Chemical Studies on Amino Acid Acceptor Ribonucleic Acids. VI. Partial Digestion of Alanine Soluble Ribonucleic Acid from Yeast with Pancreatic Ribonuclease*

Edward K. Wagner† and Vernon M. Ingram

ABSTRACT: Purified yeast alanine soluble ribonucleic acid (s-RNA) and unfractionated yeast s-RNA were partially degraded with pancreatic RNAase at 0° in the presence of Mg^{2+}. Under these conditions the s-RNA molecule is broken into large pieces approximately half molecules in size and some short oligonucleotides. Analysis of the oligonucleotides and half molecules produced by such partial digestion leads us to conclude that one-half of the alanine s-RNA molecule is more resistant to attack than the other and that this may be a general feature of yeast and *Escherichia coli* B s-RNA.

The ability of a specific soluble ribonucleic acid (s-RNA) molecule to act as an amino acid "adaptor" will depend on its nucleotide sequence and on its secondary and tertiary structure. Now that the primary structure of one s-RNA, yeast alanine s-RNA, is known (Holley *et al.*, 1965), experiments designed to probe the conformation of alanine s-RNA can be interpreted more rationally. Although there is general agreement that a typical s-RNA molecule shows a high degree of secondary and tertiary structure, the nature of the structural elements is not settled. Evidence for such definite conformations has usually been interpreted in terms of regions of a DNA-like double-helical structure with specific hydrogen-bonded base pairing of the Watson–Crick type; but different types of interaction between bases and hydrogen bonding between other base pairs are possible and might well occur in the relatively short s-RNA molecule with its high proportion of unusual bases.

On the basis of hydrogen exchange studies, Englander and Englander (1965) have concluded that about 80% of the nucleotides in a typical s-RNA molecule are hydrogen bonded. Thermal denaturation studies (Fresco *et al.*, 1963; Felsenfeld and Cantoni, 1964) have led to the conclusion that as much as 75% of the bases in unfractionated s-RNA could be paired in hydrogen-bonded double helices of the deoxyribonucleic acid (DNA) type.

Earlier work from this laboratory (Litt and Ingram, 1964) showed that the conformation of yeast s-RNA could be protected by Mg^{2+} so that attack by pancreatic ribonuclease at 0° left large segments of the molecule intact, while digesting other regions. It was concluded that these large segments were derived from "helical" regions of s-RNA. Purified alanine s-RNA showed similar behavior. This technique of partial enzymatic digestion, but with ribonuclease T_1, was used by Holley and his colleagues (Penswick and Holley, 1965; Holley *et al.*, 1965) to obtain overlap sequences from alanine s-RNA.

Keselev *et al.* (1964) studied the rate of liberation of acid-soluble nucleotides from mixed yeast s-RNA by attack with pancreatic ribonuclease at 20° in the absence of Mg^{2+}; they concluded that about one-quarter of the mixed s-RNA "molecule" was completely free of hydrogen bonding, about one-half was fully hydrogen bonded and resistant to attack, and the remainder was "imperfectly" hydrogen bonded and slowly liberated by action of the enzyme.

This paper describes studies on the products of partial pancreatic RNAase digestion of unfractionated s-RNA and of alanine s-RNA, both from yeast. The following paper deals with parallel experiments using ribonuclease T_1 and also valine s-RNA. We have related our findings to the nucleotide sequence of alanine s-RNA (Holley *et al.*, 1965) and can propose a model for the secondary structure of this molecule. Studies on unfractionated yeast and *Escherichia coli* B s-RNA show similarities in conformation.

Materials and Methods

s-RNA. Yeast and *E. coli* B soluble RNA were obtained from the General Biochemical Corp. (Chagrin Falls, Ohio). Alanine-specific s-RNA was purified from the commercial yeast s-RNA by two countercurrent distributions (Apgar *et al.*, 1962; Armstrong *et al.*, 1964).

It should be noted that in our experience there is no indication of more than one alanine-specific s-RNA in our yeast s-RNA, even after subjecting the material from the first countercurrent distribution to a second distribution. The findings of Apgar *et al.* (1962) are

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† Predoctoral Fellow of the National Institutes of Health.

