PREPARATION AND SOME PROPERTIES OF A SOLUBLE RIBONUCLEIC ACID FROM YEAST*

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SUMMARY

A method for the preparation of a soluble RNA of yeast which is capable of combining with L-leucine in the presence of ATP and enzymes has been described. The procedure involved phenol extraction of RNA from the pH 5.1 supernatant of the 78,410 × *g* supernatant from the cells, followed by its absorption and desorption from Ecteola-cellulose. The RNA obtained revealed considerable homogeneity in an electrophoretic field and sedimentation analyses gave a sedimentation constant $s^0_{20} = 4.0$. Comparative studies of the RNA with that prepared from ribonucleoprotein particles indicated that the soluble RNA has a distinct nucleotide composition characterized by the presence of pseudo-uridylic acid. Some other macromolecular properties of this RNA in solution were also investigated.

INTRODUCTION

In a previous paper[1], it was reported that in yeast cells the bulk of the RNA which is capable of combining with L-leucine is localized in the fraction soluble at pH 5.0 or pH 5.3 of the 105,000 × *g* supernatant of the homogenate. The RNA extracted from the acid-supernatant fraction was contaminated with polysaccharides which have however little effect on the [^{14}C]-leucine incorporation into RNA under the conditions previously described[1]. We have recently succeeded in purifying the RNA by means of Ecteola-cellulose, and have investigated some of its properties.

Preliminary accounts of some of the results described in this paper have been published elsewhere[2].

MATERIALS AND METHODS

Organisms: Late log-phase cells of *Saccharomyces cerevisiae*, Strain Kaneka, grown in a synthetic medium were used. For a report of the culture conditions, see the preceding paper[1].

Ecteola-cellulose: Ecteola-cellulose resin was prepared by the method of PETERSON AND SOBER[3]. The cellulose powder used was a product of "Genuine Whatman", W. and R. Balston Ltd., England.

* Soluble RNA (S-RNA) refers to any RNA which binds amino acid in the presence of amino acid activating enzymes, amino acids and ATP.

Electrophoresis: A Tiselius electrophoresis apparatus was used unless otherwise specified. All electrophoretic analyses were performed in 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1 in a field of 7.3 V/cm at 3°.

Sedimentation: Sedimentation analyses were performed in a Spinco E analytical centrifuge in 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1.

Viscosity: An Ostwald type of viscosimenter was used. All determination were made at 20°.

U.V. absorption: Curves were determined at room temperature (*ca.* 25°) with a Beckman Model DU spectrophotometer.

Nucleotide composition: RNA was hydrolyzed in 1 *N* KOH at 37° for 24 h. After neutralization with 60% perchloric acid, the individual mononucleotides were separated on Dowex-1-formate column as previously described[1,4].

PREPARATION OF RNA

Preparation of crude RNA from the pH 5.1 supernatant of the 78,410 × g supernatant fraction

The procedure described in a previous paper[1] was adopted with some modifications. About 100 g yeast cells (wet weight) were homogenized with quartz sand for 20 min in a hand mortar. The homogenate was suspended in 200 ml CHAO–SCHACHMAN'S medium[5] and centrifuged at 6,000 × *g* for 2 min. The sediment was washed once more with 200 ml of the medium and the supernatants were combined. The solution was then centrifuged for 120 min at 78,410 × *g* in a Spinco L ultracentrifuge (Rotor No. 30). The supernatant was then converted to pH 5.1 with 1 *N* acetic acid, and immediately centrifuged at 14,000 × *g* for 10 min in a Servall SS-1 centrifuge. The pH of the clear supernatant obtained was brought back to 6.8 with 0.5 *N* KOH, and then mixed with an equal volume of 90% commercial phenol[6] (w/v). All the operations described above were carried out at 0–2°. The emulsion was stirred for 1 h at room temperature (16–22°) with a mechanical stirrer. It was then centrifuged in the cold at 16,300 × *g* (Lourdes Centrifuge, Volume rotor) for 30 min. After the aqueous layer had been carefully pipetted out, the remainder was mixed with 300 ml cold water and centrifuged as above. The combined aqueous layers were centrifuged at 16,300 × *g* for 30 min to remove insoluble matter. Two volumes of ethanol were added to the still turbid solution in the presence of 2% potassium acetate[6], and the crude RNA was precipitated at —5° over 18 h. The pellet formed was homogenized with about 100 ml of cold water in a glass homogenizer, and centrifuged for 20 min at 78,410 × *g* for 30 min. The clear supernatant was then dialyzed for 5 h against 3 l of cold water. The solution was centrifuged again at 14,000 × *g* for 20 min, and the clear solution was lyophilized (or was used directly for the purification of S-RNA).

