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CHARACTERIZATION OF THE *t*RNA AND AMINOACYL-*t*RNA SYNTHETASES OF HEALTHY AND TUMOROUS CALLUS TISSUES FROM *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; *Phaseolus vulgaris*; Leguminosae; crown gall tumor tissues; *t*RNA; aminoacyl-*t*RNA synthetases.

Abstract—Aminoacyl-*t*RNA synthetases extracted from healthy and crown gall tumor tissues (induced by *Agrobacterium tumefaciens* strain B6) from *Nicotiana tabacum* (strain Wisconsin 38) grown *in vitro*, showed the same ability to charge *Phaseolus vulgaris* *t*RNA, for all the 15 amino acids tested. For each amino acid, optimal charging conditions (enzyme concentration, Mg^{2+}/ATP ratios, K^+ ion effects) have been determined with *Phaseolus vulgaris* *t*RNA and were found to be the same whether aminoacyl-*t*RNA synthetases from healthy or tumor tissues were used. In each case, valyl- and glutamyl-*t*RNA synthetases were very sensitive to an excess of Mg^{2+} and K^+ ions. Although *t*RNA's extracted from healthy and tumor tissues gave the same electrophoretic patterns, charging levels obtained with tumor *t*RNAs were generally 45% higher than those obtained with *t*RNA's from healthy tissues.

INTRODUCTION

Crown gall is a cancer induced by the bacterium *Agrobacterium tumefaciens* on wounded dicotyledons. These tumorous tissues are able to grow on a synthetic medium deprived of any plant hormone [1]. There is some evidence for the transmission of genetic material from the bacterium during tumor induction [2–7]. Srivastava [8] showed that there were more methyl groups in the nucleic acids from tumors than those from healthy tissues, but the same cytokinin content was found by Johnson *et al.* [9] for *t*RNA's from both types of tissues. In this paper, we have compared the aminoacyl-*t*RNA synthetases and the *t*RNA's from tumorous and healthy tissues of *Nicotiana tabacum* grown *in vitro*.

RESULTS

Growth of tissues

Crown gall tissues were maintained on a synthetic medium without any plant hormone, and healthy tissues on a medium complemented with NAA (naphth-l-ylacetic acid) and kinetin (6-furfurylaminopurine). After 45 days in the dark (to prevent chloroplast formation) at 25°, an inoculum of 0·5 g (wet wt) gave a callus of 5–7 g in the case of tumors, and of 1–2 g in the case of healthy tissues.

Aminoacyl-tRNA synthetases and tRNA preparation

To prevent phenol oxidation, 0·4 M borate buffer pH 7·6 was used for the extraction of aminoacyl-*t*RNA synthetases [10–11]. Extraction and purification of *t*RNA from *Phaseolus vulgaris* was made according to Burkard *et al.* [12]. The method of Guderian *et al.* [13] was used for the extraction of *t*RNA from healthy and tumor tissues from *Nicotiana tabacum*. From 100 g of fresh bean hypocotyls, 10–15 mg of *t*RNA was generally obtained; 1–2 mg and 3–5 mg of *t*RNA were obtained from 100 g of fresh tumor and healthy tissue respectively. *Phaseolus* *t*RNA was extracted from 3-day-old, dark-grown hypocotyls; 45-day-old *Nicotiana* tissue served as a source for *t*RNA and aminoacyl-*t*RNA synthetases. These *t*RNA's showed the same electrophoretic pattern on 8% polyacrylamide gels (Fig. 1); identical patterns were obtained for *Phaseolus* *t*RNA preparations (unpublished results).