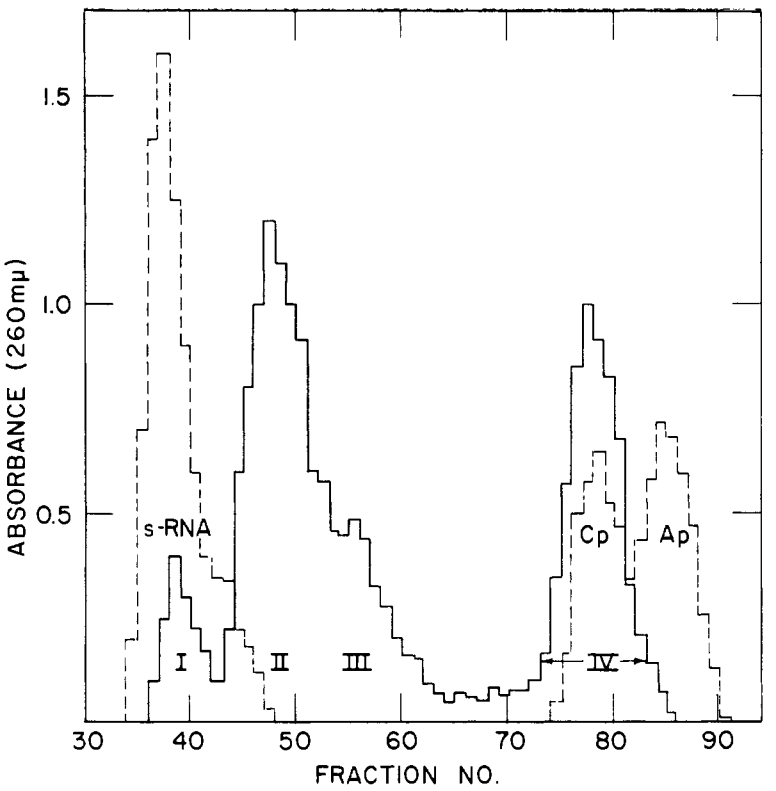

FIGURE 1: Fractionation of a typical partial digestion of mixed yeast s-RNA on Sephadex G-75. Yeast s-RNA (2 mg) were treated with 20 μg of pancreatic ribonuclease for 1 hr at 0° (see Methods). The sample was loaded on a 0.9 × 160 cm G-75 Sephadex column and eluted at 10 ml/hr with 0.1 M ammonium acetate (———). After the digest was eluted the column was rinsed with buffer for 16 hr, and a mixture containing 0.25 mg of unfractionated yeast s-RNA and 0.1 mg each of Ap and Cp was loaded on the column and eluted (- - - -).

in agreement with ours. The chemical purity of the alanine s-RNA used in the present experiments rests on the close to theoretical yields of oligonucleotides obtained by pancreatic ribonuclease (Armstrong *et al.*, 1964) as well as the high yield of oligonucleotides obtained with ribonuclease T_1 (V. M. Ingram, A. Armstrong, and H. Hagopian, in preparation). The highest alanine acceptor activity found in our preparations was 0.8 mole of alanine/mole of s-RNA. We regard our yeast alanine s-RNA to be identical with Holley's, because the same fractionation procedure was employed in its isolation and because the same two sets of highly characteristic oligonucleotides were obtained with pancreatic and T_1 ribonucleases.

Bentonite. The commercial product was treated according to Fraenkel-Conrat *et al.* (1961).

Chelex 100. Chelex 100, 50–100 mesh, was obtained as the Na^+ form from the California Corp. for Biochemical Research. It was washed with 2 N hydrochloric acid, 2 N ammonium hydroxide, and water before use.

Partial Pancreatic RNAase Digests of s-RNA. The digestion conditions used were identical with those described by Litt and Ingram (1964) except that the amount of enzyme used was lowered to 1 μg/mg of RNA in some cases. Bentonite was removed by centrifugation at 40,000 rpm for 1–1.5 hr in a Spinco Model L ultracentrifuge (Spinco Division, Beckman Instrument Corp., Palo Alto, Calif.).

Gel Filtration. Limited digests of mixed s-RNA and alanine s-RNA were applied to columns of Sephadex G-75 fine (Pharmacia, Inc., N. J.), 0.9 × 160 cm, which had been previously equilibrated with the elution buffers. The samples (50–400 μl) were carefully layered at the top and rinsed in with three equal volume washes of buffer. Flow rates were routinely between 8 and 12 ml/hr.

Fingerprinting Procedures. METHOD C. Method C of Ingram and Pierce (1962) was used to determine nucleotide compositions of fractions. This was done with Whatman 3HR, 3MM, or 3MC paper in 18 × 22 in. sheets which were previously washed with EDTA and water.

METHOD D. The fingerprinting of oligonucleotides from enzymatic digestion of s-RNA was carried out according to method D of Armstrong *et al.* (1964) using the above papers.

Nucleotide Composition of Fractions. METHOD C. Appropriate fractions from the column chromatography were pooled and dried in a rotary evaporator. The flasks containing the dry residues were than lyo-

