Synthesis of ^{125}I-Labeled *N*-3-(4-Hydroxyphenyl)propionyl Aminoacyl Transfer Ribonucleic Acids†

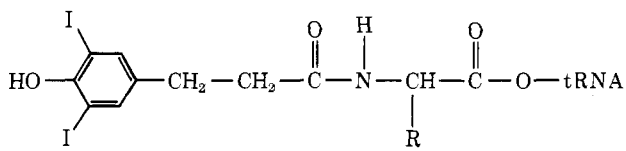
G. M. Tener,* A. D. Delaney, T. A. Grigliatti, G. J. Cowling, and I. C. Gillam

ABSTRACT: A method for the isolation and labeling to high specific radioactivity of individual isoaccepting tRNAs is described. After blocking reactive minor bases by acetylation and iodination of the crude tRNA, a single family of isoacceptors was aminoacylated. Individual isoacceptors were separated by chromatography on RPC-5 and then acylated with the 3-(4-hydroxyphenyl)propionyl ester of *N*-hydroxysuccinimide. The product was purified by chromatography on BD-cellulose and RPC-5. This derivatized tRNA was then iodinated with $^{125}I^-$ and Chloramine-T to give a product containing between 5×10^7 and 3×10^8 dpm/μg. The suitability of such labeled tRNAs for hybridization to homologous DNA in solution and cytological preparations of chromosomes is discussed with particular reference to *Drosophila melanogaster*.

Transfer RNAs are a class of stable transcription products which may be isolated in quantity from a variety of cells. Since posttranscriptional modification of a portion of their nucleotides does not prevent the formation of hybrids with homologous DNA (Landy et al., 1967), appropriately labeled tRNAs may be used as probes to examine the location, organization, and numbers of genes specifying their structure. There are, however, two principal problems to such a scheme. Firstly, the mixture of tRNAs extracted is complex, probably containing about a hundred components. Secondly, the fraction of the genome of a eukaryote representing the genes for one species of tRNA is so small that the specific activity required in a radioactively labeled tRNA probe is higher than can be normally introduced by biosynthetic labeling. This latter problem can be overcome by in vitro labeling of the tRNA.

The two extensively used in vitro procedures for labeling tRNA are that of Commerford (1971), which involves iodination of tRNA with ^{125}I, and the addition of ^{3}H-labeled amino acids using aminoacyl-tRNA synthetases. The product of the latter reaction, an aminoacyl-tRNA, may be stabilized by conversion of the amino group to a hydroxyl group (Hervé & Chapeville, 1965) or by acylating it. The advantage of the Commerford procedure is that one can obtain a product of very high specific activity (better than 5×10^6 dpm/pmol). Its disadvantage is the requirement to isolate the tRNA in a high state of purity before iodination, since for some studies even trace quantities of impurities may lead to ambiguous results.

In this paper we report a procedure which simplifies the preparation of specifically labeled tRNA. It makes use of the high specificity of aminoacyl-tRNA synthetases and simple column chromatographic procedures to produce a product of the following general structure.



In this approach crude tRNA is acetylated to block amino residues in modified bases and treated with unlabeled I_3^- to react centres of unsaturation. It is then specifically aminoacylated with a single amino acid and the individual isoacceptors separated by chromatography on an RPC-5 column. Each is then reacted with the *N*-hydroxysuccinimide ester of 3-(4-hydroxyphenyl)propionic acid and the derivatized aminoacyl-tRNA separated from unreacted tRNA on BD-cellulose. After additional purification on RPC-5 the product is iodinated with ^{125}I to give a product having a specific activity of between 5×10^7 and 3×10^8 dpm/μg (1.4×10^6 to 8.4×10^6 dpm/pmol).

Materials and Methods

Transfer RNA was extracted from *Drosophila melanogaster* adults of the Samarkand strain by the method used by Roe (1975). The resultant RNA was largely free of high molecular weight RNA and DNA. Similar flies were used as a source of crude aminoacyl-tRNA synthetases (White & Tener, 1973). Na^{125}I was obtained from Amersham/Searle Corp. Uniformly labeled [^{14}C]glycine and -phenylalanine and 2,3-[^{3}H]valine were from New England Nuclear.