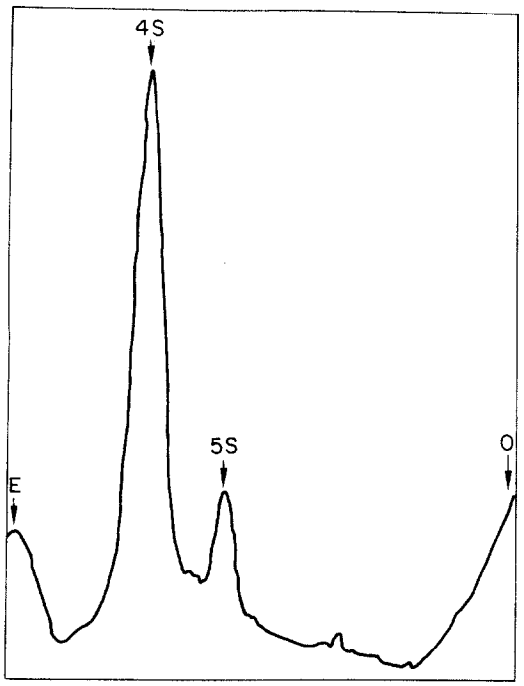

Fig. 1. Electrophoresis on 8% polyacrylamide gels of healthy and tumor tissue *t*RNA preparations. From left to right, arrows indicate successively, the origin, 5S RNA, 4S RNA, and the end of the gel.

Enzyme characterization

In this study, *Phaseolus* *t*RNA served as common substrate for both enzyme preparations. Table 1 shows that the individual and the total level of charge obtained for 15 amino acids is the same regardless of the enzyme source. Depending on the amino acid tested, optimal Mg^{2+}/ATP ratios ranged from 1 to 2·5; for a given amino acid, the enzymes from both sources had the same optimal Mg^{2+}/ATP ratios, and showed the same sensitivity to increasing concentrations of Mg^{2+} in the incubation medium. Valyl- and glutamyl-*t*RNA synthetases were the most sensitive to increasing concentrations of Mg^{2+} ions, showing an inhibition of 70–80% at a Mg^{2+}/ATP ratio of 5 (Fig. 2); Burkard *et al.* [12] have shown the same sensitivity in the case of valyl-*t*RNA synthetase from *Phaseolus vulgaris*.

At optimal Mg^{2+}/ATP ratios, only a slight stimulation (10%) of K^+ could be observed in the case of glutamyl-, isoleucyl- and lysyl-*t*RNA synthetase for KCl concentrations ranging between 0·03 M and 0·05 M. Again, valyl- and glutamyl-*t*RNA synthetase were very sensitive to increasing concentrations of K^+ ions, as they were completely inhibited by a K^+ concentration of 0·3 M

Table 1. Incorporation of amino acids on *Phaseolus vulgaris* *t*RNA

	Tumor enzymes (nmol of amino acids for 1 mg of *t*RNA)	Healthy tissue enzymes (nmol of amino acids for 1 mg of *t*RNA)
Alanine	0·80	1·09
Arginine	1·04	1·10
Asparagine	0·39	0·30
Aspartic acid	0·65	0·59
Glutamic acid	1·17	1·02
Glycine	1·50	1·58
Histidine	0·27	0·25
Isoleucine	1·05	1·21
Leucine	2·11	2·24
Lysine	2·75	2·96
Phenylalanine	1·28	1·22
Proline	0·58	0·48
Threonine	0·56	0·47
Tyrosine	1·70	1·49
Valine	1·95	1·99
*t*RNA charged (%) Total	44·97%	44·50%

Each value is the average of at least three experiments made with different enzyme and *t*RNA preparations. Percentage estimations were made, assuming MW 25000 for a *t*RNA molecule.