The crude sample of RNA obtained was contaminated with polysaccharide to a considerable extent, although this has little effect on the ^{14}C-leucine incorporation into RNA. On electrophoretic examination, polysaccharide stayed at the starting point.

Purification of the S-RNA with Ecteola-cellulose

Crude preparations of RNA derived from 3–5 runs (total absorbancy at 260 mμ through 1 cm optical path = *ca.* 2,500) were dissolved in 100 ml of the cold 0.001 *M*

potassium phosphate buffer at pH 7.1. After removal of insoluble matter by centrifugation, the clear supernatant was mixed with 7.5 g Ecteola-cellulose which had been washed well with the phosphate buffer. The mixture was shaken by hand for 5 min in an ice bath. About 90 % of the u.v. absorbing substance originally present in the crude RNA preparation was absorbed onto the Ecteola, while all the polysaccharides remained uncombined. The Ecteola–RNA complex was washed 3 times with the phosphate buffer to remove any unabsorbed material. The sediment was then mixed with 100 ml of 0.3 *M* $NaClO_4$ at pH 7.1, and centrifuged. The extraction was repeated once more. At this step, the bulk of the u.v. absorbing substances were separated from the Ecteola. The combined extract was centrifuged at 14,000 × *g* for 10 min to remove a small amount of the Ecteola particles. The RNA was precipitated from 67 % ethanol at —20° for 1 h. The precipitate was then dissolved in about 10 ml of water and dialyzed against cold water for 4 h and lyophilized. In Table I, the results of a small-scale recovery experiment are recorded.

The preparation thus obtained was a white powder, and easily soluble in water or salt solutions. No precipitation occurred in 2 *M* NaCl solution in sharp contrast to the case of the high molecular RNA derived from ribonucleoprotein particles. At this stage, electrophoretic examination indicated that contaminating polysaccharides were not detected. A small peak was however detected just ahead of the main RNA

TABLE I

RECOVERY OF U.V. ABSORBING SUBSTANCES IN ECTEOLA TREATMENT OF CRUDE S-RNA

Step	*Total absorbancy at 260 mμ*
Original crude RNA preparation	452
Not absorbed by Ecteola	63.5
Extracted with 0.3 *M* $NaClO_4$, pH 7.1	320**
Not extracted with 0.3 *M* $NaClO_4$, pH 7.1 (by difference)	68.5

1.5 g Ecteola-cellulose used.

* Measured in a cell of 1 cm optical path.
** Completely recoverable by ethanol precipitation.

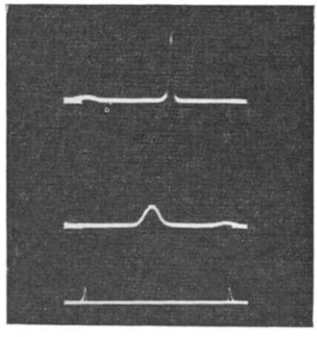

Fig. 1. Electrophoretic patterns of purified S-RNA (Prep. No. S-2): 0.6 % solution in 0.1 *M* NaCl–0.03 *M* Tris buffer, pH 7.1; photographs taken after 60 min: top, ascending pattern; bottom, descending pattern.

peak. The fast component, which presumably consisted of breakdown products of RNA, could be completely removed by a further 24 h dialysis against water or 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1. Thus an electrophoretically homogeneous sample of RNA was obtained (Fig. 1). The RNA prepared was usually monodispersed in the sedimentation analyses (Fig. 3).

The N/P ratio of the product was 1.63. The DNA contamination of the most pure sample was around 0.2 % relative to RNA as determined by microbiological assay of deoxyribonucleosides using *Lactobacillus acidophilus* R-26[7]*.

