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The Enzymic Synthesis of Amino Acyl Derivatives of Ribonucleic Acid

III. ISOLATION OF AMINO ACID-ACCEPTOR RIBONUCLEIC ACIDS FROM *ESCHERICHIA COLI**

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The existence of a ribonucleic acid fraction with the unique property of binding amino acids is now well documented (1–6). The mechanism of formation and the structure of these amino acyl ribonucleic acid derivatives have been investigated in a number of laboratories with the following conclusions. Each amino acyl RNA compound is formed by a specific enzyme that catalyzes both the synthesis of the intermediate amino acyl adenylate complex and the corresponding amino acyl RNA derivative (2, 3, 7); each amino acid is linked through its carboxyl group to a ribose-hydroxyl group of the terminal adenylic acid residue in a specific RNA chain (8–10); and each amino acid-acceptor RNA chain is terminated at the acceptor end by the identical trinucleotide sequence, RNA . . . pCpCpA (10–12) and at the other end by pGp . . . RNA (13, 14). Regarding the structural features, which differentiate RNA chains with respect to the amino acids they accept, essentially nothing is known.

In connection with our studies of the mechanism of amino acyl RNA formation and with the objective of determining the chemical and structural features necessary for the formation of a specific amino acyl RNA derivative, a procedure for the isolation of the amino acid-acceptor RNA from *Escherichia coli* was developed (2). The present report describes the details of this method and some physical and chemical properties of the purified RNA.

Since this procedure was developed, there have been a number of accounts of the isolation and properties of amino acid-acceptor RNA from animal tissues (15, 16), yeast (17–19), and bacteria (14, 20). It is of considerable interest and very likely of fundamental significance that the amino acid-acceptor RNA from these diverse cells shows striking similarities in molecular weight, secondary structure, and in nucleotide end groups and composition. How closely this similarity prevails at the more detailed level of nucleotide sequence and over-all molecular conformation remains to be seen.

EXPERIMENTAL PROCEDURE

Materials

E. coli was used as the starting material for the isolation of the amino acid acceptor RNA and for the preparation of enzyme extracts used in measuring C^{14}-amino acid incorporation into the RNA. The organisms were grown as previously described (21), and extracts were prepared in a Waring Blendor with glass beads (21). The specific amino acyl RNA synthetases were isolated and used as already described (21). DNase was a twice recrystallized preparation purchased from Worthington Biochemical Company.

The C^{14}-amino acid mixture used in the assays was derived from an acid hydrolysate of protein from *Chromatium*, strain D, grown with $C^{14}O_2$ as sole carbon source. For use in the assay, it was diluted with an unlabeled hydrolysate of *Chromatium* protein to a specific activity of 3 to 5 $\times$ 10^5 c.p.m. per μmole of carbon. DL-Leucine-1-C^{14} and DL-valine-1-C^{14} were purchased from Isotope Specialties, and unlabeled amino acids were obtained from the California Corporation for Biochemical Research.

Ecteola-cellulose, type 20, was a reagent grade material obtained from the Brown Company. Sodium lauryl sulfate, U.S.P. grade, was used without further purification.

Methods

RNA was routinely assayed for amino acid acceptor activity with the C^{14}-amino acid mixture in the presence of a dialyzed extract of *E. coli*. The standard conditions were as follows: 50 μmoles of sodium cacodylate buffer, pH 7.0, 1 μmole of $MgCl_2$, 0.2 μmole of ATP, approximately 6 $\times$ 10^5 c.p.m. of the C^{14}-amino acid mixture, 0.2 to 8 units of the acceptor RNA and dialyzed *E. coli* extract containing 75 to 150 μg of protein. The mixture, in a total volume of 0.5 ml, was incubated for 20 to 60 minutes at 30°. With these amounts of enzyme and RNA, the reaction is complete in 20 minutes; with aged and less active extracts somewhat longer incubation periods were necessary. The incubation was terminated and the amount of C^{14}-amino acyl RNA formed was determined as previously described (3). Fig. 1 shows that the amount of C^{14}-amino acid linked to the RNA was a linear function of the amount of RNA added. A unit of amino acid acceptor RNA is defined as that amount which binds 10^3 c.p.m. (equivalent to 0.5 mμmole of an amino acid with 5 carbon atoms) as amino acid and the specific activity is expressed as units per micromole of RNA nucleotide (3).

