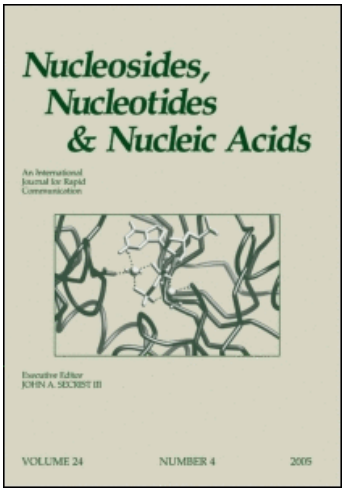

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Fluorescence Properties of Y-Nucleoside Derivatives

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FLUORESCENCE PROPERTIES OF Y-NUCLEOSIDE DERIVATIVES

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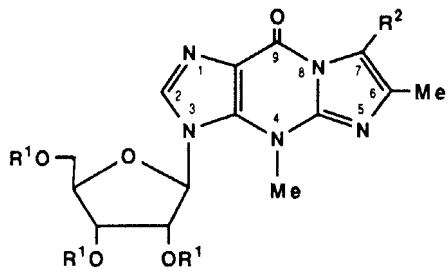
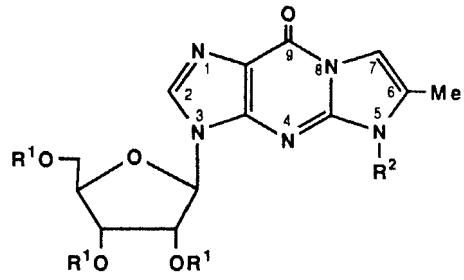
Abstract. Modified-base and modified N^3-(β-D-ribofuranoside) derivatives of the Y base exhibit subnanosecond fluorescence decays. Attachment of groups to the "unnatural" N^1 base position produces compounds showing dominant 7 - 10 ns decays. These spectral properties have been compared with those of the free Y base and suggest that the free Y base exists mainly as the N^1-H tautomer.

Introduction

Fluorescence of the hypermodified Y base at position 37 of yeast $tRNA^{phe}$ has been used to study interactions and dynamics of the polyribonucleotide for a number of years.[1,2,3,4,5,6] The spectroscopic properties of the Y base (wybutine) in $tRNA^{phe}$ which make it particularly useful for fluorescence measurements include high extinction coefficient for the major absorption band located in the 300-320 nm region and a long fluorescence decay time, about 7 ns, which predominates under conditions of high salt and Mg^{++} concentration above 1 mM.[4,5] In addition, both the absorption and emission bands are well separated from those of the normal bases, making specific interpretations of the fluorescence measurements possible over any time scale.

Recent measurements on the fluorescence decays of wybutine in $tRNA^{phe}$ have shown that 80% of the fluorescence amplitude is described by a 7-ns lifetime when 0.1 M KCl and 5-20 mM Mg^{++} is included in the buffer medium.[5,7] At a magnesium concentration of 0.02 mM, the largest amplitude decay times are about 300 ps and 2 ns. (This trend is also shown in ref. 4.)





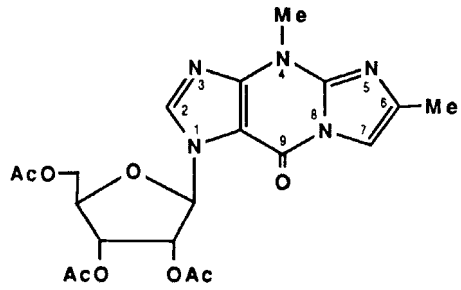
1 : $R^1 = R^2 = H$

4 : $R^1 = Ac$, $R^2 = H$

16 : $R^1 = H$, $R^2 = Me$

17 : $R^1 = Ac$, $R^2 = Me$

5b : $R^1 = Ac$, $R^2 = H$



15

FIG. 1. Structures of modified Y nucleosides used in this study. Compounds are numbered to conform with ref. 9. Me = methyl; Ac = acetate.