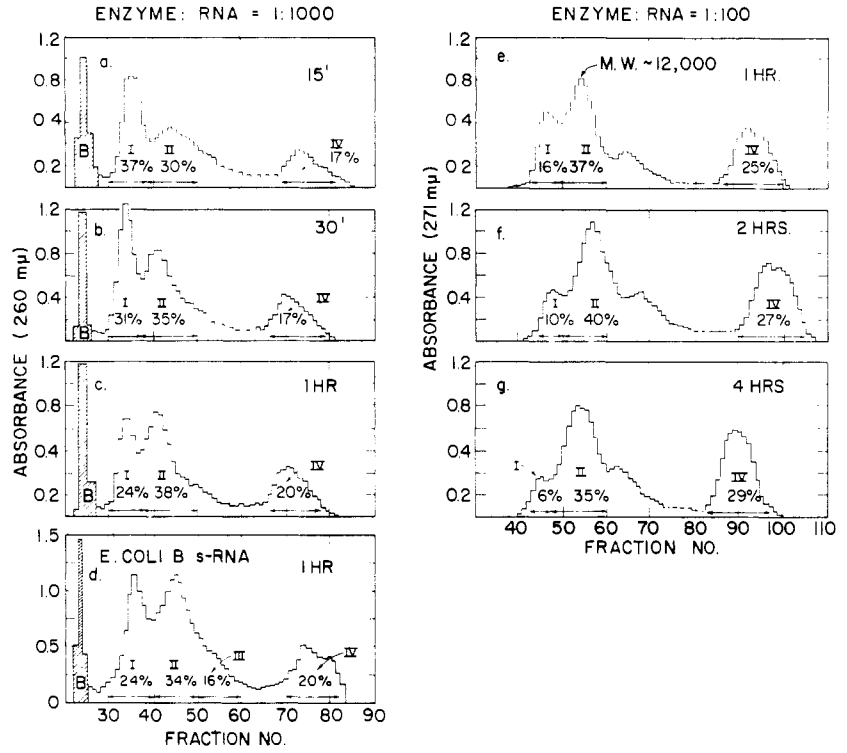

FIGURE 2: Effect of enzyme concentration on the products of partial enzymatic digestion of unfractionated yeast s-RNA. (a–c) Samples (2 mg) of s-RNA were digested for the times shown with 2 μg of pancreatic ribonuclease (see Methods). The samples were eluted from identical 0.9 × 160 cm G-75 Sephadex columns at 8–9 ml/hr with 0.1 M ammonium acetate. Fraction size was 1.4 ml. (d) Unfractionated *E. coli* B s-RNA (2 mg) was digested for 1 hr with 2 μg of pancreatic ribonuclease at 0°. Details are the same as a–c. (e–g) Samples (2 mg) of yeast s-RNA were digested at 0° for the times shown with 20 μg of pancreatic ribonuclease. The samples were fractionated in the same manner as a–d, except fraction size was 1.1 ml. The molecular weight of the fraction indicated in Figure 2e was determined according to the method described by Litt and Ingram (1964).

philized for 16–24 hr in a vacuum desiccator to remove all traces of ammonium acetate. The lyophilized residues were dissolved in 100 μl of deionized water and transferred to small test tubes. To these samples were then carefully added 50 μl of 0.907 M potassium hydroxide, and the tubes were covered with Parafilm and incubated (placed in a room) at 37° for 14–16 hr. The potassium hydroxide was removed by addition of an equimolar amount of perchloric acid and the insoluble potassium perchlorate removed by settling at 0° for about 15 min. The supernatant solutions were dried on Parafilm in a vacuum desiccator using a water pump; the dry residues were taken up in 10–20 μl of water, spotted over 0.5 in., and fingerprinted according to method C. The ultraviolet-absorbing spots of the resulting fingerprints were cut out and eluted with 100 or 200 μl of water depending on the amount of material present. The eluates were diluted to 600 μl with 0.1 M hydrochloric acid, and spectra of the nucleotides were taken and corrected with eluates from suitable blank areas.

Analysis of Oligonucleotide Fingerprints. METHOD D. The ultraviolet-absorbing spots were cut out and eluted with 100–200 μl of water, and the eluates were diluted to 600 μl with 0.1 M ammonium acetate, pH 7.0. Spectra of the spots were then taken and the samples were dried under water pump vacuum. The dry residues were hydrolyzed with potassium hydroxide as described, and the hydrolysates were then examined by electrophoresis at pH 3.0 in 20% acetic acid–NH_3 at 1.2 kv for 3–4 hr. The total amount of oligonucleotide originally present was calculated by using the sum of the extinction coefficients of the individual nucleotides.

Thermal Denaturation Studies. Studies on thermal denaturation were carried out with a Zeiss spectrophotometer PMQ II equipped with a water-jacketed cuvet holder (Zeiler Instruments, Boston, Mass.) in 0.2 M sodium chloride, 0.005 M potassium phosphate, pH 7.2, which had been autoclaved prior to use.

Results

To determine the efficiency of removal of the ribonuclease by bentonite, the effect of any remaining enzyme on the amino acid acceptor activity was measured. Pancreatic ribonuclease (2 or 20 μg) was added to a solution containing (per milliliter) 200 μmoles of

TABLE I: Nucleotide Composition of Sephadex Fractionation of a Partial Digest of Yeast Alanine s-RNA (Figure 3).[a]

	Moles/75 Nucleotides[b]											
	Cp	Ap	Gp	Up	Tp	Ip[c]	ψUp[c]	1MGp + 2DMGp[d]	Cyt + Ad[e]	pGp	Total	Total mμ moles Analyzed
Intact alanine s-RNA[f]	22	7	26	12	1	1	2	2	1	1	75	
Peak I	23	9	24	13	1	← 2.7 →		1.1	+	0.8	74	675
II[g]	16	7	18	9	1	0	1.4	1.5	0	0.7	55	1367
III[g]	2	1.8	2	3	0	← 0.1 →		0.4	0	0.2	10	240
IV[g]	2.6	1	2	2	0	0.8	0	0.3	0.5	0.1	10	243
Total II–IV	21	10	22	15	1	0.8	1.4	2.2	0.5[h]	1.0	75	1850