Ribose was measured by an orcinol method (22) with AMP as a standard; deoxypentose was determined by the diphenylamine procedure of Dische (23) with dAMP as the standard. Total phosphate was measured after ashing, by the Fiske-SubbaRow method (24) or by the procedure of Chen, Toribara,

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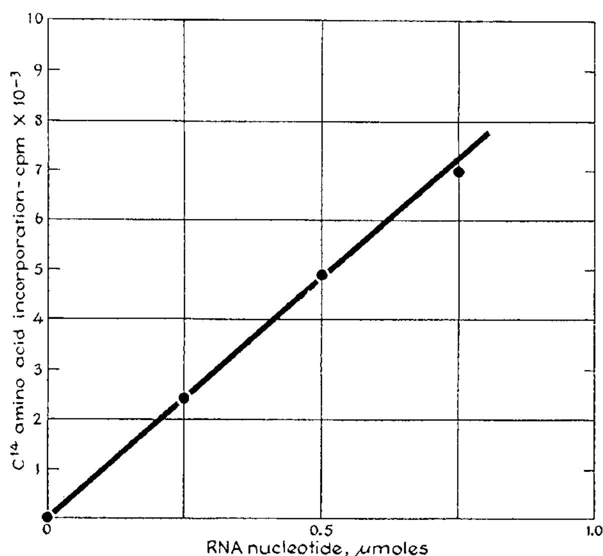

FIG. 1. C^{14}-amino acid uptake as a linear function of RNA nucleotide concentration. See text for conditions.

and Warner (25). The total RNA content of the dried cell preparation was determined by the orcinol method on the extracts obtained after heating in 0.5 N perchloric acid at 100° for 15 minutes.

RESULTS AND DISCUSSION

Preparation of Amino Acid-Acceptor RNA—Washed *E. coli* cells were dried by suspending the cell paste in a mixture of ethanol and ether (3:1) at a concentration of 67 g per liter of solvent and incubating the mixture at 37° for 60 minutes. After filtration of the suspension, the residue was washed with the ethanol-ether mixture, then with ether and brought almost to dryness by suction. The filter cake was dried in a vacuum over KOH and paraffin shavings at room temperature, and the dried powder was stored at −15°. The average yield was 24 g of powder per 100 g of cell paste.

Dried cells, 7.2 g, were suspended with the aid of a Waring Blendor in 225 ml of sodium cacodylate, pH 7.0; foaming was kept to a minimum. After the addition of 56 ml of 5% sodium lauryl sulfate (dissolved in 45% ethanol) the mixture was transferred to a 2-liter Erlenmeyer flask and heated, with constant swirling, for 7 minutes in a boiling water bath. The mixture was cooled to about 50° in an ice bath and 121 ml of 5 M NaCl were added. Rapid mechanical stirring was necessary to ensure adequate mixing of the salt solution with the viscous suspension. The mixture was kept in the ice bath for 45 minutes, centrifuged in the cold at 30,000 × *g* for 15 minutes, and the supernatant fluid removed. Subsequent steps in the isolation were carried out at 2–4°. The sediment was washed with 150 ml of 1.5 M NaCl, and the wash fluid was combined with the first supernatant solution. The precipitate which contains no significant amino acid acceptor activity and about 80% of the total RNA was discarded.[1] Two volumes of ethanol were added to the supernatant fluid, and after about an hour, the precipitate was collected by centrifugation, washed with 67% ethanol containing 0.5 M NaCl (salt-ethanol), and then suspended in 100 ml of 0.01 M sodium cacodylate buffer, pH 7.0.

To this solution (crude acceptor RNA) were added 50 μg of crystalline DNase and 2 ml of 0.5 M $MgCl_2$, making the concentration 0.01 M. The mixture was incubated at 30° for 30 minutes, and the acceptor RNA was then precipitated by the addition of solid NaCl to 1.5 M and then of 2 volumes of ethanol. After centrifugation, the precipitate was washed with 60 ml of the salt-ethanol mixture, dissolved in 50 ml of 0.6 M ammonium formate buffer, pH 4.7, and centrifuged to remove any insoluble material.

The supernatant fluid (DNase-treated RNA) was passed through an Ecteola-cellulose column (16 × 3.5 cm), which had been equilibrated with 0.6 M ammonium formate, pH 4.7; the rate was about 0.5 ml per minute. The column was washed with about 100 ml of 0.6 M ammonium formate buffer and then the RNA was eluted from the column with a linear gradient of buffer concentration. This was established by use of 250 ml of 1.6 M ammonium formate, pH 4.7, in the reservoir and 250 ml of 0.6 M ammonium formate, pH 4.7, in the mixing chamber. Fractions of 5 to 10 ml were collected, and the optical density at 260 mμ was determined. For analysis of the amino acid acceptor activity, the RNA was precipitated by the addition of 2 volumes of ethanol, and after being washed with the salt-ethanol mixture, it was dissolved in 0.01 M sodium cacodylate buffer, pH 7.0. The histogram for the chromatography of the RNA is shown in Fig. 2. A summary of the recoveries during the course of the isolation procedure is presented in Table I. About 70% of the amino acid acceptor activity and approximately 30% of the total optical density applied to the column is eluted between 0.8 and 1.0 M ammonium formate with the peak being at 0.86 M. A consistent finding has been that the early portion of the peak contains the RNA with the highest specific activity for accept-

