ISOLATION AND SOME PROPERTIES OF BAMBOO SHOOT t-RNA[1]

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In the course of biosynthesis of protein, the RNA* species existing in the soluble fraction of cell extracts plays the important role of transferring activated amino acids onto ribosomes where the amino acids are incorporated irreversibly into the grwoing peptide chain (Hoagland *et al*, 1958; Zachau *et al*, 1958; Hecht *et al*, 1959; Lipmann *et al*, 1959; Preiss *et al*, 1959; Monier *et al*, 1960; Zamecnik *et al*, 1960; Cantoni *et al*, 1662; Yu and Zamecnik, 1963). Because of the nature of its function, the RNA is generally called transfer RNA (t-RNA), although it is also sometimes referred to as soluble RNA (s-RNA) or amino acid-acceptor RNA.

In the past two decades, investigations done on this type of RNA have been extensive. It has been definitely established that they are single-stranded molecules consisting of about eighty nucleotide residues and having molecular weight of about 25,000 (Tissieres, 1959). They also differ from other types of RNA by having methylated bases (Davis *et al*, 1959; Dunn, 1959) thymine (Dunn, 1959; Davis *et al*, 1959) and an uncommon nucleoside, 5-ribosyl uridina or pseudouridine (Dunn, 1959; Davis *et a'*, 1959). Although t-RNA's have specificity toward amino acids, it has been known that they have at the 3'-hydroxyl ends a common sequence of bases, -CCA (Zachau *et al*, 1958; Hecht, 1958). These known facts are mostly, if not exclusively, obtained from studies of t-RNA's of animal or microbial origin. Literatures dealing with t-RNA's from plant sources are, to our surprise, relatively few (Glitz and Dekker, 1963). It seems, therefore, quite essential to undertake a detailed investigation of this particular, biochemically important species of RNA in plants.

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* The following abbreviations are used: RNA, Ribonucleic acid; ATP, Adenosine triphosphate; CTP, Cytidine triphosphate; Tris, Tris (hydroxymethyl) aminomethane; GSH, Reduced glutathione; DEAE, Diethylaminoethyl; TCA, Trichloroacetic acid; PCA, Perchloric acid.

It is postulated that the non-photosynthetic bamboo shoots, being a tissue of rapid growth, may possess high activity of protein synthesis and hence high content of t-RNA. Meanwhile, this popular vegetable in chinese cuisine may offer better experimental conditions since it has been shown to be devoid of starch and pectic substances (Su, 1965). The present communication describes the isolation and characterization of t-RNA from the shoots of bamboo, *Leleba oldhami*.

Materials and Methods

Unless otherwise indicated, all operations were carried out in a cold room at 2-4°.

Protein content was determined by the phenol method of Lowry *et al* (1951), using bovine serum albumin as the standard. Ribose was determined according to Dische (1955). Phosphorus was estimated by the method of Fiske and Subba-Row (1925).

Centrifugations were done either with a Servall superspeed centrifuge, KSB-1 or a Beckman Spinco, model L. UV absorption spectrum was obtained with a Perkin-Elmer 202 recording spectrophotometer. Optical densities were measured with a Beckman DU spectrophotometer.

Yeast t-RNA was purchased from Schwarz BioResearch, Inc., Orangeburg, New york. C^{14}-Chlorella protein hydrolysates were gifts from Dr. A. Tsugita of Osaka University, Japan, and Dr. B. Maruo of the University of Tokyo, Japan. Other materials used were obtained from commercial sources.

Bamboo shoots used were obtained from the local market at Nankang, Taipei. Only the edible portion was used.

