Optically Detected Magnetic Resonance of *Escherichia coli* Glutamic Acid Specific Transfer Ribonucleic Acid and Its Anticodon–Anticodon Complex with Yeast Phenylalanine-Specific Transfer Ribonucleic Acid†

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ABSTRACT: The low-temperature phosphorescence and the optically detected magnetic resonance (ODMR) spectra of *Escherichia coli* $tRNA_2^{Glu}$ and its anticodon–anticodon complex with yeast $tRNA^{Phe}$ are reported. The ODMR signals are assigned to the modified base 5-[(methylamino)-methyl]-2-thiouracil (mnm^5s^2U) located at the "wobble" position of the anticodon. The zero-field splittings (zfs) are larger than those found for 1-methyl-2-thiouracil previously studied in a 1-methyluracil host system by ODMR. They are comparable, however, to those found for neat, polycrystalline 1-methyl-2-thiouracil and for the latter dissolved in ethyl acetate solvent. In the polycrystalline sample, five traps with widely varying zfs are assigned. A very large (15–25%) reduction in the $|D|$ parameter of mnm^5s^2U is found to occur on formation of the anticodon–anticodon complex with yeast $tRNA^{Phe}$. Additional ODMR signals found in the complex are assigned to the wybutine base of yeast $tRNA^{Phe}$. The extreme sensitivity of the zfs parameters of s^2U to environmental perturbations is ascribed to the variable involvement of the heavy atom containing chromophore, C=S, in the triplet-state wave function, which is largely localized on the C=C—C=O portion of the molecule.

Thiouracils frequently occur as modified bases in the tRNAs of *Escherichia coli*. The modified base s^4U[1] is found at position 8 from the 5′-end of most tRNAs from *E. coli* (Singhal & Falls, 1979). The phosphorescence emission of the base s^4U has been used (Hélène et al., 1968; Hélène & Yaniv, 1970) in spectroscopic studies of *E. coli* tRNAs at 77 K. In order to further extend the usefulness of thiouracils as probe molecules of tRNA structure, we have used optical detection of magnetic resonance, ODMR (Clarke, 1982), to investigate the phosphorescent triplet states of m^1s^2U, m^1s^4U, and $m^1s^2s^4U$ (Taherian & Maki, 1981a; Taherian, 1981). The zfs of these triplet states is found to be unexpectedly large, with $|D|$ increasing in the order $s^2U < s^4U < s^2s^4U$. The large value of the zfs in s^4U can be understood in terms of the internal heavy atom effect introduced by the sulfur atom (Taherian et al., 1982). In a previous publication (Taherian & Maki, 1981b) we reported the ODMR study of *E. coli* $tRNA_1^{Val}$. In that work, we focused on the ODMR signals of s^4U that is present at the 8-position (5′ → 3′) of that tRNA. It was found that the zfs of the s^4U triplet state were sensitive to conformational changes resulting from removal of Mg^{2+} from solution. The phosphorescence spectrum of $tRNA_1^{Val}$ was found to be sensitive to the excitation wavelength. Blue-shifted emission produced ODMR signals that were assigned to the modified base cmo^5U, which is located at the 5′ end of the anticodon triad.