BD-cellulose (Gillam et al., 1967) was prepared as described. RPC-5 was prepared by the method B of Pearson et al. (1971). Columns were packed and run as described by White et al. (1973). *N*-Hydroxysuccinimide and its 3-(4-hydroxyphenyl)propionic ester were purchased from Pierce Chemical Co. The ester was also synthesized by the method used for other *N*-hydroxysuccinimide esters (Anderson et al., 1964) and crystallized from 1-propanol by addition of ice-cold water (Rudinger & Ruegg, 1973). The product melted 132–136 °C (uncorrected; lit. 129 °C). It is essential to check the melting point of the product mixed with 3-(4-hydroxyphenyl)propionic acid, which melts 129–130 °C. *N*-Acetoxysuccinimide was prepared in a similar manner and melted at 125–129 °C (lit. 131–132 °C).

Recovery of RNA. Fractions containing RNA eluted from chromatographic columns were filtered, if necessary, through Millipore membranes of 0.45-μm pore size and then diluted with 2 vol of 95% ethanol cooled to −20 °C. The added ethanol was mixed well with the solution and left at −20 °C for at least 1 h. If a precipitate of RNA was visible, it was removed by centrifugation. If there was no obvious precipitate, the product

† From the Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5. *Received September 7, 1977.* Supported by Grant MT-1279 to G.M.T. from the Medical Research Council, Ottawa.

[1] Abbreviations used: BD-cellulose, benzoylated DEAE-cellulose; A_{260} unit, that quantity of material, which dissolved in water to a volume of 1 mL, has an absorbance at 260 nm of 1.0; UV, ultraviolet.

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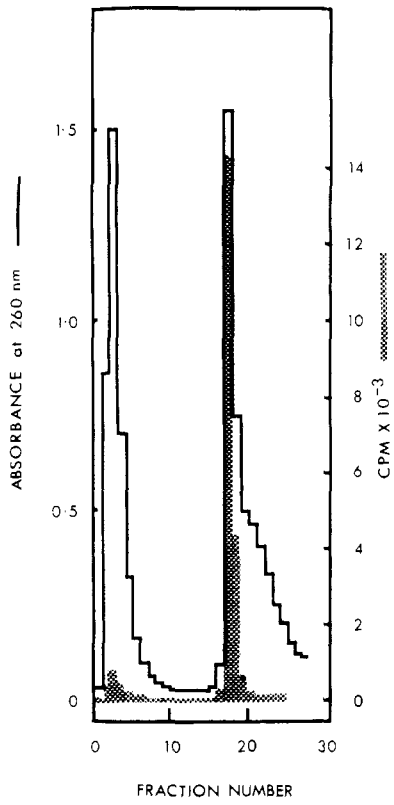

FIGURE 1: Derivatized [^{3}H]Val-tRNA$^{Val}_3$ on BD-cellulose. Pooled material from RPC-5 containing 8.6 A_{260} units and 254 000 cpm was derivatized with the 3-(4-hydroxyphenyl)propionyl ester of *N*-hydroxysuccinimide as described and recovered on a column (0.8 × 7.5 cm) of BD-cellulose. After washing with buffer A, elution with buffer B was started. Fractions of 1.5 mL were collected every 4 min. After fraction 15, the eluent was changed to buffer D. Samples of 100 μL were taken for determination of radioactivity. Pool 1 (fractions 2–7 inclusive) contained 5.9 A_{260} units and 28 000 cpm. Pool 2 (fractions 18–21 inclusive) contained 2.8 A_{260} units and 151 000 cpm. (Total recovery 102% of A_{260} units and 70% of radioactivity.)

was collected by filtration through Millipore membranes of 0.45-μm pore size. Up to 20 A_{260} units could be recovered on a membrane of 25-mm diameter before it became clogged. Supernatants obtained from centrifugations may similarly be filtered to collect traces of precipitate.

Buffers Frequently Used. The solutions contained 10 mM $MgCl_2$/50 mM acetic acid, pH 4.5 (NaOH), with the following additions; for buffer A, 0.3 M NaCl; for buffer B, 0.8 M NaCl; for buffer C, 1.1 M NaCl; for buffer D, 1.1 M NaCl and 10% (v/v) dioxane.