Results and Discussion

1. RNA Distribution:

A servey of RNA contents in various cell fractions of bamboo shoots was made by Ts'o and Sato's (1959) procedure. Bamboo shoots were sliced and homogenized in 0.02 M Tris-HCl, pH 7.6, containing 0.001 M $MgCl_2$. The homogenate was subjected to differential centrifugation so that four subcellular fractions were obtained. Each fraction was then treated with 0.5 N TCA overnight and the precipitate was washed successively with 70% ethanol, 0.1 N PCA in 70% ethanol, 1:2 ether-ethanol at 50° and 0.2 N PCA, and finally extracted with 1.0 N PCA for 72 hours. Aliquots of the extract were taken for determinations of ribose and phosphorus and also for the measurement of absorption at 260 mμ. The optical density units of the RNA was first converted into phosphorus content by the use of E(P)=11,000 (Magasanik, 1955). The amount of phosphorus thus obtained was then converted into μg of RNA by

multiplying 11. This is based on the fact that nucleic acids contain 9% phosphorus (Magasanik, 1955). The residue of each fraction was further extracted with 0.5 N PCA at 75°. Phosphorus and optical density of the hot PCA extract were also analyzed.

The results obtained are summarized in Table I. These results are comparable with those of pea epicotyls (Ts'o and Sato, 1959), but the RNA content in the supernatant fraction of bamboo shoots is about 5.3 times higher than that of pea epicotyls.

Table I. *Amount and Distribution of RNA (μg/g fresh weight)*

Fraction	Cold $HClO_4$ Estimated by			Av.	Hot $HClO_4$ Estimated by		Av.	Total	%
	U. V.	Phos.	Ribo.		U. V.	Phos.			
Nuclei	56.9	47.5	52.9	52.4	13.3	27.5	20.4	72.8	9.5
Mitochondria	102.8	95.0	105.7	101.2	11.8	17.1	14.5	115.7	15.4
Microsome	492.8	428.2	491.3	470.8	19.7	13.3	16.5	487.3	63.3
Supernatant	97.8	66.6	71.7	87.7	16.7	7.7	14.4	93.1	12.1

2. Isolation and Purification of Bamboo Shoot t-RNA:

Bamboo shoots were homogenized with 0.5 volume (v/w) of 0.02 M Tris-HCl containing 0.01 M magnesium chloride, pH 7.6, in a Waring Blendor, at top speed, for 1 minute. After squeezing through two layers of cheese cloth, the extract was centrifuged at 5,000×g for 10 minutes. The supernatant liquid was collected and centrifuged at 78,000×g for three hours. To the clear supernatant solution was added equal volume of 80% phenol. After having strirred for one hour, the mixture was allowed to separate for 24 hours. The upper aqueous phase was collected by decantation and extracted twice more with 0.5 volume of 80% phenol. To the final aqueous layer, 0.1 volume of 20% potassium acetate, pH 5.0, was added, followed by the addition of 2.5 volumes of 95% ethanol. The precipitate obtained was dissolved in 0.02 M NaCl solution. At this stage, any insoluble material was removed by centrifugation. The solution was brought to pH 10.0 with 1.0 N NaOH and kept at 37° for 30 minutes to split off amino acids esterified to the t-RNA. The solution was then neutralized with 1.0 N HCl and passed through a G-25 Sephadex column to remove any residual phenol and contaminants with small molecular weight. Since Phenol shows strong absorption at 220 mμ and the value of A_{220}/A_{260} does not exceed 0.8 for phenol free preparation of nucleic acids (Gross *et al*, 1965), this ratio was taken as a measure of the appearance of phenol in the eluates from the gel column. Accordingly, the effiuents with an A_{220}/A_{260} of less than 0.8 were pooled and the t RNA was precipitated with 2 volumes of 95% ethanol. The precipitate was dissolved in a small amount of 0.02 M

Tris-Hcl, pH 7.6, and applied on a DEAE-cellulose column previously equilibrated with 0.02 M Tris-Hcl, pH 7.6. The column was washed with three bed volumes of 0.3 M KCl in 0.02 M Tris-HCl, pH 7.6. The RNA was then eluted from the column with 1.0 M KCl in Tris-HCl, precipitated with ethanol, and dehydrated successively with ethanol, ethanol-ether (1:2), and ether, followed by drying over $CaCl_2$ in vacuum. From one kilogram of fresh bamboo shoots, about 45 mg of t-RNA could be obtained. The yield is thus about 50%.

