A FLUORESCENT NUCLEOSIDE FROM MYCOBACTERIAL tRNA

DILEEP N. DEOBAGKAR AND K. P. GOPINATHAN
Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560 012, India

TRANSFER RNAs are rich in modified nucleosides[1]. Apparently, these components are synthesized as secondary modifications of the major constituent nucleosides at the polynucleotide level[2,3]. Modified bases may be present anywhere on the tRNA molecule but their role in the maintenance of structure and function of tRNA is not established. Several tRNAs contain hypermodified derivatives of isopentenyl adenosine next to the 3'-end of the anticodon[1], and the $tRNA^{Phe}$ from several organisms (Yeast, rat liver, calf liver) contains next to the anticodon a hypermodified purine derivative, referred to as 'Y' base and exhibiting characteristic fluorescent properties[4-10]. In this communication, we report the isolation and partial characterization of a new fluorescent nucleoside from mycobacterial tRNA.

Transfer RNAs from *M. smegmatis* and *M. tuberculosis* $H_{37}R_v$ were isolated by the phenol extraction method[11]. The total tRNA was passed through DEAE-cellulose in order to free it from contaminating ribosomal RNA, DNA, oligonucleotides, polyphosphates and polysaccharides. For chemical hydrolysis, the tRNA was treated with 0·5 N KOH for 18 hr at 37° C. The hydrolysate was adjusted to pH 8·5 and digested with alkaline phosphatase to obtain the nucleosides. For enzymatic hydrolysis, the tRNA preparations were separately treated with pancreatic RNase A (10 units/50 $A_{260\,nm}$ units of tRNA) or RNase T_1 (5 units per 15 $A_{260\,nm}$ units of tRNA). Thin layer chromatography (TLC) was carried out with microcrystalline cellulose. Fluorescent measurements were carried out on a Perkin–Elmar spectrofluorimeter.

The tRNA preparations isolated from two different species of Mycobacteria, *viz., M. tuberculosis* $H_{37}R_v$ and *M. smegmatis* exhibited a high degree of fluorescence. To ascertain whether the fluorescent compound is indeed a part of the tRNA and not a contaminant associated with it, the tRNA samples were hydrolyzed (chemically or enzymatically) and gel filtration on a column of Sephadex C-25 was carried out. While the fluorescence was associated with total tRNA in the void volume when native tRNA was gel filtered, the fluorescent compound also migrated with nucleotides on hydrolysis and fractionation, strongly suggesting that the fluorescent compound may be a constituent of the tRNA. When the total tRNA from *M. smegmatis* was fractionated on a column of benzoylated DEAE-cellulose[12] the fluorescence was associated with the tRNA fractions eluting with high salt and 20% ethanol. Since, these fractions contained a mixture of several tRNAs, as evidenced by the amino acid acceptance, it is necessary to do further fractionation to assign the fluorescent compound to any particular tRNA.

To characterize the nucleosides, the tRNA digestion products were separated by TLC on cellulose (Fig. 1). The nucleosides were eluted

