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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*

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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.

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

DIGEST A. Limited Digestion of Alanine s-RNA with Ribonuclease T_1, 0°, 60 Min, Enzyme:s-RNA = 1:60. Alanine-specific s-RNA (4.6 mg) in 1.0 ml of 0.2 M ammonium acetate, pH 7.6, was treated in the absence of magnesium in ice in a 12-ml conical centrifuge tube with 80 μg of ribonuclease T_1 for 60 min. To the cold solution 50 μl of an ovalbumin solution (10 mg/ml) was added as protein collector, followed at once by 2 ml of phenol (ACS grade, Allied Chemical Co.), previously saturated at room temperature with the ammonium acetate buffer. Immediately, the mixture was agitated violently in a Vortex mixer for 60 sec and then centrifuged in a table centrifuge at room temperature. Supernatant was removed and the phenol was washed with 0.2 ml of buffer. The combined supernatant and wash were treated at once with phenol for a second and third time in a similar manner. The final aqueous solution was freed of phenol with repeated extractions with ether (five times, 4 ml), followed by removal of ether in a stream of filtered air. Previous experiments had shown that no ribonuclease T_1 activity, measured by the release of small oligonucleotides from yeast s-RNA at 37°, was detectable after this treatment. The final solution contained 4.5 mg of s-RNA with a ratio of absorbancies 260:280 mμ = 2.02; this was evaporated to a syrup on a rotary evaporator and mixed with 80 μl of water and with 10 μl of a solution of Dextran Blue (Pharmacia, Inc., New York) of arbitrary concentration in 0.5 M ammonium acetate, prior to gel filtration. The sample was applied by carefully layering it on top of the column underneath 2 cm of buffer.

DIGEST B. Alanine-specific s-RNA (4.2 mg) in 1.0 ml of 0.2 M ammonium acetate, pH 7.6, was treated at 20° for 10 min with 20 μg of ribonuclease T_1 (enzyme:s-RNA = 1:200). The rest of the reaction conditions and the isolation of the s-RNA fragments were done as described for digest A; the recovery of ultraviolet-absorbing material was very close to the theoretical.

DIGEST C. Alanine-specific s-RNA (3.8 mg) was dissolved in 0.25 ml of 0.44 M ammonium acetate, pH 7, containing 20 μmoles of magnesium acetate. After equilibration at 20°, 20 μg of ribonuclease T_1 in 0.01 ml of water was added and allowed to react for 1 hr at 25°. The ratio of ribonuclease:s-RNA was 1:175 (w/w). The s-RNA fragments were isolated as described above for digest A. After the removal of ether, the aqueous solution was passed through a column (0.9 × 5 cm) of Chelex 100 (50–100 mesh, California Corp. for Biochemical Research) in the ammonium form to remove Mg^{2+}. The recovery was 87% of the original s-RNA absorbance.

DIGESTS D AND E. Limited Digestion of Valine s-RNA with Pancreatic Ribonuclease. Valine-specific s-RNA (4.1 mg, digest D, or 2 mg, digest E) was digested at 0° in the presence of Mg^{2+} according to the method described by Litt and Ingram (1964). Reaction was with enzyme:s-RNA = 1:100 for 60 min (digest D) or with a ratio of 1:1000 for 45 min (digest E). Pancreatic ribonuclease was removed with bentonite, as described by Wagner and Ingram (1966), prior to concentration and gel filtration.

Gel Filtration. Details are given in the legends to the figures. Typically, a column of the appropriate Sephadex, 0.9 × 220 cm, was equilibrated carefully with the respective ammonium acetate solution at room temperature. The oligonucleotide sample, approximately 120 μl, was layered cautiously under the buffer solution, employing Lang–Levy constriction pipets of 100- and 20-μl capacities. The flow rate was usually 10.2 ml/hr and 10-min fractions were collected.

Fingerprinting. Method D of Armstrong *et al.* (1964) was employed, using the EDTA-treated and water-washed Whatman 3MM or 3HR paper with double wicks 12 in. wide. Electrophoresis on a cooled plate with sheets of paper 18.25 × 22.5 in. was usually for 5 hr at 2500 v and 45–65 ma with a buffer of 20% acetic acid made pH 2.7 with concentrated NH_3.

Results

Alanine s-RNA

Digest A: No Mg^{2+}, 0°, 60 Min, Enzyme:s-RNA = 1:60. The elution profile of the limited ribonuclease T_1 digest A of alanine-specific s-RNA is shown in Figure 1a. The yield of each fraction is indicated. Clearly, digestion has been very limited and therefore fraction AI (70% of the total), which is probably intact s-RNA, was redigested under conditions likely to lead to more extensive breakdown: 2 mg of fraction AI calculated as s-RNA in 1.0 ml of 0.2 M ammonium acetate, pH 7.6, ribonuclease T_1 = 10 μg (enzyme: RNA ratio 1:200, w/w), 37°, 10-min of digestion. The work-up with phenol was exactly as described for digest A in Methods, but only 77% of RNA was recovered. The same column of Sephadex G-50 from the first elution was used for the fractionation (Figure 1b).