Comment: A rapid Ecteola treatment seems to be essential for the successful preparation of S-RNA. Extensive degradation and inactivation of RNA was observed when column chromatographic fractionation of the RNA on Ecteola was tried. RNA prepared from ribonucleoprotein particles was also absorbed onto Ecteola, but could not be removed with 0.3 *M* $NaClO_4$.

The amount of contaminating DNA in the S-RNA preparation was usually less than 1 %. It was however found that one preparation (Prep. No. S-5) contained about 4 % of DNA. Because of the serious effect of the contaminating DNA on the viscosity

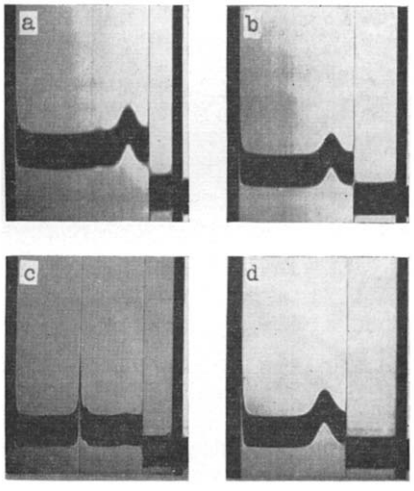

Fig. 2. Sedimentation patterns of S-RNA, H-RNA, and H-RNAhe: a, S-RNA (Prep. No. S-5) contaminated by DNA; b, same preparation as "a" after treatment with deoxyribonuclease (Prep. No. S-5b); c, H-RNA (Prep. No. H-3); d, H-RNAhe (Prep. No. H 3 he). All in 0.1 *M* NaCl–0.03 *M* Tris buffer, pH 7.1; photographs taken approximately 32 min (a), 40 min (b), 20 min (c) and 48 min (d) after reaching 56,900 rev./min; bar angle: a, 60°; b, 70°; c, 75°; d, 75°.

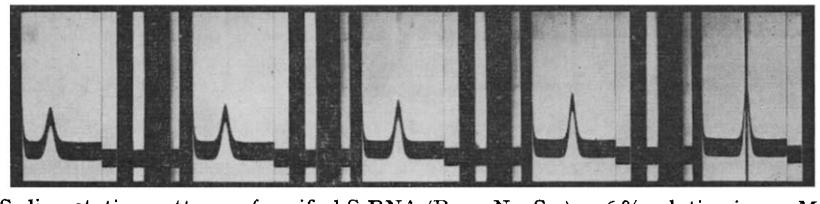

Fig. 3. Sedimentation patterns of purified S-RNA (Prep. No. S-2): 1.6 % solution in 0.1 *M* NaCl–0.03 *M* Tris buffer, pH 7.1; photographs taken at 8 min intervals at 56,900 rev./min and 24°; bar angle, 80°; synthetic boundary cell (Photograph by Dr. M. TAKANAMI).

* Generously performed by Drs. R. and T. OKAZAKI of our institute.

behavior of the S-RNA preparation, it is desirable to remove DNA from such preparation by deoxyribonuclease. Contaminating DNA appeared as a small peak (about 8 S) in the sedimentation analyses (Fig. 2a). About 0.5 mg of pancreatic deoxyribonuclease (Worthington Biochemical Corp., U.S.A.) was added to 5 ml of 0.3% solution of the same sample as shown in Fig. 2a in 0.001 *M* potassium phosphate buffer (pH 7.1) plus 0.001 *M* $MgCl_2$. The mixture was dialyzed against 3 l of the same solvent in the cold for 4 h. It was then treated with 3 g Ecteola as described above. The RNA released from Ecteola with 0.3 *M* $NaClO_4$ was recovered by ethanol precipitation. The sedimentation pattern shown in Fig. 2b revealed that the 8 S component had been completely removed without any effect on the main S-RNA peak.