Acetylation and Iodination of tRNA. The tRNA (5100 A_{260} units) was dissolved in 100 mL of 0.1 M triethanolamine/10 mM EDTA/8 M urea, pH 8.0 (acetic acid), and stirred at 25 °C. A solution of 0.5 g of *N*-acetoxysuccinimide in 20 mL of tetrahydrofuran was added and the solution was stirred for 1 h with additions of 1 N NaOH as required to maintain the pH at 8.0. It was then extracted with ethyl acetate (30 mL) and the aqueous layer was diluted with 2 mL of 1 M magnesium chloride and 400 mL of buffer A and applied to a column of DEAE-cellulose (4 × 7 cm). The column was washed with the same solution and the RNA eluted with buffer C. The yield was 4070 A_{260} units (80%) in 223 mL. To this solution was added sufficient iodine/KI solution (containing 5 and 40 g/L, respectively) to give a light straw color (about 1 mL required). It was left for 5 min and then the color was discharged by dropwise addition of sodium bisulfite solution. RNA was recovered by precipitation with ethanol, dialyzed against distilled water, and freeze-dried.

Aminoacylation of tRNA. Acetylated, iodinated tRNA was aminoacylated with a single unlabeled amino acid under the conditions described by White & Tener (1973) except that the buffer used in the incubation mixture was 3-(*N*-morpholino)-propanesulfonic acid, adjusted to the desired pH with KOH. A smaller sample, usually 10% of the other, was separately incubated with a mixture containing radioactive amino acid, usually at a lower concentration and higher specific activity than used by White & Tener (1973). After 30 min of incubation, the two were combined, diluted with ice-cold buffer A, and recovered on DEAE-cellulose as described by Yang & Novelli (1968).

RPC-5 Chromatography of Aminoacyl-tRNAs. About 200 A_{260} units of aminoacylated tRNA containing 1–5 × 10^5 cpm of ^{14}C- or ^{3}H-labeled amino acid was dissolved in 10 mL of starting buffer. The RPC-5 column (2.6 × 26 cm) had previously been washed with 1.5 M NaCl/10 mM $MgCl_2$/50 mM acetic acid, pH 4.5 (NaOH), and then with starting buffer. The sample was applied and eluted with an appropriate linear gradient of concentration of sodium chloride at the temperature described by White et al. (1973). The total volume of eluent used was 1 L. Fractions of 5 mL were collected each 6 min. Samples of 200 μL were removed from appropriate fractions for determination of radioactivity.

Derivatization of Aminoacyl-tRNA. Material recovered from the first RPC-5 column as a peak containing the required aminoacyl-tRNA isoacceptor was recovered by precipitation. The sample, usually about 20 A_{260} units, was dissolved in 3 mL of 0.1 M triethanolamine/5 mM EDTA, pH 4.5 (acetic acid), and stirred at 20 °C. Half of a total of 50 mg of the *N*-hydroxysuccinimide ester of 3-(4-hydroxyphenyl)propionic acid in 1 mL of dioxane was added and the pH of the solution was raised to 8.0 by addition of 1 N NaOH. Then the rest of the reagent was added and the solution was stirred at 20 °C with additions of alkali as required to maintain pH 8.0. After 10 min, glacial acetic acid was introduced to return the pH to 4.5 and the sample was extracted with 4 mL of ethyl acetate (further acetic acid was required to maintain pH 4.5). The aqueous layer was left under an air jet to remove traces of solvent and then the solution was diluted with buffer A and applied to a column of BD-cellulose. The column was washed with buffer A and eluted successively with buffers B and D. (Details are to be found in the legend to Figure 1.)

Separation of Derivatized Aminoacyl-tRNA by RPC-5. The pooled material eluted from BD-cellulose by buffer D was recovered and chromatographed on a smaller RPC-5 column. Details are presented in the legend to Figure 2. Before fractions were collected the eluate from the column was passed through the flow cell of an Altex monitor (Altex Scientific, Inc., Berkeley, Calif.) recording absorbance at 254 nm on a suitably sensitive scale. The fractions containing the derivatized tRNA were pooled and the product recovered and redissolved in 1 mL of 0.2 M sodium acetate (pH 3.8).

Determination of Radioactivity. Fractions (200 μL) of eluate were taken into glass vials of nominal capacity 3.7 mL and diluted to 400 μL by addition of water before 3.4 mL of scintillation fluid was added. The latter was composed of Triton N-101/xylene (Lieberman & Moghissi, 1970) plus fluors, to which 4 mL of 12 N HCl had been added per liter. Radioactivity was determined in an ambient temperature scintillation counter.