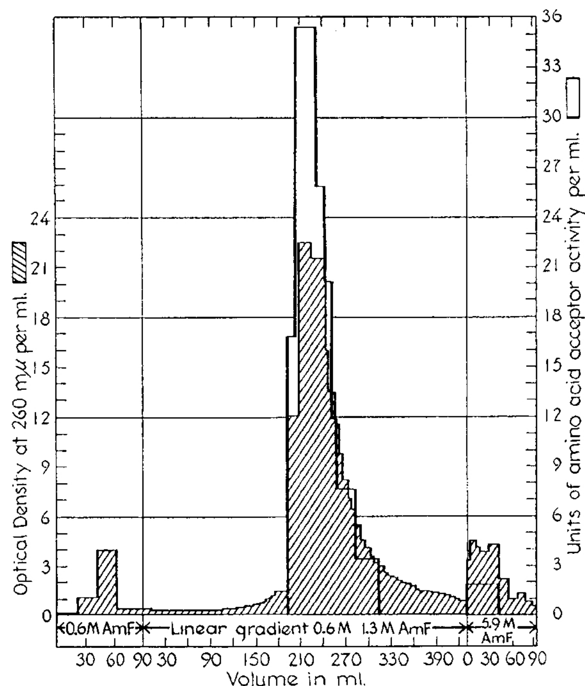

FIG. 2. Chromatography of amino acid-acceptor RNA on Ecteola-cellulose.

[1] The RNA contained in this precipitate was subsequently isolated by phenol extraction (1). Although no further characterization of this RNA fraction was attempted, its nucleotide composition (Table II) was identical to that reported for *E. coli* ribosomal RNA (26).

Table I

Isolation of amino acid-acceptor RNA from E. coli

RNA fraction	Yield of amino acid acceptor activity	Nucleic acid content	Specific activity
	units/g cells	*µmoles/RNA nucleotide/ g cells*	*units/µmole RNA nucleotide*
Crude	392	93	4.2
DNase treated	396	73	5.4
Ecteola	266	24	11.0

ing amino acids. For example, fractions recovered from the leading portion of the peak have 50 to 75% higher specific activity than those fractions from the later part of the peak when assayed either with the amino acid mixture or with leucine, valine, isoleucine, or methionine. Rechromatography of the once-chromatographed RNA yields a single peak emerging at an ammonium formate concentration of 0.83 M with an increase in specific activity of about 20%.

We have also used this procedure on 20 g batches of dried cells with slight modifications but essentially similar results. The various components were increased proportionately, and the sodium lauryl sulfate extraction was performed in a stainless steel beaker, immersed in a boiling water bath, with a stirrer driven by an overhead motor to ensure adequate mixing during the heating. Five such batches of the crude acceptor RNA fraction (representing about 10 mmoles of RNA nucleotide) were combined and subjected to the DNase step and then chromatography on a 37- × 4.5-cm column. The buffer gradient for elution was between 0.6 M and 2.1 M ammonium formate (1200 ml). The peak of acceptor RNA activity was eluted at an ammonium formate concentration of about 0.9 M. In some runs as much as one-third of the optical density applied to the column was removed with the 0.6 M ammonium formate wash but this material was always inactive as an amino acid acceptor.

Analysis of the total RNA content of the dried cell preparation gave values which averaged about 525 µmoles of nucleotide per g. Basing our calculations on the recovery of 24 µmoles of RNA nucleotide in the amino acid acceptor fraction (Table I) and correcting for the yield (68%), we estimate that the amino acid acceptor RNA comprises between 5 to 10% (6.7% in this instance) of the total cellular RNA. Using a value of 3 µmoles of RNA nucleotide as being equal to 1 mg of RNA, we estimate that the acceptor RNA constitutes about 1% of the dry weight of *E. coli*. This value is somewhat higher than the value of 300 mg of acceptor RNA per 300 g wet weight of cells reported by Tissières (20).

Purity of Acceptor RNA Preparation—Estimations of protein in the acceptor RNA by the method of Lowry *et al.* (27) gave values which were equivalent to between 7 to 9 µg of serum albumin per µmole of nucleotide (approximately 2 to 3% on a weight basis). However, this estimate is undoubtedly high, since guanine reacts in the Lowry procedure to yield a color with the same spectrum as that found with proteins. Guanine, 1 µg, yields a color equivalent to about 7 to 8 µg of serum albumin.[2] Similar values for the protein content of RNA, as determined by this method, were noted by Tissières (20).

In contrast to the acceptor RNA preparations obtained by phenol extraction of mammalian tissue (1, 7), the present RNA preparation did not contain significant amounts of bound amino acids. Although no direct estimate was made of the amino acid content of the acceptor RNA preparations described here, the fact is that at least 98% of the ability to accept a number of amino acids was lost after exposure of the RNA to periodate under conditions in which bound amino acids protect against periodate inactivation (9). Moreover, there was no significant increase (<5%) in the amount of amino acid which could be incorporated after dilute alkaline treatment of the RNA, a procedure which removes bound amino acids (9). If, under the conditions in which the cells were grown, the RNA were saturated with amino acids, then it seems most likely that these bound amino acids were removed during the initial extraction step, since all other procedures have routinely been used for the purification of amino acyl RNA derivatives.