Similarly, when *E. coli* $tRNA^{glu}$ is bound to $tRNA^{phe}$, the predominant decay time is about 100 ps. These fluorescence decays are accompanied by mobility changes of the Y base, as measured by fluorescence anisotropy decays. The details of the mechanisms by which the fluorescence properties of the Y base are modified are not yet understood. In view of the potential utility of the fluorescence method to selectively probe dynamics and interactions of the transfer RNA, we have undertaken a study of the fluorescence properties of Y base derivatives which have recently been synthesized by simple, efficient methods.[8,9] This study directly determines the dependence of the base fluorescence properties upon the chemical structure of the nucleoside.

We present in this paper fluorescence properties of the nucleosides of Fig. 1 and the free Y base. It should be kept in mind that the Y base whose fluorescence in $tRNA^{phe}$ has been quoted contains aminoacid $CH_2CH_2(NHCO_2CH_3)CO_2CH_3$ at the C^7 position of the base.

Experimental

Y nucleosides were synthesized in high yield and characterized as described in references 8 and 9. Samples were freshly dissolved in spectroscopic grade dimethylsulfoxide (DMSO, Merck) for fluorescence measurements. The choice of DMSO as solvent was made on the basis of the good solubility of all compounds and our desire to eliminate solvent effect differences in the fluorescence of the samples. For comparison with a solvent of lower dielectric constant, the fluorescence of compound **4** was measured in dichloromethane.

Samples were maintained at 20° C throughout the measurements, which were completed on each sample within one hour of preparation. Fluorescence and anisotropy decay measurements were carried out as described in reference 2, using the method of picosecond time-resolved single photon counting. The excitation wavelength was 300 nm and the emission was filtered through a 3 mm Schott KV 370 glass filter. The system response time (full width at half maximum) was 75 ps for these measurements. Analysis of data was performed using methods described in ref. 5. In each case, however, no more than three exponential decay components were allowed to fit the fluorescence decay data. This gave fits adequate in all cases to make relative comparisons and comparisons with previous data of refs. 5-7.

Results

The fluorescence decays of compounds **1**, **4**, **5b**, **16**, and **17** in DMSO (Fig. 2 and Table 1) are dominated by subnanosecond decay times. The decay times and amplitudes were found by nonlinear least squares fits. For these compounds 88% or more of the fluorescence amplitude is described by the respective (**1**, **4**, **5b**, **16**, **17**) decay times of 240, 530, 90, 170, or 340 ps. Compound **15** and the free Y base, on the other hand, show almost single exponential decays of 10 and 7 ns, respectively. The more hydrophobic solvent dichloromethane relatively increases the longer-lived components for compound **4**, though the effect is not dramatic.

Discussion

We observe two classes of Y-base derivative fluorescence. One class is dominated by subnanosecond decay times, the other by decay times of 7-10 ns. The rapid fluorescence decays observed for the Y base derivatives **1**, **4**, **5b**, **16**, and **17** in DMSO contrasts sharply with the predominant 7 ns decay observed in the case of $tRNA^{Phe}$ (in water with Mg^{++}). Compound **15**, with ribose attached to the "unnatural", thermodynamically-preferred[9] N^1 rather than the "natural" N^3 position of the ring, and the free base show long lifetimes.