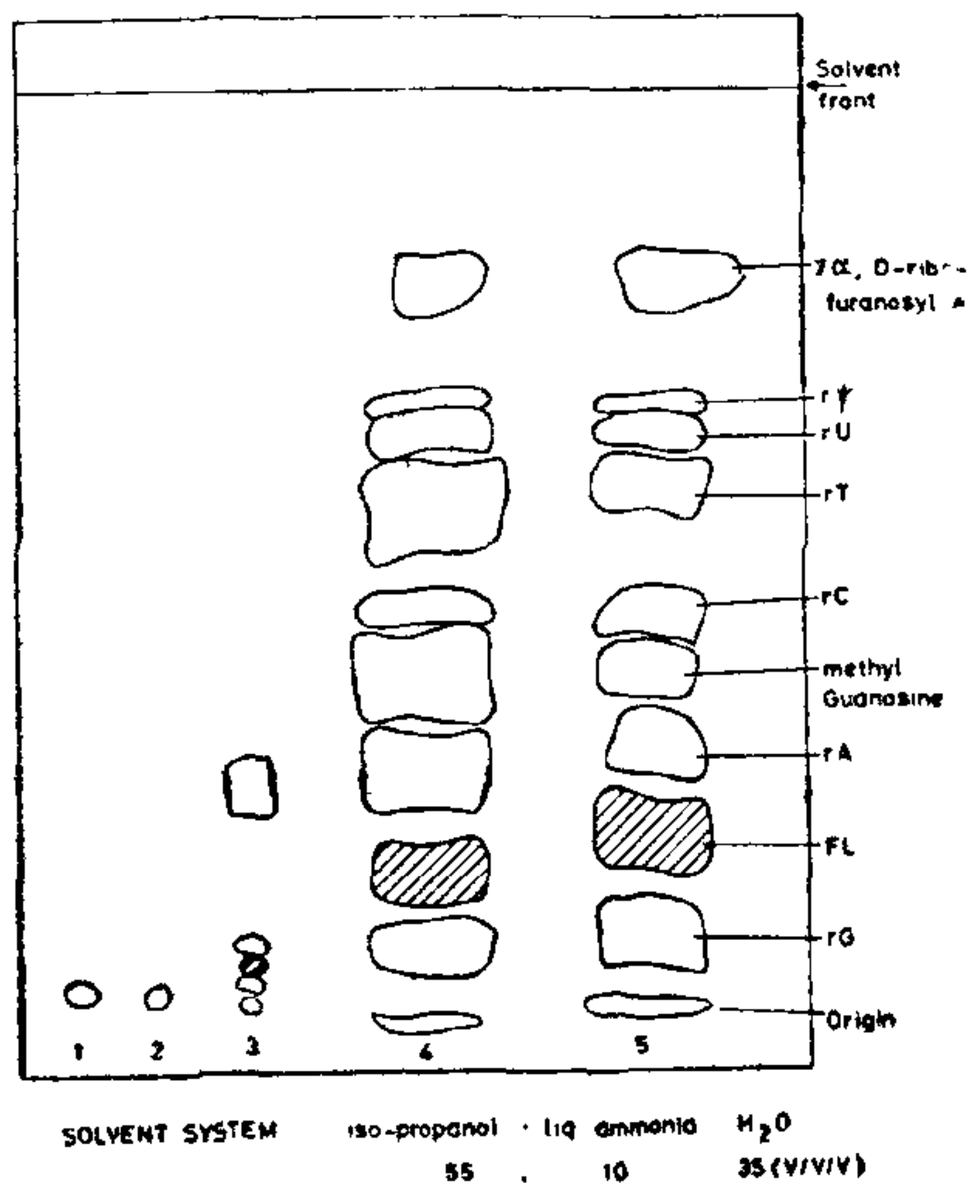

FIG. 1. Thin Layer Chromatography on cellulose. Different tRNA samples, as listed below, were chromatographed using microcrystalline cellulose; 1, *E. coli* total tRNA, untreated; 2, *M. smegmatis* total tRNA, untreated; 3, *M. smegmatis* total tRNA, alkaline hydrolysate; 4, *M. smegmatis* total tRNA, alkali hydrolysis followed by alkaline phosphatase treatment; 5, *M. tuberculosis* $H_{37}R_v$ total tRNA, alkali hydrolysis followed by alkaline phosphatase treatment. (FL = Fluorescent nucleoside.)

using 0·1 N HCl and the ultraviolet spectra were recorded. Tentative identifications of the nucleosides were carried out based on the ultraviolet spectral characteristics at different pH conditions and cochromatography with available nucleosides as reference markers. In addition to the normal

constituents of tRNA (U, C, A and G), the modified bases like ribo T, methyl G, pseudo U, and 7, α D-furanosyl A were also present. The fluorescent spot was located near the guanosine residue and showed the characteristic mobility change of nucleotide to nucleoside conversion on treatment with phosphatase. The ultraviolet absorption and the fluorescent spectra of the isolated nucleoside are presented in Figs. 2 and 3. The fluorescent spectra of the isolated compound and the total tRNA were identical.

