SELECTIVE MODIFICATION OF YEAST SERYL-t-RNA AND ITS EFFECT ON THE ACCEPTANCE AND BINDING FUNCTIONS.

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Summary: Yeast seryl-t-RNA contains N^6-(Δ^2-isopentenyl) adenosine adjacent to the presumed anticodon. This component can be selectively modified by treatment of the RNA with aqueous iodine. This treatment does not affect the acceptance of serine residues but it does interfere with the binding of seryl-t-RNA to the ribosome-messenger RNA complex.

Introduction: Transfer RNA's contain several minor nucleosides. Because of their structural variety and because any minor nucleoside may be restricted to a few molecular locations in the t-RNA fraction, they may serve as focal points for the study of t-RNA structure-function relationships. An opportunity for employing a minor nucleoside in such an experimental approach has presented itself with the finding of N^6-(Δ^2-isopentenyl)adenosine (IPA) in yeast t-RNA (Hall et al, 1966, Biemann et al, 1966). More specifically, Zachau and coworkers (1966) have shown that IPA is located, adjacent to the presumed anticodon, in the sequence of seryl-t-RNA I and II. IPA by virtue of its very reactive side chain enables a chemical modification of the seryl-t-RNA molecule to be made at this location.

Experimental: General. Unfractionated yeast t-RNA was prepared according to Holley (1963). IPA was synthesized (Hall et al, 1966). Poly U and poly (U, C) (1:1) were obtained from Miles Laboratories. C^{14}-L-serine (120µc/µm) and C^{14}-L-phenylalanine (360 µc/µm) were obtained from Schwarz Biochemicals. Amino acyl synthetases were prepared by standard procedures as modified by Hoskinson and Khorana (1965). In addition, just before use, the enzyme solution (4ml) was passed through a sephadex G-25 column; (1.5 x 30 cm.); the enzyme was eluted with 0.05 M Tris-SO_4, 0.001 M EDTA, pH 7.5. Washed ribosomes were isolated from E. coli-B (Nirenberg, 1963).

Reaction of iodine with IPA: To a solution of 3.3 mg of IPA (0.01 mmole) in a 0.5 ml of water at 25° was added a solution of 12.8 mg of I_2 (0.1 mmole) and 30 mg of KI in 1.0 ml of water. A precipitate immediately appeared which redissolved on addition of a few drops of ethanol. Paper chromatography of this mixture in 2-propanol: conc. NH_4OH:H_2O (7:1:2) showed that all the IPA had disappeared and a new compound was present. The ultraviolet absorption spectra of this product are similar to those of an N^1-(alkyl substituted)adenosine.

Reaction of iodine with yeast t-RNA: To a solution of 500 mg of t-RNA in 200 ml of 0.01 M Tris, pH 7.0, was added 1.2g of KI and 150 mg of I_2. The mixture was shaken for 30 minutes at 25° to dissolve the iodine and kept for a total of 4 hours at 25°. The solution was dialyzed against distilled water for 14 hours and lyophilized. The sample was hydrolyzed enzymically to its constituent nucleosides (Hall, 1964), and the mixture was resolved by partition chromatography on a Celite column (2.54 x 58 cm) according to the general method of Hall (1962); solvent system, ethyl acetate: 1-propanol: H_2O (4:1:2). In

this system IPA is eluted as a sharp peak in the first hold-back volume; i.e. from an untreated control sample of t-RNA (500 mg), 300 µg of IPA was obtained. This analytical system would enable the detection of at least 10 µg of IPA. No IPA was detected in the hydrolysate of the iodine-treated t-RNA.

Amino acid acceptor activity of iodine treated t-RNA: t-RNA (100 mg) was treated with iodine as described above. The reaction mixture was dialyzed but not lyophilized. The pH of the solution was adjusted to 8.8 and the solution was incubated for one hour at 37°, then dialyzed against distilled water for 8 hours. A control sample of yeast t-RNA was processed identically with the exception that iodine was omitted from the initial incubation mixture.

AMINO ACID ACCEPTOR ACTIVITY OF IODINE-TREATED t-RNA

t-RNA	Radioactivity Incorporated	
	Serine cpm	Phenylalanine cpm
Iodine-treated	918	2,280
Untreated	872	2,325
No t-RNA	43	81

The reaction mixture contained in a volume of 0.125 ml, pH 7.3, sodium cacodylate, 10 µm, $Mg(OAc)_2$, 1.0 µm, KCl, 1.0 µm; ATP 1.5 µm; C^{14}-amino acid and 19 other amino acids, 6.8×10^{-4} µm of each; RNA 1.70 A^{260} units; and syntetase preparation (0.07 mg protein). The solution was incubated at 37° for 15 minutes. 25 µl was pipetted on to a paper disc which was washed (Hoskinson and Khorana, 1965) and counted in a liquid scintillation counter. Counting efficiency was 65%.

Binding of iodine-treated t-RNA to the ribosome-messenger RNA complex: For use in the binding assay the stripped samples of t-RNA were charged with either C^{14}-L-phenylalanine or C^{14}-L-serine on a scale 40 times that described above and the purification was carried out, according to Ehrenstein and

Lipmann (1961). The binding assay was carried out according to Nirenberg and Leder (1964).

