The 5′-Linked Termini of Plant Ribosomal Ribonucleic Acids*

John M. Halloin,† Clifford J. Pollard, Veryl E. Becker,‡ and Edison R. Fowlks§

ABSTRACT: Adenosine and uridine were the only nucleosides that resulted from the alkaline hydrolysis of unfractionated (18 S + 28 S) ribosomal ribonucleic acid from a fungus (*Agaricus campestris*) and four higher plants: cabbage, cauliflower, parsnip, and spinach. Small amounts of cytidine (5% of the total nucleoside) were found in addition to adenosine and uridine in alkaline hydrolysates of wheat germ ribosomal ribonucleic acid. Periodate oxidation of whole cabbage ribosomal ribonucleic acid (18 S + 28 S), followed by amine cleavage yielded adenine and uracil only. Since these techniques liberate nucleosides and free bases, respectively, from 5′-linked end groups of ribonucleic acid, these ribosomal ribonucleic acids appear to terminate preferentially as adenosine and uridine. Experiments on the individual ribosomal ribonucleic acid subunits from cauliflower, after separation of the ribosomal ribonucleic acid components by sucrose density gradient centrifugation, revealed that adenosine and uridine also terminate both subunits. The efficacy of identifying the nucleosides resulting from the 5′-linked termini of ribonucleates after alkaline hydrolysis and formation of radioactive derivatives is given. Nucleosides were identified spectrophotometrically and by the formation of ^{32}P-labeled phosphates and tritiated trialcohols, formed by the reduction of the products of periodate oxidation of the nucleosides with tritiated sodium borohydride.

Interesting and valuable information has been revealed by studies on terminal groups of ribonucleic acids. Adenosine has been identified as the 5′-linked terminal nucleoside of RNA from tobacco mosaic virus (Sugiyama and Fraenkel-Conrat, 1961, 1963; Whitfeld, 1962, 1965; Singer and Fraenkel-Conrat, 1963; Steinschneider and Fraenkel-Conrat, 1966) and of bacteriophages MS2 (Lee and Gilham, 1965; Sugiyama, 1965) and f_2 (Lee and Gilham, 1965). The findings of a predominance of guanosine 3′,5′-diphosphate as the 3′-linked terminal group of tRNA (Singer and Cantoni, 1960; Herbert and Canellakis, 1960; Zillig *et al.*, 1960; Hudson *et al.*, 1965) and of 23S rRNA of *Escherichia coli* (Nichols and Lane, 1967; Takanami, 1967), and adenosine as the 3′-linked terminus of TMV-RNA (Sugiyama and Fraenkel-Conrat, 1963) and the 16S rRNA of *E. coli* (Nichols and Lane, 1967; Takanami, 1967) are probably related to the recent observation that purines initiate the ribonucleic acid chain in *in vitro* studies with RNA polymerase (Maitra and Hurwitz, 1965). That this is not a universal phenomenon is suggested, however, by findings of a predominance of uridine at the 3′-linked termini of rRNAs from yeast and four species of bacteria (Sugiura and Takanami, 1967), the 5S rRNA from *E. coli* (Rosset *et al.*, 1964; Brownlee and Sanger, 1967) and the 16S subunit from L cells (Lane and Tamaoki, 1967). Furthermore, cytidine was shown to be the 3′-linked terminus of 28S rRNA from L cells (Lane and Tamaoki, 1967).

It has been found that unfractionated rRNA of *E. coli* has mostly adenosine and uridine at the 5′-linked terminus (Lane, 1962; Midgley and McIlreavy, 1966; McIlreavy and Midgley, 1967), and that the 16S subunit terminates primarily as adenosine, whereas the 23S subunit terminates primarily as uridine (Nichols and Lane, 1967; McIlreavy and Midgley, 1967). Other studies have shown that rRNA from L cells has mostly adenosine at the 5′-linked terminus of the 16S subunit and uridine at that terminus of the 28S subunit (Lane and Tamaoki, 1967) and that with rabbit reticulocyte rRNA there are essentially two types of 5′-linked termini on the 30S subunit, one being -pyrimidine-p-U and the other -G-p-U, whereas the 17S subunit terminates as -pyrimidine-p-A (Hunt, 1964, 1965).

Wheat germ rRNA, the only plant rRNA studied prior to our investigation, also provides the only example to date of an RNA on which no preferential termination at either terminus has been observed, as all four nucleoside diphosphates were derived from the 3′-linked terminus in alkaline hydrolysates (Singh and Lane, 1964; Lane, 1965) and all four nucleosides were similarly derived from the 5′-linked terminus (Lane and Allen, 1961; Singh and Lane, 1964; Lane, 1965; Lee and Gilham, 1965). Information concerning the termini of rRNA has been reviewed by Madison (1968).

It is apparently impracticable to probe in a direct manner the function of rRNA by existing methods. In view of the fact that sequences common to all rRNA

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† Present address: Department of Plant Pathology, University of Wisconsin, Madison, Wis. 53706.