In this paper we extend our ODMR investigations to another *E. coli* tRNA, $tRNA_2^{Glu}$, which is one of the few tRNAs from this organism that does not contain the base s^4U. Instead, $tRNA_2^{Glu}$ has the modified base 5-[(methylamino)methyl]-2-thiouracil, mnm^5s^2U, at the 5′ end of the anticodon, often called the "wobble" position. The presence of a modified base at this position appears to be a well-documented structural property of tRNA molecules, having only three known exceptions (Singhal & Falls, 1979). Bases in this position are modified in a characteristic manner; a uridine at this position, for instance, is often modified by sulfur substitution at the 2-position and/or attachment of a bulky group at position 5 (Nishimura, 1972; McCloskey & Nishimura, 1977). Whereas we found previously that the zfs parameters of s^4U were relatively insensitive to changes in local environment, the zfs of s^2U are found in the present study to be extremely sensitive to the local environment. The zfs of mnm^5s^2U in *E. coli* $tRNA_2^{Glu}$ are found to differ greatly from the model system of m^1s^2U in crystalline m^1U studied earlier (Taherian & Maki, 1981a). The variation of the zfs is indeed a genuine environmental perturbation, and not due to the substituent in the 5-position, since we find also that the large zfs changes are mimicked by m^1s^2U when dissolved in ethyl acetate or when studied as a neat polycrystalline sample. We also have studied the effect on the zfs of mnm^5s^2U of forming the anticodon–anticodon complex between yeast $tRNA^{Phe}$ and *E. coli* $tRNA_2^{Glu}$, whose anticodons are complementary (Eisinger, 1971a,b). This complex has been studied previously by ODMR spectroscopy (Luk, 1975).

Materials and Methods

m^1s^2U was prepared by published procedures (Vorbrueggen & Strehlke, 1973). The product was chromatographed over a silica gel column by using ethyl acetate as the eluent. White crystals, which separated after concentrating the solution, were purified further by recrystallization from methanol. Phosphorescence and ODMR spectroscopic measurements were made on the neat polycrystalline material and on 10^{-4} M

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[1] Abbreviations: s^nU, *n*-thiouracil; m^1s^nU, 1-methyl-*n*-thiouracil; $m^1s^2s^4U$, 1-methyl-2,4-dithiouracil; ODMR, optical detection of triplet-state magnetic resonance; zfs, zero-field splittings; *D* and *E*, zero field splitting parameters; cmo^5U, 5-(carbomethoxy)uridine; mnm^5s^2U, 5-[(methylamino)methyl]-2-thiouracil; EG, ethylene glycol; EPA, ethyl ether–isopentane–ethanol (5:5:2); AM-PMDR, amplitude-modulated phosphorescence–microwave double resonance; yW, wybutine; Gm, 2′-methoxyguanosine.

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Table I: Phosphorescence Origins and Zero-Field Splittings for Uracil and Some Sulfur Derivatives

sample	origin (nm)	ν_1 (GHz)[a] [$\Delta\nu_1$ (MHz)]	ν_2 (GHz)[a] [$\Delta\nu_2$ (MHz)]	ν_3 (GHz)[a] [$\Delta\nu_3$ (MHz)]	$\lvert D\rvert$ (cm^{-1})	$\lvert E\rvert$ (cm^{-1})
poly(rU)[b]		~0.65	5.48 [370]	6.13 [410]	0.194	0.011
m^1s^2U in m^1U[c]	391.2	4.365 [2]	6.500 [35]	10.872 [20]	0.2895	0.0728
m^1s^2U (neat)						
trap A	397.7	4.73 [21]	7.17 [200]	11.59 [130]	0.308	0.079
trap B	397.8	4.64 [21]	8.40 [130]	13.10 [160]	0.358	0.077
trap C	397.9	3.78 [230]	9.11 [130]	12.86 [140]	0.367	0.063
trap D	398.0	5.66 [230]	8.22 [160]	13.84 [220]	0.369	0.094
trap E	398.2	4.69 [21]	9.87 [165]	14.52 [200]	0.406	0.078
m^1s^4U in m^1U[c]	475.0	3.001 [4.4]	16.57 [107]	19.70 [100]	0.605	0.0500

[a] Numbers in brackets are the line widths at half-maximum intensity. [b] Data are from Luk (1975). The sample is dissolved in ethylene glycol–H_2O and is complexed with Ag^+ to enhance the phosphorescence intensity. [c] Data are from Taherian & Maki (1981a).

