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Incorporation of Dicarboxylic Amino Acids into Soluble Ribonucleic Acid

Norman Coles, Martha W. Bukenberger, and Alton Meister

From the Department of Biochemistry, Tufts University School of Medicine, Boston

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Although it is known that many amino acids are incorporated into soluble ribonucleic acid to yield amino acyl RNA derivatives, little information is available concerning the dicarboxylic amino acids. In the present work, enzymatic formation of glutamyl RNA and aspartyl RNA was observed, and experiments were carried out to determine which of the two carboxyl groups of these amino acids were linked to RNA, or whether both were involved. Although an α-glutamyl-RNA linkage would be consistent with results obtained with other amino acids, the finding of γ-glutamyl RNA might be of significance in relation to the function of widely distributed enzymes that catalyze γ-glutamyl transfer. Enzyme preparations from baker's yeast and rat liver were incubated with soluble RNA, ATP, Mg^{++}, and C^{14}-glutamic or aspartic acids. The C^{14}-amino acyl RNA derivatives obtained were treated with ammonia under several sets of conditions and the radioactive products found after ammonolysis were examined. Aspartyl RNA gave mainly isoasparagine and small amounts of aspartic acid, but no asparagine. Ammonolysis of glutamyl RNA gave isoglutamine and small amounts of glutamic acid; neither glutamine nor pyrrolidone carboxylic acid was formed. Similar results were obtained on ammonolysis of ribonuclease-treated amino acyl RNA preparations. The evidence indicates that α-aspartyl RNA and α-glutamyl RNA were formed by the enzymes employed here. The rates of hydrolysis of aspartyl RNA and glutamyl RNA were determined at 37° at *p*H values between 6.65 and 8.65. At *p*H 7.25, these derivatives, glycyl RNA, and arginyl RNA were hydrolyzed at similar rates, while valyl RNA exhibited significantly greater stability.

It is now generally believed that amino acyl RNA derivatives formed by amino acid–activating enzymes in the presence of soluble RNA, ATP, and amino acids are involved in the incorporation of amino acids into proteins and probably in the synthesis of proteins. Amino acyl RNA formation has been observed in systems containing activating enzymes and soluble RNA (Holley, 1957; Berg and Ofengand, 1958; Hoagland *et al.*, 1958; Weiss *et al.*, 1958; Schweet *et al.*, 1958), and studies in this laboratory have provided evidence that the amino acyl moiety of synthetic tryptophanyl adenylate is specifically transferred by an activating enzyme to RNA (Wong *et al.*, 1959; Wong and Moldave, 1960). The available data are consistent with the belief that the amino acyl moieties are attached to terminal adenosine residues of soluble RNA, and that they are probably linked to a 2′ or 3′ hydroxyl group; isolation of amino acyl adenosine after treatment of amino acyl RNA with ribonuclease has been reported in studies with leucyl RNA and valyl RNA (Zachau *et al.*, 1958, Preiss *et al.*, 1958).

Although a number of reports on the formation and reactions of amino acyl RNA compounds have appeared, relatively little information is available

concerning the dicarboxylic amino acids. Prior to the present studies, incorporation of radioactivity from C^{14}-glutamate into soluble RNA had been reported by Fraser *et al.* (1959), and similar observations were made by Coles (1960). Although an α-glutamyl–RNA linkage would be consistent with observations made on other amino acids, the finding of γ-glutamyl RNA might be of significance in relation to the function of the widely distributed enzymes that catalyze γ-glutamyl transfer (Hanes *et al.*, 1950, 1952; Waelsch, 1951, 1952).

Experimental

DL-Aspartic acid-4-C^{14} (3.2 μc/μmole), DL-glutamic acid-1-C^{14} (3 μc/μmole), L-arginine-HCl-U-C^{14} (2.5 μc/μmole), L-valine-1-C^{14} (6.9 μc/μmole), and glycine-1-C^{14} (17.5 μc/μmole) were purchased from New England Nuclear Corporation. ATP was obtained from the Sigma Chemical Company, and crystalline ribonuclease was obtained from Worthington Biochemical Company. Soluble RNA was isolated from baker's yeast, essentially as described by Monier *et al.* (1960), except that the treatment with charcoal was omitted, and the RNA was incubated at 37° for 30 minutes at *p*H 10 before treatment with 2-methoxyethanol.