‡ Present address: Department of Botany and Plant Pathology, Oregon State University, Corvallis, Ore.

§ Present address: Department of Molecular Biology, University of California, Berkeley, Calif.

may be responsible for biological activity, it was deemed desirable, as an initial effort, to identify the terminal groups of rRNA from several plant species. Also, since it has been amply documented that alkaline hydrolysis of ribonucleate chains results in the production of nucleosides solely from the 5′-terminal groups, it was thought of value to utilize techniques for the identification of such groups through the formation of radioactive derivatives after hydrolysis of the phosphodiester chain. It was toward these objectives that this study was directed.

The central finding reported here demonstrates that plant rRNA chains terminate preferentially as adenosine or uridine, and at least in cauliflower, there is no apparent preference of one over the other in the individual subunits. The six species examined here represent only a minute fraction of the diversity of biological material. In view of the consistency of the data it seems improbable that the results are fortuitous.

Some insight into the biological relevance of our findings may be related to the occurrence of enzymes capable of attaching the end groups found here (adenosine or uridine) to rRNA (Klemperer, 1963a,b; Page *et al.*, 1967). In fact, the significance of our findings cannot be assessed at the present time because of a general dearth of information on the biosynthesis, structure, and function of rRNA. An aspect of rRNA is brought into question as a result of the experiments of Hunt (1965), Lane (1965), and Midgley and McIlreavy (1967) as well as our own, namely, the question of homogeneity of the phosphodiester chains. Although the concept of single, homogenous chains in the individual subunits appears to be generally accepted, to our knowledge there is no evidence to rule out the existence of heterogeneous rRNA chains, as is the case for tRNA.

Materials and Methods

Preparation, Separation, and Hydrolysis of RNA. Plant tissues used in these studies, cauliflower (*Brassica oleracea*, L. var. botrytis, L.), cabbage (*B. oleracea*, L. var. capitata), mushroom (*Agaricus campestris*, L. ex Fr.), and wheat germ (*Triticum aestivum*, L.), were purchased from local commercial sources.

The following procedures were employed for the preparation of cytoplasmic rRNA. Equal quantities of chilled plant tissue and homogenizing medium (w/v) were homogenized in the cold in a Waring Blendor at one-half line voltage for 2 min. The homogenizing medium comprised 0.01 M magnesium chloride, 0.01 M calcium chloride, 0.25 M sucrose, and 0.05 M Tris (pH 7.8). Usually 300–400 g of tissue was homogenized in each batch. The homogenate was filtered through two layers of cheesecloth and the filtrate was utilized as homogenizing medium for two to three more successive batches of plant tissue under the conditions described above. The final filtrate from the cheesecloth was refiltered through four layers of facial tissue, the residue was discarded, and the filtrate was centrifuged at 15,000*g* for 15 min. The supernatant solution was recentrifuged at 105,000*g* for 30 min with a no. 40 rotor in a Spinco Model L ultracentrifuge. The microsomal pellet which resulted was then homogenized in a hand-driven all-glass homogenizer with solution A (1×10^{-3} M magnesium chloride, 5% sodium dodecyl sulfate, 0.1 M sodium acetate, and 0.28 M lithium sulfate (pH 6.0) prepared after McCarthy and Hoyer, 1964). After dissolution of the pellet the resulting solution was homogenized with an equal volume of 80% phenol, the aqueous layer collected after centrifugation, and the phenol deproteinization step repeated. The ribonucleic acid was precipitated from the aqueous layer in the cold by the addition of an equal volume of ethanol. sRNA and other contaminants were removed by treating the precipitate twice with cold 3 M sodium acetate (pH 6.0) (Kirby, 1964); the rRNA was collected by centrifugation. In a typical experiment 3–4 kg of cauliflower yielded approximately 250 mg of rRNA. Yields from cabbage, spinach, and parsnip were much lower. Mushrooms gave higher yields of RNA than higher plants. sRNA was prepared from the precipitate which resulted from treatment of the postmicrosomal fraction (105,000*g* supernatant solution) with an equal volume of ethanol. It was extracted and deproteinized by the same method used for rRNA; however, the material *soluble* in cold 3 M sodium acetate (pH 6.0) was collected by ethanol precipitation.

RNA was also prepared directly from tissue by the technique utilized to extract cytoplasmic rRNA from the microsomal pellet. Solution A and 80% phenol, two volumes of each per gram of tissue, were employed. The RNA was of high apparent optical purity (optical density 260/280 = 1.95–2.15). Estimations of RNA were based on the assumption that 1 mg of RNA gives 24 optical density units at 260 mμ.