solutions in ethyl acetate (Fluka, Puris.). *E. coli* MRE 600 $tRNA_2^{Glu}$ and brewer's yeast $tRNA^{Phe}$ were obtained as lyophilized powders from Boehringer-Mannheim. Aqueous solutions were prepared by dissolving the tRNAs in 10 mM $MgCl_2$ and annealing the solution for $^1/_2$ h at 80 °C. Under these conditions, the tertiary structure will be melted (Crothers et al., 1974). The samples were cooled slowly, over a period of 30 min, to room temperature and subsequently dialyzed against cacodylate buffer (30 mM cacodylate, 100 mM NaCl, 10 mM $MgCl_2$, pH 7) for 2 days at room temperature. Other samples of the tRNAs were prepared similarly but omitting the thermal melting and annealing steps. The phosphorescence, the ODMR, and the effects of association via anticodon complexes were indistinguishable from those measured for the heated tRNAs. Although we cannot rule out completely the formation of some cleavage fragments by heating with Mg^{2+}, they apparently are not present in sufficient quantities to affect the spectroscopic observations. The tRNA concentrations were determined by UV absorption spectroscopy using a molar extinction coefficient of 6.3×10^5 at 260 nm (Eisinger, 1971a) and found to be 5.4×10^{-5} M ($tRNA_2^{Glu}$) and 1.5×10^{-4} M ($tRNA^{Phe}$). the tRNA solutions were mixed with equal volumes of ethylene glycol (EG, Matheson Coleman and Bell, chromatoquality) for low-temperature spectroscopic studies. The anticodon–anticodon complex was formed by mixing appropriate volumes of the aqueous tRNA solutions and keeping the mixture at 0 °C overnight before addiiton of EG. Ethylene glycol may have some influence on tRNA structure. We have found in previous work, however (Hoover et al., 1974), that anticodon–codon recognition is not affected in the presence of 50% EG.

Low-temperature phosphorescence and ODMR studies were carried out by using apparatus and methods described previously (Maki & Co, 1976; Maki et al., 1978; Taherian & Maki, 1981a,b). Phosphorescence spectra were measured either at 77 K or at the temperature of pumped liquid He (1.1–1.2 K). ODMR measurements were made under the latter conditions. AM-PMDR measurements (ElSayed et al., 1970; Olmstead & ElSayed, 1974) were carried out at 1.1–1.2 K by using previously described procedures (Davis & Maki, 1982).

Results

(A) 1-Methyl-2-thiouracil (Neat Polycrystalline Sample). The phosphorescence spectrum of m^1s^2U, shown in Figure 1, is characterized by a well-resolved 0,0-band at 398.2 nm with a full width at half-maximum intensity of ca. 50 cm^{-1}. There is an underlying broad emission, but well-resolved vibronic bands are found at 0,0–723 and at 0,0–1617 cm^{-1}. Except for the broad underlying emission, the spectrum is very similar to that observed previously for m^1s^2U as a guest in polycrystalline m^1U (Taherian & Maki, 1981a). There is, however, a red shift of 450 cm^{-1} in neat m^1s^2U relative to its emission from the m^1U host. The ODMR spectrum of m^1s^2U in the m^1U host reported previously (Taherian & Maki, 1981a) consists of three narrow bands (4.365, 6.500, and 10.872 GHz) and no evidence of site heterogeneity. When the phosphorescence of neat m^1s^2U is monitored at its phosphorescence 0,0-band with relatively broad (60-cm^{-1}) slits, very complex ODMR spectra are observed, as shown in Figure 2. By using narrower monochromator slits (5 cm^{-1}) and varying the detection wavelength throughout the 0,0-band, we could obtain simpler spectra. The zfs of five distinguishable sites could be assigned from the behavior of the relative intensities of the ODMR signals with detection wavelength. These are given in Table I where they are compared with the single site observed in the m^1U host system. The approximate 0,0-band emission maxima of these sites obtained from the wavelength dependence of the signal intensities are given as well. It should be noted that $|D|$ increases with increasing red shift of the emitting site but that E varies less and apparently is not directly related to the red shift. The much larger zfs observed for the triplet state of m^1s^4U in a polycrystalline m^1U host also are included in Table I for comparison.