The fraction AIe (Figure 1b) was first evaporated to dryness using an oil pump at about 40° to remove ammonium acetate and then a fingerprint (see Figure 1b) was prepared as described under Methods. The other fractions, AIb–d, were also dried, redigested completely with ribonuclease T_1 in 0.2 M ammonium acetate, pH 7.6, and then fingerprinted in a similar manner with a reduced electrophoresis time of 2.5 hr. In all these fingerprints, spots were eluted and their spectra were recorded on a Cary Model 14 in 0.1 M ammonium acetate. Oligonucleotides were mostly identified by their base composition together with the data of Ingram and Sjöquist (1963), Holley *et al.* (1965), and V. M. Ingram, A. Armstrong, and H. Hagopian (in preparation). In this way the data in Table I were compiled.

Fractions AII and AIII (Figure 1a) were concentrated and desalted by evaporation. Attempts to fractionate these materials by paper electrophoresis in 7 M urea–7% acetic acid, adjusted to pH 3.0 with ammonia, were not successful. Fraction AIV was examined by paper electrophoresis only (washed 3MM paper) in 20% acetic acid made to pH 2.7 with ammonia. It

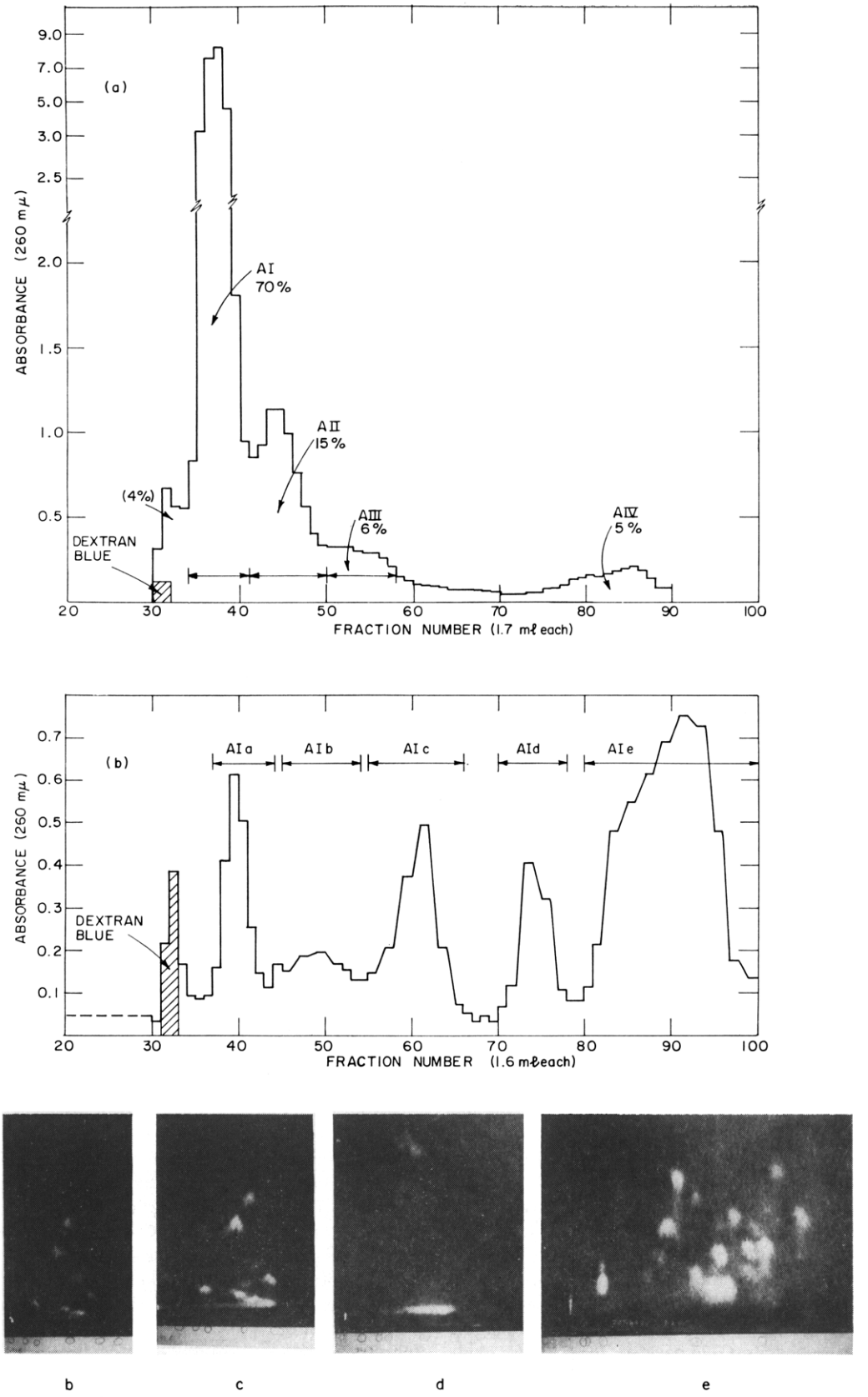

FIGURE 1: (a) Alanine s-RNA, limited digest (A) with ribonuclease T_1. (a) Gel filtration on Sephadex G-50 in 0.5 M ammonium acetate (see Methods). (b) Gel filtration on Sephadex G-50 in 0.5 M ammonium acetate of peak AI re-digested with ribonuclease T_1 (see text).