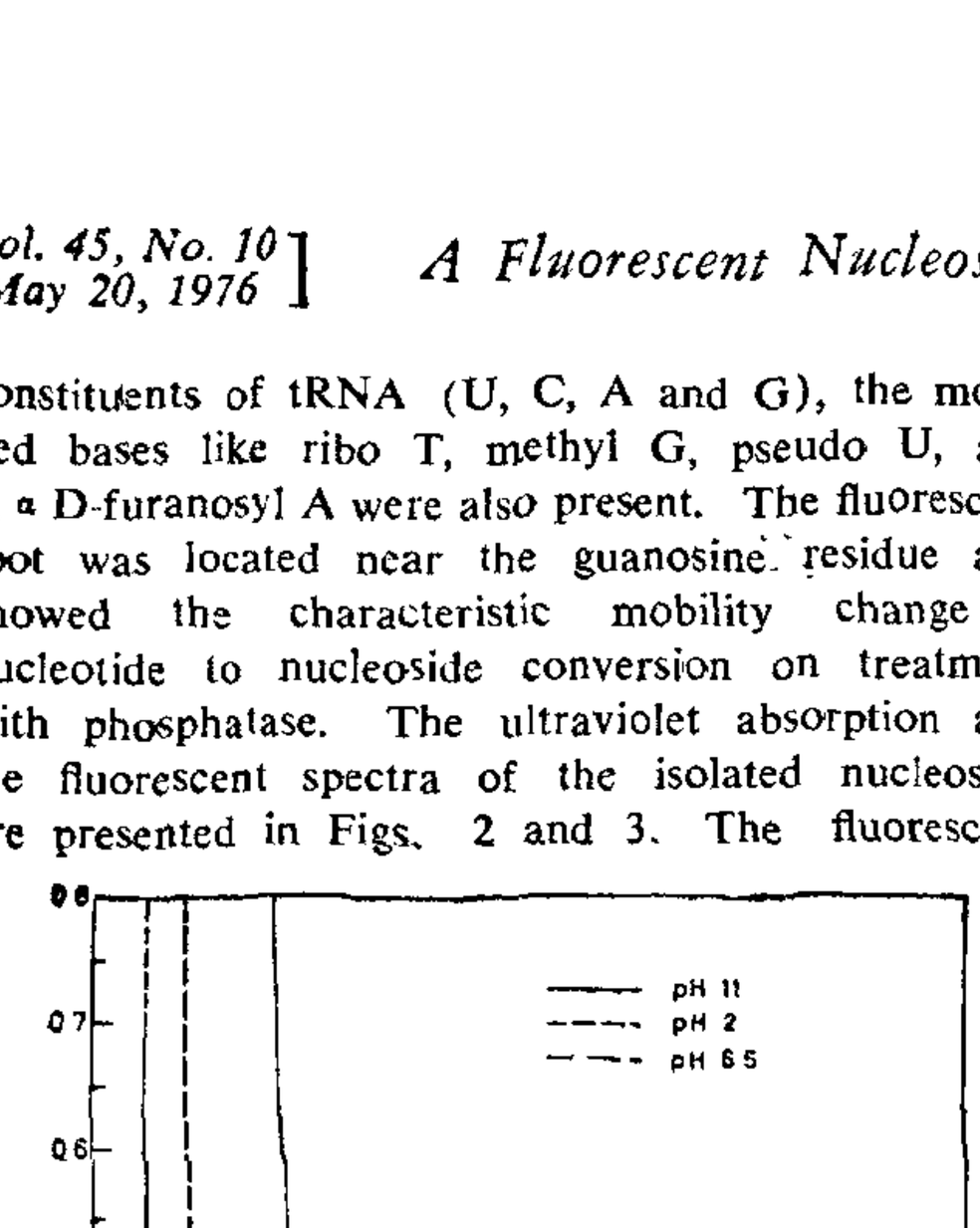

FIG. 2. Ultraviolet absorption spectra of the fluorescent nucleoside from tRNA.