Separations of RNA were effected by sucrose density gradient centrifugation. From 12 to 50 optical density units (260 mμ) per bucket was centrifuged in ordinary runs whereas approximately 100 optical density units/bucket was centrifuged in preparative runs. Linear gradients (4–20% sucrose) were prepared either in 0.15 M sodium chloride–0.015 M sodium citrate (pH 7.0) or in a solution of 0.05 M sodium chloride, 0.05 M sodium acetate, and 0.0001 M magnesium chloride (pH 5.3), and centrifuged for 13–18 hr at 23,000 rpm with a Spinco 25.1 rotor. Recycling of the RNA subunits was effected without precipitation of the RNA after dialysis overnight against the buffer used to prepare the gradients.

Unless otherwise noted, nucleic acids were hydrolyzed in 0.3 N KOH at 37° for 21–24 hr, adjusted to pH 7.0 in the cold with Dowex 50-X2-400 H^+, the mixture was centrifuged, the resin was washed with water, and the diluted hydrolysate was applied to a 2 × 15 cm column of Rexyn 201, Cl^- (Fisher Scientific Co. quaternary amine resin). In the hydrolyses the ratio of RNA:KOH did not exceed 1 mg of RNA/ml of KOH solution. Material which came off the column during loading, during a water wash, and before 0.003 N HCl began to elute cytidylic acid, was taken as the nucleoside fraction. The combined fractions were evaporated to dryness at 60° under reduced pressure, the residue was extracted with boiling pyridine, and the pyridine was evaporated at reduced pressure. Small quantities of concentrated NH_4OH were added to the residue and reevaporated to ensure total removal of the pyridine.

All nucleic acid derivatives were located on paper

chromatograms with ultraviolet light. For quantification the areas were eluted with 0.1 N HCl. Spectra obtained were usually those expected of pure products. However, in experiments with the nucleoside fraction from rRNA, other ultraviolet-absorbing material was often present in the guanosine area of chromatograms of system C (see below). This material differed from guanosine in its chromatographic mobility; in acidic solvents it was resolved into several spots.

Chromatography and Electrophoresis. Paper chromatography was carried out by the descending method in the following solvent systems: (A) isopropyl alcohol–concentrated hydrochloric acid–water (65:16:19, v/v), (B) 1-butanol–formic acid–water (77:10:13, v/v), (C) 75% ethanol–10% saturated ammonium sulfate impregnated paper, (D) isopropyl alcohol–1% ammonium sulfate with paper impregnated with 1% ammonium sulfate (50:25, v/v), (E) water-saturated butanol, (F) water-saturated butanol with ammonia-saturated atmosphere, (G) isopropyl alcohol–concentrated ammonia–0.1 M boric acid (70:10:20, v/v), (H) isobutyric acid–concentrated ammonia–water (66:1:33, v/v), and (I) *t*-butyl alcohol–formic acid–water (70:15:15, v/v).

Since this work has involved the separation of a variety of nucleic acid derivatives, we have elected to list several generalities and pertinent facts concerning the chromatography of these substances. (1) Congeners of all the common purine and pyrimidine bases are separated in systems C and D; the rate of migration in decreasing order being U > A > G > C. Separations are also effected in system A; the order being U > C > A > G. (2) For the purpose of this report the 2′- and 3′-monophosphates are considered as a single congener since these isomers of a given nucleoside are not separated in the systems above. After chromatography in system C (14-hr duration) the distances traversed by adenosine 2′(3′)-phosphate, adenosine 5′-phosphate, uridine 2′(3′)-phosphate, and uridine 5′-phosphate were 24, 20, 34, and 29 cm, respectively. In system D the order of migration is the same but the 2′- and 3′-esters of adenosine overlap with the 5′-ester of uridine. (3) In systems E and F the rate of migration of the adenosine trialcohol is 1.4 and 2.4, respectively, times the rate of migration of the uridine trialcohol. The uridine trialcohol overlaps the cytidine trialcohol in these systems whereas the guanosine derivative migrates slowest. Separation of all four derivatives may be accomplished as mentioned above (systems C and D). (4) System I allowed the separation of nucleotides from *p*-nitrophenyl phosphate and P_i. Nucleotides remained near the origin, whereas *p*-nitrophenyl phosphate and P_i migrated more rapidly. Paper electrophoretic separation of nucleotides was achieved in a Beckman RD-2 electrophoresis unit, using the system of Chandra and Varner (1965), as modified by Becker and Pollard.