Preparation of RNA from ribonucleoprotein particles

About 170 g yeast cells (wet weight) were ground with quartz sand for 20 min. The homogenate produced was suspended in 200 ml CHAO–SCHACHMAN's medium[5] and centrifuged at 6,000 × *g* for 20 min. The sediment was washed with 200 ml of the medium. The combined supernatants were centrifuged for 20 min at 12,000 × *g* in a Servall SS-1 centrifuge. The supernatant was then centrifuged for 120 min at 78,410 × *g* in a Spinco L ultracentrifuge (Rotor No. 30). After decanting the clear supernatant, a slightly yellowish, transparent pellet was collected. It was then homogenized with 80 ml distilled water, and mixed with an equal volume of 90% commercial phenol (w/v). All operations were carried out at 0–2°. The mixture was stirred for 1 h at room temperature (16–22°), centrifuged in the cold at 16,300 × *g* for 1 h, and then the water layer was pipetted out. The remainder was mixed with 50 ml of cold water and centrifuged as above. The RNA was precipitated from 67% ethanol and 2% potassium acetate. The pellet was dissolved in 100 ml of cold water and centrifuged at 78,410 × *g* for 30 min to remove some turbid materials. The RNA was again precipitated as above, washed with 70% ethanol 3 times, dissolved in 30 ml of cold water and finally lyophilized. This sample was designated as H-RNA (high molecular RNA).

Preparation of heat-degraded H-RNA

The method described by TAKANAMI[8] was followed for the preparation of heat-degraded H-RNA. About 100 mg of H-RNA preparation were dissolved in 20 ml 0.2 *M* NaCl, and heated at 90° for 1 h. During the heat treatment, a small amount of contaminating material (presumably proteins) was precipitated. After cooling, the solution was centrifuged at 10,000 × *g* for 10 min to obtain a clear solution. The RNA was precipitated with 2 volumes of ethanol, washed with 70% ethanol 3 times, dissolved in 10 ml of cold water and lyophilized. The sample was called hereafter H-RNAhe.

RESULTS

Sedimentation

The conditions of the sedimentation analyses are given in the legends of Fig. 2 and Fig. 3. As may be seen in the photographs, the S-RNA preparation is usually quite homogeneous, giving a sedimentation constant of $s^0_{20} = 4.0$ S extrapolated from the values obtained at three sample concentrations (Fig. 4). This value is rather close to the mean sedimentation constant of rat liver S-RNA (3.45 S) given in a preliminary

note of GOLDTHWAIT[10], but is considerably higher than that of HOAGLAND *et al.*[11] for the S-RNA of the pH 5 enzyme fraction of ascites tumor (1.85 S). The molecular weight of our RNA preparation calculated from the sedimentation constant and the intrinsic viscosity ($[\eta]$ = 0.05) by the MANDELKERN–FLORY equation[9] is around 23,000, assuming a partial specific volume = 0.58, and a flexible-chain molecular model (Table II). In a preliminary note on this study[2], it was thought reasonable to assume that the molecular shape of S-RNA is a rigid rod because of the relatively small sedimentation constant and the low intrinsic viscosity value in salt solution. However, it is now found that the viscosity behavior in salt and in water (described in the next section) rather conforms to a flexible chain molecule of S-RNA.

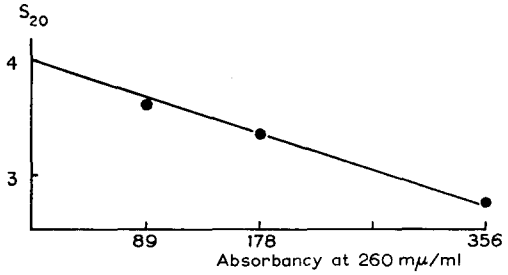

Fig. 4. Concentration dependency of sedimentation constant of S-RNA (Prep. No. S-2).

Similar experiments were also carried out with H-RNA preparations as well as with H-RNAhe for comparison. As seen in Fig. 2c, H-RNA preparations gave three boundaries, the main peak giving the sedimentation constant of 22 S and the two small peaks 19 S and 4–5 S respectively. In this case, the sedimentation constants were not extrapolated to zero concentration. It was found that the proportion of the 22 S to the 19 S component varied to some extent in different preparations. Fig. 2d shows a sedimentation pattern for H-RNAhe.

As observed by TAKANAMI[8] on rat liver ribosomal RNA, the treatment of H-RNA by heat yielded a monodisperse peak of about 4 S. The molecular weight calculated from the sedimentation constant and intrinsic viscosity was about 27,000, comparable to that of S-RNA (Table II).

TABLE II

MOLECULAR WEIGHTS OF S-RNA, H-RNA AND H-RNAhe

All measurements were carried out in 0.1 *M* NaCl–0.03 *M* Tris buffer, pH 7.1.