The pentose to phosphate ratios in the purified RNA preparations have ranged from 0.96 to 1.08. Based on the phosphate content, the extinction coefficient, $E(P)$ at 260 mµ in the presence of 0.2 M NaCl is 7.7×10^3 at pH 7.0 and 8.8×10^3 at pH 12. In most preparations, the deoxypentose content was less than 4%. In certain instances however the RNA preparations obtained from the Ecteola-cellulose column during the large scale procedures were contaminated with deoxypentose-containing oligonucleotides produced during the DNase treatment. Such oligodeoxynucleotides were removed by treatment with the Lehman phosphodiesterase (28). This enzyme converts oligodeoxynucleotides and single-stranded polydeoxynucleotides to mono- and dinucleotides which are readily removed by dialysis against 0.2 M NaCl.

The spectrum of the purified acceptor RNA preparation is characteristic of the nucleic acids (Fig. 3). At pH 7.0 in 0.2 M

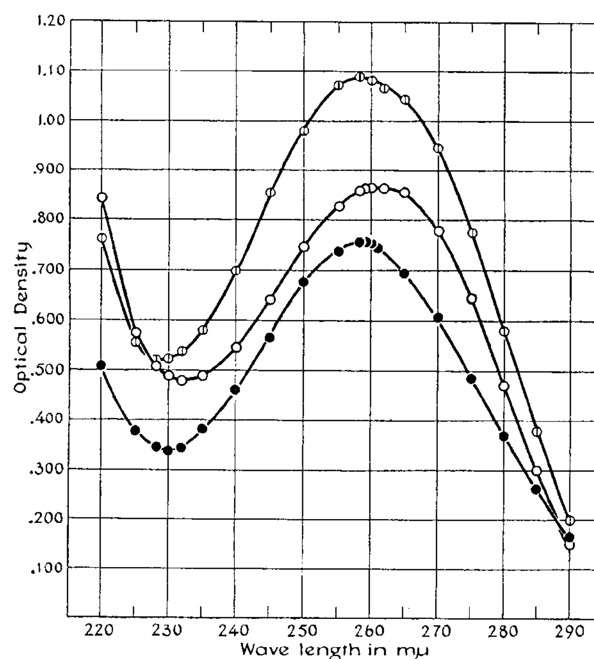

Fig. 3. Absorption spectra of amino acid-acceptor RNA. All curves were normalized to a single concentration of the RNA. ●——●, 0.2 M NaCl-0.001 M sodium cacodylate buffer, pH 7.0; ○——○, 0.2 M NaCl-0.01 N KOH; ⦶——⦶, alkaline hydrolysate in 0.2 M NaCl at pH 7.0. The RNA was hydrolyzed in 0.5 M NaOH for 18 hours at 37°, neutralized with HCl buffered with sodium cacodylate, pH 7.0, and then diluted to the salt concentration indicated.

[2] A. D. Kaiser, private communication.

NaCl, it has a peak at 259 mμ and a minimum at 230 mμ. The ratio of the optical density at 260 to 280 mμ in various preparations was 2.0 to 2.3 and the ratio at 260 to 230 mμ was 2.2 to 2.3. The optical density of the RNA is about 15% higher at pH 12 (0.01 N KOH) than at pH 7.0, although the optical density is restored to the original value when the pH is returned to neutrality. After hydrolysis of the RNA with 0.5 N NaOH for 18 hours at 37°, there is an irreversible increase of 44% in the optical density at 260 mμ.

Nucleotide Composition and End-Group Analysis—The results of chromatographic analysis of an alkaline hydrolysate of the amino acid-acceptor RNA are shown in Table II. With each of the two RNA preparations examined, more than 95% of the nucleoside recovered was adenosine. The adenosine to nucleotide residue ratio was 1:89 in one case and 1:93 in the other. If we accept that nucleosides arise only from the terminal nucleotide with free 2'- and 3'-hydroxyl groups on the ribose and that there is only one such residue per polynucleotide chain, then it is possible to estimate the minimal molecular weight. This estimate, based on an average chain length of 91 nucleotides and on the nucleotide composition, yields a value of 31,000 ± 1,500. In this calculation, it is assumed that all the RNA molecules contain a terminal adenylic acid residue unesterified at the 3'-position. Any chains with the 3'-hydroxyl group esterified

TABLE II

Nucleotide analysis of purified amino acid-acceptor RNA

Digests were prepared by incubating the RNA with 0.3 N NaOH at 37° for 18 hours after which the mixture was adjusted to between pH 6 to 7 with dry Dowex 50-H^+. The hydrolysate was then adjusted to about pH 9 to 10 with NH_4OH and adsorbed onto a Dowex 1-formate column (10 × 2 cm^2). The nucleosides were eluted with 0.01 M ammonium formate buffer, pH 3.7, and the nucleotides were eluted with a parabolic gradient established with 500 ml of 0.035 M ammonium formate, pH 3.7, in each of two mixing chambers and 500 ml of 0.6 M ammonium formate, pH 3.7, in the reservoir chamber. The elution was carried out at 4° with a flow rate of 0.2 ml per minute. Fractions of about 6 ml were collected and the optical density at 250, 260, and 280 mμ was determined. The components listed in the table were eluted in the following order: adenosine, cytidylic, the unidentified compound(s),* uridylic, adenylic, and guanylic acids. Concentrations were determined with extinction coefficients published by Beaven *et al.* (29). Recoveries from the column ranged between 93 and 98% of the optical density applied to the column.

