BIOSYNTHETIC STUDIES OF THE Y BASE IN YEAST PHENYLALANINE tRNA. INCORPORATION OF GUANINE

Huimin J. Li, Koji Nakanishi*
Department of Chemistry, Columbia University
New York, N. Y. 10027

and

Dezider Grunberger, I. Bernard Weinstein
Institute of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10027

Received October 10,1973

SUMMARY: Biosynthetic studies of the Y base in yeast phenylalanine tRNA employing a guanine requiring yeast mutant have shown that the main building block of this base is derived from guanine.

In almost all tRNAs, thus far sequenced, there is a modified nucleoside adjacent to the 3′ end of the anticodon (1). Phenylalanine tRNAs isolated from eukaryotic cells, including yeast, wheat germ, beef-, rat-, and chicken- livers, contain at this position a highly modified hydrophobic, fluorescent base. The base from baker's yeast (Saccharomyces cereviseae) was termed by its discoverers, RajBhandary, et al., the "Y" base (2). Thiebe and Zachau (3) found it can be excised from tRNA by incubation at pH 2.9, without breaking the polynucleotide chain.

The Y base present in Saccharomyces cereviseae $tRNA^{Phe}$ was assigned the structure indicated in Fig. 1 (4). This was subsequently supported by findings of Thiebe, et al. (5). Further micro-degradations and a synthesis (6) established the full structure, including its stereochemistry. This

Copyright © 1973 by Academic Press, Inc.
All rights of reproduction in any form reserved.

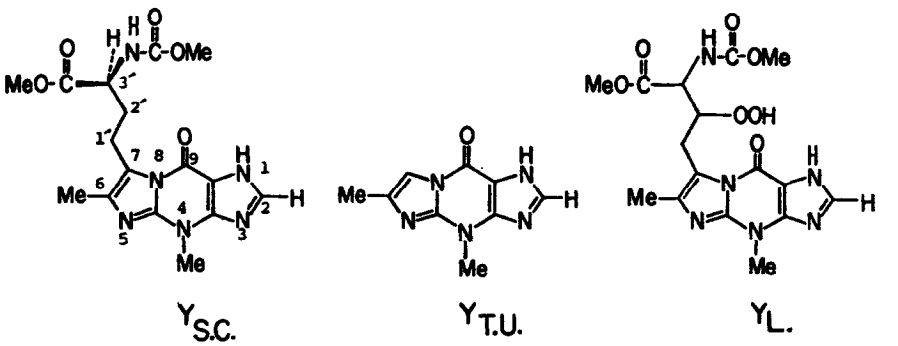

Figure 1.
Structure of the fluorescent bases from phenylalanine tRNA of Saccharomyces cereviseae ($Y_{S.C.}$), Torulopsis utilis ($Y_{T.U.}$) and of mammalian liver ($Y_{L.}$).

structure represents the most highly modified nucleic acid base characterized thus far (1), and essentially consists of a tricyclic imidazo-purine nucleus to which is attached a complex 4-carbon side-chain. Kasai, et al. (7) have established the structure of the corresponding base from Torulopsis utilis which interestingly lacks the side-chain (see Fig. 1). A further modification is the peroxy Y structure (Fig. 1) assigned to the corresponding base found in mammalian liver (8,9), and probably wheat germ (10).

Thiebe, et al. have shown that the sugar moiety of Y nucleoside is ribose, and there is evidence that the Y base is not an artifact of the acid hydrolysis procedure used for its excision (3,5,11). Furthermore, recent syntheses of ribosides having the Y base skeletons indicate that the ribose moiety in Y nucleoside is attached to position N-3 of the imidazo-purine nucleus (12).

Because of the unusual structural and biochemical aspects of the Y base and related compounds, we are currently investigating its biosynthetic pathway. In the present study we demonstrate, by employing a guanine requiring mutant of yeast,

that the fundamental building block of the Y base is guanine (13).

MATERIALS AND METHODS: A mutant of Saccharomyces completely blocked in GMP synthetase, strain Q1230 (a generous gift from Dr. Uwe Reichert), was grown on minimal medium (14) modified by addition of 20g/l glucose, 1g/l $(NH_4)_2 SO_4$ and 5g/l Bactopeptone, supplemented with 60 mg guanine/l. An inoculum was grown overnight and then transferred to 1 liter of the same medium to which 190 μC [8-^{14}C] guanine (35μC/μmole, purchased from Schwarz/Mann Co.) was added. The mixture was shaken vigorously at 29°C, and cell growth was followed by absorbance at 650 nm. The cells were harvested by centrifugation just prior to onset of the "stationary phase."