Periodate Oxidation–Amine Cleavage of RNA. The most effective conditions which we have found for the production of free bases from terminal groups of RNA were based on the procedure of Neu and Heppel (1964). Thus 200–300 mg of RNA was dissolved in 50 ml of 0.1 M sodium bicarbonate. The solution was made 0.05 M with respect to sodium periodate, 0.4 M with respect to methylamine hydrochloride, adjusted to pH 9.0 with dilute sodium hydroxide, and incubated for 90 min at 45°. The RNA was then precipitated in the cold by the addition of an equal volume of ethanol containing 1% ethylene glycol and 50–100 mg of magnesium chloride. The RNA was collected by centrifugation, washed twice with cold 75% ethanol, and the washings were combined. These were evaporated to dryness under reduced pressure, dilute ammonia was added to the residue, and the solution was reevaporated. The residue was extracted with three portions (20–30 ml) of boiling pyridine and the pyridine was removed under reduced pressure. Extraction of the residue with successive portions of benzene and diethyl ether removed some extraneous material. The residue was dissolved in 5 ml of water, placed on a 1 × 7 cm column of Rexyn 101 H^+ (Fisher Scientific Co. sulfonic acid resin), and the column was developed with 1 N HCl (Cohn, 1949). Fractions of 9 ml each were collected. Uracil was not retained and appeared in the first two fractions. Cytosine and guanine were eluted in fractions 5–10 and 11–16, respectively. Adenine was then eluted with 2 N HCl; it began to appear in the fourth fraction. The fractions were evaporated to dryness and chromatographed on paper in system A. Relevant R_F values for this system are guanine, 0.34; adenine, 0.51; cytosine, 0.62; and uracil, 0.80. Recoveries of free bases (50–100 μg each) added to the oxidation–cleavage mixture in the absence of RNA were greater than 60% and averaged 85%; no preferential losses being observed.

Formation of ^{32}P-Labeled Phosphate Esters. Experiments employing polyphosphate had as their basis the techniques of Weiss *et al.* (1958). A dried sample of the nucleoside fraction resulting from the alkaline hydrolysis of 3–30 mg of RNA was reacted with 40 μl of 0.1 M [^{32}P]H_2PO_4 (specific activity 0.75 mCi/μmole) and approximately 5 mg of phosphorus pentoxide. The mixture was allowed to stand for 2 hr in an 80° incubator, 4 ml of water was then added, and the mixture was placed in a boiling-water bath for 45 min. Upon cooling, the sample was diluted with water to 10 ml, centrifuged, and the residue was discarded. The nucleoside phosphates were adsorbed onto approximately 50 mg of Norit A charcoal and washed 20 times with water by centrifugation. After desorption with successive portions of 80% ethanol–1% NH_4OH and 50% ethanol–1% NH_4OH and subsequent removal of the solvents, the products were cochromatographed with authentic samples of nucleoside 3′(2′-), 5′-, and diphosphates. Quantitative recoveries of phosphate esters were realized.

Experiments employing ^{32}P-labeled cyanoethyl phosphate and dicyclohexylcarbodiimide were based on the method of Tener (1961). In both procedures it was established that 0.5 μg of the pyrimidine nucleosides could be phosphorylated and the nucleotides detected with relative ease. The formation of the 3′(2′)- and 5′-phosphates was used to assess the reality of the phosphorylations.

Enzymatic phosphorylation of nucleosides was accomplished through the transfer of ^{32}P from [^{32}P]*p*-nitrophenyl phosphate by nucleoside phosphotrans-

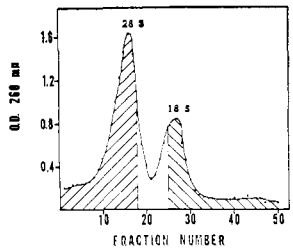

FIGURE 1: Sedimentation profile of a typical preparation of cauliflower RNA. Linear sucrose gradient (4–20%) in 0.15 M sodium chloride–0.015 M sodium citrate (pH 7.0). Centrifugation at 23,000 rpm for 16.5 hr with Spinco 25.1 rotor. Shaded areas collected as purified subunits.

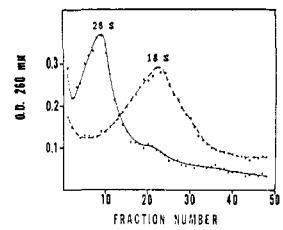

FIGURE 2: Sedimentation profiles of individual RNA subunits from cauliflower. RNA species centrifuged simultaneously in separate sucrose gradients prepared in 0.05 M sodium acetate, 0.05 M sodium chloride, and 0.0001 M magnesium chloride (pH 5.3). Spinco 25.1 rotor; 23,000 rpm for 15 hr.

ferase isolated from carrot leaves, as described by Becker and Pollard (1969). Nucleosides were incubated for 6 hr, at 37°, with 0.1 ml of 0.1 M sodium acetate (pH 5.1), 50 μl of [^{32}P]*p*-nitrophenyl phosphate (15.8 mg/ml, activity = 10,000 cpm/μg), and 0.1 ml of a nucleoside phosphotransferase preparation. Nucleotides were separated from P_i and *p*-nitrophenyl phosphate by chromatography in system I. Radioactivity was determined in these and in the following experiments with a Packard 3003 Tri-Carb scintillation spectrometer. Segments of chromatograms were counted in 15 ml of toluene containing 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene and 2,5-diphenyloxazole (0.3 and 5 g per l., respectively).