Nucleoside or nucleotide	Amino acid-acceptor RNA		"Ribosomal RNA"
	Preparation I	Preparation II	
	moles/100 moles recovered nucleotide		
Adenosine	1.12	1.08	not detected
Adenylic acid	18.4	18.6	25.6
Uridylic acid	16.4	18.1	20.9
Unidentified component*	4.10	2.94	not detected
Guanylic acid	31.5	30.4	31.2
Cytidylic acid	28.5	28.4	22.3
Purines/pyrimidines	1.04	1.00	1.31
Adenine + uracil + unidentified component*/guanine + cytosine	0.67	0.70	0.87

* This material was eluted from the column immediately preceding the peak containing 2'- and 3'-UMP. The ratio of the optical density at 280 mμ to 260 mμ was 0.64 and 0.73, respectively. It had an absorption maximum at 268 mμ in 0.1 N HCl and in 0.1 N KOH. At the alkaline pH, there was a decrease in the absorption at 268 mμ of about 15% and the appearance of a distinct shoulder in the region of 290 mμ. Insufficient material was available to identify this compound(s) or to determine whether it was a derivative of the pseudouridylic acid type of compound (30). To estimate its concentration, the extinction coefficient of uridylic acid was used.

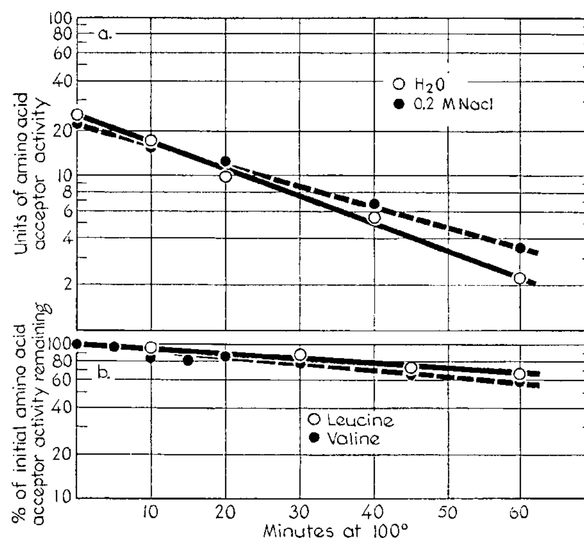

FIG. 4. Effect of heating on amino acid-acceptor activity of RNA. (*a*) Acceptor RNA, which had been dialyzed for 48 hours against 3 changes of distilled water, was diluted in water or 0.2 M NaCl to a concentration of 6.8 μmoles of RNA nucleotide per ml. One milliliter was heated in a boiling water bath for the times indicated, cooled by immersion in an ice bath and stored at −10° for about 24 hours. The samples were then thawed and assayed with a dialyzed *E. coli* extract and a C^{14}-amino acid mixture as described in "Methods." (*b*) RNA nucleotide in water, 1.8 μmoles, was heated as above for the times indicated and then cooled in ice and assayed within 60 to 90 minutes for acceptor activity with leucine and valine, as described previously (3).

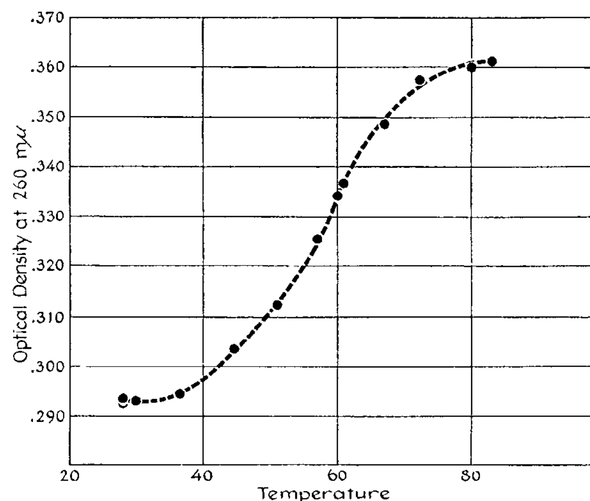

FIG. 5. Effect of temperature on the ultraviolet absorption of amino acid-acceptor RNA. RNA was dissolved in 0.1 M sodium cacodylate buffer, pH 7.0. Readings were made as described by Doty *et al.* (33). Approximately 10 minutes were allowed for the sample to come to thermal equilibrium at each temperature.

would appear as nucleotides after alkaline hydrolysis and therefore make our estimate of the chain length and molecular weight too high. Nevertheless, the molecular weight is only about 20% higher than that estimated by Tissières (20) from sedimentation, viscosity, and diffusion parameters and also is somewhat higher, or of the same order as, the estimates for acceptor RNA from mammalian liver (15) and yeast acceptor RNA preparations (17, 18).