[a] See Methods for details. [b] Does not include MeIp and DHUp which cannot be analyzed by alkaline hydrolysis. [c] Fingerprinting by method C does not resolve Ip and ψUp. Presence or absence of these nucleotides was determined by drying the eluate from the corresponding spot, dissolving in 1 N HCl, sealing in a melting point capillary tube, and heating at 100° for 1 hr. This hydrolysate was then dried, spotted on Whatman No. 3HR paper, and chromatographed as in method C. This treatment allows separation and identification of ψUp and hypoxanthine. [d] The methylated guanine nucleotides do not separate from each other. [e] From the acceptor end of the molecule. [f] Holley *et al.* (1965). [g] These values were derived by assuming that these three peaks together should contain 75 nucleotides/mole of s-RNA digested. [h] The poor yield of nucleotides in this analysis may be due to the adsorption of the bases to the Sephadex column as noted in the Results section and as described by Gelotte (1960).

ammonium acetate, 10 μmoles of magnesium acetate, and 2 μmoles of Tris–acetate, pH 7.5. After addition of 2 mg of bentonite, the pH was adjusted to 5.5–6 and the bentonite and adsorbed ribonuclease were removed by centrifugation as described in Methods. The supernatant solutions were adjusted to pH 7.5–8 and 30 μg of unfractionated yeast s-RNA was added; the solutions were incubated for 30 min at 37° and then assayed for amino acid acceptor activity (Ingram and Pierce, 1962). Unfractionated yeast s-RNA treated in this way retained more than 90% of its alanine acceptor activity. Omission of the bentonite treatment resulted in a 100% loss of acceptor activity.

Fractionation of Partial Digestions of Unfractionated s-RNA. Earlier work by Litt and Ingram (1964) had shown that the products of partial pancreatic RNAase digestion of s-RNA could be divided into high and low molecular weight material by gel filtration with Sephadex G-75. Columns (0.9 × 160 cm) of Sephadex G-75 gave good fractionation of the partial digest products into intact s-RNA, high molecular weight oligonucleotides, and small oligonucleotides, provided that 0.1 M ammonium acetate was used (Figure 1). Higher ionic strength (0.5 M ammonium acetate) gave less effective separation between the intact s-RNA and large oligonucleotides.

It is interesting to note that purine nucleotides are adsorbed to the gel matrix at low ionic strength (0.1 M ammonium acetate); thus adenylic acid elutes later than cytidylic acid. In 0.5 M ammonium acetate the purine-containing oligonucleotides of a complete pancreatic digest of s-RNA elute later on Sephadex G-75 than the free pyrimidine nucleotides.

Redetermination by the technique of Yphantis (1960) of the molecular weights of the first two peaks (Figure 1) obtained by gel filtration of the partial digests confirmed the earlier results of this laboratory (Litt and Ingram, 1964) (also see Figure 2e). Since the method used for determination of molecular weight would not show heterogeneity in size of the s-RNA fragments, we looked for small material in the early fractions on gel filtration.

Haselkorn and Doty (1961) reported that RNA can be irreversibly denatured by heating in formaldehyde. In order to confirm this for our partial digestions, half molecules obtained by partial digestion of yeast s-RNA were dissolved in 0.1 M sodium chloride, 0.005 M potassium phosphate, pH 7.2, 0.5% formaldehyde, and subjected to thermal denaturation as described in the Methods section. Upon cooling the maximal optical density did not fall as it does when formaldehyde is absent (see below).

In order to see if disruption of the secondary structure of the products of the partial digestion would change their behavior upon gel filtration, a partial digest was lyophilized and dissolved in a buffer composed of 0.1 M sodium chloride, 0.005 M potassium phosphate, pH 7.2, 0.5% formaldehyde. The sample was sealed in a capillary tube, heated to 60° for 30 min, and then loaded onto a Sephadex G-75 column; the elution buffer was 0.1 M NaCl, 0.005 M potassium phosphate, pH 7.2, 0.5% formaldehyde. The elution

profile of this digest was identical with those obtained with the untreated digests in 0.1 M ammonium acetate; thus, there is no great degree of contamination of the high molecular weight material with short oligonucleotides. We can also conclude that secondary structure of the products, large or small, of the partial ribonuclease digestion plays little or no role in the Sephadex fractionation.

Effect of Variation of Conditions of Digestion on the Products. Several experiments were carried out to determine the effect of temperature, time of digestion, magnesium concentration, and enzyme concentration on the proportions of large and small material produced by partial digestion. A tenfold increase in the concentration of magnesium (from 10 to 100 μmoles/ml) has little if any influence on the proportion of high molecular weight oligonucleotides produced. The concentration of magnesium normally used in our partial digestions (10 μmoles/ml) provide a twofold excess over the number of phosphate groups present in the s-RNA; this apparently is enough for maximal protection.