Preparation of Yeast Enzyme.—Pressed baker's yeast (60 g) was homogenized in 60 ml of the medium of Chao and Schachman (1956) containing 100 mg of glutathione and 2 drops of octyl alcohol. Homogenization was carried out in the presence of 120 ml of glass beads (Minnesota Mining and Manufacturing Company; type 120-5005) for 10 minutes at maximum speed with a Servall Omnimixer. The metal bowl was immersed in crushed ice during homogenization. The subsequent steps in the preparation of the enzyme were carried out according to Monier *et al.* (1960). The final enzyme preparation contained 4–6 mg of protein per ml. Only freshly prepared enzyme was active with aspartate; however, the activity toward glutamate was stable for several weeks when the enzyme was stored at −10°.

Preparation of Glutamyl and Aspartyl RNA with the Yeast Enzyme.—The incorporation of C^{14}-amino acid into RNA was determined in reaction mixtures consisting of C^{14}-glutamate (0.084 μmole; 250,000 cpm) or C^{14}-aspartate (0.078 μmole; 250,000 cpm), ATP (10 μmoles), $MgCl_2$ (10 μmoles), RNA (0.2 mg), enzyme preparation (0.1 ml), and 2 - amino - 2(hydroxymethyl) - 1,3 - propanediol–HCl buffer (50 μmoles; *p*H 7.6) in a final volume of 1 ml. After incubation of the mixture at 37° for 20 minutes, the reaction was stopped by the addition of 5 ml of ice-cold 5% trichloroacetic acid. The precipitate was collected on a Millipore Filter (Millipore Filter Corporation; Bedford, Mass.; type DA, 25 mm in diameter, pore size 0.65 μ). After washing with 5% trichloroacetic acid (3 washes of 5 ml each), the filter was attached to a stainless steel planchet with rubber cement and then dried and the radioactivity counted in an automatic gas flow counter. The values were corrected for background. In preliminary studies it was established that incorporation of glutamate and aspartate was dependent upon the presence of ATP, Mg^{++}, and RNA; all of the incorporated radioactivity was solubilized by treatment of the precipitate with alkali (*p*H 10) or with ribonuclease. Under these conditions, 250 and 450 cpm of C^{14}-glutamate and C^{14}-aspartate, respectively, were incorporated. The corresponding control and "zero time" values were less than 10 cpm.

Larger volumes of the reaction mixture given above were used for subsequent experiments. In these, the RNA concentration was increased to 2–3 mg per ml, which resulted in a several-fold increase in incorporation (per ml). The large-scale reactions were stopped by drop-wise addition of 1.6 M acetic acid; when the solutions became turbid, 2.5 volumes of cold ethanol were added and the mixture was allowed to stand at −10° for at least 4 hours. The precipitate was collected by centrifugation and washed once with a solution containing 75 volumes of ethanol and 25 volumes of 0.1 M potassium acetate buffer (*p*H 5.0). The precipitate was suspended in 0.1 M ammonium acetate (*p*H 5.5) and dialyzed for 18 hours against 3 liters of the same buffer at 4°. After additional dialysis against distilled water for several hours, insoluble material was removed by centrifugation. The supernatant solution, which contained the amino acyl RNA, was lyophilized. Valyl, glycyl, and arginyl RNA preparations were also obtained by this procedure.