Reduction Products of Periodate-Oxidized Nucleosides. In this phase of our studies nucleosides were oxidized with periodate and the resulting dialdehydes were reduced with sodium borohydride. The unlabeled trialcohols, the name these reduction products shall be called henceforth, were prepared as described by Khym and Cohn (1960). The minor modifications adopted for formation of the labeled derivatives were as follows. The nucleoside fractions were oxidized with 50 μl of 0.1 M sodium periodate for 20 min; the isolated dialdehydes were reduced with 1–2 mg of tritiated sodium borohydride (1.4 μCi/mg, Nuclear-Chicago Radiochemical Division) in 0.1 ml of water for 4–12 hr. The reaction was stopped by the addition of excess acetone and the mixture was evaporated to dryness under reduced pressure. Six more successive portions (15–20 ml) each of acetone and of methanol were added to the flask and removed under reduced pressure. Portions of water were added and removed in the same manner until only traces of radioactivity appeared in the distillate. After elution of the trialcohols from Rexyn 101, H^+, they were adsorbed onto charcoal; carrier unlabeled trialcohols were added at this time. The charcoal was washed ten times with water by centrifugation and the products were desorbed with portions of 80% ethanol–1% NH_4OH and 50% ethanol–1% NH_4OH. After evaporation of the ethanolic solution the residue was washed with four to five portions of benzene followed by two portions of diethyl ether. From 70 to 90% recoveries of the trialcohols from the charcoal adsorption-elution steps were realized when the products were desorbed from 0.05 to 0.005 N HCl solutions. The trialcohols were then chromatographed in either systems C, E, or F. Usually the first chromatographic separation resulted in a general distribution of activity over the whole of the chromatogram with some concentrations of radioactivity at the origin in systems E and F, with the front in system C, and in the areas of the adenosine and uridine derivatives. These areas of the adenosine and uridine derivatives were eluted, readsorbed onto charcoal, eluted, and rechromatographed.

Results

Sedimentation Profiles of rRNA. Figure 1 gives the sedimentation pattern on a typical preparation of rRNA. The distribution of the RNA moieties was found to be 67.1% 28 S and 32.9% 18 S for cauliflower RNA. Of importance to these studies was the virtual absence of material in the t- or sRNA areas (4–5 S). Figure 2 demonstrates the profiles of 28S and 18S cauliflower RNA upon recycling the material represented by the shaded areas of Figure 1.

Nucleosides Resulting from Alkaline Hydrolysis of Unfractionated rRNA (18 S + 28 S). The major finding of this aspect of the studies was the isolation of adenosine and uridine only from the nucleoside fraction resulting from the hydrolysis of rRNA from cauliflower, cabbage, spinach, parsnip, and mushrooms (Table I). The nucleosides were estimated after chromatography in system A. The predominance of adenosine over uridine was consistently noted in experiments with cauliflower RNA; however, the reverse was true with rRNA from the other species. Some experimental facts validating the results are as follows. (a) From 50 to 90% recoveries were obtained after subjecting mixtures of nucleosides (50–100 μg each) to the procedures; the average being around 75%. No preferential losses were noted. (b) Sucrose gradient profiles revealed that only 18S + 28S subunits were present. (c) Our data on partially degraded beef liver RNA and on sRNA from cabbage and cauliflower demonstrated that all nucleosides may result from alkaline hydrolysis; the latter results preclude contamination by sRNA as a source of nucleosides produced by rRNA. It is known that 28S RNA from higher plants differs in base composition from the 18S component. Furthermore they are species specific (Pollard, 1964). From the results obtained with RNA extracted directly with solution A–phenol, it appears that all cauliflower cellular rRNA, including that from organelles, terminates as adenosine and uridine since RNA

TABLE I: Nucleosides Resulting from the Hydrolysis of RNA in Typical Experiments.

Source of RNA	Wt (mg)	Expt	Micromoles of Nucleoside Produced				% of Expected Yield
			Adenosine	Guanosine	Cytidine	Uridine	
			18S + 28S rRNA				
Cauliflower	550	5	0.328	n.d.[a]	n.d.	0.189	95
Cauliflower[b]	252	1	0.258	n.d.	n.d.	0.122	151
Spinach	116	1	0.002	n.d.	n.d.	0.048	43
Parsnip	116	1	0.049	n.d.	n.d.	0.086	116
Cabbage	455	3	0.091	n.d.	n.d.	0.318	90
Mushroom[c]	730	1	0.308	n.d.	n.d.	0.691	137
			sRNA				
Cauliflower	20	2	0.002	0.003	0.077	0.004	22
Cabbage	20	2	0.049	n.d.	0.055	n.d.	13
			Partially Degraded RNA[d]				
Beef liver	214	1	0.072	0.097	0.055	0.077	140

[a] n.d. means not detected. [b] Extracted by direct treatment of tissue with solution A–80% phenol. 18S + 28S present only. [c] *Agaricus campestris*. [d] Extracted by direct treatment of tissue with solution A–80% phenol. RNA was distributed throughout sucrose gradient.

from all sources would be extracted under these conditions.