Penswick and Holley (1965) reported conditions for limited digestion of yeast alanine s-RNA with ribonuclease T_1 which produced half molecules, but no small oligonucleotides. In order to determine whether similar results could be obtained using the pancreatic enzyme, partial digestions were carried out using only 1 μg of enzyme/mg of s-RNA (a ratio close to that of Penswick and Holley). It is evident (Figure 2a–c) that even during short digestion times considerable amounts of small oligonucleotides are formed. It thus appears that magnesium-stabilized yeast s-RNA molecules have some unprotected areas which are attacked rapidly by pancreatic ribonuclease.

Increase by a factor of 10 in the amount of pancreatic ribonuclease does not appreciably change the elution pattern of the partial digests. Thus, even after 4 hr there is a large amount of high molecular weight oligonucleotides and even some intact s-RNA left (Figure 2f).

Studies on Alanine-Specific s-RNA from Yeast. A 1-hr digest of 2 mg of alanine s-RNA at 0° with 20 μg of pancreatic ribonuclease gave the elution profile on Sephadex G-75 which is shown in Figure 3. The appropriate fractions were pooled; base analyses were carried out as described in Methods (Table I). Thymidylic, pseudouridylic, and inosinic acids are specifically localized into either high or low molecular weight products; however, both the methylated guanylic acids and guanosine 3′,5′-diphosphate[1] are found in both high and low molecular weight oligonucleotides.

After another partial digestion identical with the one shown in Figure 3, material from peak II was

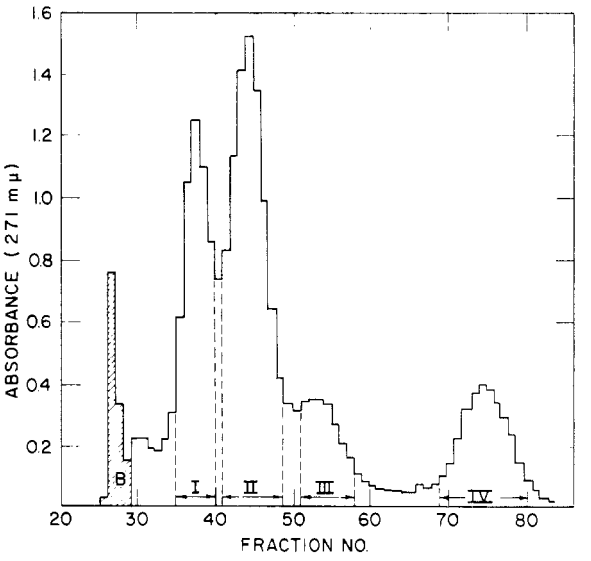

FIGURE 3: Elution profile of a representative 1-hr partial digestion of yeast alanine s-RNA. Yeast alanine s-RNA (2 mg) was digested for 1 hr with 20 μg of pancreatic ribonuclease (see Methods). The sample was eluted at 10 ml/hr with 0.1 M ammonium acetate from a 0.9 × 160 cm G-75 Sephadex column. Fraction size was 1.4 ml. The amount of digested s-RNA was calculated by summing the absorbances of all fractions from peaks II–IV. The absorbance of peak II was increased by 14% to take into account the hyperchromicity of this material (see text and Figure 5b). The ratios of peaks II, III, and IV to total digested s-RNA were, respectively, 66, 13, and 20%.

hydrolyzed completely with pancreatic ribonuclease and fingerprinted according to method D. The fingerprint (Figure 4a) was analyzed (Table II) as described in Methods. Except for the complete absence of any of the oligonucleotide IpGpCp, the oligonucleotide composition is not strikingly different from that of intact alanine s-RNA. To obtain a better analysis of the methylated guanylic acids in the fraction of small oligonucleotides, a partial digestion was carried out on 5 mg of alanine s-RNA (stabilized with Mg^{2+}) at 0° using 1 μg of RNAase/mg of RNA. The peak containing short oligonucleotides (corresponding to peak IV of Figure 3) was digested completely with pancreatic ribonuclease to hydrolyze 2′,3′-cyclic phosphate groups which would interfere with the analysis of small amounts of oligonucleotides. The fingerprint (method D) of this redigested material is shown in Figure 4b and its analysis is given in Table II. No nucleotides other than those present in alanine s-RNA were seen. The significance of these findings in relation to structural models for alanine s-RNA is discussed below.

Analysis of Partial Digests of Mixed s-RNA from Yeast and E. coli B. Material from fraction II of a 1-hr partial pancreatic ribonuclease digest of 2 mg of unfractionated yeast s-RNA (Figure 2c) was subjected to complete

[1] Abbreviations used: Tp, ribosylthymidine phosphate; ψUp, pseudouridine 3′-phosphate; 1MGp, 1-methylguanosine 3′-phosphate; 2DMGp, 2-dimethylguanosine 3′-phosphate; DHUp, 5,6-dihydrouridine 3′-phosphate; MeIp, methylinosine 3′-phosphate; C!, cytidine 2′,3′-cyclic phosphate; ψ, pseudouridylic acid; diHUp, dihydrouridylic acid.

