PHOSPHORESCENCE FROM SULFUR-CONTAINING BASES IN ESCHERICHIA COLI t-RNA

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Transfer ribonucleic acids (t-RNA) are known to contain a number of unusual bases among which are methylated derivatives, pseudo-uracil, dihydro-uracil, inosine ... (Miura, 1967). It has been recently shown that E. coli-t-RNA contains thioderivatives of normal bases such as 2-thiopyrimidine (Carbon et al, 1965) and 4-thiouracil (Lipsett, 1965-1966). Among the physical methods which could be used to identify unusual bases of t-RNA, emission spectroscopy seems to be very convenient.

We wish to present here the results of a preliminary study of E. coli valine-specific t-RNA which shows that 4-thiouracil in this RNA is responsible for a phosphorescence of short lifetime. A short report on the phosphorescence of E. coli t-RNA recently appeared (Steiner et al, 1967) but no mention was made of the emission from thiopyrimidines.

MATERIALS AND METHODS

Valine-specific t-RNA was purified from total E. coli B t-RNA purchased from General Biochemicals. Two phase column chromatography, as described by Kelmers (1966), was run at 37°C, the t-RNA eluted by a sodium chloride gradient in 10^{-2}M sodium acetate (pH 4.5). Valine acceptor activity was separated into a $t\text{-RNA}_{val\ I}$ minor peak and a $t\text{-RNA}_{val\ II}$ major peak eluted first and second

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respectively. The t-$RNA_{val\ II}$ peak fractions were pooled and rechromatographed twice on hydroxylapatite columns at pH 6.8 and 5.8 according to Muench and Berg (1966). The peak fractions, having a constant acceptance activity of 1.5 mμmoles per A_{260} O.D. unit, were desalted and concentrated.

Emission measurements have been carried out at the temperature of liquid nitrogen with a Jobin-Yvon apparatus equipped with two quartz prism monochromators, a xenon XBO 250 W lamp and a 1P28 photomultiplier. Aqueous solutions were frozen in 2 mm i.d. quartz tubes. Excitation and emission spectra have been corrected for monochromator transmission, lamp and photomultiplier responses. Phosphorescence decay-times were measured with a Tektronix 549 storage oscilloscope equipped with a polaroid camera. A cary 14 spectrophotometer has been used for absorption measurements.

RESULTS

When excited with ultra-violet light at 77°K, normal bases and nucleotides (A,C,T,U,G) fluoresce and phosphoresce. The fluorescence and phosphorescence characteristics (spectra,yields and lifetimes) have been reported (Gueron et al, 1967, Hélène and Michelson, 1967). Interactions between bases in polynucleotides and nucleic acids lead to a modification of luminescence characteristics which has been ascribed to excimer formation (Eisinger et al, 1966, Hélène and Michelson, 1967) or energy transfer phenomena (Hélène, 1966, Eisinger and Shulman, 1967, Hélène et al, 1968). Base pairing as in poly(A+U) or poly(G+C) is associated with a luminescence quenching (Eisinger et al, 1966, Hélène, 1966).

The absorption spectrum of E. coli t-$RNA_{val\ II}$ shows an absorption band above 300 nm, the λ max. of which is about 335 nm (Figure 1). Previous studies of unfractionated E. coli t-RNA and of tyrosine-specific t-RNA have led to the conclusion that this band was mainly due to 4-thio UMP (Lipsett, 1965, 1966). In t-$RNA_{val\ II}$, this absorption is about 2% of the absorption at 260 nm and should correspond to one thio UMP per t-RNA molecule. Heat-denaturation of t-$RNA_{val\ II}$ in 0.05 M cacodylate buffer, pH 7, was followed by adsorption spectrophotometry at two wavelengths (260 and 330 nm). As already observed in t-RNA_{tyr} (Lipsett, 1966), there is a sharp transition in the 330 nm absorption with a T_m of 52°C while there is a gradual hyperchromicity at 260 nm over a wide temperature range with a T_m of 62°C (Figure 1). This suggests that the region containing the 4-thio U has a less stable secondary structure than the rest of the t-RNA molecule.

When excited at 77° K in the wavelength range 300-350 nm, t-$RNA_{val\ II}$ emits a phosphorescence the spectrum of which is shown in Figure 2. The

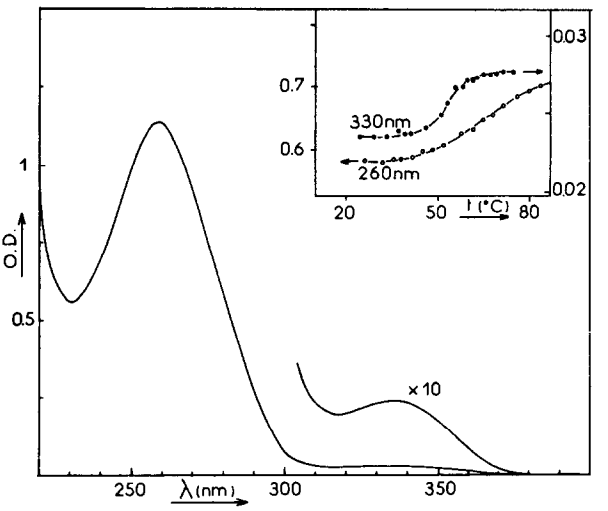

Figure 1 : Absorption spectrum of a 2.7 x 10^{-5} M solution of t-RNA$_{val}$ II in 0.05 M cacodylate buffer, pH 7, obtained in a 1 cm light path cell.