Preparation of the Rat Liver Enzyme.—Fresh rat liver (60 g) was homogenized at 0° in a glass homogenizer with 4 volumes of a solution containing 0.35 M sucrose, 0.035 M $KHCO_3$, 0.025 M KCl, 0.004 M $MgCl_2$, and 0.02 M K_2HPO_4 (adjusted to *p*H 7.2 with HCl) for 2 minutes, and then centrifuged at 10,000 × *g* for 15 minutes at 0°. The supernatant solution was centrifuged for 2 hours at 100,000 × *g*, and this supernatant solution was adjusted to *p*H 5.2 by cautious addition of M acetic acid. The precipitated material was collected by centrifugation and dissolved in 40 ml of 0.1 M 2-amino-2-(hydroxymethyl)-1,3-propanediol–HCl buffer (*p*H 7.6). The final preparation contained 10–20 mg of protein per ml.

Preparation of Glutamyl RNA and Aspartyl RNA with the Rat Liver Enzyme.—Incorporation of C^{14}-amino acid into RNA was determined in pilot experiments with reaction mixtures consisting of C^{14}-amino acid (0.25 μmole; 500,000 cpm), ATP (5 μmoles), $MgCl_2$ (5 μmoles), RNA (2.5 mg), enzyme preparation (2–5 mg of protein), and 2-amino - 2 - (hydroxymethyl) - 1,3 - propanediol–HCl buffer (*p*H 7.6; 50 μmoles) in a final volume of 0.6 ml. Controls were carried out in which ATP, RNA, and enzyme were separately omitted; a "zero time" control and an experiment in which enzyme was inactivated by heating at 100° for 5 minutes were also performed. After incubation of the reaction mixture for 30 minutes at 37°, 2.4 ml of cold 0.4 M perchloric acid was added. The

precipitated material was washed by centrifugation four times with 5 ml of 0.4 M perchloric acid, and then transferred to a planchet with ethanol and the radioactivity counted. The values were corrected for background and self-absorption. Under these conditions, 500–1100 cpm were found in the precipitate in various experiments; the control values were lower than 50 cpm. All of the incorporated radioactivity was rendered soluble by incubation of the precipitates for 15 minutes at 37° at *p*H 10. Larger volumes (6–18 ml) of such reaction mixtures were employed in subsequent experiments; the precipitates were washed twice with cold water to remove some of the adherent perchloric acid. In several experiments, the RNA was isolated from the reaction mixtures by the phenol extraction procedure of Kirby (1958).

Ammonolysis.—Amino acyl RNA preparations were treated with a large excess of ammonia under several sets of conditions. The following procedures were used with 0.1–0.5 ml volumes of solutions or suspensions containing 10–100 mg of RNA. (*a*) The sample was treated with 7 ml of an aqueous solution previously saturated with ammonia gas at 0°. After standing for 90 minutes at 0°, the solution was evaporated to dryness with a stream of air. (*b*) The sample was treated with 7 ml of 50% aqueous methanol previously saturated at 0° with ammonia gas. After standing for 90 minutes at 0°, the solution was evaporated to dryness. (*c*) The sample was treated with 5 ml of absolute methanol previously saturated at 0° with ammonia gas. After standing for 3 hours at 26°, the solution was evaporated to dryness. (*d*) The sample was treated with 8 ml of a solution prepared by treating methanol at −75° with ammonia gas until the initial volume of the solution had doubled. The temperature of the mixture was allowed to rise gradually from −75° to 26°; after 18 hours, the solution was evaporated. (*e*) The sample was treated with 8 ml of liquid ammonia at −35°. The material was placed at 26° until the ammonia evaporated. (*f*) The sample was treated with 5 ml of N ammonium hydroxide at 26°; after standing at 26° for 3 hours, the solution was evaporated to dryness.

After ammonolysis and evaporation of the solution, 0.2 ml of water was added to the residual material and the solutions obtained were used for determinations of amino acids and amino acid amides as described below. In the experiments with the liver enzyme, there was much insoluble material in the residue obtained after ammonolysis. In these studies, the insoluble material was thoroughly extracted with water and the extracts were combined and evaporated to low volume. In some experiments, the residues obtained after ammonolysis were suspended in a small amount of water and dialyzed at 4° against several changes of water; the dialysates were concentrated and passed through a column of Dowex-50 (H^+ form). Under these conditions, all of the radioactivity was adsorbed by the column; it was subsequently eluted with 5 N ammonium hydroxide. The eluates were evaporated to dryness and employed for analysis.