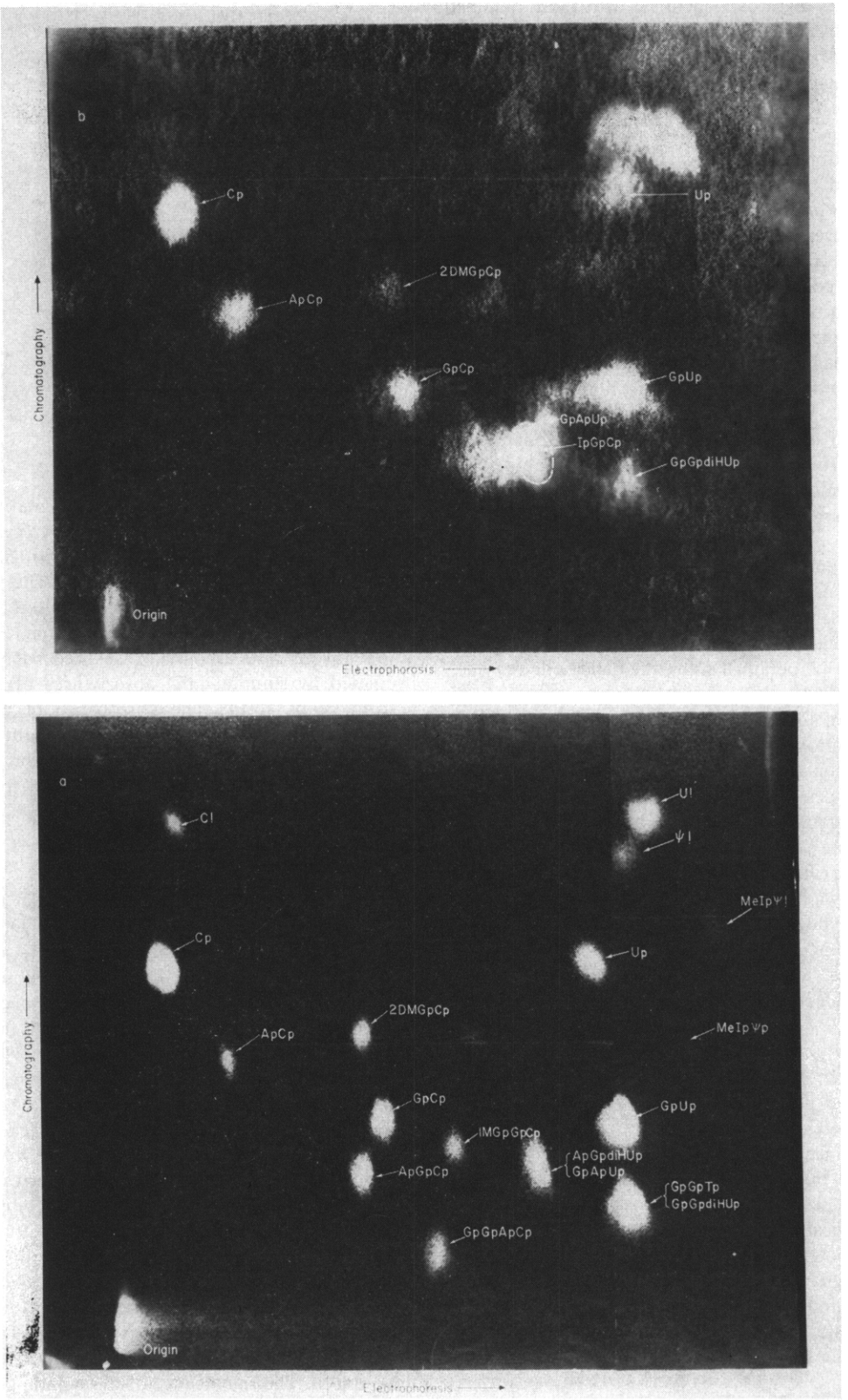

FIGURE 4: Fingerprints of total pancreatic ribonuclease digestion of the products of partial digestion of yeast alanine s-RNA. (a) Peak II of Figure 3 was digested with pancreatic ribonuclease and fingerprinted according to method D (see text and Methods). (b) Peak IV of a G-75 Sephadex fractionation of a partial digestion of 5 mg of yeast alanine s-RNA was redigested with pancreatic ribonuclease and fingerprinted according to method D. The smear of ultraviolet-absorbing material above Up is an impurity in the bentonite.

TABLE II: Oligonucleotide Composition of Peaks II and IV from a Partial Digest of Alanine s-RNA (Figure 4).[a]

Oligonucleotide	Peak II (mμmoles/mg of digest)	Moles[b]	Peak IV (mμmoles/mg of digest)	Alanine s-RNA (moles/molecule)[c]
Cp + C!	115	12	32	13
ApCp	9.5	(1.0)	4	1
2DMGpCp	14.5	1.5	1.8	1
GpCp	21.5	2.25	4.2	2
ApGpCp	10.5	(1.1)	++	1
1MGpGpCp	9.5	(1.0)	?	1
GpGpApCp	6.5	0.7	0	1
(ApGp)Up[d]	16.5	1.7	3	1
(ApGp)diHUp[d]				
ψUp	8.5	(0.9)	0	1
Up + U!	69	7	10	6
MeIp ψUp	5.5	0.6	0	1
GpUp	39	4	10	4
GpGpTp[d]	16	1.7	0	1
GpGpdiHUp[d]			2.2[f]	1
IpGpCp	0	0	11.4	1