The results of others (Lane, 1962; Lane and Allen, 1961; Lee and Gilham, 1965; Singh and Lane, 1964), who found all four nucleosides in alkaline hydrolysates of wheat germ rRNA, appeared to be different from our findings of only adenosine and uridine in hydrolysates of plant rRNA. In an attempt to explain this difference, wheat germ rRNA was isolated by direct phenol extraction and hydrolyzed both by the methods employed in our laboratory and those described by Singh and Lane (1964). Their techniques differed from ours as follows. (a) The homogenizing medium was 0.05 M phosphate buffer (pH 7.0), rather than solution A. (b) rRNA was precipitated with 1.0 M sodium chloride rather than 3.0 M sodium acetate. (c) Hydrolysis was effected with 1.0 M rather than 0.3 M sodium hydroxide and at room temperature rather than at 37°. (d) The hydrolysate was neutralized with 3 N HCl rather than with Dowex 50 H^+ resin.

Two preparations (I and II) of wheat germ rRNA were made by the procedures employed in our laboratory. Only adenosine and uridine were detected in the hydrolysate from preparation I, whereas 5% of the total nucleoside in preparation II was cytidine, although adenosine and uridine again predominated. The hydrolysate of wheat germ rRNA, prepared and hydrolyzed by the methods of Singh and Lane (1964) (preparations III and IV), were found to contain all four nucleosides, as had been previously reported (Lane, 1962; Lane and Allen, 1961; Lee and Gilham, 1965; Singh and Lane, 1964). These results are presented in Table II.

The sedimentation profile of the RNA from preparation III was identical with that shown in Figure 1; it was therefore unfragmented and was not contaminated by sRNA. Possibly, the use of strong alkali in the hydrolysis as well as the use of acid to neutralize the hydrolysate, conditions known to produce additional nucleosides, caused the appearance of all of the nucleosides in the studies on wheat germ RNA mentioned above and in preparations III and IV.

The yield of nucleosides from preparation I was approximately that which would be expected from molecules with an average weight of 10^6, and only adenosine and uridine were detected. Preparation II yielded 44% more nucleosides than expected and in this case, in addition to adenosine and uridine, cytidine was detected. Yields of nucleosides from preparations III and IV were, respectively, 99 and 76% greater than expected, and in both cases, all four nucleosides were detected.

Ten per cent of the nucleoside fraction from preparation II was incubated with purified carrot leaf–nucleo-

TABLE II: Summary of Data on the 5′-Linked Termini of Wheat Germ rRNA.

Prepn	Mole % of Nucleoside				% of Expected Yield
	A	G	C	U	
I	60	n.d.[a]	n.d.	40	114
II	49	n.d.	5	46	144
III	10	18	31	41	199
IV	28	17	25	30	176

[a] n.d. means not detected.

TABLE III: Reaction of [^{32}P]*p*-Nitrophenyl Phosphate and Nucleoside Phosphotransferase with the Nucleoside Fraction from Wheat Germ rRNA.

Activity	Nucleotide			
	AMP	GMP	CMP	UMP
cpm	74	7	161	554
Per cent	9	1	20	70

side phosphotransferase and [^{32}P]*p*-nitrophenyl phosphate, spotted on chromatography paper, and the chromatogram was developed with system I for 37 hr. Elution of the nucleotide area, followed by chromatography in system C, gave the distribution of radioactivity shown in Figure 3. Subsequent electrophoresis of each of the nucleotide samples individually, showed the distribution of radioactivity presented in Table III. Radioactivity in the guanosine monophosphate area of the chromatogram shown in Figure 3 migrated with cytidine monophosphate in the electrophoretic separation, and was probably due to overlap of the two spots on the chromatogram. These results provide qualitative support for the identity of nucleosides reported for this RNA preparation, as the monophosphates of adenosine, cytidine, and uridine formed the major portion of the product. The relative amounts of radioactivity, however, are probably misleading, as this criterion showed cytidine to be twice as abundant as adenosine, whereas spectral evidence showed that adenosine was more than ten times as abundant as cytidine.