RNA	*Preparation No.*	S^{0}_{20}	$[\eta]$ *(g/100 ml)*	*Molecular* weight*
S-RNA	S-2	4	0.050	23,000
H-RNA	H-3	(22**)	0.54	(1,100,000)
H-RNAhe	H-3he	4	0.057	27,000

* Calculated from the equation of MANDELKERN AND FLORY[9].
** Main component only. No correction for concentration dependency was made.

Viscosity

Samples were dialyzed overnight against a cold medium. In 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1, the viscosity of S-RNA was found to be much lower than

that of the H-RNA. In both cases, no definite concentration-dependency was observed (Table III).

A remarkable difference between S-RNA and H-RNA was however found when the measurements were made in water as shown in Table III. In the case of H-RNA, the viscosity in water was much higher than in the salt solution and showed a considerable concentration-dependency (*cf.* ref. 12). With a concentration of 0.06 g RNA/100 ml, for example, the viscosity in water is about 22 times as much as that in salt solution. By contrast, the viscosity of S-RNA of a comparable concentration in water was only about 3.8 times as much as that in salt solution.

TABLE III

REDUCED SPECIFIC VISCOSITY OF S-RNA, H-RNA AND H-RNAhe IN SALT SOLUTION AND IN WATER

RNA	*Preparation No.*	*Salt**		*Water*	
		concentration (g/100 ml)	*η (sp./C)*	*concentration (g/100 ml)*	*η (sp./C)*
S-RNA	S-2	0.22	0.05		
		0.13	0.05		
S-RNA	S-5b**	0.14	0.068	0.13	0.21
		0.07	0.068	0.065	0.26
H-RNAhe	H-3he	0.24	0.057	0.13	0.27
		0.12	0.057	0.065	0.32
H-RNA	H-3	0.28	0.54	0.37	3.5
		0.13	0.54	0.22	4.7
		0.065	0.51	0.11	7.4
		0.033	0.59	0.055	11.3
				0.028	17.6

* 0.1 *M* NaCl–0.03 *M* Tris buffer, pH 7.1.

** Treated with deoxyribonuclease. Original S-5 preparation was contaminated with DNA. See "Comment" under "Preparation of RNA" in text.

The results obtained for the H-RNA are in harmony with those reported by other workers[12,13], suggesting that the RNA in solution exists in the form of a randomly-coiled flexible chain. Viscosity measurements on S-RNA indicated that the molecule in solution is also flexible to a considerable extent, although the difference of the viscosity of S-RNA in salt solution from that in water is much less as compared with H-RNA. The apparent less electroviscous behavior of S-RNA does not necessarily mean that the S-RNA is less flexible than H-RNA, but is rather due to the much smaller molecular size of S-RNA. In this connection, it is noteworthy that the H-RNAhe behaved in a similar way as the S-RNA.

U.V. absorption spectra

The u.v. absorption spectra of the S-RNA and that of the H-RNA were examined under three different conditions as shown in Figs. 5 and 6, and Table IV. The main differences of the spectra of S-RNA from those of H-RNA can be summarized as follows: (1) a slightly lower $\varepsilon(P)$ value around the region at 260 mμ under the three conditions studied, and (2) a considerable flattening of the top of the maximum in degraded samples especially in 0.5 *N* KOH. Such spectral differences should be at least in part due to the compositional differences between S-RNA and H-RNA. A higher content of cytidylic acid in S-RNA could explain the shift of spectrum

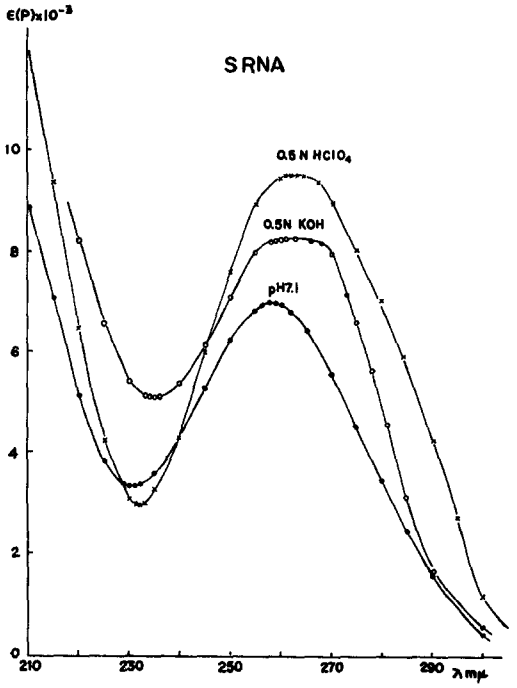

Fig. 5. U.V. absorption spectra of S-RNA (Prep. No. S-2) in 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1, in 0.5 *N* KOH and in 0.5 *N* $HClO_4$.