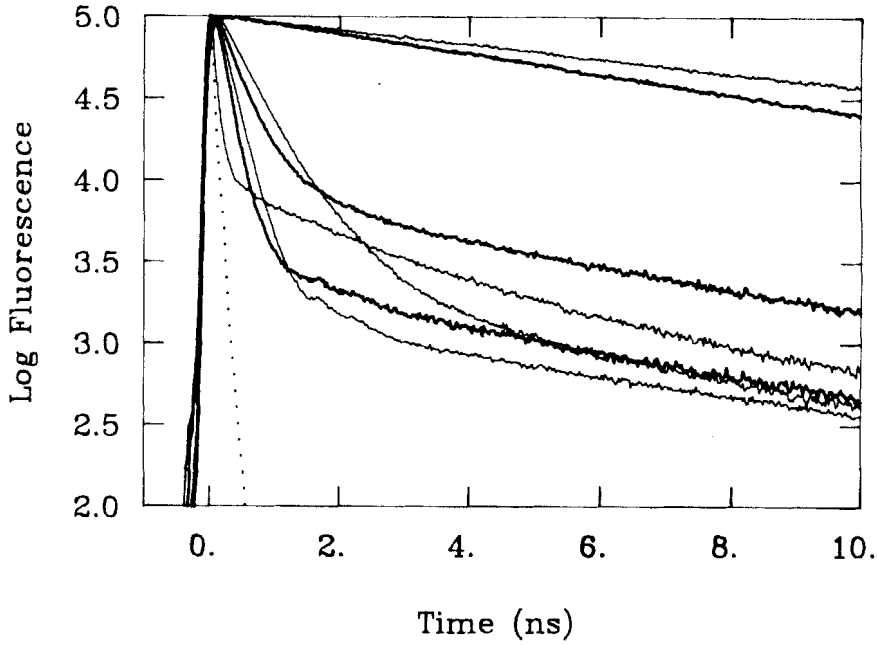


FIG. 2. Fluorescence of six Y nucleosides and the free Y base. Curves, top to bottom at 4 ns: compound 15, free base, 17, 5b, 4, 16, and 1. Data was recorded and fit to 39 ns, but is shown only to 10 ns for clarity.

Binding and conformational effects can greatly increase the amplitudes of the faster decay components of the $tRNA^{phe}$ fluorescence[5,7] which then more resembles the fluorescence of the N^3 Y nucleosides **1**, **4**, **5b**, **16**, and **17**, but the mechanism for this change is not clear. The presence of the "tail" at C^7 of the Y base in $tRNA^{phe}$ may be involved in the lifetime distribution change, but we note that substitution at C^7 of methyl for H has only moderate effects. Compare **1** with **16** and **4** with **17** in Table 1. It is noteworthy in any case that the decay times determined for all of the present Y base compounds and for $tRNA^{phe}$ lie in three classes: a fast, subnanosecond time, a 1-2 ns time, and a 7-10 ns time.

Chemical modifications on the Y base ring itself or even on the more remote ribose ring periphery affect the electronic structural properties of the Y base. This can be seen from ^{15}N NMR and optical absorption measurements[9] and from the present fluorescence decay data. The decay rate of the Y-base excited electronic state seems to depend most sensitively upon blocking of the N^1 site vs. the N^3 site. N^4 demethylation, acetylation of the ribose, and methylation of C^7 produce progressively smaller effects, measured

TABLE 1. Fluorescence decay parameters of Y nucleosides.

Compound*	Fluorescence Decay Time (ns)	Amplitude	Residual r.m.s.
1	0.24	0.977	
	1.08	0.014	1.26
	7.39	0.009	
4	0.53	0.950	
	2.05	0.040	1.13
	8.95	0.009	
4†	0.81	0.825	
	1.68	0.165	1.06
	8.73	0.010	
5 b	0.09	0.878	
	2.17	0.099	1.16
	8.28	0.023	
1 6	0.17	0.975	
	1.72	0.015	1.09
	7.31	0.011	
1 7	0.34	0.900	
	1.61	0.051	1.09
	6.89	0.049	
1 5	2.61	0.040	1.10
	10.12	0.960	
Free base††	7.01	1.000	1.07

* Compounds numbered in accordance with ref. 9.
† Measured in dichloromethane; all others in DMSO.
†† Base with Me at N^4 and C^6 and H at N^1 (or N^3). See Fig. 3.

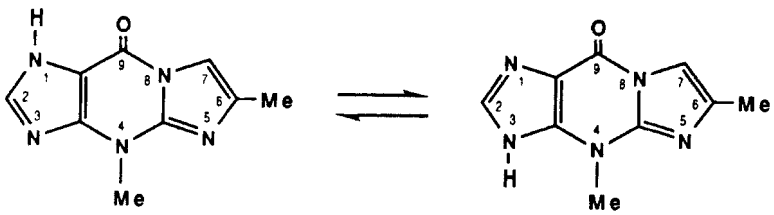


FIG. 3. Scheme 1: Possible tautomeric structures of the free Y base studied in this work. See Discussion.

in terms of lifetime and amplitude of the fastest decay rate. The fluorescence differences produced by all the chemical modifications are clear and easy to quantitate, nevertheless. The present data can, in fact, be used to infer unknowns of chemical structure. The details of the dependence of fluorescence on structure and conformation are problems we cannot yet completely answer, but the case of the free Y base is reasonably transparent, as is seen from arguments in the following paragraph.

