EFFECT OF DEAMINATION OF THE TERMINAL ADENOSINE OF TRANSFER RIBONUCLEIC ACID ON ITS AMINO ACID ACCEPTOR ABILITY[1,2]

Chien-chung Li and Jong-Ching Su

Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan, Republic of China

Received August 28, 1967

Formation of aminoacyl-t-RNA complex, the important intermediate in protein synthesis, has been known to depend on the presence of a specific terminal nucleotide fragment consisting of -pCpCpA at the 3'-hydroxyl end of the t-RNA molecule (Hecht et al, 1958; Daniel and Littauer, 1963; Moldave, 1965). On activation, amino acid is esterified with the 2'- or 3'-hydroxyl group of the ribose moiety of the terminal adenosine of t-RNA (Preiss at al, 1959; Daniel and Littauer, 1963).

Hecht et al (1958) pointed out that when the CMP units were removed from the terminal trinucleotide segment, little incorporation of amino acid into the t-RNA was observed. Using a purified rat liver enzyme, Daniel and Littauer (1963, 1965) were able to convert normal t-RNA molecules into RNA--pXpCpU and RNA--pXpC forms. The RNA--pXpCpU chains could no longer serve as amino acid acceptors in the presence of ATP. On the other hand, RNA--pXpC chains, which could receive an AMP residue in the presence of ATP, incorporated amino acids to the same extent as that of the normal chains but at a slower rate. These experimental findings may lead to the conclusion that the terminal adenosine is an absolute requirement for the amino acid acceptability of t-RNA. By treatment of t-RNA with periodate which cleaves the ribose of the terminal adenosine between the 2' and 3' positions, Preiss et al (1959) has demonstrated that the amino acid acceptor function of t-RNA is completely destroyed. So far, however, selective modification of the base portion of the terminal adenosine of t-RNA has never been attempted. The possibility exists that this terminal adenosine may be altered to some extent without impairing the amino acid acceptor ability of the t-RNA.

In the present communication, the authors present evidence that when the terminal adenosine of t-RNA is converted into inosine by an algal adenylate de-

---

[1] Paper No. 67 of the Scientific Journal Series, Institute of Botany, Academia Sinica.

[2] This work was supported by the National Council on Science Development of the Republic of China.

aminase (Su et al, 1966) which specifically removes the 6-amino group of adenosine and adenylate compounds with free 2'- and 3'-hydroxyl groups, no change of the amino acid acceptability of t-RNA is observed.

Unfractionated E. coli t-RNA was purchased from Schwarz BioResearch Inc., Orangeburg, New York. Since more than 80% of the terminal adenosine was intact in this commercial product (Table 1), it was used as such without attempting to repair the lost adenyl group. Deamination of t-RNA was carried out as follows: In a total volume of 3.0 ml, the reaction mixture contained about 70 mg of t-RNA, 6,000 units (Su et al, 1966) of algal adenylate deaminase, 180 μmoles of NaCl, 90 μmoles of $CaCl_2$, and 90 μmoles of Tris-acetic acid, pH 5.0. The mixture was incubated at 37° for 4 hours after which the t-RNA was isolated by the phenol method (Kirby, 1956). It was finally dissolved in distilled water and stored in the cold.

The extent of selective deamination was determined by a combination of alkali hydrolysis and column chromatography (see legend of Table 1). Each nucleoside fraction was identified by the shape of its absorption spectrum or by paper chromatography, using 1 M sodium bicarbonate as the developing solvent. The R_f values

Table 1

Distribution of the terminal nucleoside of t-RNA

t-RNA	C (%)	A (%)	I (%)	Deamination (%)
Untreated	17	83		
Deaminase treated	17	9	74	89
Control*	17	83		

Five to 6 mg of t-RNA was hydrolyzed in 0.3 N KOH at 35° for 16 hours. In the case of deaminase treated t-RNA, the sample was first mixed with 0.005 M EDTA to remove the residual calcium ions introduced at the deamination step and then precipitated and dried before subjecting to alkali hydrolysis. The hydrolysate was neutralyzed with perchloric acid and the $KClO_4$ precipitate was removed by centrifugation. The hydrolysate was then applied, at room temperature, on a small Dowex-1 (Cl^- form) column (0.6 x 6.0 cm) previously washed with distilled water. The column was again washed with distilled water and the washings were collected in 1-ml fractions. While the nucleotides in the hydrolysate are adsorbed on the resin, the nucleosides released from the 3'-termini of t-RNA are eluted by water, in the appearance order of C → A → I. By measuring the absorption spectra of each fraction with a recording spectrophotometer and by applying Loring's equations (1958), a fairly good separation can be obtained.

*t-RNA treated as in the deamination reaction, only the deaminase was added at the end of the incubation.

of cytidine, adenosine, and inosine in a 6-hour decending run at room temperature in this system was 0.77, 0.52, and 0.69 respectively. As is seen in Table 1, nearly 90% of the terminal adenosine has been converted into inosine by the treatment of the algal enzyme under the specified conditions. Moldave (1965) has suggested that since the cytidylate-adenylate sequence at the amino acid-binding terminus was susceptible to enzymatic degradation, these nucleotides could exist as an exposed nonhydrogen-bonded single chain. The present finding that the terminal adenosine is deaminated by the algal enzyme is a further confirmation of his suggestion.

Amino acid acceptability of the deaminase treated and untreated t-RNA samples are shown in Table 2. Assays using 1-C^{14}-glycine was also performed. The results are shown in Table 3.