With regard to the over-all nucleotide composition of amino acid-acceptor RNA, two features are noteworthy. The first is that a compound representing approximately 3 to 4% of the total nucleotide was eluted from the column just preceding uridylic acid, whereas this material was not detected in the ribosomal RNA hydrolysate. Although authentic pseudouridylic acid was eluted in this region of the chromatogram, this material had somewhat different spectral properties than those reported for pseudouridylic acid (30) (Legend to Table II). Because of the limited amounts of this material, no further characterization was carried out. More recent analyses of the amino acid-acceptor RNA of *E. coli* have shown that pseudouridylic acid and thymine ribonucleotide comprise about 3% of the total nucleotides (31). This unusually high content of pseudouridylic acid has also been reported for the acceptor RNA from mammalian tissue and from yeast (17, 19). The second feature of the amino acid-acceptor RNA is the relatively close correspondence between the purine and pyrimidine content. Moreover, the total adenylic acid is almost equal to the total uridylic acids (assuming the unknown component to be a derivative of uridylic acid) and the guanylic acid is roughly equal to the cytidylic acid. This type of nucleotide equivalence, which resembles that found in DNA, has also been reported by other investigators (14, 15, 31, 32) but it has not been observed with the RNA isolated from ribosomes (26).

Effect of Heating on Purified Amino Acid-Acceptor RNA—At 100°, the amino acid-acceptor RNA is slowly inactivated in its ability to accept amino acids (Fig. 4*a* and *b*). This occurs at about the same rate whether the heating is carried out in distilled water or in 0.2 M NaCl at pH 7.0. Inactivation of the specific leucine- and valine-specific RNA chains was slower than the total population of amino acid-acceptor RNA chains.

Because the amino acid acceptor activity of the RNA was only slowly inactivated by heating at 100°, another criterion for examining the effect of heating on the structure of the RNA was investigated. Prompted by the studies of Doty *et al.* (33), we examined the effect of heating on the optical density of the RNA (Fig. 5).[3] Commencing at about 40°, there is a hyperchromic shift at 260 mμ which attains a maximum at about

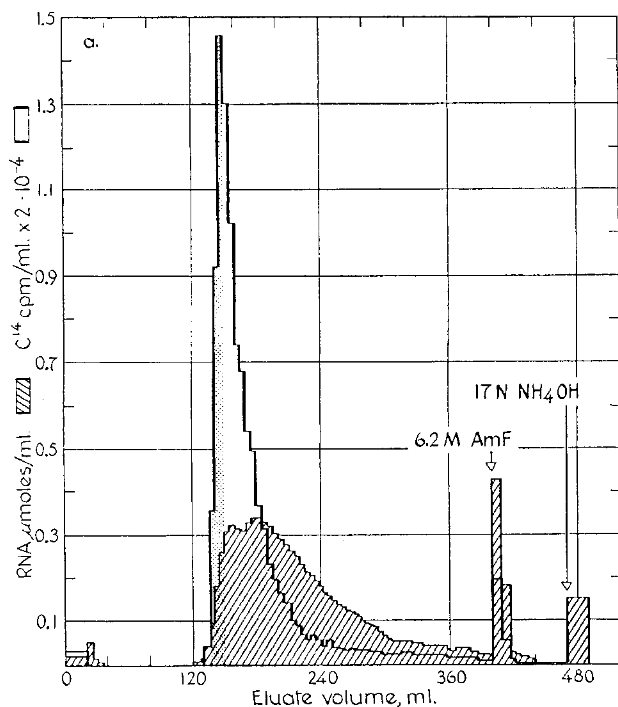

Fig. 6A.