Experiment with Periodate Oxidation–Amine Cleavage of Unfractionated rRNA. Our experiences with this technique appear to corroborate qualitatively the data obtained with alkaline hydrolysis. In six experiments with cauliflower rRNA (200–300 mg) using the Neu–Heppel (1964) procedure, only adenine was detected in each instance. For example, in one experiment employing 285 mg of RNA approximately 5 μg of adenine was isolated, whereas in another 206 mg of RNA yielded 10 μg of adenine. Note from Table I that much more adenosine resulted from alkaline hydrolysis of a similar quantity of RNA. Significantly, in all experiments no other free base except traces of uracil was observed. Employing cabbage rRNA, where uracil occurs as an end group three times as frequently as adenine, more logical qualitative results and higher yields, perhaps due to a greater mastery of the technique, were obtained. Thus from 274 mg of cabbage rRNA 23 μg of uracil and 25 μg of adenine were isolated. Materials with absorption maxima around 260 mμ in acid were detected in both the cytosine and guanine areas. At most it corresponded to 3–4 μg of these compounds, and since the spectra were distinctly different from those of cytosine and guanine they probably did not represent the compounds in question. Low yields of adenine and uracil were also obtained when the technique of Steinschneider and Fraenkel-Conrat (1966) was applied to cauliflower rRNA.

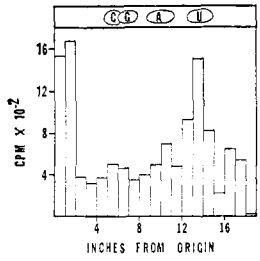

FIGURE 3: Radioactivity on chromatogram, developed in system C, of nucleoside monophosphates resulting from phosphorylation of wheat germ rRNA nucleoside fraction with [^{32}P]*p*-nitrophenyl phosphate and nucleoside phosphotransferase. The nucleotides were chromatographed first in system I, to remove ^{32}P and [^{32}P]*p*-nitrophenyl phosphate. The drawing at the top represents the ultraviolet-absorbing areas of unlabeled standards with which the sample was cochromatographed.

Terminal Groups of Cauliflower 28S RNA. By utilizing three techniques it was possible to show that adenosine and uridine terminate the 28S subunit. In the first place alkaline hydrolysis of 89 mg of 28S RNA resulted in the production of 9.8 μg of adenosine and 15.6 μg of uridine. No other nucleosides could be detected. It should be noted here that the total amount of nucleosides produced is the amount that would be expected from RNA chains with molecular weights of *ca.* 10^6.

Labeling of the nucleoside fraction from 13 mg of 28S RNA with polyphosphate corroborated that uridine is an end group. The appearance of radioactivity in uridine 2′(3′)-monophosphate, uridine 5′-monophosphate, and uridine 2′(3′),5′diphosphate was demonstrated. Since these experiments were similar to the ones done with the 18S subunit and the methods of proof were similar, we shall only relate experiments done with the 18S subunit.

Additional evidence that both nucleosides terminate the 28S subunit was obtained through reduction of the periodate-oxidized nucleoside fraction with tritiated sodium borohydride. Histograms representing radioactivity found in the adenosine and uridine products after the second chromatographic separation in system E are shown in Figure 4. Similar localization of activity in the expected areas was noted when the products were re-

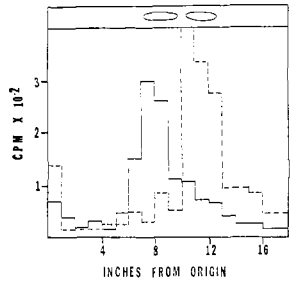

FIGURE 4: Radioactivity in tritiated trialcohols formed from cauliflower 28S RNA nucleosides. Uridine trialcohol (solid line) and adenosine trialcohol (broken line) after second chromatography in adjacent lanes in system E. Encircled areas represent ultraviolet absorption of cochromatographed unlabeled standards.

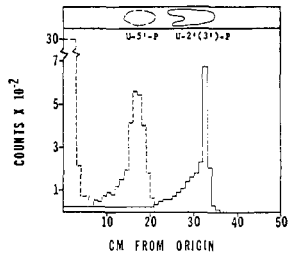

FIGURE 5: Chromatography of uridine monophosphates resulting from phosphorylation of cauliflower 18S RNA nucleoside fraction with [^{32}P]polyphosphate. Isomers chromatographed successively on paper in systems C and G. Encircled areas represent ultraviolet-absorbing areas of unlabeled standards. Counts per 10 min for the 5′ isomer (broken line) and counts per minute for the 3′(2′) isomers (solid line).

chromatographed in systems C and F. The activity shown in Figure 4 represents the terminal groups of approximately 3 mg of 28S RNA.

Terminal Groups of the 18S Subunit. Evidence obtained by phosphorylation with [^{32}P]cyanoethyl phosphate and dicyclohexylcarbodiimide, by phosphorylation with [^{32}P]polyphosphate, and by formation of the tritiated trialcohol derivatives constituted proof of the mixed endings of the 18S subunit.

In the experiment with [^{32}P]cyanoethyl phosphate the nucleosides resulting from alkaline hydrolysis of 2.5 mg of RNA were employed. Radioactivity was retained in uridine 2′(3′)-monophosphate after successive steps of adsorption onto charcoal, chromatography in system C, ion-exchange chromatography on Rexyn 201, Cl^-, and chromatography in system H. No evidence for the production of adenosine phosphates was obtained. We were unable, however, to phosphorylate microgram quantities of authentic adenosine by this technique.