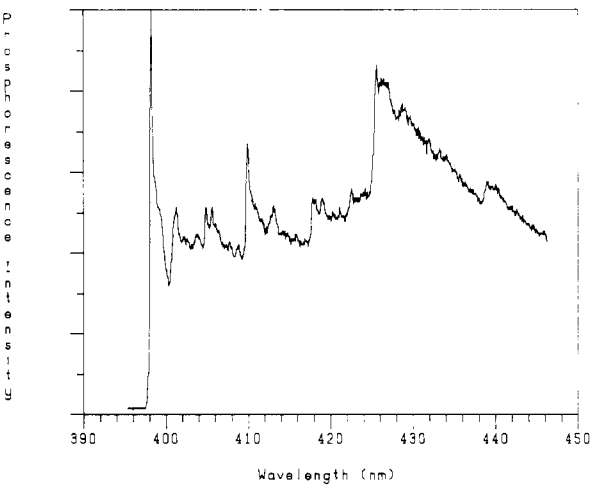

FIGURE 1: Phosphorescence spectrum of neat, polycrystalline m^1s^2U at 4.2 K. Excitation is at 312 nm and the emission is monitored with a monochromator slit width of 0.16 nm.

(B) 1-Methyl-2-thiouracil in Ethyl Acetate. The phosphorescence spectrum of m^1s^2U in ethyl acetate at 4.2 K is broad and poorly resolved, resembling that of s^2U in EPA glass (Pownall et al., 1978). The origin occurs at 392 nm, which is close to that of m^1s^2U in the m^1U host. The ODMR spectrum of m^1s^2U in ethyl acetate is complex, but it bears some resemblance to that obtained from neat m^1s^2U with broad slits. The individual transitions are broader, but at least 11 transitions are resolved in the frequency range between 4.3 and 16.5 GHz. It was not possible, however, to make unique

Table II: Zero-Field Splittings of *E. coli* $tRNA_2^{Glu}$ and Its Anticodon–Anticodon Complex with Yeast $tRNA^{Phe}$

sample	ν_1 (GHz)[a] [$\Delta\nu_1$ (MHz)]	ν_2 (GHz)[a] [$\Delta\nu_2$ (MHz)]	ν_3 (GHz)[a] [$\Delta\nu_3$ (MHz)]	$\|D\|$ (cm^{-1})	$\|E\|$ (cm^{-1})
$tRNA_2^{Glu}$					
site I	4.4 [300]	9.3 [600]	12.9 [700]	0.370	0.073
site II	4.4 [300]	11.1 [800]	14.7 [800]	0.430	0.073
anticodon–anticodon complex					
mnm^5s^2U signals	4.3 [550]	7.3 [1250]		0.315	0.072
yW signals	0.81 [200]	1.69 [240]	2.51 [200]	0.0700	0.0135

[a] Numbers in brackets are the line widths at half-maximum intensity.

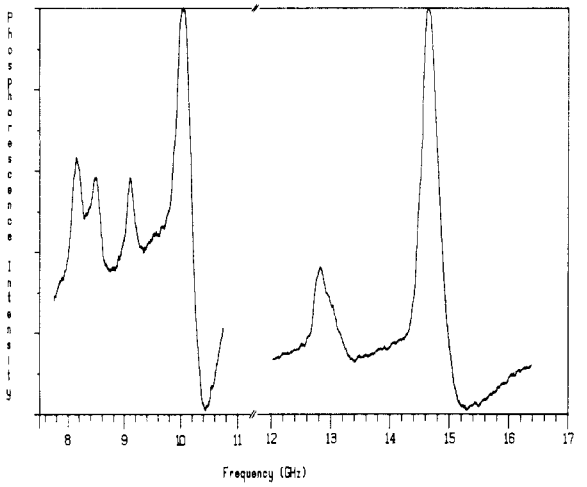

FIGURE 2: ODMR spectra of neat, polycrystalline m^1s^2U at 1.1 K. The sample is excited at 312 nm and the 0,0-band is monitored with a monochromator bandwidth of 0.96 nm. The signals are the result of 2048 scans with each range swept at a rate of 12.5 MHz/ms.