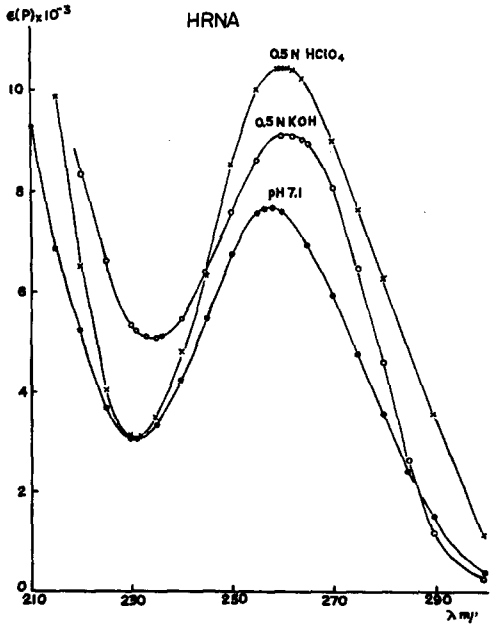

Fig. 6. U.V. absorption spectra of H-RNA (Prep. No. H-1) in 0.1 *M* NaCl–0.03 *M* Tris buffer at pH 7.1, in 0.5 *N* KOH and in 0.5 *N* $HClO_4$.

toward the direction of longer wave length under acidic conditions. Similarly, the predominant occurrence of pseudo-uridylic acid in S-RNA should cause a shift in the same direction under alkaline conditions. Both RNA preparations show a similar hyperchromic effect upon degradation by acid or alkali.

TABLE IV

OPTICAL PROPERTIES OF S-RNA AND H-RNA

Measurements unless otherwise stated were made at room temperature (25°).

Condition	*Sample*	*λmax.*	*ε(P)*	*% increase*	*λmin.*	*ε(P)*	*% increase*
0.1 *M* NaCl–	S-RNA*	258	6948	—	230	3330	—
0.03 *M* Tris, pH 7.1	H-RNA**	258	7620	—	231	3100	—
0.5 *N* KOH	S-RNA	262	8240	18	234	5000	51
	H-RNA	261	9120	20	235	5310	70
0.5 *N* $HClO_4$,	S-RNA	262	9400	35	232	2870	—13
70°, 20 min	H-RNA	261	10300	35	232	3080	— 0.7

* Preparation No. S-2; ** No. H-1.

Nucleotide composition

The nucleotide composition of S-RNA and H-RNA was determined on a Dowex-1-formate column. As seen in Table V, Figs. 7 and 8, the composition of S-RNA is characterized by the presence of a considerable amount of pseudo-uridylic acid, and by a higher cytidylic acid and lower uridylic and adenylic acid contents when compared with the composition of H-RNA. The results confirm those of the preceding papers[1,14]. The first peak in the chromatogram of S-RNA probably represents adenosine which was released from the terminal group of the RNA by alkali[15]. The u.v. spectrum of this peak was similar to that of adenine. No extra peaks were detected in the chromatogram of H-RNA.

TABLE V

NUCLEOTIDE COMPOSITION OF S-RNA AND H-RNA

Sample	*Moles per 100 moles nucleotides*				
	Adenylic	*Guanylic*	*Cytidylic*	*Uridylic*	*Pseudo-uridylic*
S-RNA (S-4)*	19.7	30.0	23.0	22.0	5.4
H-RNA (H-1)*	25.6	29.0	18.6	26.8	trace

* Preparation No.