^{125}I-Labeled Iodination of Derivatized Aminoacyl-tRNA. The following solutions were placed into a 1.8 mL polyethylene tube with a conical bottom (Bel-Art Products 19929): 6 to 50 μL of the tRNA solution (20–40 pmol), 10 μL of 1 mM gua-

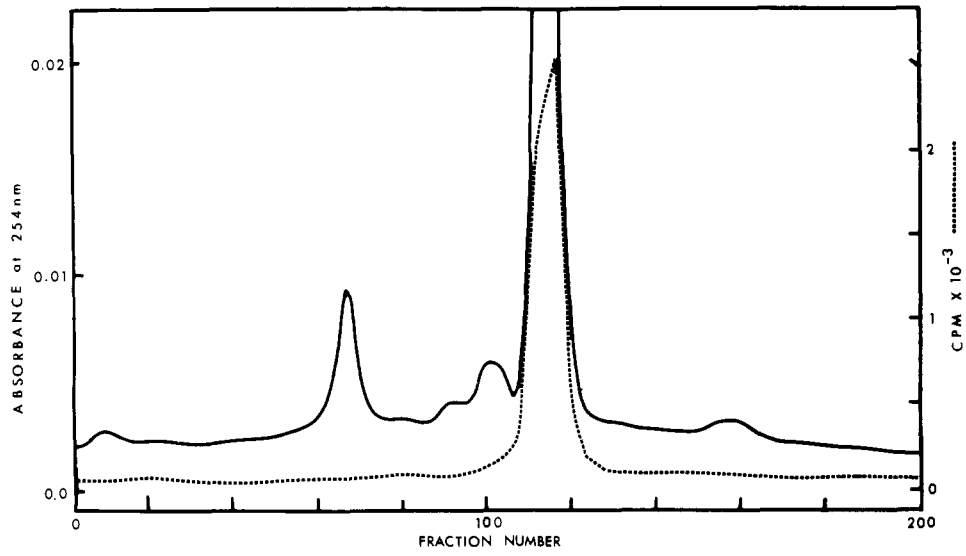

FIGURE 2: Rechromatography of derivatized [^{3}H]Val-tRNA$^{Val}_3$ from Figure 1 on RPC-5. RNA was recovered from pool 2 of Figure 1 and applied to a column (0.9 × 22 cm) of RPC-5 in 0.6 M NaCl/0.1 M $MgCl_2$/0.05 M acetic acid, pH 4.5 (NaOH). It was washed in with the same buffer and eluted with a linear gradient of concentration of sodium chloride from 0.6 M to 1.2 M containing the same buffer and magnesium chloride concentrations. A total volume of 100 mL of eluent was used and fractions of 0.5 mL were collected every 3 min. The column was jacketed at 37 °C. Samples of 100 μL were taken from selected tubes for determination of radioactivity.

nosine 5′-phosphate, 1–5 μL of Na^{125}I (1 to 2 mCi in NaOH solution), and 5 μL of freshly prepared 1 mM Chloramine-T in water. The reaction was left at room temperature for 5 min, then 5 μL of 0.1 M sodium sulfite was added and the reaction left for 5 more min. About 1 mL of 0.3 M NaCl, 30 mM sodium acetate, pH 5, was added, with mixing, followed by 50 μL of a solution of *Escherichia coli* tRNA (80 μg), and the resulting solution applied to a 0.7 × 3 cm column of DEAE-cellulose. The column was washed with 75 mL of the same salt solution to remove unreacted iodide and the product eluted with 1.5 M NaCl, 0.15 M sodium acetate, pH 5. Fractions of 0.5 mL were collected, most of the radioactivity eluting in the 2nd, 3rd, and 4th tubes. The peak fractions were pooled and applied to a column of Sephadex G-25 equilibrated with 0.15 M NaCl, 15 mM sodium acetate (pH 5), 20 mM EDTA. The fraction eluting at the void volume and containing the labeled RNA was stored at 4 °C. The recovery and specific activity were determined from the absorbance at 260 nm of the *E. coli* tRNA added as carrier.