TABLE I: Oligonucleotides from Alanine s-RNA by Partial Digestion with Ribonuclease T_1.[a]

	Frequency in Alanine s-RNA (moles)	Digest A, Figure 1				Digest B, Figure 2a	Digest C, Figure 3	
		AIV	AI Partially Redigested AIc[b]	AId[b]	AIe	BIV	CIV (expt 414)	CIV (expt 384)
Gp + Gp!	8	Trace	24	–	–	+	10	±
		"pGp" half of s-RNA						
pGp	1	[c]	–	–	20	+	–	+
CpGp[d]	4	8	–	–	37	+	16	++
UpGp	1	15	–	–	7	+	2	+
(UpApGp + DHUpCpGp)	1	10	–	–	–	+	22	+
Up1MGpGp	1	–	–	–	14	–	–	+
Cp2DMG!	1	–	6	17	–	–	–	–
CpUpCpCpCpUpUpIp	1	–	4	18	–	–	–	–
		"CCA" Half of s-RNA						
ApGp	2	–	–	–	14	–	–	–
(ApGpUpCpUpCpCp)Gp!	1	–	13	–	–	–	–	–
UpCpUpCpCpGp	1	–	7	–	16	?	–	–
Tpψ UpCpGp	1	–	5	–	–	?	–	–
ApUpUpCpCpGp	1	–	4	–	–	+	–	–
ApCpUpCpGp	1	–	5	–	10	+	–	–
UpCpCpApCpCyt	1	–	3	–	12	+	–	+

[a] Yields in millimicromoles. [b] Fractions AIc and d were completely digested with ribonuclease T_1 before analysis by fingerprint. [c] This sign in a column indicates absence of that particular oligonucleotide. [d] May include CpGp!

gave CpGp, UpGp, and a mixture of UpApGp and DHUpCpGp (Table I).

Experiment B: No Mg^{2+}, 24°, 10 Min, Enzyme: s-RNA = 1:200. A limited digest with ribonuclease T_1 was made at a higher temperature, 24°, at which presumably the structure of the s-RNA would be more open and subject to hydrolysis. In this way it was hoped to locate new sites of enzymatic attack. In order to restrict the extent of hydrolysis, the time of digestion and the concentration of ribonuclease T_1 were drastically reduced. The profile of the gel filtration (Figure 2a) is similar to that from digest A and the proportions of the fractions are also similar; only the amount of totally included small oligonucleotides is significantly increased from 5 to 10%.

Redigestion of the split product with largest molecular size (fraction BI in Figure 2a) was done with ribonuclease T_1 at 37°, no Mg^{2+}, for 3 min, enzyme: s-RNA = 1:150, in the usual ammonium acetate buffer. Gel filtration on Sephadex G-25 allowed most of the material to pass unretarded, but refractionation of this on Sephadex G-75 yielded some more small pieces and also some "half molecules" (fraction BI β in Figure 2b).

Fraction BI in Figure 2a is presumably undegraded alanine s-RNA, since its melting curve (determined as described in the previous paper; Wagner and Ingram, 1966) closely resembles the melting behavior of untreated mixed yeast s-RNA and that of alanine s-RNA itself. In addition, fraction BI still had some alanine acceptor activity, which, although much less than the original alanine s-RNA, was similar to the activity which normally remains when untreated alanine s-RNA is passed through a Sephadex column of this type. Fraction BIV (Figure 2a) was directly fingerprinted, giving the oligonucleotides shown in Table I.

Experiment C: Mg^{2+} Present, 25°, 60 Min, Enzyme: s-RNA = 1:175. Two limited digests of this type were made, with similar results, one of which is illustrated in Figure 3. It was hoped that Mg^{2+} would stabilize the secondary structure of the alanine s-RNA and thereby enable us to define the susceptible region more clearly. That some stabilization occurred is indicated by the fact that in digest C approximately the same small proportion (8%) of the original s-RNA appeared as completely included small oligonucleotides, although the time of digestion was six times longer than in experiment B.

Some unaltered alanine s-RNA (16%) is still present as fraction CI. In addition to alanine acceptor activity, this fraction has a melting profile similar to the biphasic curve for intact alanine s-RNA described previously by Fresco *et al.* (1963). Its molecular weight, in 0.5% formaldehyde solution, is approximately 24,000 as