3. Properties of Bamboo Shoot t-RNA:

(1) Absorption Spectrum—The absorption spectrum of t-RNA in the ultraviolet region is given in Fig. 1.

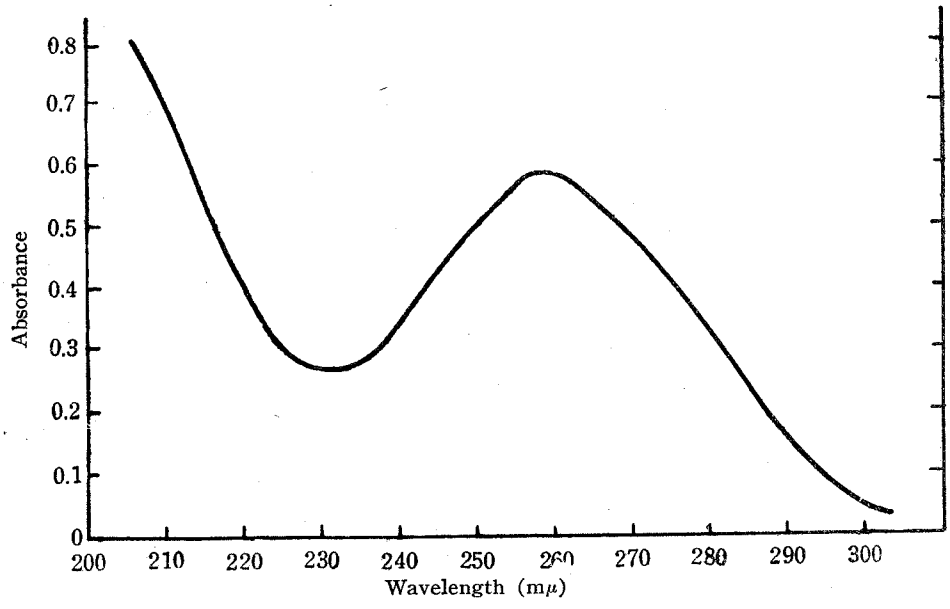

Fig. 1. Ultraviolet absorption spectrum of t-RNA (29.0 μg/ml in distilled water, light= 1cm). The maximun of the absorption lies at 258.5 mμ.

(2) Phosphorus and Protein Content, and Specific Extinction Coefficient—The results of estimation of phosphorus and protein, together with the specific extinction coefficient in distilled water are shown in Table II. Absorption ratios are also included in the table.

Table II. *Phosphorus and Protein Contents and Spectral Data of t-RNA*

Phos.	Prot.	$E^{1\%}_{1cm}$ at 258.5 mμ based on P content of 9%.	A_{230}/A_{260}	A_{250}/A_{260}	A_{280}/A_{260}
8	2.5	236	0.446	0.884	0.504

(3) Sedimentation Properties in a Sucrose Gradient Column and Determination of Base Composition—Sucrose gradient centrifugation was performed

according to Martin and Ames (1961). The 5 to 20% sucrose gradient contained 0.2 M NaCl. Centrifugation was carried out with a Beckman Spinco model L SW-25 rotor at 25,000 r. p. m. for 12 hours. Cytochrome *c* was used as the marker substance. After removal from the rotor, the tubes were punctured with a syringe needle and 5-drop fractions were collected. Usually 41 fractions were obtained. Cytochrome *c* and t-RNA were assayed, after proper dilution, by taking optical density readings at 415 and 260 mμ respectively. From the data obtained, the sedimentation constant of bamboo shoot t-RNA was calculated.