Results

Separation of Aminoacyl-tRNAs on RPC-5. The initial RPC-5 separation of the isoaccepting aminoacyl-tRNAs was performed as described previously (White et al., 1973). In a few cases the use of larger columns gave better resolution of some of the minor species normally occurring as shoulders on the major peaks. Fractions containing the major species of the aminoacyl-tRNAs were pooled for recovery. Such peaks contained from 5 to 20 A_{260} units and all together accounted for about 60% of the initial radioactivity.

Aminoacylation of Acetylated, Iodinated tRNA. The rate and extent of aminoacylation of the treated material were generally similar to those of the untreated. In the case of valine the reacted tRNA accepted more amino acid than the control. Heat treatment (80 °C for 5 min) in the incubation medium just before aminoacylation increased the acceptance of the control more than that of the reacted tRNA, although the latter still accepted more amino acid per A_{260} unit (results not shown). Subsequent chromatography of aminoacylated tRNA showed the expected pattern of peaks. Thus there is no evidence that new species accept valine after acetylation and iodination. We have observed a slight reduction in the acceptance of lysine by tRNA$^{Lys}_5$ in the acetylated, iodinated tRNA. It was reported by Faulkner & Uziel (1971) that iodination of tRNA^{Phe} from *E. coli* did not affect its acceptor activity appreciably.

Results of experiments to isolate specific tRNAs are discussed individually below.

tRNA$^{Val}_3$. Figure 1 shows the separation achieved between derivatized Val-tRNA$^{Val}_3$ and underivatized tRNAs upon BD-cellulose. The incomplete recovery of radioactivity (70%) may be attributed in part to hydrolysis of labeled amino acid, particularly during derivatization at pH 8 and to general losses in handling. The extent of derivatization, as judged from the proportion of total radioactivity eluted by buffer D, is good. Chromatography of the derivatized tRNA on RPC-5 (Figure 2) showed a single major peak of UV absorbance coincident with the radioactivity. The peak was symmetrical and well separated from the small, early-eluting peak representing traces of tRNAs that were not derivatized. This major peak contained a total of 0.4 A_{260} unit and 107 000 cpm. The minor peaks, devoid of radioactivity, are of unknown origin.

R. J. Dunn in this laboratory has recently shown (unpublished) that under different conditions of chromatography tRNA$^{Val}_3$ may be resolved into two components, tRNA$^{Val}_{3a}$ and tRNA$^{Val}_{3b}$. These two isoacceptors elute as a single symmetrical peak under the conditions used here but both should have been present in this product.

tRNA$^{Val}_4$. The isolation of derivatized Val-tRNA$^{Val}_4$ from material separated originally on RPC-5 from the same batch of Val-tRNA used above was closely similar. The principal peak of UV absorbance was again coincident with that of radioactivity. Due to the slight increase in concentration of sodium chloride used to start the gradient, the peak eluted at a lower fraction number.

tRNA$^{Gly}_{3a}$. The initial separation of labeled Gly-tRNA on the large column of RPC-5 gave slightly better resolution of the isoacceptors than reported earlier (White & Tener, 1973). Gly-tRNA$^{Gly}_1$ and Gly-tRNA$^{Gly}_2$ were resolved into two peaks and Gly-tRNA$^{Gly}_3$ showed a small shoulder on its trailing side. The major peak was called 3A and the minor 3B.

Pooled material containing peak 3A was processed in the standard way and the derivatized Gly-tRNA$^{Gly}_{3a}$ gave the expected coincident peaks of UV absorbance and radioactivity when eluted from the second RPC-5 column. However, on either side of this peak other UV-absorbing peaks were also present. These contained about as much material as was found in the desired peak.

tRNA$^{Phe}_2$. This species represents by far the greatest part of total acceptance for phenylalanine (White et al., 1973) and is not unusually strongly bound to BD-cellulose. Its isolation was like that of the other acceptors studied. A principal peak of radioactivity coincided with a peak of UV absorbance but, as for tRNA$^{Gly}_{3a}$ above, there were other UV absorbing peaks. These seemed to contain small amounts of radioactivity.

Iodination of Isolated Derivatized Aminoacyl-tRNAs. Derivatized aminoacyl-tRNAs incorporated labeled iodine under mild conditions, i.e., incubation at room temperature for a few minutes. The incorporation normally achieved corresponded to about 0.75 mol of iodine per mol of tRNA or 1.2 $\times 10^8$ cpm per μg. The products hybridized with homologous DNA in solution at the same rate and to the same level as did the same tRNAs purified by other means and labeled by the method of Commerford (1971) (results to be published elsewhere).