From 80 to 90% of the radioactivity initially incorporated into the RNA was recovered in the final solution after ammonolysis. A loss of 10 to 20% of the initial radioactivity is not unexpected in view of the number of manipulations involved. As stated above, all of the incorporated radioactivity was rendered soluble by treatment with alkali; thus, significant incorporation into protein did not occur. The labeled aminoacyl RNA preparations obtained with the yeast enzyme contained little if any protein, since most of the enzyme was precipitated and was removed after dialysis. In several of the experiments with the liver enzyme, the labeled RNA was isolated by the phenol extraction procedure of Kirby (1958); similar results were obtained when ammonolysis was carried out directly on the washed perchloric acid precipitate (containing both protein and RNA).

Paper Electrophoresis.—Paper electrophoresis was carried out with an apparatus similar to that of Markham and Smith (1952) on strips (2.5 × 50 cm) of Whatman No. 3 MM paper. Authentic samples of unlabeled aspartic acid, asparagine, and isoasparagine, or of glutamic acid, glutamine, and isoglutamine, were added to the sample. After electrophoresis, the amino acid bands were located by treatment of the dried strips with ninhydrin; the strips were then cut into 1-cm sections which were counted in an automatic gas flow counter. Aspartic acid, asparagine, and isoasparagine were separated by electrophoresis (40 v/cm) for 2 hours in 0.05 M potassium phthalate buffer of *p*H 3.0; these amino acids moved 8–11, 14–17, and 25–29 cm, respectively, from the origin (in the direction of the cathode) under these conditions. Glutamic acid, isoglutamine, and glutamine were separated by electrophoresis (40 v/cm) for 90 minutes in a 0.05 M sodium diethylbarbiturate buffer of *p*H 8.5; the respective mobilities (in the direction of the anode) were 20–21, 15–17, and 2–7 cm.

Preparation of 2,4-Dinitrophenyl Amino Acids.—In several experiments, aliquots of the residue obtained after ammonolysis were treated with 0.4 ml of 1-fluoro-2,4-dinitrobenzene (5% in 95% ethanol) and sodium bicarbonate (5 mg). After being shaken in the dark for 2 hours the mixture was extracted three times with ether and then acidified with hydrochloric acid. After five additional extractions of the acidified solution with ether, the ethereal extracts were combined and evaporated to dryness. The residue was dissolved in a small volume of ethanol and applied to paper, and chromatography was carried out with a solvent consisting of cresol, 0.1 M sodium borate (*p*H 9.2), and phenol (50:50:7). The R_F values for the DNP-derivatives of glutamic acid, glutamine, and isoglutamine were, respectively, 0.38, 0.50, and 0.75. The DNP-derivatives of aspartic acid, asparagine, and isoasparagine were separated by paper electrophoresis (40 v/cm) for 2 hours in 0.05 M potassium phthalate buffer of *p*H 4.0; the re-

spective mobilities (distance from origin) under these conditions were 15–16, 12–13, and 9–10 cm.