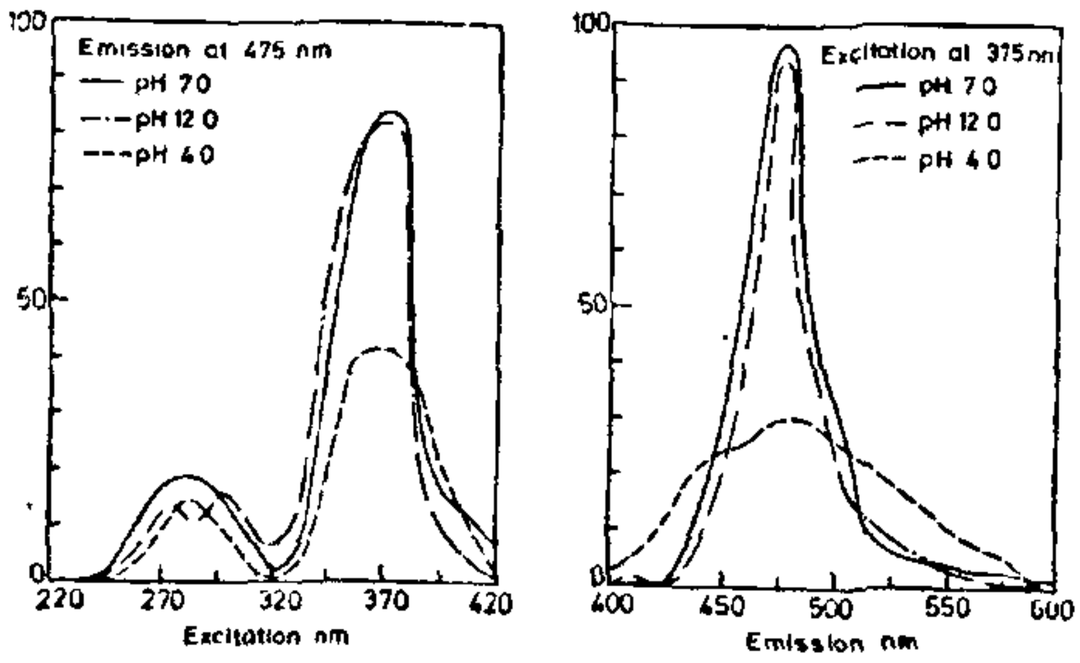

FIG. 3. Fluorescent spectra of nucleoside.

The low mobility of the fluorescent nucleoside on TLC under the conditions used and the patterns of ultraviolet spectra suggested that this might be a modified purine derivative. The nucleoside was however distinctly different from the fluorescent 'Y' base and all other modified nucleosides reported in literature till now. Further experiments for the complete characterization of the nucleoside and its role if any, in the regulation of the tRNA functions, are under way.

The authors are grateful to the Department of Atomic Energy, India, for the financial assistance.

1. Nishimura, S., *Progr. Nucl. Acid. Res. Mol. Biol.*, 1972, 12, 50.
2. Bernhardt, D. and Darnell, J., *J. Mol. Biol.*, 1969, 42, 43.
3. Moshowitz, D. B., *Ibid.*, 1970, 50, 143.
4. Blobstein, S., Grunberger, D., Weinstein, I. B. and Nakanishi, K., *Biochemistry*, 1973, 12, 188.
5. Dudock, B. S., Katz, G., Taylor, E. K. and Holley, R. W., *Proc. Natl. Acad. Sci. U.S.A.*, 1969, 62, 941.
6. Fink, L. M., Goto, T. and Weinstein, I. B., *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1969, 28, 409.
7. Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D. and Weinstein, I. B., *J. Amer. Chem. Soc.*, 1970, 92, 7617.
8. —, Blobstein, S., Funamizu, M., Furutachi, N., Van Lear, G., Grunberger, D., Lanks, K. W. and Weinstein, I. B., *Nature New Biol.*, 1971, 234, 107.
9. Raj Bhandary, U. L. and Chang, S. H., *J. Biol. Chem.*, 1968, 243, 598.
10. Thiebe, R. and Zachau, H. G., *Europ. J. Biochem.*, 1968, 5, 546.
11. Capecchi, M. R., *J. Mol. Biol.*, 1966, 21, 173.
12. Deobagkar, D. N., *Ph.D. Thesis*, submitted to the Indian Institute of Science, Bangalore, India, 1975.