The present procedure is applicable to tRNAs and is an alternative to the more general method of Commerford (1971) by which cytidine residues in non-aminoacylated RNA are directly iodinated. The Commerford method labels all nucleic acids in the sample and therefore requires that the material used be carefully purified if misleading results are not to be obtained. We have used RNA labeled by the Commerford method to confirm (Grigliatti et al., 1973) the location of the genes for 5S RNA in *Drosophila* (Wimber & Steffensen, 1970). In the same way we have also used purified tRNA$^{Lys}_5$ to localize its genes in *Drosophila* (Grigliatti et al., 1973). The tRNA samples used were isolated by repeated chromatography on various supports under different conditions and were judged to be highly purified by several criteria. However, autoradiographs of chromosomes hybridized with radioiodinated material prepared from tRNA$^{Lys}_5$ showed heavy labeling over the site for 5S RNA genes. This is interpreted as being due to the presence in the purified tRNA of traces of material capable of hybridizing to the 5S RNA genes. Presumably this represents various fragments of 5S RNA which copurify with the tRNA. These fragments are iodinated and hybridize preferentially to the 5S RNA genes in spite of their low concentration. Their hybridization is favored by the large number of copies of the genes for 5S RNA which occur in the genome. Such fragments of RNA corresponding to any highly redundant gene may be persistent minor contaminants of tRNAs during purification and may cause erroneous conclusions to be reached in hybridization studies. In the alternative procedure described here the purification of tRNAs of interest is simplified to a standard series of steps. Such components as 5S or mRNAs or their fragments do not accept aminoacyl groups and so will not undergo the shift in their position of elution upon derivatization nor will such fragments be iodinated under the mild conditions used for preparing the derivatized tRNA. A potential disadvantage of this method, however, is that if the derivatizing reagent used is not entirely specific for the aminoacyl group but reacts to some extent elsewhere in the tRNA, the products will also bind to BD-cellulose and be shifted to later elution from RPC-5. They will then also be readily iodinated. Once highly reactive groups have been blocked by acetylation the main source of such contaminants is likely to be products of transesterification from the *N*-hydroxysuccinimide ester to hydroxyl groups of the RNA.

It is difficult to prove that the acylating reagent is completely free of side reactions but experience with the original reagents (Gillam et al., 1968) and in these studies suggests there is little such side reaction. The derivatization of aminoacyl-tRNA is performed in aqueous solution using triethanolamine buffer to minimize the possibility of reaction with hydroxyl groups on the tRNA.

A second limitation of this method is the lability of the derivatized aminoacyl group which carries the label. The derivatized ester, though more stable than before derivatization, is still labile to hydrolysis, particularly to hydrolysis catalyzed by hydroxide ion. The label is lost quite rapidly at pH values above 7 but below pH 6.5 the labeled tRNAs are stable enough for most hybridization experiments.

The stability of iodinated derivatized aminoacyl-tRNA may be illustrated by the example of tRNA$^{Phe}_2$. After incubation for 4 h at 65 °C in hybridization medium (0.15 M NaCl, 20 mM EDTA, 50 mM sodium acetate, pH 5.0), 8% of the initial radioactivity was lost as low molecular weight material separable by gel filtration on Sephadex G-25.

Transfer RNAs uniformly carry the 3′-hydroxyl terminus -CCA, which extends beyond the double stranded stem region. At least in the case of *E. coli*, the genes for certain tRNAs do not include this amino acid accepting terminus. This may also be true for *Drosophila*. In such cases a hybrid formed from a tRNA and DNA carrying the corresponding gene may leave the -CCA terminus (and amino acids attached to it) as a single stranded appendage. This non-hydrogen-bonded region may be susceptible to cleavage by nucleases and thus the label at this terminus could be lost. A similar problem may be present when the Commerford procedure is used. The Commerford procedure labels cytidine residues but, in tRNAs, the marked secondary structure leads to a preferential labeling of the exposed -CCA terminus (Schmidt et al., 1973). When this sequence does not correspond to part of the gene the labeled region will again be left exposed. However, we have not observed the loss of label from hybrids prepared from tRNAs labeled by either method after pancreatic RNase digestion (S. Hayashi, unpublished).