Products of Ammonolysis of Glutamyl RNA and Aspartyl RNA Preparations.—The only radioactive products detected after ammonolysis of aspartyl RNA prepared with the yeast enzyme were isoasparagine and aspartate. As indicated in Table I, ammonolysis by procedures (*a*) to (*e*) resulted in the formation of considerable quantities of isoasparagine. From 88 to 100% of the recovered radioactivity was associated with this product. The remainder of the radioactivity was accounted for as aspartic acid. Procedure (*f*) gave only aspartic acid. Isoasparagine was cleanly separated from asparagine in these studies, and it may therefore be concluded that no asparagine was formed. The identity of the radioactive isoasparagine was confirmed by two-dimensional paper chromatography, using in one direction *tert*-butanol–methyl ethyl ketone–concentrated NH_4OH–water (50:50:15:25), and in the other *sec*-butanol–90% formic acid–water (100:15:25), followed by radioautography. Further confirmation was obtained by conversion of the products of ammonolysis by procedure (*d*) to the corresponding DNP derivatives; all of the radioactivity moved on electrophoresis with authentic DNP-isoasparagine. Similar results were obtained with aspartyl RNA prepared with the rat liver enzyme. Thus, in three separate experiments approximately 80% of the recovered radioactivity was associated with isoasparagine and no asparagine was found. The isoasparagine formed in the experiments with yeast and liver enzymes was hydrolyzed with 2 N hydrochloric acid for 2 hours at 100° and then resubjected to electrophoresis; all of the radioactivity was found in the aspartic acid area.

TABLE I

AMMONOLYSIS OF C^{14}-ASPARTYL-RNA

Conditions of Ammonolysis[a]	Radioactivity Recovered After Ammonolysis and Electrophoresis: Aspartic Acid (cpm)	Isoasparagine cpm	Isoasparagine % of Total	Total cpm
a; yeast enzyme	80	780	91	860
b; yeast enzyme	120	920	88	1040
c; yeast enzyme	0	540	100	540
d; yeast enzyme	0	650	100	650
e; yeast enzyme	20	610	97	630
f; yeast enzyme	590	0	0	590
d; liver enzyme	520	2380	82	2900
d; liver enzyme	120	480	80	600
d; liver enzyme	130	620	83	750

[a] See the text.

Table II summarizes the experiments carried out with glutamyl RNA prepared with the liver and yeast enzymes. The major radioactive product in these experiments was isoglutamine. After elution of this material from the paper strips and hydrolysis in 2 N hydrochloric acid at 100° for 2 hours, 95% of the original radioactivity was recovered as radioactive glutamic acid. In one experiment a small amount of radioactivity (95 cpm) was found in the "glutamine area"; however, hydrolysis of this material with hydrochloric acid followed by electrophoresis did not give detectable quantities of radioactive glutamic acid, and about 50% of the original radioactivity was again found in the "glutamine area." Under the conditions of electrophoresis employed, a number of monoaminomonocarboxylic acids would be expected to exhibit mobility similar to that of glutamine. The small amount of radioactivity found in the "glutamine area" may probably be ascribed to the presence of a substance or substances other than glutamine.

TABLE II

AMMONOLYSIS OF C^{14}-GLUTAMYL-RNA[a]

Source of Enzyme	Radioactivity Recovered After Ammonolysis and Electrophoresis: Glutamic Acid (cpm)	Isoglutamine cpm	Isoglutamine % of Total	Total cpm
Liver	740	2230	73	3065[b]
Liver	50	825	94	875
Yeast	260	3330	93	3590

[a] Ammonolysis by procedure (*d*); see text. [b] Includes 95 cpm associated with unidentified material (see text).

In the experiments with glutamic acid, paper chromatography was also carried out as described by Krishnaswamy *et al.* (1960) on the material obtained after ammonolysis in an attempt to detect the formation of pyrrolidone carboxylate. In these experiments, the C^{14}-glutamic acid employed contained less than 0.15% of pyrrolidone carboxylic acid. The chromatographic studies revealed no more than traces of pyrrolidone carboxylate equivalent in amount to those found in control experiments. Similar results were obtained when the supernatant solutions obtained during the washing of the RNA precipitates were examined.