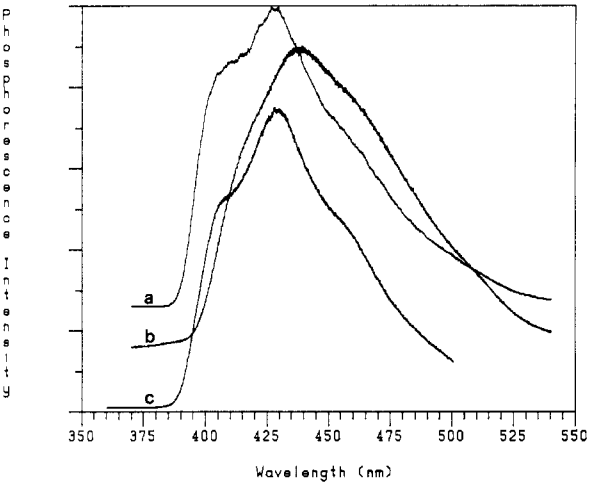

FIGURE 3: Phosphorescence spectra of (a) *E. coli* $tRNA_2^{Glu}$, (b) yeast $tRNA^{Phe}$, and (c) the anticodon–anticodon complex of the two tRNAs. The temperature is 4.2 K, and excitation is at 310 nm.

assignments of zfs to individual sites by optical selection because of the broad, unstructured character of the phosphorescence. It is clear, however, that several discrete emitting sites are present having variations in zfs even larger than those observed in neat m^1s^2U.

(C) E. coli $tRNA_2^{Glu}$. The phosphorescence spectrum of *E. coli* $tRNA_2^{Glu}$ is independent of excitation wavelength, indicating that there is only one emitting base. The spectrum, shown in Figure 3, is broad and poorly resolved, resembling that of m^1s^2U in ethyl acetate. The origin of the phosphorescence is at 388 nm, somewhat to the blue of m^1s^2U in ethyl acetate. The decay of the phosphorescence at 1.2 K when monitored at 406 nm with 3-nm slits could be fit to a sum of three exponential components (3.8 ms, 66%; 33 ms, 17%; 200 ms). These decay lifetimes are similar to, although somewhat shorter than, those found (Taherian & Maki, 1981a) for m^1s^2U in m^1U by using transient ODMR methods. The phosphorescence of *E. coli* $tRNA_2^{Glu}$ is accordingly assigned to the minor base, mnm^5s^2U, which occupies the wobble position of the anticodon, 5′-mnm^5s^2U-U-C-3′.

The ODMR spectrum of *E. coli* $tRNA_2^{Glu}$ contains a prominent relatively low frequency line at 4.40 GHz. This frequency is close to ν_1 frequency of m^1s^2U given in Table I. This signal is accordingly assigned as the ν_1 transition of mnm^5s^2U in the tRNA. Higher frequency transitions are found in the region 8–18 GHz; these are shown in Figure 4. Four broad signals can be resolved in this frequency range. One possible assignment of these signals is to $|D|-|E|$ and $|D| + |E|$ transitions from two resolved sites. The value of E is nearly the same for both sites, so only one $2|E|$ signal is resolved at ca. 4.40 GHz. Thus, the two sites are distinguished by a difference in the value of D. The ODMR frequencies and zfs parameter assignments are included in Table II. It should be noted that this assignment of the transitions of *E.*