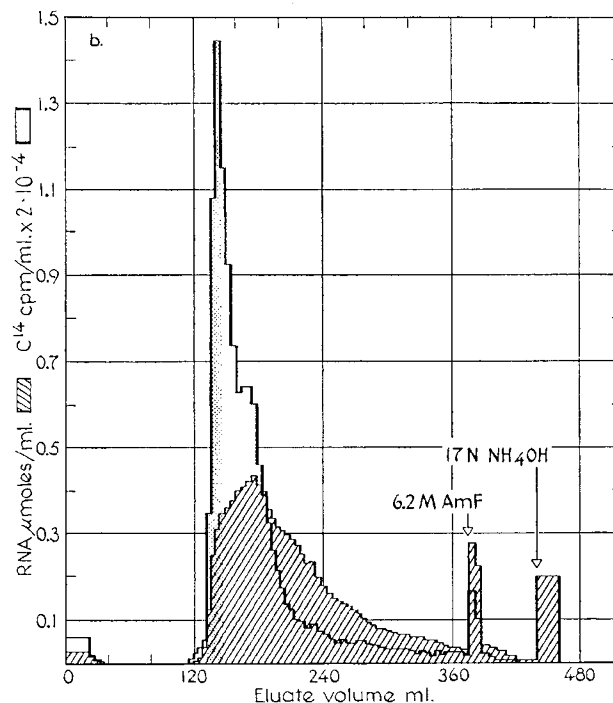

Fig. 6B

Fig. 6. Chromatography of C^{14}-valyl and C^{14}-leucyl RNA on Ecteola-cellulose. (*a*) C^{14}-valyl RNA containing 0.75 mμmole of valine per μmole of RNA nucleotide was prepared as already described (3). RNA nucleotide, 75 μmoles, was adsorbed to an Ecteola-cellulose column, (formate form, 10.3 × 1.1 cm^2). A linear buffer gradient was established with 200 ml of 1.47 M ammonium formate buffer, pH 4.7, in the reservoir and 200 ml of 0.65 M buffer in the mixing chamber. Fractions of about 5 ml were collected at 0.3 ml per minute and both the optical density at 260 mμ and the C^{14} content were determined. We recovered 88% of the C^{14} and 70% of the optical density. The fractions shown by the dotted portion of the main peak, which represented about 20% of the total radioactivity and had a specific activity of 3.7 mμmoles of valine per μmole of RNA nucleotide, were pooled, concentrated, and treated as described in Table III. (*b*) C^{14}-leucyl RNA nucleotide, 85 μmoles, containing 1.0 mμmoles of leucine per μmole of RNA nucleotide, was adsorbed to the same type of column mentioned above (10.6 cm × 1.1 cm^2). The gradient, rate of elution, fraction size, and method of analysis were as described above. We recovered 87% of the C^{14} and 68% of the optical density. The material taken for further study (dotted portion of the main peak) contained 19% of the total radioactivity and 3.8 mμmoles of leucine per μmole of RNA nucleotide.

[3] We express our gratitude to Dr. Charles Dekker, Department of Biochemistry, University of California, Berkeley, for allowing us to use his equipment.

Table III

Partial separation of leucine- and valine-specific acceptor RNA chains

The C^{14}-valyl and C^{14}-leucyl RNA were recovered from the pooled fractions indicated in Fig. 6, *a* and *b*, respectively, by alcohol precipitation. The amino acids were removed in the presence of the appropriate amino acyl RNA synthetase, PP, and AMP (3). By this procedure, 98% of the valine and 94% of the leucine were removed. This method produced no inactivation of leucine or valine acceptor sites when the original RNA was similarly treated. The RNA preparations were then assayed for their ability to accept the amino acids shown in the table with the standard assay (3).

Amino acid tested	Original RNA	RNA purified as:	
		Leucyl RNA	Valyl RNA
	mμmoles amino acid incorporated/μmole RNA nucleotide		
Leucine	0.95	3.43	1.33
Valine	0.56	0.85	3.50
Isoleucine	0.41	1.72	1.30
Methionine	0.26	1.58	1.50
Ratio of incorporation of leucine to valine	1.70	4.00	0.38

80°. The total increase in the optical density is about 23% and the midpoint of the transition is at 59°. On cooling, the original optical density is restored. According to Doty *et al.* (33), such temperature-induced hyperchromic shifts indicate the existence of a secondary structure resulting from hydrogen-bonded bases arranged in helical regions. The fact that the transition is reversible (in our experiments with respect to the ultraviolet absorption properties) makes it difficult to determine whether a specific structure is or is not required for biological activity. In an attempt to study this problem, we have determined the ability of the acceptor RNA to bind leucine and valine at elevated temperatures. With 20- to 50-fold excess of the leucyl- and valyl RNA synthetases (so that heat inactivation of the enzymes during the incubation does not effect the final yield of amino acyl RNA) and the conditions already described (3), the *yield* of leucyl- and valyl-RNA formed at 25, 30, 37, 47, and 55° was determined. Essentially no difference in the *yield* of leucyl- and valyl RNA (less than 10%) was observed up to and including 55°. These experiments show that at a temperature at which one-half the maximal hyperchromic shift occurs there is little or no difference in the amount of leucyl- or valyl-RNA formation. Although these results suggest that a specific secondary structure of the RNA is unessential for the enzymatic formation of a specific amino acyl RNA derivative, we can not eliminate the possibility that the enzymes react with that portion of the molecules with the native configuration and that the amino acyl RNA has a somewhat higher temperature transition range.