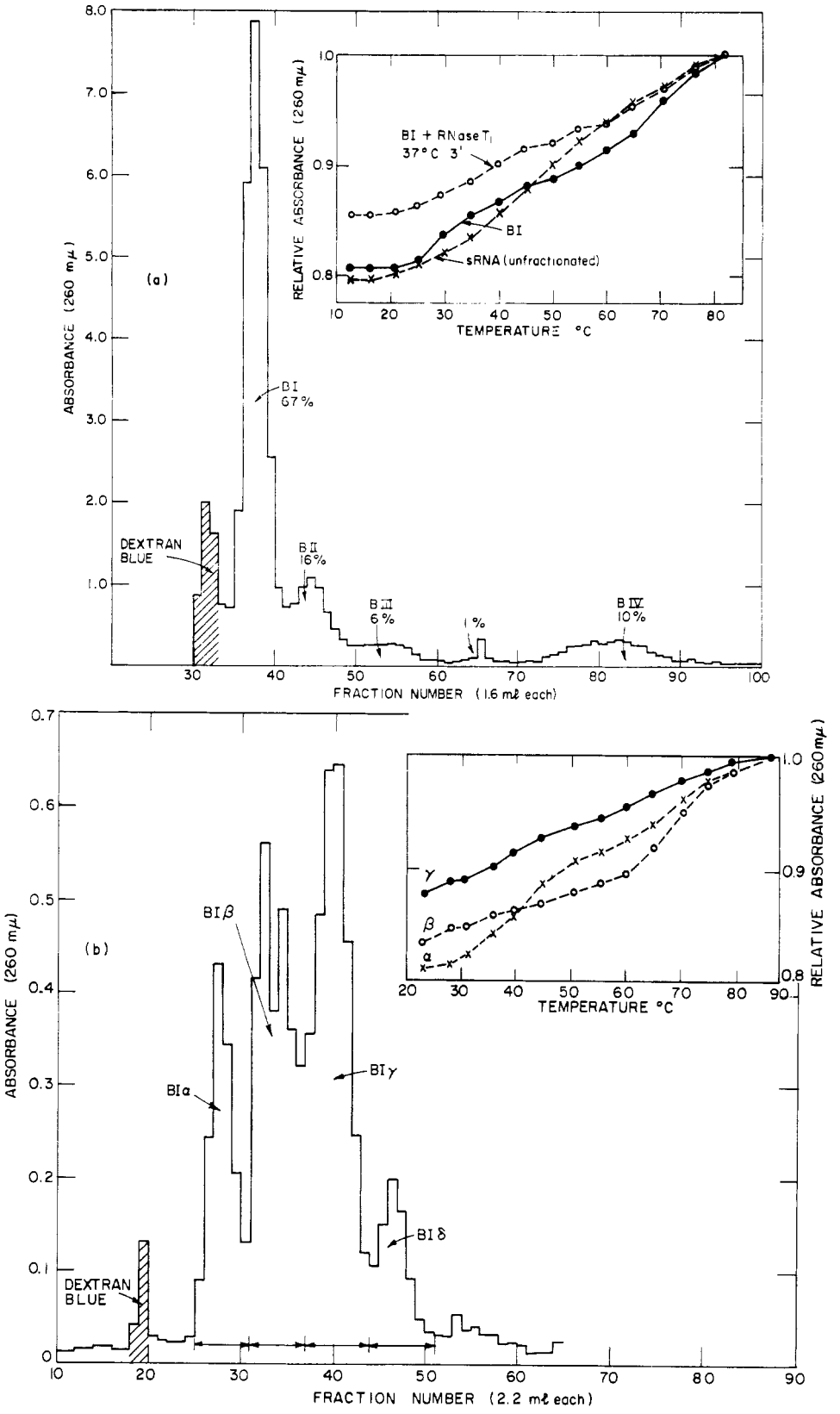

FIGURE 2: Gel filtration: (a) of alanine s-RNA, limited digest with ribonuclease T_1 (digest B, no Mg^{2+}, 24°, 10 min, enzyme:s-RNA = 1:200). Sephadex G-50, 0.5 M ammonium acetate. The dextran blue fraction accounted for 13% of total A_{260} recovered. Other fractions are given in terms of per cent total A_{260} after subtracting this fraction. (b) Of peak BI from (a) after resubmitting to partial degradation (see text) and gel filtration on G-25 to remove small molecules. Sephadex G-75, solvent–0.1 M ammonium acetate. Inserts are melting curves measured in 0.1 M potassium phosphate, pH 6.95, containing 0.1 M NaCl.