Table 2

Amino acid acceptability of normal and selectively deaminated t-RNA

t-RNA	CPM/μmole RNA nucleotide (Berg et al, 1961)
Untreated	9,020
Deaminase treated	8,980
Control*	8,700

The assay procedure was essentially that of Ofengand et al (1961). CTP was excluded from the reaction mixture. Radioactive amino acid mixture used was U-C^{14}-Chlorella protein hydrolysate. Aminoacyl-t-RNA synthetase was prepared according to Ishida and Miura (1965) with only minor modifications. Radioactivity counting was carried out with a windowless gas flow counter.
*see Table 1.

Table 3

The capacity of glycyl-t-RNA formation of normal and terminal adenosine-deaminated t-RNA

t-RNA	CPM/μmole RNA nucleotide
Untreated	2,340
Deaminase treated	2,300

It can be seen from the results listed in Tables 2 and 3 that the capacity of the deaminase treated t-RNA as an amino acid acceptor is exactly the same as

that of the untreated sample. It follows then that the terminal adenosine may not be an absolute requirement for the amino acid acceptor function of t-RNA since the terminal adenosine can be replaced by inosine, without losing amino acid acceptability.

One may argue that the aminoacyl-t-RNA formed by the deaminated t-RNA may be an expression of the capacity of the portion of t-RNA which has not been deaminated and the portion with its terminal adenosine lost. That this is not the case is clearly indicated by the following two experiments. In the first one, the incorporation values in the presence and absence of CTP were compared. It was found that it was about 10% higher when CTP was included in the reaction mixture than when it was absent. The second experiment was aimed to show that the terminal inosine of the deaminase treated t-RNA was really esterified with amino acid. The amino acid incorporating reaction was performed, using cold amino acid mixture. All constituents in the reaction system were scaled up about 40 times. At the end of the incubation, the aminoacyl-t-RNA as well as the untreated t-RNA were isolated as in the deamination reaction. After drying over $CaCl_2$, it was dissolved in 0.01M ammonium acetate buffer, pH 5.0. One μmole of sodium metaperiodate was added and the mixture was left over at room temperature for 20 minutes, with ocasional stirring. Then 2 μmoles of glucose were added and it was left at room temperature for another 20 minutes. The aminoacyl-t-RNA and the t-RNA with its terminal ribose oxidized, if any, were isolated by alcohol precipitation, dissolved in 0.005 M EDTA, reprecipitated, dried and then subjected to alkali hydrolysis. The inosine fraction obtained from the hydrolysate was identified as before and compared with the dialdehyde inosine prepared by periodate oxidation of inosine. The results are listed in Table 4. Unfortunately, the cytidine and the adenosine peaks were masked by a heavy protein peak when they

Table 4

Properties of inosine terminus obtained from periodate oxidized aminoacyl-t-RNA

Substance	R_f in 1 M $NaHCO_3$	Absorption maximum in H_2O (mμ)
Inosine	0.69	249.5
Inosine in dialdehyde form	0.75	247.5
Sample	0.68	249.5

were eluted from the anion exchanger column and thus the distribution expressed in percentage as that shown in Table 1 could not be obtained. Nevertheless, the results derived from the periodate oxidation experiment clearly show that the terminal inosine of the deaminase treated t-RNA can actually be esterified with amino acid.

Of course it is quite possible that the present conclusion may not be universally valid. For instance, t-RNA's from other sources may show different results. Further more, it is possible that replacement of the terminal adenosine by inosine may stimulate the rate of aminoacyl-t-RNA formation of certain amino acid specific t-RNA's while at the same time reduce that of others so that the apparent incorporation rate is the same as that of the normal sample when unfractionated t-RNA is used. Nevertheless, it is safe to conclude that the terminal adenosine of t-RNA is not necessarily an absolute requirement for its amino acid acceptor function.

Acknowledgements

The U-C^{14}-Chlorella protein hydrolysate used in this work was generous gifts from Dr. B. Maruo of the University of Tokyo and Dr. A. Tsugita of Osaka University, Japan. The authors wish to show their gratitude to Dr. H. W. Li, director of the institute, for his encouragement.

References

Berg, P., Bergmann, F. H., Ofengand, E. J., and Dieckmann, M., J. Biol. Chem. 236 1726, (1961).
Daniel, V., and Littauer, U. Z., J. Biol. Chem. 238 2102, (1963).
Daniel, V., and Littauer, U. Z., J. Mol. Biol. 11 692, (1965).
Hecht, L. I., Stephenson, M. L., and Zamecnik, P. C., Biochem. Biophys. Acta 29 460, (1958).
Ishida, T., and Miura, K. I., J. Mol. Biol. 11 341, (1965).
Kirby, K. S., Biochem. J. 64 405, (1956).
Loring, H. S., in Chargaff, E., and Davidson, J. N., (Editors), The Nucleic Acids, Vol. 1 Acad. Press, Inc., New York, 201, (1955).
Moldave, K., Ann. Rev. Biochem. 34 419, (1965).
Ofengand, E. J., Dieckmann, M., and Berg, P., J. Biol. Chem. 236 1741, (1961).
Preiss, J., Berg, P., Ofengand, E. J., Bergmann, F. H., and Dieckmann, M., Proc. Nat. Acad. Sci. U. S. 45 319, (1959).
Su, J. C., Li, C. C., and Ting, C. C., Biochemistry 5 536, (1966).