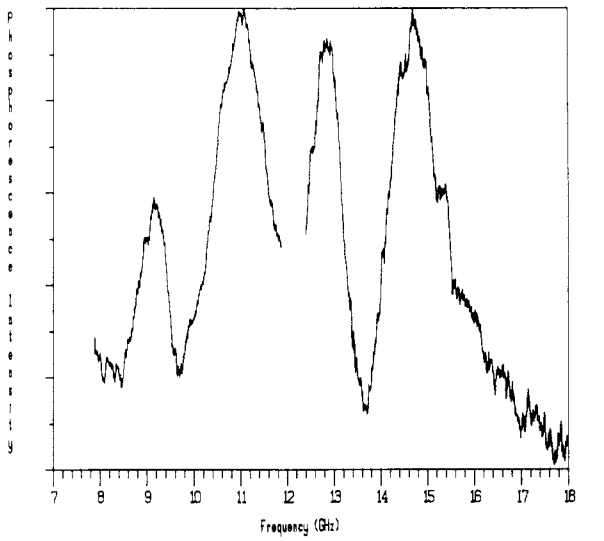

FIGURE 4: ODMR spectra of *E. coli* $tRNA_2^{Glu}$ at 1.1 K. The sample is excited at 312 nm with the phosphorescence monitored at 410 nm. The region between 8 and 12 GHz is swept at 8.8 MHz/ms, and 3600 scans have been averaged. The 12–18-GHz region is swept at 11.2 MHz/ms and is averaged for 1260 scans.

coli $tRNA_2^{Glu}$ does not provide particularly good consistency between the three ODMR transitions for which $\nu_1 + \nu_2 = \nu_3$ should hold. The signals are broad, however, and there can be a significant apparent breakdown of this relationship in heterogeneous samples (Maki & Co, 1976). In order to confirm the assignments of the two highest frequency ODMR transitions to distinct emitting sites of the tRNA, we measured the AM-PMDR spectra (ElSayed et al., 1970; Olmstead & ElSayed, 1974) obtained by coherently modulating each of these transitions in turn. The AM-PMDR spectra obtained each resemble the phosphorescence of the tRNA, but the

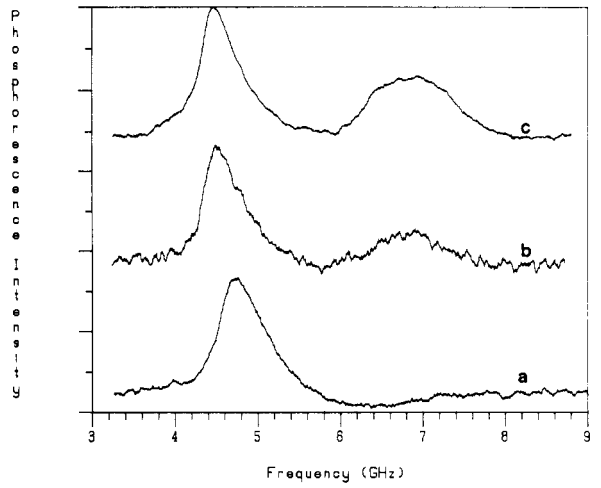

FIGURE 5: ODMR spectra at 1.1 K of (a) *E. coli* $tRNA_2^{Glu}$, (b) the anticodon–anticodon complex with $tRNA^{Phe}$:$tRNA_2^{Glu}$ = 1:1, and (c) the anticodon–anticodon complex with $tRNA^{Phe}$:$tRNA_2^{Glu}$ = 2:1. The samples were excited at 310 nm, and the phosphorescence was monitored at 430 nm. The microwave sweep rate was 9.2 MHz/ms.

spectrum obtained by modulating the higher frequency ODMR signal is shifted to the red by ca. 620 cm^{-1} relative to the other. This confirms the assignment of the two highest frequency transitions to distinct mnm^5s^2U sites and shows that the site with the larger $|D|$ has the lower triplet-state energy relative to the ground state.