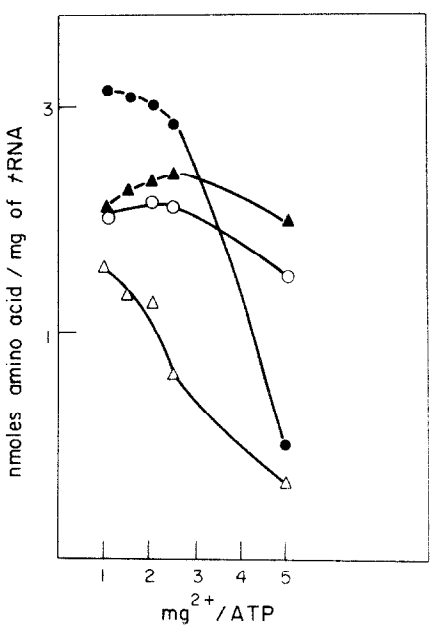

Fig. 2. Effect of increasing Mg^{2+}/ATP ratios on aminoacylation of *Phaseolus* *t*RNA by tumor tissue enzymes: (●) Valine, (△) Glutamic acid, (▲) Glycine, (○) Phenylalanine.

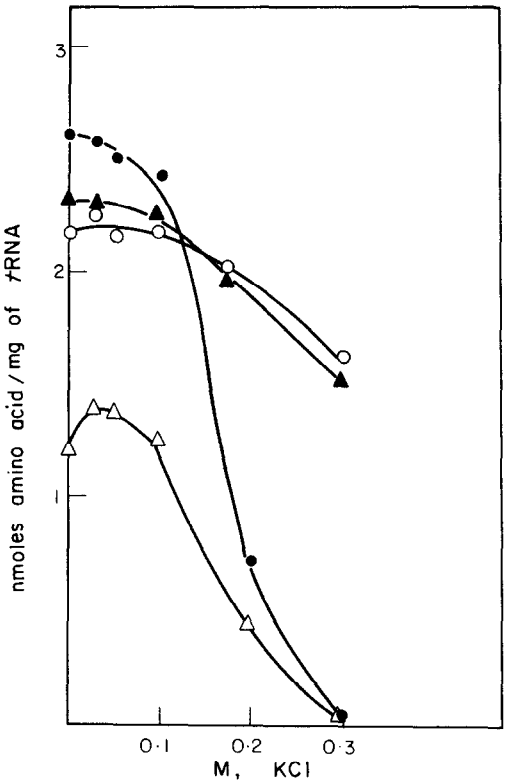

Fig. 3. Effects of increasing KCl concentrations on aminoacylation of *Phaseolus* tRNA by tumor tissue enzymes: (●) Valine, (△) Glutamic acid, (▲) Glycine, (○) Phenylalanine.

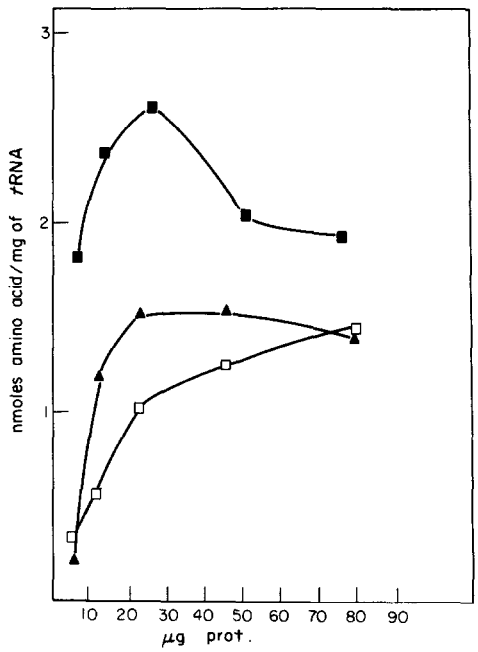

Fig. 4. Effects of tumor enzyme concentration on the aminoacylation of *Phaseolus* tRNA: (■) Lysine, (▲) Glycine, (□) *Iso*leucine.

(Fig. 3). These results are in agreement with those obtained by Lea and Fowden [14].