The electronic properties of the free Y base should be interpreted as a manifestation of the tautomeric structures, N^3-H *versus* N^1-H (Scheme 1, Fig. 3). Since compounds **4** and **15** can be considered as the N^3- and N^1-blocked tautomers, the fluorescence properties of the Y base should be compared to those of **4** and **15**. Table 1 shows similarities to the fluorescence properties of the N^1-tautomer **15** [96% of the fluorescence amplitude with long decay time 10 ns] , but not to wyosine triacetate **4**. This suggests a similarity of the electronic structures of **15** and the free Y base, i.e., that the free Y base shown in scheme 1 most probably exists mainly as the N^1-H tautomer (>95%) while the population of the N^3-H tautomer presumably is small (<5%)[10]. The chemical evidence in support of the N^1-H tautomeric structure of the Y base stems from the fact that the glycosylation of the Y base by 1', 2', 3', 5'-tetra-O-acetyl-β-D-pentofuranose under acidic conditions gave exclusively the N^1-isomer **15**; no trace of the naturally-occurring isomeric Y nucleoside **4** was detectable.[11,12] Furthermore, N^1-isomer **15** was the only isomeric product formed when wyosine-triacetate **4** was subjected to a treatment of anhydrous $AlCl_3$ in dry CH_2Cl_2, while the the N^1-isomer **15** was found to be completely stable under the latter condition.[9] It should also be noted that attempts to methylate the Y base under a neutral condition (CH_3I in dimethylformamide at room temperature in the dark) gave exclusively the N^1-methyl isomer of the Y base (Glemarec and Chattopadhyaya, unpublished data). These considerations clearly suggest that the glycosylation site (N^3) of the naturally occurring wyosine **1** and its triacetate **4** is not thermodynamically favored and may partly explain the inherent instability across the glycosidic bond.

Acknowledgements

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REFERENCES

1 Eisinger J., Feuer B. and Yamane T. (1970) Proc. Natl. Acad. Sci. USA **65**, 638-644.

2 Bujalowski W., Gräser E., McLaughlin L.W. and Porschke D. (1986) Biochemistry **25**, 6365-6371.

3 Wells B.D. (1984) Nucleic Acids Res. **12**, 2157-2170.

4 Wells B.D. and Lakowicz J.R. (1987) Biophys. Chem. **26**, 39-43.

5 Claesens F. and Rigler R. (1986) Eur. Biophysics J. **13**, 331-342.

6 Rigler R., Claesens F. and Nilsson L. (1986) Chemica Scripta **26b**, 103-107.

7 Claesens F., Thesis (1987) Dept. of Medical Biophysics, Karolinska Institute, Stockholm, Sweden., pp. 11-26 and pp. v.1-v.27.

8 Bazin H., Zhou X.-X., Glemarec C. and Chattopadhyaya J. (1987) Tetrahedron Letters **28**, 3275-3278.

9 Glemarec C., Wu J.-C., Remaud G., Bazin H., Oivanen M., Lönnberg H. and Chattopadhyaya J. (1988) Tetrahedron **44**, 1273-1290.

10 Our ^{15}N- and ^{13}C-NMR data also support this approximate population of tautomers: C. Glemarec, G. Remaud and J. Chattopadhyaya, unpublished.

11 Blobstein S.H., Gebert R., Grunberger D., Nakanishi K. and Weinstein B. (1975) Arch. of Biochem & Biophys. **167**, 668-673.

12 Kasai H., Goto M., Ikeda K., Zama M., Mizuno Y., Takemura S., Matsuura S., Sugimoto T. and Goto T. (1976) Biochemistry **15**, 898-904.