Test for the ability of the RNA to combine with L-*leucine*

In Table VI is shown the result of an experiment in which the ability of the crude and purified S-RNA preparations to combine with L-leucine was tested under the conditions described previously[1]. The purified S-RNA combined L-leucine in the proportion of 2.86 mμM per mg RNA. The value was about 40% higher than that of the crude S-RNA preparation. The fast moving component (see p. 113) was isolated

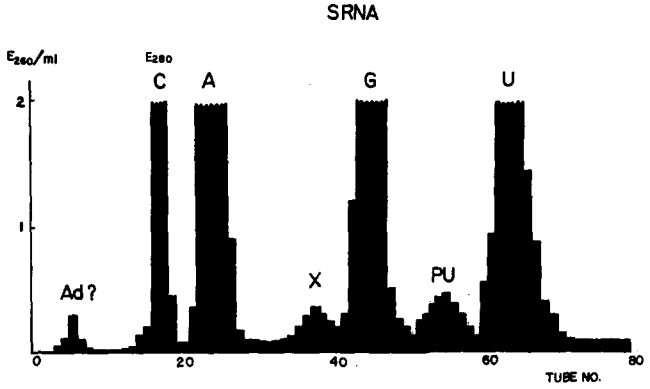

Fig. 7. Chromatographic separation of individual mononucleotides of S-RNA (Prep. No. S-4): Ad(?) = adenosine?; C = cytidylic acid; A = adenylic acid; X = unidentified; G = guanylic acid; PU = pseudo-uridylic acid; U = uridylic acid.

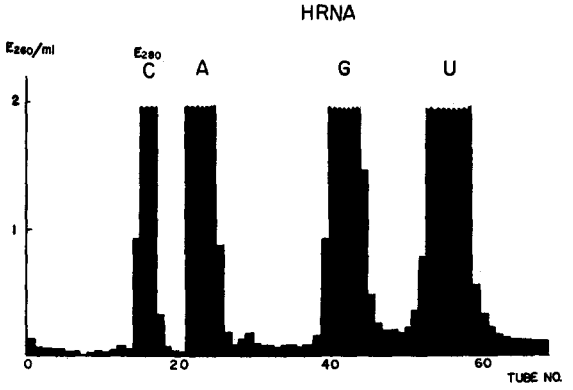

Fig. 8. Chromatographic separation of individual mononucleotides of H-RNA (Prep. No. H-1). For symbols, see Fig. 7.

TABLE VI

INCORPORATION OF [^{14}C]-L-LEUCINE INTO CRUDE AND PURIFIED S-RNA, H-RNA AND H-RNAhe

The system contained 0.2 ml of test material, 5 μM ATP, 3 μM $MgCl_2$, 50 μM Tris buffer (pH 7.6), 0.3 ml of dialyzed yeast cell extract, and 0.41 μM L-leucine (^{14}C-labeled) in a total volume of 1 ml (*cf.* ref. 1). After incubation for 15 min at 30°, the RNA (and protein) was precipitated with cold 67% ethanol–0.5 *M* NaCl[16], washed with this solution 4 times, with absolute ethanol twice, with ether twice, and dried. The radioactivity of the powder was measured as described previously[1].

Samples	*L-leucine linked to RNA mμM/mg RNA*
Crude RNA preparation	2.02
Purified RNA preparation (S-2)*	2.86
Fast moving component	0.00
Substances unabsorbed by Ecteola	0.00
Purified S-RNA (S-8)*	2.90
Purified S-RNA + 0.5 μM CTP	2.55
H-RNA (H-3)*	0.00
H-RNA + 0.5 μM CTP	0.00
H-RNAhe (H-3 he)*	0.00
H-RNAhe + 0.5 μM CTP	0.00

* Preparation No.

by the removal of the peak using a Spinco H electrophoresis-diffusion apparatus*, and was found to be inactive. Substances unabsorbed by Ecteola (see p. 112) were also found to be inactive. These results suggest that the lower value of the crude S-RNA as compared with the purified preparation is due not to the inhibitory action of contaminating materials, but rather to the presence of inactive u.v. absorbing substances which can be removed during the purification procedure.

The ability of H-RNA and H-RNAhe to combine with L-leucine was also tested. As shown in Table VI, both RNAs were found to be completely inactive even in the presence of CTP[15].