[a] See Methods for details. [b] The recoveries of ψUp, ApCp, ApGpCp, and 1MGpGpCp were averaged and set equal to 1.0. [c] Holley *et al.* (1965). [d] These oligonucleotides do not separate when fingerprinted according to method D (see Figure 4). [e] ++ present, but not analyzed. [f] Identified by base hydrolysis (see Methods) followed by electrophoresis. The electropherogram showed an ultraviolet-absorbing spot only in the region where a standard sample of Gp ran and its spectrum in 0.1 N HCl was identical with Gp. In the region corresponding to Up a spot was found that did not absorb ultraviolet light, but gave a positive reaction when dipped in the organic phosphate reagent of Burrows *et al.* (1952).

TABLE III: Nucleotide Composition of Sephadex Fraction of a Partial Digest of Unfractionated *E. coli* B s-RNA (Figure 2e).[a]

	Moles/75 Nucleotides[b]										Total mμ moles Analyzed
	Cp	Ap	Gp	Up	Tp	ψUp	MeGp's	2MeAp	pGp	Total	
Intact *E. coli* B s-RNA[c]	22	15	24	11	0.8	1.6	0.15	0.2	No data	75	
Peak I	22	15	25	10	1.3	1.6	0	0	0.45	75	476
II[d]	13	8	14	7	0.55	0.57	0	0	0.28	43	664
III[d]	5.4	3.4	9	3.9	0	0	0	0	0.54	22	365
IV[d]	0.6	1.8	2.4	4.5	0	0	0	0	0	9.3	123
Total II–IV	19	13	25	15	0.55	0.57	0	0	0.82	74.3	1152

[a] See Methods for details. [b] The total number of nucleotides in the average *E. coli* B s-RNA molecule was assumed to be 75. [c] Dunn *et al.* (1960). [d] Treated as in Table I (see footnote *g* for details).

digestion with RNAase T_1 and the digest was fingerprinted according to method D. The fingerprint was qualitatively identical with a corresponding fingerprint of a RNAase T_1 digest of intact unfractionated yeast s-RNA. It was interesting to find the oligonucleotide TpψUpCpGp which Zamir *et al.* (1965) have reported to be present in all species of yeast s-RNA, in a yield (30 mμmoles) comparable to the amount of pGp in fraction II (20 mμmoles).

A partial digest of 2 mg of unfractionated *E. coli* B s-RNA yields an elution profile on Sephadex G-75 that is qualitatively identical with one of unfractionated yeast s-RNA (Figure 2d). The nucleotide composition of peaks I-IV of this partial digestion was analyzed

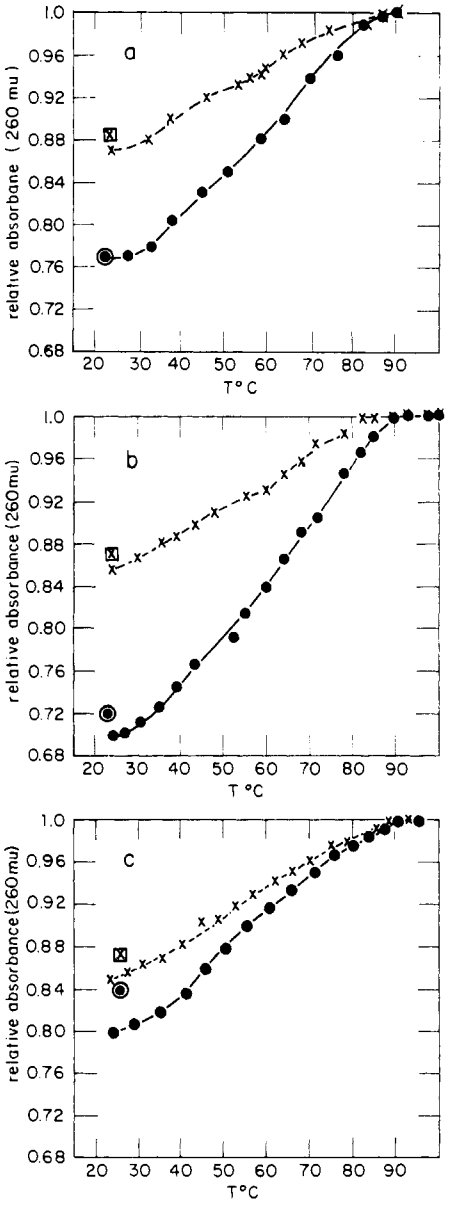

FIGURE 5: Melting profiles of (a) unfractionated yeast s-RNA, (b) yeast alanine s-RNA, and (c) unfractionated *E. coli* B s-RNA; and the half molecules produced by their partial digestion. Intact s-RNA (-•-•-). Relative absorbance after quick cooling to room temperature from maximum temperature ⊙. Half molecules produced by partial pancreatic ribonuclease digestion of s-RNA (see text). (- × - × - ×) Relative absorbance after quick cooling from the maximum temperature to room temperature ⊠.

as described for alanine s-RNA (Table III). It is interesting to note that all the Tp and ψUp, as well as the guanosine 3′,5′-diphosphate, are found in the high molecular weight material, a situation similar to unfractionated yeast s-RNA.