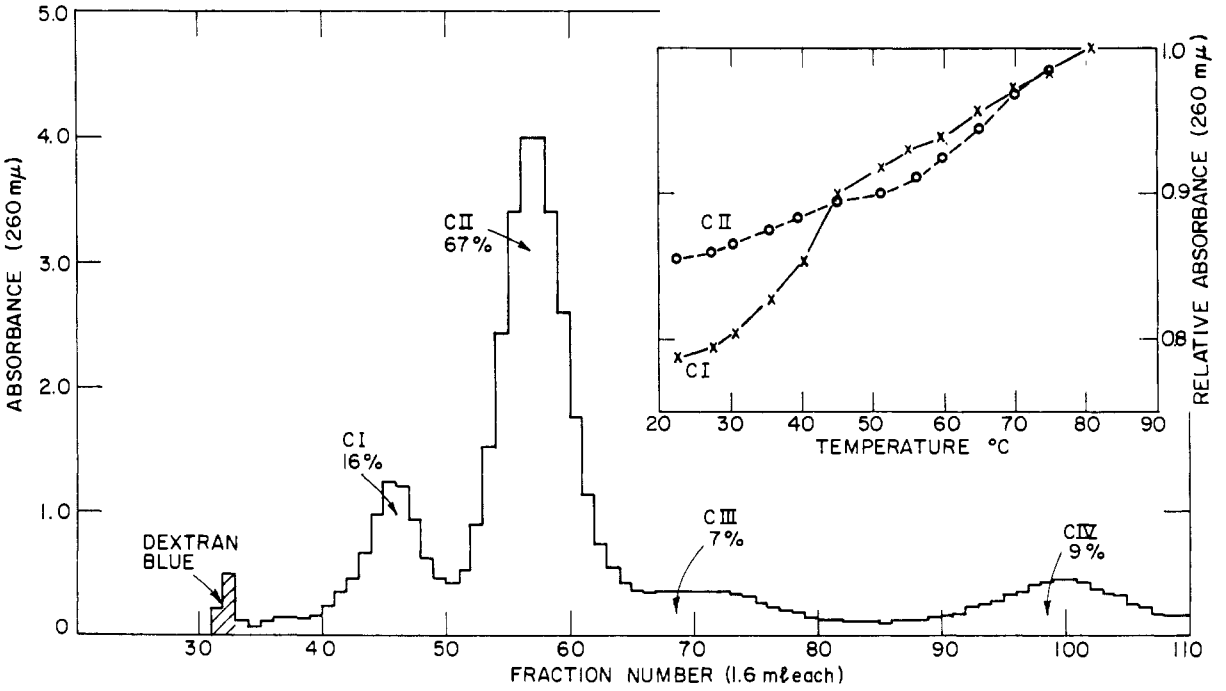

FIGURE 3: Gel filtration of alanine s-RNA, limited digest with ribonuclease T_1 (digest C, Mg^{2+} present, 25°, 60 min, enzyme:s-RNA = 1:175); column, Sephadex G-75; solvent, 0.1 M ammonium acetate; insert, melting curves of fractions CI and CII in 0.01 M potassium phosphate, pH 6.95, containing 0.1 M NaCl.

determined by the method of Yphantis (1960).

On the other hand, fraction CII apparently contains half molecules, judging from its position on the column and from a determination of its molecular weight in 0.5% formaldehyde which gave a value of approximately 10,000. Fraction CII still retains considerable secondary structure as measured by its melting profile. Compared with fraction CI (intact s-RNA), the hyperchromicity (22–81°) has decreased from 21.5 to 14.5%. The melting curve is hardly biphasic anymore; apparently most of the decrease in hyperchromicity occurred in the lower melting phase of the original s-RNA. Those regions of the secondary structure which melt out first are apparently also the first to be digested away with ribonuclease T_1.

Fraction CIII, like other similar gel filtration fractions (Figures 1–3), appears to be composed of at least two large oligonucleotides which on complete digestion with ribonuclease T_1 give complex fingerprint patterns. Not enough material was available for further studies. The fingerprints of fraction CIV showed a relatively simple pattern (Table I). The small oligonucleotides released are derived from the "pGp" half of the molecule. An exception is a small amount of the terminal oligonucleotide from the amino acid acceptor end. Each oligonucleotide obtained from fraction CIV was identified by its spectrum, its position on the fingerprint, and its base composition.

Valine s-RNA

Partial Pancreatic Ribonuclease Digest. When valine s-RNA was partially digested with pancreatic ribonuclease with an enzyme:s-RNA ratio of 1:100 (digest D), the gel filtration pattern (Figure 4a) showed that little intact valine s-RNA remained, but that there was again a major fraction in the position on the column where "half molecules" are expected, Fraction DII. However, degradation had clearly been more extensive than under comparable conditions with alanine s-RNA, and in particular the material with a size intermediate between "half molecules" and small

TABLE II: Valine s-RNA: Partial Pancreatic Digest Yields of Small Oligonucleotides (Fraction DIV, Figure 4a).

	mμM	Expected Moles/78 Nucleotides in a Complete Digest[a]
IpApCp	14	1
(ApGp)Up	12	3
ApCp	30	2
GpCp	7	4
GpUp	20	1
Cp + C!	187	(?)
Up + U!	75	6

[a] From Armstrong *et al.* (1964).