The method described offers a standardized procedure to obtain labeled tRNAs for use as tools to localize the corresponding genes in homologous DNA within chromosomes or in separated fragments and to determine the number of tRNA genes in the genome. These results will be reported elsewhere. Extension of the procedure to examine the isoacceptors present in small quantity is under investigation.

Supplementary Material Available

Three additional figures and their legends describing the chromatography of derivatized aminoacyl-tRNAs on RPC-5 for the isoacceptors Val$_4$, Gly$_{3a}$, and Phe$_2$ (5 pages). Ordering information is available on any current masthead page.

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Shape of Protein L11 from the 50S Ribosomal Subunit of *Escherichia coli*†

Lallan Giri,* Jan Dijk, Harald Labischinski, and H. Bradaczek

ABSTRACT: Protein L11 from the 50S ribosomal subunit of *Escherichia coli* A19 was purified by a method using nondenaturing conditions. Its shape in solution was studied by hydrodynamic and low-angle x-ray scattering experiments. The results from both methods are in good agreement. In buffers similar to the ribosomal reconstitution buffer, the protein is monomeric at concentrations up to 3 mg/mL and has a molecular weight of 16 000–17 000. The protein molecule resembles a prolate ellipsoid with an axial ratio of 5-6:1, a radius of gyration of 34 Å, and a maximal length of 150 Å. From the low-angle x-ray diffraction data, a more refined model of the protein molecule has been constructed consisting of two ellipsoids joined by their long axes.

The topographical studies of ribosomal components performed by immune electron microscopy and neutron scattering experiments (reviewed by Brimacombe et al., 1978) have given some preliminary evidence about the shape of ribosomal proteins.

More directly, hydrodynamic studies and low-angle x-ray scattering experiments on proteins S1 (Laughrea & Moore, 1977; Giri & Subramanian, 1977), S3, S4, S5, S7, and S20 (Rohde et al., 1975; Paradies & Franz, 1976; Österberg et al., 1976a,b), S8 (Giri et al., 1977) as well as on L6 (Giri et al., 1977), L7/L12 (Wong & Paradies, 1974; Österberg et al., 1976b), L7/L12-L10 (Österberg et al., 1977a), and L18 and L25 (Österberg et al., 1976a) have shown that both globular and elongated shapes occur in the *E. coli* ribosome.

Protein L11 from the 50S subunit has been shown to play an important role in the function of the *E. coli* ribosome. It is involved in the binding of chloramphenicol (Dietrich et al., 1974), thereby establishing its presence at the A site of the 50S subunit and its proximity to protein L16. Partial reconstitution experiments have shown that L11 is involved in the peptidyl transferase activity of the 50S subunit (Nierhaus & Montejo, 1973). Protein L11 has further been identified by photoaffinity labeling as one of proteins involved in EF-G-dependent GDP binding (Maassen & Möller, 1974). It has been cross-linked to the functionally important proteins L7/L12 and L10 (Expert-Bezançon et al., 1975, 1976). Recently, it was reported that protein L11, prepared under nondenaturing conditions, binds specifically to 23S ribosomal RNA (Littlechild et al., 1977). By immune electron microscopy two antibody binding sites on the 50S subunit were found for L11 (Tischendorf et al., 1975). These authors suggested that protein L11 might therefore have an elongated shape in situ.

We have studied the shape of protein L11 which was prepared by a nondenaturing purification procedure. Protein L11 was found to have an elongated shape resembling a prolate ellipsoid with an axial ratio of 5-6:1 and a maximum length of 150 Å. Refinement of the model suggested the possibility of two separate structural domains in the molecule.

Experimental Procedures

Protein Preparation. Protein L11 was obtained from *Escherichia coli* strain A19 by a nondenaturing purification method (Dijk, J., & Ackermann, I., submitted for publication). Briefly, this was accomplished by extracting the 50S subunits with 1 M or 2 M LiCl in the presence of 0.01 M Hepes,[1] pH

† From the Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann (L.G. and J.D.), Ihnestrasse 63-73, 1 Berlin 33 (Dahlem), West Germany, and Institut für Kristallographie, Freie Universität (H.L. and H.B.), Takustrasse 6, 1 Berlin 33, West Germany. *Received August 24, 1977.*

[1] Abbreviations used: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; CM, carboxymethyl; $NaDodSO_4$, sodium dodecyl sulfate.

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