DISCUSSION

The function of S-RNA in the transfer of activated amino acids to microsomal ribonucleoprotein particles has been related to the presence of a specific terminal -pCpCpA group[15], and to the occurrence of pseudo-uridylic acid in the molecule[1]. One of the basic requirements for the full understanding of the function on a structural basis would be the preparation of a RNA sample which is (1) homogeneous, (2) unaltered, and (3) having a functional activity not different from that in living cells. The principal purpose of this study has been to establish a technique whereby a RNA sample which satisfies the above requirements can be prepared. The experiments described in this paper have led to the preparation of an active RNA sample which is homogeneous when judged by electrophoretic examination and sedimentation analyses. It is however not known whether the preparation is homogeneous with respect to other criteria. In recent years, it has been suggested that individual amino acids link to the corresponding specific RNA[16-18]. We have not yet tested whether the RNA obtained here is leucine-specific. This is however not likely, because the choice of L-leucine for the activity test was only incidental. Detailed information on the structural basis of the function cannot be obtained until better fractionation of the preparation can be made. The "authenticness" of our sample is also not known. However, a comparison of various molecular properties of the S-RNA preparation and of H-RNA or of H-RNAhe seems, at the present state of development in this field, to have a considerable significance for understanding the nature of the active and inactive molecules.

It has recently been suggested that the high molecular RNA prepared by phenol or detergent methods from animal microsomes or from ribonucleoprotein particles may be composed of smaller sub-units[8,12]. This suggestion stems from the preparation of a "stabilized RNA" by heating high molecular RNA[8,12,19]. We have also prepared such a RNA (H-RNAhe) by heating a solution of H-RNA obtained from yeast ribonucleoprotein particles. If the above explanation is correct, good agreement between the sedimentation constant of S-RNA and that of H-RNAhe could be taken to indicate that the basic unit of all RNA's would have an S value around 4.

The extent of the electroviscous effect in S-RNA is much less than that in H-RNA. As the molecular weight of S-RNA is estimated to be about one fiftieth of that of H-RNA, difference of viscosity behavior between S-RNA and H-RNA does not necessarily show that the H-RNA is much more flexible than S-RNA. The probable explanation is that the S-RNA molecule exists in solution mainly in the form of

* Kindly performed by Dr. K. TAKATA of our institute.

randomly-coiled, flexible chains* like in the case of the H-RNA molecule. In this respect, it is of considerable interest to note that H-RNAhe behaves in a relatively similar way to S-RNA in sedimentation and viscosity measurements. In other words, the macromolecular properties in solution are similar for S-RNA and the "basic unit" (H-RNAhe) of high molecular RNA (H-RNA), so far as the sedimentation and viscosity behavior is concerned.

As indicated in Table V, only RNA capable of combining L-leucine can be classed as S-RNA; neither H-RNA nor H-RNAhe has such an ability. The results imply that the molecular properties observed here in S-RNA, H-RNA, and H-RNAhe have no immediate relation to the amino acid combining activity. HECHT *et al.*[15] reported that the presence of the terminal –pCpCpA group of S-RNA is essential for combination with amino acids, and even after removal of the end group from S-RNA, it is easily reformed in the presence of CTP, ATP, and the pH-5 enzymes so that the S-RNA again becomes able to combine with amino acids. In the case of H-RNAhe (and also H-RNA), the ability to combine with L-leucine is completely lacking even in the presence of CTP and ATP. The results strongly suggest that RNA's other than S-RNA have neither a –pCpCpA terminal group nor the ability to form it. What causes such differences between S-RNA and other inactive RNA's we do not know. One possibility is that the presence of one particular nucleotide or a sequence of several nucleotides next to the terminal –pCpCpA of S-RNA is required for the formation of such a specific end group. In this connection, it is of considerable importance to mention that S-RNA differs from inactive RNA's in its nucleotide compositions. The remarkable feature of the nucleotide composition of S-RNA lies in the occurrence of several additional ribonucleotides such as pseudo-uridylic acid[1,14,20], and methylated guanine nucleotides[21,22] etc. Although their significance is still completely unknown, it is not improbable that these components play a role in forming the specific terminal group of S-RNA.

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* Recent optical rotation measurements generously performed for us by Dr. A. MAEDA of the Chemical Institute have shown that the $[\alpha]D$ value for S-RNA was $+148°$, and the rotation was abolished upon alkaline hydrolysis. If the optical rotation value really can be taken as a measure of the helical content as has been discussed by DOTY *et al.*[23], the S-RNA, like H-RNA[23], has a considerable amount of helical region in the molecule.

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