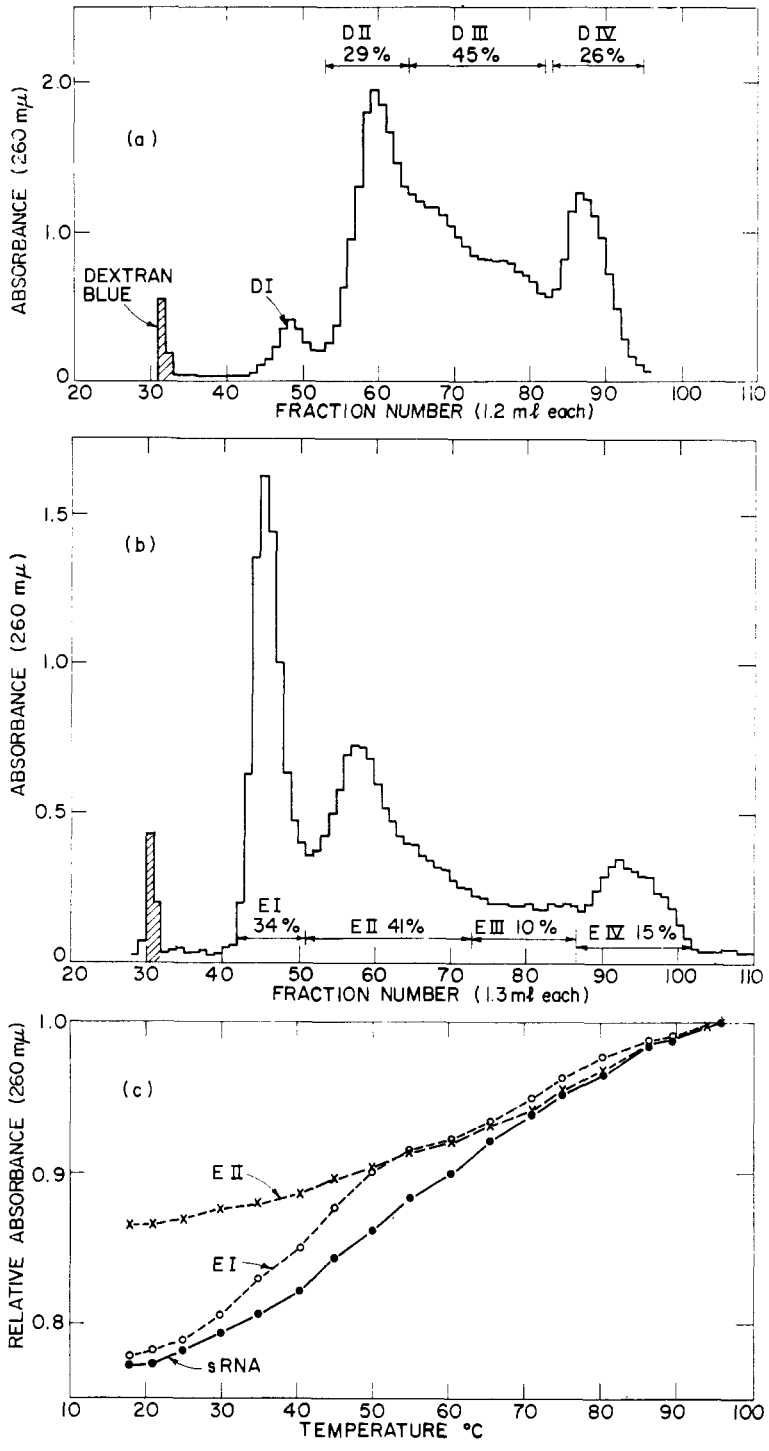

FIGURE 4: Gel filtration of limited pancreatic ribonuclease digest of valine s-RNA. Column, Sephadex G-75; solvent, 0.1 M ammonium acetate. (a) Digest D, Mg^{2+} present, 0°, 60 min, enzyme:s-RNA = 1:100. (b) Digest E, Mg^{2+} present, 0°, 45 min, enzyme:s-RNA = 1:1000. (c) Melting curve of fractions EI and EII from (b), in 0.01 M potassium phosphate, pH 6.95, containing 0.1 M NaCl.

oligonucleotides (DIII, Figure 4a) was unusually high. The fraction of small oligonucleotides, DIV, one-quarter of the total RNA, was separated on a fingerprint (see Methods) into mono- and oligonucleotides, previously obtained from valine s-RNA by pancreatic ribonuclease (Armstrong *et al.*, 1964). The yields of oligonucleotides in fraction DIV and their structures are given in Table II. No 5-methylcytidylic acid was found. Among the oligonucleotides, the yields of IpApCp, GpUp, and ApCp are seen to be higher

