Structure Determination of the Fluorescent Base from *Geotrichum candidum* Phenylalanine tRNA

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Synopsis. The chemical structure of the fluorescent base purified from *Geotrichum candidum* phenylalanine tRNA was determined as peroxywybutine by the use of MS, UV, and NMR measurements.

Modified nucleosides found in tRNA have been considered to play an important role in the functioning of tRNA. Hypermodified fluorescent bases, classified as "W," are located at the position adjacent to the 3′ side of the anticodon. The chemical structures of these fluorescent bases were determined as follows: α-(carboxyamino)-4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butyric acid dimethyl ester for wybutine obtained from yeast phenylalanine tRNA ($tRNA^{Phe}$),[1] 4,9-dihydro-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine for wye from *Torulopsis utilis* $tRNA^{Phe}$,[2] and α-(carboxyamino)-4,9-dihydro-β-hydroperoxy-4,6-dimethyl-9-oxo-1*H*-imidazo[1,2-*a*]purine-7-butyric acid dimethyl ester for peroxywybutine from rat liver $tRNA^{Phe}$.[3] Peroxywybutine was also found in wheat germ and bovine liver $tRNA^{Phe}$ by chromatographical[4] and spectrometrical[5] measurements respectively.

In this study, the hydrophobic and fluorescent base was purified from $tRNA^{Phe}$ of *G. candidum*[6,7] that is one of the simplest eukaryotic organisms and is classified as an aquatic fungus. The chemical structure of the base was determined to be peroxywybutine by the use of MS, UV, and NMR measurements.

Experimental

Preparation. Wet *G. candidum* cell[7] (10 kg) gave 4 g of crude tRNA by phenol extraction followed by the procedure of Miyazaki.[8] The crude tRNA was applied on a benzoylated DEAE-cellulose column and chromatographed as described by Gillam *et al.*[9] The rechromatography of the fraction eluted at 10% ethanol using the same column, as described by Litt,[10] showed a slight difference in the separation of $tRNA^{Phe}$ as compared with yeast $tRNA^{Phe}$. The specific activity of the $tRNA^{Phe}$ fractionated increased to 16 times that of the crude tRNA. The fraction containing 120 mg of $tRNA^{Phe}$ in 50 ml of water was incubated at 37 °C for 3.5 h at pH 2.9.[11,12] After neutralization, the fluorescent base liberated was extracted by ethyl acetate and purified by chromatography on Toyo No. 51A paper with a developing solvent prepared as the upper phase of a mixture of ethyl acetate : 1-propanol : water=4:1:2. The R_f value of the fluorescent base was 0.43. The base was extracted from the paper with water doubly distilled in a glass vessel. The base was then extracted again with the same volume of distilled chroloform six times and dried to an amorphous powder.

Measurements of Mass, UV, and NMR Spectra. The mass spectrum was measured with JMS-D300 on a lined JMA-2000 mass data system operated at 70 eV; PFK standard; $m/\Delta m$=5000; error, ±5 milli mass unit; 190 °C. The UV spectra were measured in water at pHs 2.42, 6.56, and 11.72 with the use of a Hitachi 200-20 spectrophotometer. The NMR spectrum was measured with JNM-PFT-100A in $CDCl_3$ containing 1% of C_2D_5OD, using TMS as the internal standard.

Results and Discussion

Table 1 shows the data of high-resolution MS of the base and elemental compositions. The ion peaks **1** and **c** are the parent and the base peaks respectively. The degradations of ions from the parent ion to the ion **2** and also from the ion **a** to the ion **b** correspond to a reduction of the mass unit equivalent to one oxygen atom. Fragmentations of the base can reasonably be presented by two pathways. The oxygen atom liberated would be originally found in the hydroperoxide group of the base, since hydroperoxides are known to lose an oxygen atom in mass spectrometry.[13] The results obtained by MS in this experiment show that the base contained the same fragment ion of $C_5H_8O_4N$ and the ion **c** as compared with those of wybutine previously reported,[1] though it contains two more oxygen atoms in the ion **a**. These results may be compared to the papers[3,5] reported for the chemical structure of peroxywybutine with quite a close agreement.

The UV spectra measured at each pH were very close to those of wybutine, and the absorption maxima were observed at 232 and 285 nm at an acidic pH, at 235, 262, and 311 nm at a neutral pH, and at 235, 265, and 303 nm at a basic pH. The comparison of the absorption maxima between the base measured in this experiment and the synthetic analogous compounds previously reported[1,14] suggests that the chromophore structure of this base is similar to that of wybutine.

In the NMR spectrum, the detection of four signals at 2.29, 3.71, 3.76, and 3.95 ppm was made possible by a scan repeated 17200 times. These peaks were close to those assigned to 6-C Me, methyl esters, and 4-N Me respectively, as reported for wybutine,[1,15] wye,[2] and peroxywybutine.[16]

As a result, discussions of the data obtained by MS, UV, and NMR lead to the conclusion that the fluorescent base purified from *G. candidum* $tRNA^{Phe}$ has the structure of the peroxide of wybutine.

Wye, wybutine, and peroxywybutine, all classified as "W," share the same 4,6-dimethylimidazopurine as a nucleus and have various side chains at the C-7 position of the nucleus. Wye and wybutine are found in simpler eukaryotic organisms, such as *Torulopsis utilis* and yeast $tRNA^{Phe}$'s respectively. On the other hand, peroxywybutine is found in such higher organisms as mammalian liver, *Lupinus luteus*, and wheat germ $tRNA^{Phe}$'s. It is interesting to find that $tRNA^{Phe}$ isolated from an aquatic fungus of *G. candidum* also gave the fluorescent base peroxywybu-

Table 1. Pertinent ions observed in the MS of the fluorescent base from *G. candidum* $tRNA^{Phe}$

Ion peaks	*m/e*	Elemental composition (mol wt)	Relative abundance/%
1	408.1358 (M^+)	$C_{16}H_{20}O_7N_6$ (408.1390)	3
2	392.1409	$C_{16}H_{20}O_6N_6$ (392.1441)	10
a	262.0989	$C_{11}H_{12}O_3N_5$ (262.0941)	14
b	246.1027	$C_{11}H_{12}O_2N_5$ (246.0992)	7
c	216.0886	$C_{10}H_{10}ON_5$ (216.0886)	100

tine, though recently Kasai *et al.*[17] reported a mass spectrum showing no peroxywybutine for rat liver $tRNA^{Phe}$.

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