BINDING OF t-RNA's TO RIBOSOME-MESSENGER RNA COMPLEX

t-RNA	Aminoacyl-t-RNA Bound to Ribosomes			
	Untreated		I_2-Treated	
	cpm	μμm	cpm	μμm
Phenylalanyl-	1730	3.6	1780	3.7
Without poly U	142	0.29	156	0.32
Seryl-Exp. 1	676	3.4*	238	1.15
Exp. 2	616	3.1	217	1.05
Without poly (U,C)	132	0.75	71	0.41

The reaction mixture contained in 0.05 ml of buffer (0.1 M tris(OAc), 0.02 M $Mg(OAc)_2$, 0.05 M KCl, pH 7.1), 2.7 A_{260} units of washed ribosomes, 25 mμm of base residues of poly U or poly (U,C) and the charged t-RNA sample; phe-t-RNA untreated 1802 cpm (3.75 μμm), I_2-treated, 1920 cpm (3.77 μμm); ser-t-RNA, untreated, 2615 cpm (13.0 μμm), I_2-treated, 2590 cpm (12.95μμm). Specific activity (cpm/A_{260} unit) of each sample was: phe-t-RNA untreated, 3340, I_2-treated, 3370; ser-t-RNA untreated 2415, I_2-treated 2572. Solution incubated for 15 minutes at 24°. *The lower efficiency for binding of seryl-t-RNA using the poly (U,C) template has been noted by Bernfield and Nirenberg (1965).

Discussion: The overall reaction of iodine with IPA is more complex than that of a simple addition of iodine across the double bond of the side chain. The changed ultraviolet absorption spectra of the principal product indicate that the purine ring participates in the reaction and this may, in some way be related to the facile interaction of the N^1 position with the allyl double bond of the side chain, reported previously (Hall et al, 1966). At this stage in our study it is clear that under these conditions, all the IPA is essentially changed. The reaction of iodine with an IPA residue when it is an integral part of a t-RNA molecule, may or may not follow the same course, but, the IPA per se disappears.

A question central to this study is whether iodine under the reaction conditions reacts with other nucleoside residues.

The four major nucleosides, in model experiments, were unaffected by the treatment and further, Brammer (1963) found that the infectivity of tobacco mosaic virus RNA was not diminished by treatment with aqueous iodine. With respect to the other minor nucleosides, it is known that sulfur-containing nucleosides can be oxidized by iodine (Carbon et al 1965) although this reaction is reversible (Goehler et al 1966). Yeast seryl-t-RNA does not contain a sulfur-nucleoside (Zachau et al, 1966) and phenylalanyl-t-RNA contains neither a sulfur-nucleoside nor IPA (RajBhandary and Stuart, 1966). The lack of effect of iodine on the functions of phenylalanyl-t-RNA represents additional evidence that iodine has little or no effect on other nucleosides or on polynucleotide structure in general. Therefore, we conclude that the iodine treatment modifies the primary structure of seryl-t-RNA only at the point of location of the IPA residue.

Preliminary studies show that modification of the IPA in the ser-t-RNA molecule does not affect quantitatively acceptor activity but does reduce the capacity of seryl-t-RNA for binding to the ribosome-messenger RNA complex. Whether this is primarily due to the structural change in the side chain or to a change in secondary structure at this point in the RNA molecule or a combination of both is an open question. There are other data which suggest that these two t-RNA functions are disassociated. Treatment of t-RNA with B. subtilis RNA'se (Nishimura and Novell, 1965) or with nitrous acid (Carbon, 1965) causes a loss of transfer function for some amino acids but not a corresponding loss of acceptor activity.

In conclusion, the data show that a specific t-RNA molecule can be modified at a defined position and this capability may prove useful in a number of studies.

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References:

Bernfield, M.R. and Nirenberg, M,W, (1965), Science 147, 479.
Biemann, K., Tsunakawa, S., Sonnenbichler, T., Feldman, H., Dutting, D., and Zachau, H.G., (1966), Angew. Chem. 78, 600.
Brammer, K.W., (1963), Biochem. Biophys. Acta 72, 217.
Carbon, J.A., (1965), Biochem. Biophys. Acta 95, 550.
Carbon, J.A., Hung, L., and Jones, D.S., (1965), Proc. Nat. Acad. Sci. 53, 979.
Ehrenstein, G., and Lipmann, F., (1961), Proc. N.A.S. (U.S.) 47, 941.
Goehler, B., Kaneko, I and Doi, R.H., (1966), Biochem. Biophys. Res. Comm. 24, 446.
Hall, R.H., Stasiuk, L., Robins, M.J., and Thedford, R., (1966), J. Am. Chem. Soc. 88, 2614.
Hall, R.H., (1964), Biochemistry 3, 769.
Hall, R.H., (1962), J. Biol. Chem. 237, 2283.
Holley, R.W., (1963), Biochem. Biophys. Res. Comm. 10, 186.
Hoskinson, R.M., and Khorana, H.G., (1965), J. Biol. Chem. 240, 2129.
Nirenberg, M.W., (1963), Methods in Enzymology, Vol. VI. Colowick and Kaplan Eds. Academic Press, New York, pg 17.
Nishimura, S., and Novelli, G.D., (1965), Proc. Nat. Acad. Sci. (U.S.) 53, 178.
RajBhandary, U.L., and Stuart, A., (1966), Fed. Proc. 25, 520.
Zachau, H.G., Dutting, D., and Feldmann, H. (1966), Angew. Chem. 78, 392.