The tRNA was isolated and purified according to the phenol extraction procedure of Zubay (15). The yield of radioactive tRNA was 10.9 mg with a specific activity of 6.15 X 10^5 d.p.m./mg. Unlabeled carrier yeast tRNA (199.6 mg) was obtained similarly from a 20 l culture and mixed with the radioactive tRNA. To remove contaminants the mixture of tRNAs was first extracted with freshly distilled $CHCl_3$ containing 1% ethanol and then ethyl acetate and re-precipitated by addition of 3 volumes of ethanol plus 0.1 volume of 1M potassium acetate pH 5.0 buffer. The tRNA precipitate was dissolved in 6 ml 0.1 M sodium phosphate buffer pH 2.9 and then incubated at 37°C for one hour, left at room temperature overnight, and again incubated at 37°C for an additional two hours. The reaction mixture was neutralized to pH 6.8 by adding 0.1 N NaOH, and the excised Y base was extracted into ethyl acetate, thus leaving the tRNA minus Y in the aqueous layer. The Y base was further purified by silica gel tlc, using the upper phase of ethyl acetate : 1-propanol : water (4:1:2). The strongly

fluorescent band with R_f 0.35 (the same as synthetic dl Y) was eluted with water-saturated ethyl acetate to yield ca. 23.90 µg and its radioactivity measured by liquid scintillation counting. The uv, fluorescent, and mass spectra of

Table 1. Radioactivity in nucleotides and the Y base of yeast Q1230 tRNA.

Yeast Q1230 tRNA was labeled in vivo with [8-^{14}C] guanine and the radioactivity of the Y base and of the major nucleotides were determined as described in the text.

Compound	Specific Radioactivity (d.p.m./µ mole)	o/o
3′-GMP	46000	100
3′-AMP	2100	4.5
3′-UMP	0	0
3′-CMP	480	1
Y	36700	80

the Y base obtained from strain Q1230 were identical to those previously reported for the Y base from baker's yeast (4).

The tRNA minus Y was precipitated with ethanol and subjected to T_2 RNase hydrolysis at 37°C overnight to give 3′-mononucleotides. These were separated by two dimensional cellulose tlc using isobutyric acid/0.5N NH_4OH (5 : 3) and 2-propanol/HCl/H_2O (170 : 41 : 39) solvent systems, eluted with H_2O and their specific activities determined.

RESULTS AND DISCUSSION:

Table 1 indicates the specific activities of the purified nucleotides and the Y base. When the specific activity of GMP is taken as 100, it is seen that the activities of AMP and CMP are extremely low. This indicates that in this mutant there was negligible conversion of the ^{14}C guanine precursor or its carbon atoms to either A, C or U. On the other hand, the Y

base had approximately 80% of the specific activity of the G residues. These results unambiguously show that the precursor of the Y base in phenylalanine tRNA is guanine. Our results are also entirely consistent with the structure of the Y base since the tricyclic nucleus is closely related in its structure to that of guanine.

It is of interest that guanine rather than adenine is the precursor since in most tRNAs whose code words begin with U, the modified base adjacent to the 3′ end of the anticodon is a derivative of adenine (1). An interesting exception to this is a yeast leucine tRNA, $tRNA_3^{Leu}$, which apparently also has a modified guanine residue in the corresponding position of the anticodon (16).

By analogy with the synthesis of other minor bases in tRNA (1) it is likely that the Y base is formed by modification of a guanine residue which is already part of the polymeric structure of the tRNA. Further steps in the biosynthetic pathway of Y base are under current investigation.

This research was supported by NIH grants CA 02332, 11572 and the Leukemia Society of America.

References:

1. Söll, D., Science, 173, 293 (1971).
2. RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G., Proc. Nat. Acad. Sci. U.S., 57, 751 (1967).
3. Thiebe, R., and Zachau, H. G., Europ. J. Biochem., 5, 546 (1968).
4. Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D., and Weinstein, I. B., J. Amer. Chem. Soc., 92, 7617 (1970).
5. Thiebe, R., Zachau, H. G., Łaczynskyj, L., Biemann, K., and Sonnenbichler, J., Biochim. Biophys. Acta, 240, 163 (1971).
6. Funamizu, M., Terahara, A., Feinberg, A., and Nakanishi, K., J. Amer. Chem. Soc., 93, 6706 (1971).
7. Kasai, H., Goto, M., Takemura, S., Goto, T., and Matsuura, S., Tetrahedron Lett., 2725 (1971).
8. Nakanishi, K., Blobstein, S., Funamizu, M., Furutachi, N., van Lear, G., Grunberger, D., Lanks, K. W., and Weinstein, I. B., Nature New Biology, 234, 107 (1971).
9. Blobstein, S., Grunberger, D., Weinstein, I. B., and

Nakanishi, K., Biochemistry, 12, 188 (1973).
10. Yoshikami, D., and Keller, E. B. Biochemistry, 10, 2969 (1971). The fluorescent base from wheat germ has chromatographic properties similar to that from beef liver (9), and therefore is presumably peroxy Y- (8,9).
11. Gebert, R., Blobstein, S., Grunberger, D., Weinstein, I. B., and Nakanishi, K., unpublished studies.
12. Blobstein, S., and Nakanishi, K., to be published. The N-3 position of the imidazo-purine corresponds to the N-9 position of the purine skeleton.
13. Preliminary report: Grunberger, D., Li, H. J., Pulkrabek, P., Weinstein, I. B., Nakanishi, K., Proc. 9th Int. Congress Biochem., Stockholm, p. 175 (1973).
14. Wickerham, J. Bact., 52, 293 (1946).
15. "Procedures in Nucleic Acid Research" edited by Cantoni, G. L., and Davis, D. R., (Harper and Row, Publishers, New York and London) p. 455.
16. Kowalski, S., Yamane, T., and Fresco, J. R., Science, 172, 385 (1971).