Treatment of Aspartyl RNA and Glutamyl RNA Derivatives with Ribonuclease.—C^{14}-Aspartyl RNA (140 mg; 42,000 cpm) was dissolved in water and treated with 0.7 mg of crystalline pancreatic ribonuclease (final volume 0.7 ml) for 10 minutes at 26°. The entire digest was then applied to a 1 × 28 cm section of a sheet of Whatman No. 3 MM paper, and electrophoresis was carried out at 35 v/cm for 3 hours with 0.05 M ammonium acetate buffer (*p*H 3.2). After electrophoresis, radioautography revealed a narrow radioactive band 4 cm from the origin in the direction of the cathode. This band contained 38,600 cpm or 92% of the original radioactivity. A portion of the strip was eluted with 0.1 N potassium hydroxide; the eluate contained 3.58 μmoles of adenosine (based on absorbancy at 260 mμ) and 2.75 mμmoles of C^{14}-aspartic acid. These findings are consistent with incomplete separation of an aspartyl-nucleoside from other nucleoside- and nucleotide-containing material. Treatment of a portion of the paper strip containing 1,000 cpm with ammonia by procedure (*d*), followed by paper electrophoresis of the products, gave isoasparagine (860 cpm) and aspartic acid (50 cpm); no asparagine was detected. The identity of isoasparagine was confirmed by two-dimensional paper chromatography and radioautography.

Glutamyl RNA was treated with ribonculease, and electrophoresis was carried out as described for aspartyl RNA. All of the radioactivity was found in a band which corresponded closely to that observed with the aspartyl-RNA. After ammonolysis, only isoglutamine was found; neither glutamic acid nor glutamine was detected.

Hydrolysis of Amino Acyl RNA Derivatives.— The aspartyl and glutamyl RNA derivatives appear to be relatively stable in acid solution at low temperature. They undergo hydrolysis at significant rates at 37° and values of *p*H from 6.65 to 8.65 (Table III). Within experimental error the hydrolyses follow first-order kinetics. The velocity of hydrolysis increases very rapidly with increase of *p*H. The rates of hydrolysis of aspartyl RNA and glutamyl RNA are not markedly different from those of the corresponding glycyl and arginyl derivatives; on the other hand, the glycyl, arginyl, glutamyl, and aspartyl derivatives were hydrolyzed much more rapidly than that of valine (Fig. 1).

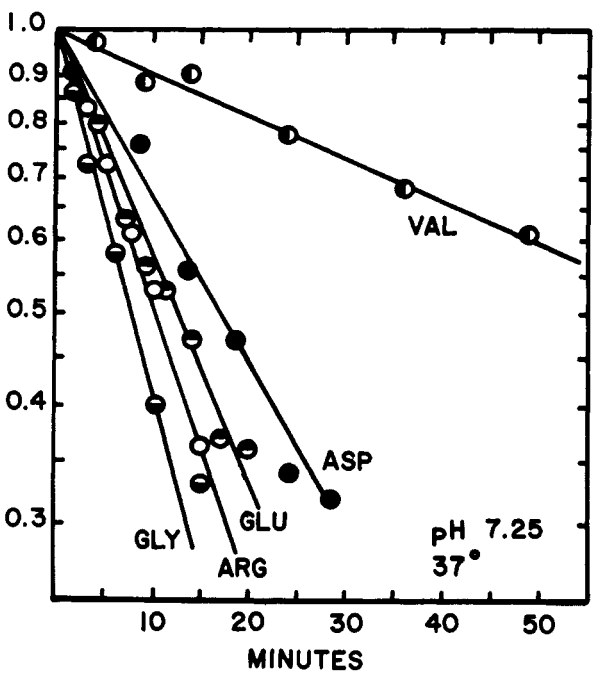

FIG. 1.—Hydrolysis of several amino acyl RNA derivatives at *p*H 7.25 and 37°; the experiments were carried out as described in Table III; the ordinate scale is logarithmic.