For a tRNA concentration of 0·2 mg/ml in the incubation medium, charging was maximum with 0·3 mg of proteins per ml of medium, but for Asp, Ile, Thr, and Tyr, 0·6 mg/ml were necessary to reach saturation. When the protein concentration was greater than 0·2 mg/ml, a decrease of incorporation was observed for lysyl-tRNA synthetase (Fig. 4).

tRNA's characterization

Table 2 shows, for 15 amino acids, the extent of charging obtained for tumor and healthy tissue tRNA with homologous synthetase preparations, under optimal conditions. (The extent of charging was calculated from plateau values obtained in kinetic studies). In each case, low levels of incorporation were found for healthy tissue tRNA preparations. Electrophoresis of these tRNA preparations on 12% polyacrylamide gels in the presence of 6 M urea [15] failed to reveal any nuclease-nicked molecules (unpublished results). Addition of CTP to the incubation medium, tested for some amino acids, in the presence of homologous or yeast synthetase preparations (the latter containing nucleotidyl transferase activity) did not enhance the extent of charging (Table 3).

Table 2. Incorporation of amino acids on tumor and healthy tissue tRNA by homologous synthetase preparations

	Tumor tRNA (nmol of amino acids for 1 mg of tRNA)	Healthy tissue tRNA (nmol of amino acids for 1 mg of tRNA)
Alanine	1·71	0·72
Arginine	1·54	0·96
Asparagine	0·82	0·47
Aspartic acid	0·71	0·52
Glutamic acid	1·70	0·87
Glycine	1·99	0·92
Histidine	0·29	0·21
Isoleucine	1·39	0·95
Leucine	2·14	1·28
Lysine	3·53	2·40
Phenylalanine	1·64	0·96
Proline	0·87	0·45
Threonine	1·02	0·71
Tyrosine	1·29	0·54
Valine	1·97	1·33
tRNA charged (%) Total	56·52%	35·70%

Table 3. Effect of the addition of CTP in the incubation medium on the charging of healthy tissue *t*RNA with homologous or yeast aminoacyl-*t*RNA synthetases preparations

Homologous enzymes	−CTP (nmol of amino acids for 1 mg of *t*RNA)	+CTP (nmol of amino acids for 1 mg of *t*RNA)
Glycine	0·90	0·90
Alanine	0·99	1·05
Arginine	0·70	0·74
Phenylalanine	0·40	0·41
Yeast enzymes		
Isoleucine	0·51	0·47
Valine	1·35	1·48

The incubation medium contained, when added, 3 μmol of neutralized CTP. Yeast aminoacyl-*t*RNA synthetase preparations were a gift of Dr. G. Keith.

DISCUSSION

There are a few possible explanations for the differences observed in the extent of charging between tumor and healthy tissue RNA's. Contaminating RNA species may be present in healthy tissue *t*RNA preparations, or the *t*RNA molecules may be nicked or partially denatured. Electrophoresis on 8% polyacrylamide gels revealed the presence of only 5S RNA as a contaminant of the *t*RNA preparations, and in approximately identical proportions in *t*RNA preparations from both sources. It is therefore unlikely that the smaller acceptor activity of healthy tissue *t*RNA is due to a higher content in contaminating RNA species, or in degradation products, since electrophoresis of healthy tissue *t*RNA on 12% polyacrylamide gels in the presence of 6 M urea revealed no degradation.

Attempts to enhance the acceptor activity of these *t*RNA preparations by denaturation and renaturation in the presence of Mg^{2+} [16] were unsuccessful (unpublished results). The CCA–OH terminus was proved to be intact, since the addition of CTP in the incubation medium did not change the degree of charging, even in the presence of yeast enzyme preparations that contain *t*RNA nucleotidyl transferase activity [17]. Therefore, we can exclude the possibilities that the lower acceptor activity of healthy tissues *t*RNA is due to the presence of incomplete CCA–OH terminal triplets, or to partial denaturation of the tridimensional configuration. Work is in progress to determine possible differences in isoacceptor *t*RNA's from those tissues.