Figure 5 illustrates that radioactivity was present in uridine 2′(3′)-monophosphates and uridine 5′-monophosphate after phosphorylation of the nucleoside fraction from 18S RNA with [^{32}P]polyphosphate. These samples were subjected to successive chromatographic separations in systems C and G. In contrast, when an equal quantity of the phosphorylated nucleoside fraction was chromatographed with cytidine 3′(2′)-phosphates only 15 cpm above background was found in the final product. Likewise, radioactivity was not present as cytidine phosphates after phosphorylation of the nucleoside fraction from cauliflower 28S RNA. Purine nucleoside phosphates are not produced by the polyphosphate procedure.

Finally, Figure 6 demonstrates the distribution of radioactivity upon rechromatography of the adenosine trialcohol formed by reduction with tritiated borohydride of the periodate-oxidized nucleoside fraction from the 18S RNA. The activity represents the adenosine found as terminal groups in approximately 2.3 mg of 18S RNA. The uridine product was also highly radioactive.

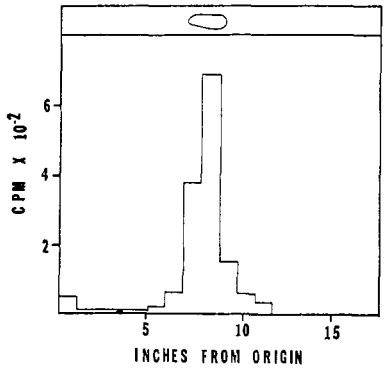

FIGURE 6: Chromatography of tritiated adenosine trialcohol formed from cauliflower 18S RNA nucleoside fraction. Third chromatographic separation of the product in system E. Ultraviolet absorption of unlabeled standard encircled.

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A Computerized Calibration of the Circular Dichrometer*

Joseph Y. Cassim† and Jen Tsi Yang

ABSTRACT: A least-squares parametric curve-fitting method is used to calibrate a circular dichrometer against a standardized spectropolarimeter. An application of this technique is demonstrated by determining the "calibrated" molar circular dichoic absorption, $\epsilon_l - \epsilon_r$, of two camphor derivatives in solution. Maximal values of +2.20 for *d*-10-camphorsulfonic acid in water, +1.54 for *d*-camphor in methanol, and +1.69 for *d*-camphor in dioxane are found at 291, 296, and 300 mμ, respectively. It is concluded that these values may be used as standards for calibration provided some precautions regarding solute and solvent purity are taken.

Circular dichroism is readily becoming a popular technique for conformational studies of biopolymers in solution (see, for example, Beychok, 1968; Yang, 1969; Yang and Samejima, 1969). It complements optical rotatory dispersion which has virtually monopolized the literature over the last decade. Several commercial circular dichrometers are now capable of high-precision measurements over a wide region of the spectrum. However, there is still uncertainty in the calibration of these instruments because of the lack of a universally acceptable standard. In contrast, spectropolarimeters can be calibrated with standard sucrose solutions or with quartz control plates of known rotations. In this communication we present a method of calibration based on a computer program developed by Thiéry (1968). In essence the method consists of calibrating the circular dichrometer against a calibrated spectropolarimeter.

* From the Cardiovascular Research Institute and the Department of Biochemistry, University of California San Francisco Medical Center, San Francisco, California 94122. *Received November 19, 1968.* This work was aided by National Institutes of Health Program Project Grant No. HE-06285 from the National Heart Institute, and U. S. Public Health Service Grants GM-K3-3441 and GM-10880.

† Present address: Academic Faculty of Biophysics, College of Biological Sciences, The Ohio State University, Columbus, Ohio 43210.

Theory

Circular dichroism and optical rotatory dispersion are closely knit phenomena whose interdependency is contained in the Kronig–Kramers relations (Moffitt and Moscowitz, 1959; Moscowitz, 1962; Emeis *et al.*, 1967). Therefore, theoretically it is possible to effect a circular dichroism to optical rotatory dispersion transformation through an evaluation of

$$[M(\lambda)] = (2/\pi)\int_0^\infty [\Theta(\lambda')][\lambda'/(\lambda^2 - \lambda'^2)]d\lambda' \quad (1)$$

where $[M(\lambda)]$ is the molar rotation at wavelength λ, $[\Theta(\lambda')]$ is the molar ellipticity at wavelength λ', and, λ and λ' are the main variable and parameter of integration, respectively. (An analogous equation can be used to transform optical rotatory dispersion to circular dichroism.) However, eq 1 requires that the integration be carried over all the optically active bands. In practice, for obvious reasons this can never be realized. This difficulty may be circumvented by applying eq 1 over a definite domain and representing bands outside this domain with the well-known Drude equation. It is necessary that these bands be sufficiently distant from the domain to warrant the applicability of the Drude equation in the region of the domain. Under these condi-