Base Composition was determined according to the method of Katz and Comb (1963). Only the relative amount of the four nucleotides, adenylic acid guanylic acid, cytidylic acid, and uridylic acid, were determined. The results are summarized in Table III. While the mole ratio of uridylic acid is very high, that of adenylic acid is surprisingly low. This seems to be one of the characteristic figure of bamboo shoot t-RNA.

Table III. *Nucleotide Composition and Sedimentation Constant*

Nucleotide composition (mole/100 moles)	Adenylic acid 10.2	Uridylic acid 34.8	Guanylic acid 27.9	Cytidylic acid 27.0
Pu/Py	0.62			
6-amino/6-keto	0.59			
S_{20}, w	4.4			

(4) Biological Activity of t-RNA—The ability of bamboo shoot t-RNA to bind amino acids was studied by the procedure of Berg and Ofengand (1958). Aminoacyl-t-RNA synthetase was prepared from yeast and from bamboo shoots by the following method: The homogenate was centrifuged at 10,000×g for 10 minutes. The supernatant was collected and subjected to centrifugation at 78,000×g for 2 hours. The upper two-thirds of the supernatant was pipetted out and mixed with DEAE-cellulose to form a loose paste. After standing for at least 10 minutes, the paste was filtered by suction and washed several times with the same buffer used in the preparation of the homogenate until the washings became colorless. The resultant filter cake was then mixed with 0.3 M KCl in 0.02 M Tris-HCl, pH 7.6 to form once more a loose paste, This paste was then filtered by suction. The filtrate was used as enzyme. The experimental conditions and results are presented in Table IV.

Though significant, the activity of the aminoacyl-t-RNA synthetase preparation obtained from bamboo shoots is rather too low. This is due either to the very low cocentration of the enzyme protein in this plant or the enzyme from this plant is extraordinarily unstable. Nevertheless, the results shown in Table IV clearly indicate that bamboo shoot t-RNA is biologically active.

Table IV. *Formation of Aminoacyl-t-RNA Complex**

t-RNA	Aminoacyl-t-RNA synthetase	cpm/unit t-RNA
Yeast	Yeast	1,100
Bamboo shoots	Yeast	667
Bamboo shoots	Bamboo shoots	103

* The complete reaction mixture contained, in a total volume of 0.65 ml, 100 μmoles of Trish-HCl, pH 7.6, 5 μmoles of $MgCl_2$, 5 μmoles of KCl, 5 μmoles of GSH, 3 μmoles of ATP, 0.3 μmole of CTP, 0.5 μc of C^{14}-Chlorella protein hydrolysate 2-6 units (O. D. 280× vol. in ml) of emzyme solution, and 4-8 units (O. D. 260× vol. in ml) of t-RNA. The mixture was incubated at 37° for 20 minutes. Aminoacyl-t-RNA was then precipitated by the addition of 4 ml of a precipitating solution consisted of 67% ethanol in 0.5 N NaCl. The precipitate was washed three times with the same solution and then dissolved in 1 ml of 1.5 N NH_4OH, transferred to a stainless steel planchet, dried, and counted in a windowless gas flow counter.

Gathering from the results obtained, the following conclusions may be reached: (a) Protein synthesis in bamboo shoots must be very active since the t-RNA content is high. (b) Bamboo shoo t-RNA can be prepared in quantity. Consequently, this plant may serve as a good source for obtaining various t-RNA's. (c) The nucleotide composition of bamboo shoot t-RNA is strikingly different from those of t-RNA's isolated from other sources, though other properties are similar.

竹筍傳遞核酸 (t-RNA) 之分離及其性質

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竹筍中 t-RNA 之含量爲其全部 RNA 含量之 12.1%，較豌豆上胚軸中之 t-RNA 含量，約高 5.3 倍。自竹筍中分離出 t-RNA 並測定其各種性質之結果，知其核苷酸組成頗爲特殊，但其他性質則與其他來源之 t-RNA 者類似。

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