EXPERIMENTAL

Plant tissue cultures. Crown gall tissue from *Nicotiana tabacum* (induced by *Agrobacterium tumefaciens* strain B6), and healthy callus tissue were a gift from Professor Morel (I.N.R.A. laboratories, Versailles, France). Tumor tissues were grown on the following synthetic medium: Skoog [18] macronutrients, Heller [19] micronutrients, $FeSO_4 \times 7H_2O$ 13·9 mg/l., Na_2EDTA 18·6 mg/l., myo-inositol 10 mg/l., calcium panthotenate 1 mg/l., nicotinic acid 1 mg/l., pyridoxine 1 mg/l., thiamine 1 mg/l., biotine 10^{-7} M, agar 0·55% (w/v). The healthy tissues were grown on the same medium, except that we used Skoog micronutrients [18]; this medium was complemented with NAA (naphth-1-acetic acid) $2 \cdot 10^{-7}$ M, and kinetin 10^{-7} M.

Radioisotopic material. ^{14}C-labeled amino acids were obtained from the Radiochemical Centre, Amersham and were adjusted to a sp. act. of 20 mCi/mM.

Determination of proteins and tRNA concns. Protein concns were determined by the spectrophotometric method of Layne [20]. The *t*RNA conc was determined assuming that 1 mg of *t*RNA in H_2O were equivalent to an extinction value of 24 at 260 nm [21].

Enzyme preparations. Aminoacyl-*t*RNA synthetases were extracted as previously described [11]: Tissues (50 g) were ground in borate buffer pH 7·6, 0·4 M, 2-mercaptoethanol 5 mM. The slurry was filtered through "tergal" tissue ("Tripette et Renaud" 45μ) and centrifuged for 30 min at 35000 *g*. The proteins of the supernatant were fractionated with $(NH_4)_2SO_4$ between 40 and 70% satn. The 70% pellet was resuspended in 2 ml of Tris HCl buffer pH 7·4 50 mM, 2-mercaptoethanol 5 mM, glycerol 10% (w/v), and filtered through a Sephadex G75 column (40 × 1·5 cm) equilibrated with the same buffer. The fractions from the first protein peak were pooled and conc by dialysis against 100% glycerol. The enzyme preparations were stored at −20°.

tRNA isolation. The method of Burkard *et al.* [12] was used for the extraction of *t*RNA from *Phaseolus vulgaris*; the *t*RNA from *Nicotiana tabacum* tissues was extracted following the method of Guderian *et al.* [13]: after the deproteinization step, the *t*RNA was purified by precipitation of high MW *r*RNA in 1 M NaCl, and DEAE-cellulose chromatography. The *t*RNA was deacylated in Tris HCL buffer pH 8·5 1 M for 90 min at 37°.

Assay procedure. Incubation mixture [12] contained: cacodylate–HCl buffer pH 7·4 50 μmol, bovine serum albumin 10 μg, neutralized ATP 10 μmol, magnesium acetate 1·2·5 μmol, KCl 3 μmol, 2-mercaptoethanol 0·1 μmol, 1-^{14}C amino acids 0·01 μmol, *t*RNA 16–25 μg, enzyme preparation 20–50 μg, in a total volume of 0·1 ml.

After 30 min at 30°, aliquots of 0·08 ml were put on Whatman 3 MM paper discs. After 30 min in cold 10% TCA (trichloracetic acid), the discs were washed 3× in 5% TCA for 10 min, and 2× in alcohol. The discs were dried and counted with 5 ml of 0·4% PPO (2-5-diphenyloxazole) in toluene in a Beckman LS 100 C liquid scintillator.

Gel electrophoresis. Electrophoresis on 8% polyacrylamide gels was performed according to the procedure of Thiollais [22]. After electrophoresis, the gels were scanned directly at 260 nm in a Beckman Acta II spectrophotometer or stained with methylene blue.

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