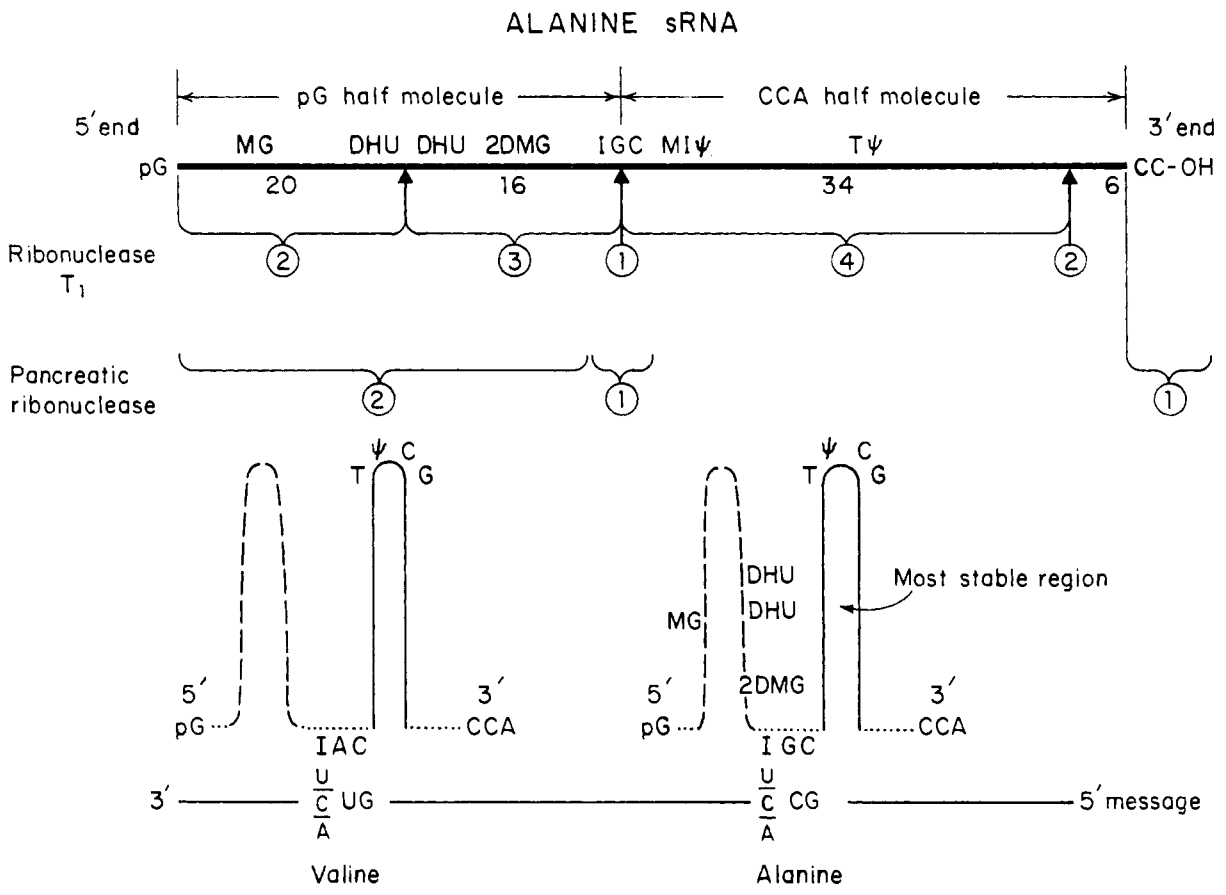

FIGURE 5: Schematic representation of alanine s-RNA indicating the first, second, third, and fourth "levels" of enzymatic attack. Hypothetical double-loop models of alanine and valine s-RNA are shown in relation to the RNA message, based on the assumption that inosinic acid (I) is involved in reading the message.

than the others *in relation to the expected molar ratios.* Presumably IpApCp, GpUp, and ApCp are located in regions of the molecule more vulnerable to enzymatic attack.

A more restricted digestion (digest E) for a shorter time and with an enzyme:s-RNA ratio of 1:1000 gave the gel filtration profile in Figure 4b. About one-third of the material remains intact and has valine acceptor activity (peak EI). Not enough of peak EIV was obtained for definitive analysis. Peak EII, a major component, no longer has this activity and also occupies the position usually ascribed to "half molecules." The melting behavior (Figure 4c) of peak EI shows the expected biphasic curve for intact valine s-RNA (Fresco *et al.*, 1963) and a hyperchromicity of 22% under the conditions used. Although fraction EII retains the melting behavior of the upper or more stable region of the melting curve of the valine s-RNA (Figure 4c), it has clearly lost most of the less stable regions in its molecule which normally contribute to the lower phase of the curves; correspondingly, the hyperchromicity of fraction EII is reduced to 13.5%.

Discussion

The information obtained in this and the previous paper (Wagner and Ingram, 1966) can be discussed in terms of the nucleotide sequence of yeast alanine s-RNA (Holley *et al.*, 1965) and with the aid of a model (Figure 5). According to Penswick and Holley (1965), the first point of enzymatic attack on alanine s-RNA with ribonuclease T_1 is in the very center of the chain leading to the isolation of two half molecules, an experiment which so far we have not been able to repeat. This initial attack takes place in the trinucleotide sequence IpGpCp which is therefore likely to be in an exposed part of the secondary structure of the s-RNA. We find that this trinucleotide is easily released by partial digestion of alanine s-RNA with pancreatic ribonuclease in the presence of Mg^{2+} at 0°. In the model in Figure 5, it is suggested that this exposed IpGpCp sequence *could* play the role of anticodon to combine specifically with an alanine RNA code word. F. H. C. Crick (unpublished data) has proposed models which use the hypoxanthine base for hydrogen bond-

ing to the three alternative bases, A, C, or U, in the RNA code word, the so-called "wobble" hypothesis. In this way, he seeks to use the same alanine s-RNA with three of the four degenerate RNA code words for alanine: GpCpUp, GpCpCp, and GpCpAp.

Somewhat more extensive degradation, as shown in Figure 5, under the conditions of digests A and C in the present paper begins to liberate small quantities of short oligonucleotides which are derived, in so far as they can be located in the Holley sequence, from the first 20 nucleotides near the pG end of the alanine s-RNA. At the same time, the terminal hexanucleotide begins to be liberated from the amino acid acceptor end of the molecule. The results of the partial digestion of alanine s-RNA with pancreatic ribonuclease (Wagner and Ingram, 1966) are in agreement with these deductions. Under our conditions of digestion (digests A and C), 70–80% of the alanine s-RNA molecules which have been attacked (subtracting first the remaining undegraded alanine s-RNA) are present as half molecules. Since much more than half of the nucleotides are found in the "half molecule" fraction, they must be composed of both loops (Figure 5) in unknown proportions. These half molecules, presumably now separate from each other and shorn of their "pGp" and "CCA" terminal sequences, retain considerable secondary structure as detected by melting behavior, although they have lost most of the less stable portions of their structure. Such structure as the half molecules do retain is apparently all in covalently bonded chains, since heating in formaldehyde does not alter the molecular weight or the behavior on Sephadex and since quick cooling restores the original optical density. Apparently, in unfractionated yeast and *Escherichia coli* s-RNA, the "half molecules" obtained with pancreatic ribonuclease (Figure 2a–c in previous paper, Wagner and Ingram, 1966) retain the same T_m, more or less. In the case of alanine or valine s-RNA (Figures 2–4) the T_m of the "half molecules" is not much different from the relevant T_m of the biphasic curve of an intact s-RNA.

Both alanine and valine s-RNA from yeast show biphasic melting profiles (Figures 2–4; Fresco *et al.*, 1963). These have been taken as evidence by various workers for the existence of two types of structural regions in the s-RNA molecule, one in which less stable and one in which more stable structural elements predominate. Within these structural regions melting out is to some extent cooperative. Partial enzymatic digestion of valine s-RNA with pancreatic ribonuclease (Figure 4c) and of alanine s-RNA with ribonuclease T_1 (Figure 3) removes the less stable structural regions preferentially, as might be expected.