TABLE III

HYDROLYSIS OF ASPARTYL RNA AND GLUTAMYL RNA AT SEVERAL VALUES OF *p*H AT 37°

	Aspartyl-RNA		Glutamyl-RNA	
*p*H	k (min.$^{-1}$)	$t_{1/2}$ (min.)	k (min.$^{-1}$)	$t_{1/2}$ (min.)
6.65	0.0129	54	0.0168	41
6.95	0.0255	27	0.0414	17
7.25	0.0410	17	0.0555	12.5
7.65	0.130	5.3	0.135	5.1
8.15	0.272	2.5	0.460	1.5
8.65	1.10	0.63	1.31	0.53

Aliquots of reaction mixtures consisting initially of C^{14}-aspartyl or glutamyl-RNA (equivalent to 1000–1300 cpm) in 6 ml of 0.1 M 2-amino-2-(hydroxymethyl)-1,3-propanediol-HCl buffer were removed at various time intervals and treated with 10 volumes of ice-cold 5% trichloroacetic acid; the amino acyl RNA was collected on a Millipore filter and counted as described in the text. The first-order rate constants (k) and half-life values were calculated from values obtained for the first two-thirds of the reaction. The *p*H values were determined at 37°.

DISCUSSION

The present studies show that yeast and liver preparations can catalyze the formation of aspartyl RNA and glutamyl RNA, and that the corresponding amino acid α-amides are formed after treatment of the acyl RNA derivatives with ammonia. No asparagine was found in the experiments with aspartic acid, and neither glutamine nor pyrrolidone carboxylic acid was found in the studies with glutamic acid. The evidence therefore indicates that the α-carboxyl groups of these amino acids rather than the ω-carboxyl groups are involved in the linkage to RNA. It is known that reaction of *N*-carbobenzoxy-γ-glutamyl azide (and of the analogous β-aspartyl derivative) with amino acid esters under certain conditions gives both α- and ω-peptide products (Sachs and Brand, 1953; Otani and Meister, 1957). The possibility that β-aspartyl RNA or γ-glutamyl RNA might participate in rearrangement reactions on ammonolysis to yield the corresponding α-amides cannot be unequivocally excluded. However, we were able to account for virtually all of the incorporated radioactivity as the α-amide and the corresponding dicarboxylic amino acid, whereas in the cited studies with the *N*-carbobenzoxy-ω-dicarboxylic amino acyl azides, both α- and ω-derivatives were formed. It is pertinent to note that ammonolysis of the β-methyl ester of aspartic acid gives only asparagine (Beecham, 1954). It appears probable that γ-glutamyl RNA, like the γ-ethyl and γ-methyl esters of glutamic acid, would yield pyrrolidone carboxylic acid on ammonolysis (Bergmann and Zervas, 1933; Coleman, 1951; Beecham, 1954), rather than glutamine. For this reason we looked for, but failed to find, pyrrolidone carboxylic acid in the experiments with glutamic acid. Glutamine might be formed during ammonolysis of glutaminyl RNA either by hydrolysis of the acyl RNA itself or by hydrolysis of α-aminoglutarimide, which is known to be formed on treatment of glutamine esters with alkali (Sondheimer and Holley, 1954, 1957). Bates and Lipmann (1960) reported that ammonolysis of soluble amino acyl RNA followed by treatment with 1-fluoro-2,4-dinitrobenzene gave DNP-glutamine, and they concluded from this observation that γ-glutamyl RNA was present in the original preparation; this reasoning is contrary to that given above.

Although the present studies provide no evidence for the synthesis of γ-glutamyl RNA by the enzyme preparations employed here, the existence of an enzyme capable of catalyzing the synthesis of this compound cannot be excluded. Studies in this laboratory have shown that the purified enzyme from sheep brain that catalyzes glutamine synthesis

(Pamiljans *et al.*, 1962) does not catalyze synthesis of glutamyl or glutaminyl RNA.

We found that glutamyl RNA and aspartyl RNA are considerably less stable to hydrolysis than valyl RNA, and that they exhibit approximately the same stability as arginyl RNA and glycyl RNA. Whether valyl RNA or the other derivatives studied here are more typical of the amino acyl RNA derivatives not yet examined can be decided only by experiment. It is of interest that, in general, peptide bonds adjacent to valyl (or leucyl) residues are more stable to hydrolysis than those adjacent to other amino acyl residues (Greenstein and Winitz, 1961).

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