Sedimentation Coefficient of Amino Acid-Acceptor RNA—The sedimentation coefficient of the amino acid-acceptor RNA in 0.1 M sodium cacodylate buffer, pH 7.0, at concentrations of 0.068 and 10 μmoles of RNA nucleotide per ml (with ultraviolet and schlieren optics, respectively) was 3.9 and 3.8. Since at the time these experiments were performed the only published value available was 1.8 S for rat liver amino acid-acceptor RNA (1), it seemed possible that our values reflected the properties of the bulk of the RNA rather than the specific amino acid-acceptor component. Sedimentation of C^{14}-valyl RNA was therefore carried out with a separation cell (34) and the procedure described by Schachman (35). C^{14}-valyl RNA at a concentration of 2.2 μmoles of nucleotide per ml, in 0.01 M potassium acetate buffer at pH 4.4, containing 0.2 M NaCl, was centrifuged so that in one run approximately 50% and in another run approximately 68% of the RNA remained in the upper compartment of the separation cell. The average $s_{20,w}$ of the radioactivity was 4.5 ± 0.2, whereas that of the ultraviolet and schlieren boundary was 4.2 ± 0.6 and 3.7 ± 0.1, respectively. It is therefore clear that the component to which the valine is bound sediments with the bulk of the ultraviolet-absorbing component. The somewhat lower $s_{20,w}$ values obtained from the schlieren boundaries may be due to the difficulty in estimating the movement of the small boundary resulting from the low concentration of valyl RNA used.

Attempt to Isolate Amino Acid-specific RNA Chains—Evidence presented in the previous paper (3) led to the conclusion that amino acid-acceptor RNA is composed of a population of polynucleotide chains each specific for a particular amino acid. This hypothesis was based on the finding that each amino acid was bound to a specific site and that these sites were the terminal nucleotides of each RNA chain. Further support for this idea was achieved by a partial physical separation of the RNA chains specific for leucine from those specific for valine.

In separate experiments, C^{14}-valyl and C^{14}-leucyl RNA were chromatographed on Ecteola-cellulose columns as shown in Fig. 6*a* and *b*. The leading fractions of each peak (dotted portion of each peak) which possessed the highest specific activity and accounted for approximately 20% of the radioactivity applied to the column were pooled and the RNA was recovered by alcohol precipitation. The leucine-C^{14} or valine-C^{14} was removed and the RNA was tested for its capacity to accept a number of amino acids (Table III). The data show that the RNA preparations selected for their acceptor activities for valine or leucine have about 7 and 3.5 times higher activity for accepting these amino acids than does the original RNA fraction. More significantly, however, the marked alteration in the ratio of leucine to valine acceptor activity in the isolated fractions suggests that the chromotography resulted in a separation of the leucine- and valine-specific RNA chains. It should be pointed out that these same fractions are also enriched about 3- to 4-fold for isoleucine and methionine acceptor activity. We interpret these results as indicating that although the RNA chains specific for leucine and valine normally chromatograph towards the center or trailing portion of the peak, the addition of the amino acid changes their chromatographic properties so that they are eluted somewhat earlier and thus overlap the fractions specific for isoleucine and methionine.

Although these experiments suggest that a partial physical separation of the leucine and valine acceptor RNA activity can be achieved, they also demonstrate the limited usefulness of this approach for isolating an RNA that is specific for a *single* amino acid. Separation of acceptor activities for different amino acids by chromatography have also been reported by Smith *et al.* (36). More recent studies by Holley *et al.* (37) with countercurrent distributions have demonstrated the separation of certain specific amino acid acceptor activities. Our own and these latter studies (36, 37), however, suffer from the difficulty of

attempting to separate one specific type of RNA chain from perhaps as many as 20 others with very similar physical properties. A more effective approach would appear to be that exploited by Brown *et al.* (38) and by Zamecnik *et al.* (39). In these two cases the separation depends on selective adsorption of a specific amino acyl RNA (38) or on the alteration of all but a single class of amino acid-specific RNA chains (39).

SUMMARY

Amino acid-acceptor ribonucleic acids have been isolated from *Escherichia coli* by extraction of dried cells with sodium lauryl sulfate, fractionation with salt and ethanol precipitation, and chromatography on Ecteola-cellulose.

Alkaline hydrolysis of the acceptor ribonucleic acid yields a single nucleoside, adenosine, and roughly equivalent amounts of adenylic and uridylic acids and of guanylic and cytidylic acids. The minimal molecular weight, based on the nucleotide composition and on the assumption of a single terminal nucleotide unesterified in the 2'- and 3'-hydroxyl groups, is 31,000 ± 5%.

The optical density at 260 mμ of the ribonucleic acid in 0.2 M NaCl, pH 7.0, increases (about 23%) as the temperature is raised between 40 and 80° and returns to the original value on cooling. When the ribonucleic acid is tested for its ability to accept leucine and valine at temperatures up to 55°, no difference is found in the yield of leucyl- and valyl ribonucleic acid suggesting that a specific secondary structure may be necessary for the amino acyl ribonucleic acid synthetases.

The $s_{20,w}$ for valylribonucleic acids is 4.5 ± 0.2 S which is essentially the same as the sedimentation coefficient of the major portion of the ultraviolet-absorbing material of the ribonucleic acid.

Attempts to isolate ribonucleic acid chains specific for leucine or valine resulted in partial resolution of the two types of ribonucleic acid chains, although there was little or no separation of either of these from acceptor ribonucleic acid chains specific for isoleucine or methionine.

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