We conclude that the "pGp" half molecule is more easily digested than the "CCA" half molecule (Figure 5) and that the latter persists longer during digestion. The reason why the TpψUpCpGp loop in the molecule (Figure 5) is not attacked by pancreatic ribonuclease is not clear; perhaps the loop is too "tight." What kind of structure exists, particularly in the relatively resistant "CCA" half molecule, cannot be guessed at from our observations. Base pairing of the Watson–Crick type has been suggested (Felsenfeld and Cantoni, 1964; Fresco *et al.*, 1963; Henley *et al.*, 1966) and if this is the only kind of structure, then, as illustrated by Holley *et al.* (1965), more base pairs and fewer unpaired regions can be drawn into the "CCA" half molecule. On the other hand, it has been pointed out by F. H. C. Crick (unpublished data) that hydrogen bonding is possible between base pairs other than the usual deoxyribonucleic acid (DNA) pairs. This idea together with the presence of pseudouridylic acid and other unusual bases should be part of our thinking when trying to describe secondary structure in s-RNA. Finally, it is interesting that the most stable portion of the alanine s-RNA molecule has the longest run of purines (seven) and might be stabilized in part by the stacking of purine bases. Our present ideas concerning the general arrangement of an s-RNA in space (Figure 5) is in agreement with the recent discussion of the configuration of unfractionated s-RNA by Henley *et al.* (1966).

The brief studies with valine s-RNA allow us to conclude that the general features of its secondary structure are similar to the secondary structure of alanine s-RNA. It is also possible to point to the presence of relatively stable "half molecules" (Figure 4a,b) and to the melting behavior of such fractions (Figure 4c). The ready release of the IpApCp sequence, which is probably unique in valine s-RNA, suggests to us a similar over-all structural model for this s-RNA (Figure 5), but we cannot go further at present.

A partial ribonuclease T_1 digest of unfractionated yeast s-RNA gave a gel filtration profile similar to those seen in Figures 1–3, although the proportions of the fractions show a somewhat more extensive degradation to small oligonucleotides. The general features are, however, similar and once again the peak containing the so-called "half molecules" shows reduced hyperchromicity with more pronounced loss of the structural regions which are less stable and melt early. A peak in gel filtration corresponding to "half molecules" is observed no matter whether alanine or valine s-RNA is partially digested with pancreatic ribonuclease in the presence of magnesium or whether alanine s-RNA is partially digested with ribonuclease T_1, with or without magnesium. For these reasons, we think that the relatively stable "CCA half molecule" is a general feature of s-RNA structure. The behavior of unfractionated *E. coli* s-RNA is apparently similar (Wagner and Ingram, 1966).

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Topography of Nucleic Acid Helices in Solutions. I. The Nonidentity of Polyadenylic–Polyuridylic and Polyinosinic–Polycytidylic Acid Helices*

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ABSTRACT: The effect of diquaternary ammonium salts of the general structure, $R_1R_2R_3N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$, I, on the helix–coil transition of polyadenylic–polyuridylic (poly-A–poly-U) and polyinosinic–polycytidylic acid (poly-I–poly-C) helices are reported. Helix stability was measured by absorbancy–temperature profiles.

The results may be summarized. (1) Maximum stabilization by I occurs at $n = 3$. The observed stabilization appears to be due to electrostatic interactions between adjacent phosphate anions, and the results are consistent with the Watson–Crick model. (2) Increasing the hydrophobic nature of the substituted salts, I, results in a decreased stability of the helices, and the effects are dependent on n for a given change in R_3, where $R_1 = R_2 = Me$. (3) Polar substituents stabilize both helices. (4) Finally, differences in the behavior of poly-I–poly-C and poly-A–poly-U toward I strongly suggest that in the former the bases are packed more closely.

It has been generally accepted that double-stranded nucleic acids have a uniform and unique structure, *i.e.*, the Watson–Crick double helix (1953). Although the dimensions of the Watson–Crick model are well known, the full physical consequences of such a structure in solutions have not yet been exploited. For example, the following stereochemical and physical characteristics of the double-stranded helix are predicted: (1) a highly polar surface, (2) a 7-A separation of adjacent negatively charged oxygen atoms on the same chain, and (3) the identity of all nucleic acid helices.

In an attempt to elucidate the structure and/or topography of nucleic acid helices in solution we have studied the effect of diquaternary ammonium salts of the general structure $R_1R_2R_2N^+(CH_2)_nN^+R_1R_2R_3 \cdot 2Br^-$ on the helix–coil transition of two model helices, polyadenylic–polyuridylic (rA–rU) and polyinosinic–polycytidylic (rI–rC) complexes. In this paper we report the effect of constant concentration of I on T_m, the transition temperature for the conformational changes helix–coil, as measured by absorbancy–temperature profiles.

Experimental Section

Polyadenylic (ϵ_p^{257} 8730),[1] polyuridylic (ϵ_p^{260} 8310),[1] polycytidylic (ϵ_p^{268} 4860),[1] and polyinosinic acids (ϵ_p^{258} 7980)[1] were obtained from Calbiochem, lot no. 52830, 52995, 46113, and 46114, respectively. Stock solutions of the homopolymers were made in 0.1 M Tris–HCl buffer, pH 7.25, and stored at 0–4° at 1.10–1.30 $\times 10^{-3}$ mole of P/l. Aliquots were removed and diluted with 0.1 M Tris–HCl buffer or with a solution of the diquaternary ammonium salts, I, in 0.1 M Tris–

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[1] In 0.1 M Tris–HCl buffer, pH 7.25, at 25°.