Insert : Heat denaturation of t-RNA$_{val}$ II. Absorbancy was followed at 330 nm (filled circles; right scale) and at 260 nm (open circles; left scale) in a 1 cm light path cell. The solvent was 0.05 M cacodylate buffer, pH 7. The curves at 330 and 260 nm were respectively obtained with the 0-0.1 and the 0-1 slidewires of the Cary 14 spectrophotometer. The solution was diluted twice with buffer for the 260 nm measurements.

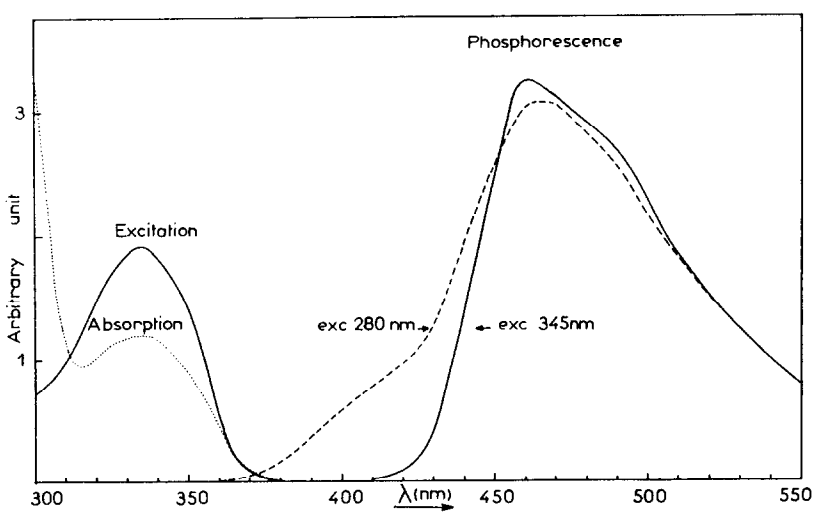

Figure 2 : Phosphorescence and excitation spectra of t-RNA$_{val}$ II at 77° K. The phosphorescence spectra were obtained by excitation at 345 nm (full line) and 280 nm (broken line). The excitation spectrum of the phosphorescence was obtained with the emission monochromator set at 470 nm. Phosphorescence and excitation spectra were corrected (see text). The absorption spectrum of t-RNA$_{val}$ II at room temperature is also included for comparison (dotted line).

corrected excitation spectrum of this phosphorescence is similar to the absorption spectrum of 4-thio UMP either isolated or incorporated in t-RNA (Figure 2). The phosphorescence lifetime is 3.5 ms in water and 4 ms in a water-propyleneglycol mixture (1v/1v). This value is quite short when compared with that of normal nucleotides : the shortest phosphorescence lifetime is that of TMP (0.3 s) and the longest is that of AMP (2.7 s) (Hélène, 1966 - Gueron et al, 1967). We have investigated the phosphorescence at 77°K of three other sulfur-containing bases, namely 2-thiouracil, 2-thiothymine and 6-thioguanine. In each case, the phosphorescence yield is very high and the phosphorescence lifetime is short (Table 1). For 2-thiothymine and 6-thioguanine we have been able to detect electron spin resonance signals (Hmin.) of the triplet state from which the phosphorescence is emitted (Table 1). Therefore, the lowest triplet is a ($\pi - \pi^*$) state and the short phosphorescence lifetime can be ascribed to spin-orbit coupling enhancement by sulfur atoms, similar to that observed for example in halogeno-derivatives of polycyclic hydrocarbons (see for example El-Sayed, 1965). A complete account of these results will be published soon.

TABLE 1 : Triplet-state characteristics of thioderivatives in water-propylene glycol mixture (1v/1v) at 77°K.

	Phosphorescence		Electron spin resonance (= 9229 MHz)	
	λ max.(nm)	τ (ms)	Hmin.(gauss)	D^* (cm^{-1})*
t-RNA$_{val\ II}$ (exc. 335 nm)	462	4	-	-
2-thiouracil	428	17	-	-
2-thiothymine	455	23	1260	0.171
6-thioguanine	468	40	1448	0.127

* D^* is a measure of electron spin-spin interaction (see Van der Waals and De Groot, 1959, 1960).

$$D^* = \left[\frac{3}{4}(h\nu)^2 - 3(g\beta Hmin)^2\right]^{1/2}$$

When excited at 280 nm, where all bases absorb light, the phosphorescence of t-RNA$_{val\ II}$ is the superposition of several components. One of them has a short lifetime and is probably due to 4-thio UMP. Another one is very long lived ($\simeq$ 1.5 s) but the low intensity does not allow precise measurements. The long-lived component probably originates from purine bases. A fluorescence is also detected by excitation at 280 nm (λ max. $\simeq$ 350 nm).

From this preliminary study, it can be concluded that sulfur-containing bases should be easily identified in t-RNA by phosphorescence measurements. Complex formation between t-RNA$_{val\ II}$ and valyl-t-RNA-synthetase does not greatly modify the short lifetime phosphorescence of t-RNA. This seems to imply that 4-thio UMP does not directly participate in the recognition site.

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