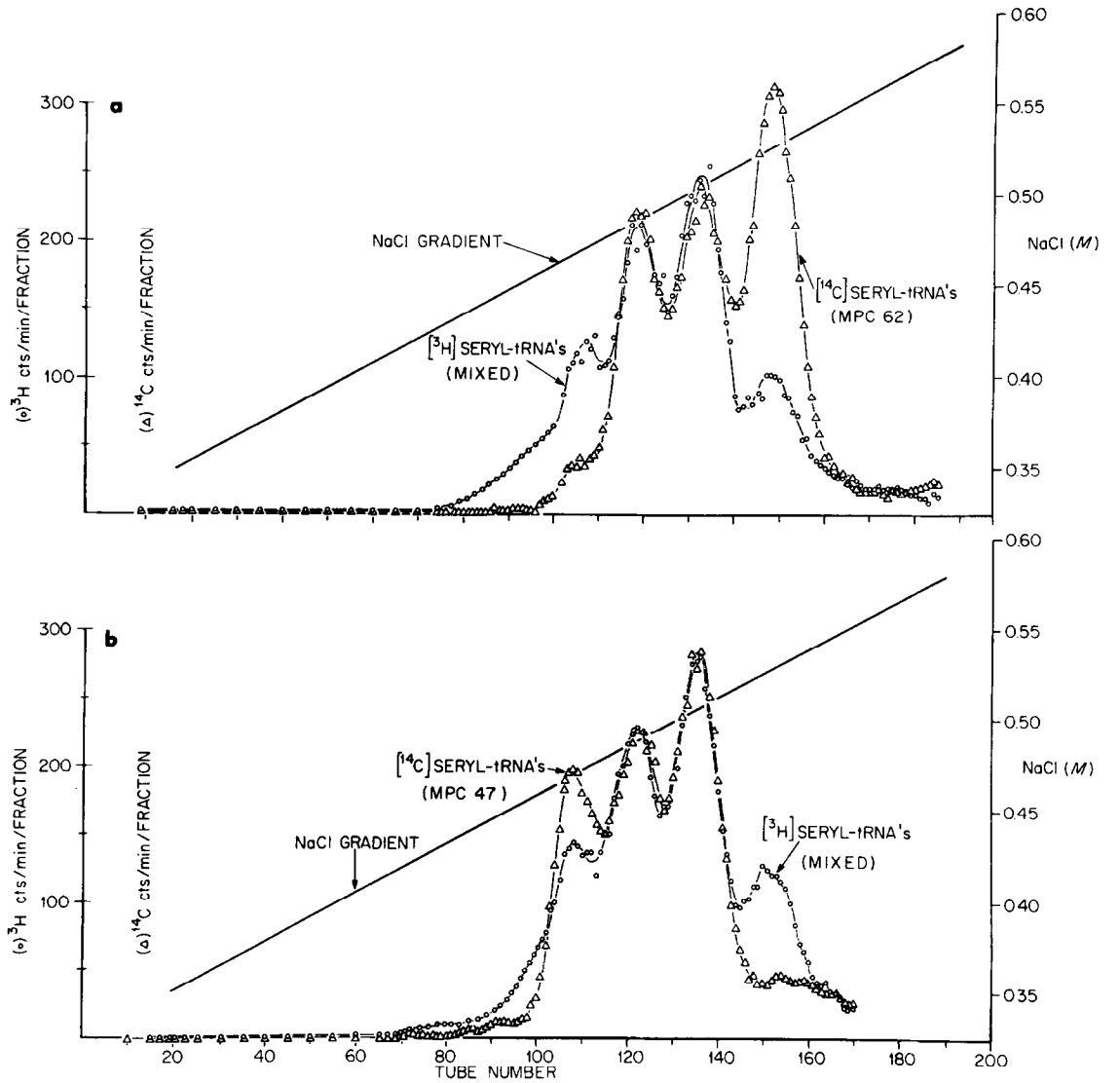

Figure 2. Co-chromatographs comparing [^{3}H] seryl-tRNA's from mixed tumor tissue (MPC 47:MPC 62 = 2:1) with [^{14}C] seryl-tRNA's from MPC 62 and MPC 47. (a) Mixed = 4.5 A_{260}; MPC 62 = 1.5 A_{260}. (b) Mixed = 5.0 A_{260}; MPC 47 = 1.5 A_{260}.

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pellet of the two tissue homogenates; (b) when the synthetases and tRNA's from the two tumor tissues were cross-reacted; and (c) after tRNA's lacking the pCpCpA terminus (about 5% of the total) have been repaired by tRNA-adenylyl transferase (EC-2.7.7.20). The four serine tRNA's accept serine equally well from BALB/c mouse liver synthetases, show

the same stability of the ester linkage with serine, and yield similar seryl-oligonucleotides from T_1 ribonuclease digests. Preliminary results of binding experiments with polyribonucleotides and ribosomes suggest that they differ in codon recognition, but further experiments are needed to establish this finding. A complete account of these results will be published elsewhere (Yang and Novelli, in preparation).

Recent studies with methylated albumin-kieselguhr column chromatography suggest that different mammalian tissues have different patterns of certain tRNA's (Taylor et al., 1967). The present study clearly shows that mouse plasma cell tumor tissue which synthesizes different proteins may show differences in the chromatographic profiles of tRNA. The finding of differences in seryl-tRNA's is especially interesting since serine is one of the few amino acids found to differ significantly in amount in the active fragment of different antibody immunoglobulins (Koshland, 1966). However, it remains to be established that the present finding in seryl-tRNA's is directly related to the synthesis of different myeloma immunoglobulins. It also remains to be determined whether this kind of change in isoaccepting tRNA's is merely a response to the necessity for translating different genetic messages or whether it serves to modify the translation of a genetic code and thus alters the protein primary structure (Potter et al., 1965; Mach, 1967).

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