Thermal Denaturation Studies. Since the stability of s-RNA to pancreatic RNAase attack is thought to be due to secondary and tertiary structure, thermal denaturation studies have been carried out on the products of partial digestion of s-RNA. The large oligonucleotides from partial digests of unfractionated yeast s-RNA do display hyperchromicity with a T_m similar to intact s-RNA, but the degree of hyperchromicity is only half of that found in the intact s-RNA (Figure 5a). Alanine-specific s-RNA of yeast and unfractionated *E. coli* B s-RNA behaved similarly (Figure 5b,c). Addition of 0.5% formaldehyde did not change the melting profile dramatically, but it did increase the degree of hyperchromicity by about one-third.

Discussion

The analyses of the products of partial digestion of alanine s-RNA show that the high molecular weight oligonucleotides ("half molecules") contain all the thymidylic, pseudouridylic, and methylinosinic acids present in the intact s-RNA. In addition, about two-thirds of the 1MGp and 2DMGp are found here. Small but significant and equimolar amounts of 2DMGpCp and GpGpDHUp are found in the small oligonucleotides. About one-half of the pGp is found in low molecular weight material. It has been shown that there are no significant amounts of small oligonucleotides clinging to the high molecular weight oligonucleotides. Also, the high molecular weight fragments possess a considerable degree of secondary and/or tertiary structure.

When these results are compared with the nucleotide sequence of alanine s-RNA, it appears that all the minor bases found in the small oligonucleotides, *i.e.*, the easily attacked regions, are located in the "pGp" half molecule. Examination of the small oligonucleotides from partial pancreatic ribonuclease digests of alanine s-RNA (peak IV in Figure 3; Table II) revealed that it contained all the IpGpCp. The molar yield of this trinucleotide was found to be five times that of any other oligonucleotide in that fraction, except GpUp, which occurs four times in the alanine s-RNA sequence, and ApCp, which occurs once, but very close to the "CCA" end of the s-RNA. One can interpret these findings to indicate that partial pancreatic ribonuclease digestion of Mg-stabilized alanine s-RNA preferentially liberates the IpGpCp sequence from the center of the molecule and, less rapidly, the ApCp sequence near the "CCA" end and the CpUp sequences near the "pGp" end of the s-RNA.

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Chemical Studies on Amino Acid Acceptor Ribonucleic Acids. VII. Partial Ribonuclease Digestion of Alanine and Valine Soluble Ribonucleic Acid from Yeast*

Ann Armstrong, Helen Hagopian, Vernon M. Ingram, and Edward K. Wagner†

ABSTRACT: Purified yeast alanine-specific soluble ribonucleic acid (s-RNA) was partially degraded with ribonuclease T_1 under conditions which stabilized the conformation; one of the yeast valine s-RNA's was stabilized and partially digested with pancreatic ribonuclease. Examination of the oligonucleotide fragments released and of the properties of the resulting large fragments suggests the localization of secondary structure in two loops of nucleotide sequences, one more stable than the other.

The preceding paper of this series (Wagner and Ingram, 1966) described experiments on the partial enzymatic degradation of yeast alanine-soluble ribonucleic acid (s-RNA) with pancreatic ribonuclease. A brief review of the literature was also given. The present paper deals with further experiments along similar lines in which yeast alanine s-RNA was partially degraded with ribonuclease T_1 and one of the yeast valine s-RNA's was partially digested with pancreatic ribonuclease. Some features of the secondary and tertiary structure of s-RNA can be deduced from the findings.

Materials and Methods

Alanine-Specific Yeast s-RNA. Commercial yeast s-RNA (General Biochemicals, Inc., Chagrin Falls, Ohio) was purified by two countercurrent distributions (Apgar *et al.*, 1962; Armstrong *et al.*, 1964). The identity of the alanine s-RNA with that of Holley's (Apgar *et al.*, 1962) is discussed in paper VI of this series (Wagner and Ingram, 1966).

Valine-Specific Yeast s-RNA was purified in the same manner. The final distribution pattern showed two peaks close together (Armstrong *et al.*, 1964); the major, slower moving fraction was used in the present experiments.

Ribonuclease T_1. The enzyme was prepared according to the method of Takahashi (1961) or it was purchased from Calbiochem, Inc., Los Angeles, Calif.

* From the Division of Biochemistry, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts. *Received May 10, 1966.* This investigation was supported by a grant (AM-08390) from the National Institute for Arthritis and Metabolic Diseases, U. S. Public Health Service.

† Predoctoral Fellow of the National Institutes of Health.

[1] Abbreviations used: Gp!, guanosine 2',3'-cyclic phosphate; DHUp, 5,6-dihydrouridine 3'-phosphate; ψUp, pseudouridine 3'-phosphate; Tp, ribosylthymidine phosphate; Cyt, cytidine.