(*D*) *Anticodon–Anticodon Complex of Yeast* $tRNA^{Phe}$ *and E. coli* $tRNA_2^{Glu}$. The phosphorescence spectrum of the complex formed with the ratio $tRNA^{Phe}$:$tRNA_2^{Glu}$ = 2:1 is compared in Figure 3 with those of *E. coli* $tRNA_2^{Glu}$ and yeast $tRNA^{Phe}$ for the same excitation wavelength of 310 nm. It is clear that the phosphorescence resembles that of *E. coli* $tRNA_2^{Glu}$ and is thus mainly due to mnm^5s^2U. The phosphorescence of yeast $tRNA^{Phe}$ has been shown previously (Hoover et al., 1974) to originate from the highly modified base wybutine (yW), which occupies the position adjacent to the 3′ end of the anticodon, 5′-Gm-A-A-3′. ODMR signals of yW are found below 3 GHz (Hoover et al., 1974; Luk, 1975). The phosphorescence of the anticodon–anticodon complex formed between the tRNAs at the ratio $tRNA^{Phe}$:$tRNA_2^{Glu}$ = 1:1 contains significant long-lived components (1–2 s) in addition to the short-lived decay of mnm^5s^2U. The presence of these long-lived components suggests that yW contributes to the decay. This is confirmed by characteristic yW ODMR signals given by the anticodon–anticodon complex, which will be described below.

The effect on the ODMR signals of mnm^5s^2U in the 4–8 GHz signal region when the anticodon–anticodon complex is formed is shown in Figure 5. As the complex is formed, a new ODMR signal appears at 7.3 GHz that is not present in *E. coli* $tRNA_2^{Glu}$, itself. Its intensity increases as the ratio $tRNA^{Phe}$:$tRNA^{Glu}$ is increased from 1:1 to 2:1. At the same time, the higher frequency signals observed in $tRNA_2^{Glu}$ (Figure 4) vanish into the noise, but the ν_1 signal is unaffected. The 7.3-GHz signal is close in frequency to that assigned to the ν_2 transition of m^1s^2U in polycrystalline m^1U (Table I). Since this frequency is larger than those of yW or of any other bases that give ODMR signals in these tRNAs (Luk, 1975) and has a short response time characteristic of a heavy atom perturbed base, we assign it to mnm^5s^2U as its ν_2 frequency in the complex. Although no ν_3 transition is observed, these results suggest that the formation of the anticodon–anticodon complex leads to the reduction of $|D|$ to a value that is comparable to that observed from m^1s^2U in the m^1U host. The ODMR frequencies and zfs assignments are given in Table II.

Additional signals are observed in the range below 3 GHz when the phosphorescence of the anticodon–anticodon complex is monitored at 1.2 K. The signal frequencies are given in Table II. The two higher frequency transitions were assigned previously by Hoover et al. (1974) to yW in yeast $tRNA^{Phe}$. The ν_1 transition could not be observed in the tRNA or in the excised yW. Formation of the anticodon complex with *E. coli* $tRNA_2^{Glu}$ induces the appearance of the ν_1 signal as well as a change in the polarity of the ν_3 signal from a decrease to an increase in phosphorescence intensity. These effects have been observed previously by Luk (1975).

Discussion

The modified base mnm^5s^2U is found at a strategic location in the tRNA molecule, namely the wobble position of the anticodon triad. Modification of this base is believed to have important biological implications, to the extent that the absence of such modifications is considered to be lethal (McCloskey & Nishimura, 1977). Minor uridine bases at the wobble position are believed to exercise control in codon recognition processes (Ishikura et al., 1971; Sekyia et al., 1969). An anticodon group containing s^2U shows increased specificity of codon recognition in that base pairing with A is enhanced while that with G is excluded. This behavior is attributed to the reduced ability to sulfur to form hydrogen bonds (Yoshida et al., 1970; Agris et al., 1973). In addition, the side chain at position 5 is believed to have a major role in determining the three-dimensional structure of the anticodon loop (Berman et al., 1978; Sen & Ghosh, 1976; Hillen et al., 1978). Crystal structures of bases that have attached groups at the 5-position (Berman et al., 1978; Hillen et al., 1978) show that these have a strong influence on the hydrogen bonding of the 4-keto group of uracil. In addition, this group has been shown to alter the stacking pattern of neighboring groups on the base at the wobble position. For these reasons, the effect of binding the anticodon of *E. coli* $tRNA_2^{Glu}$ to the complementary anticodon of yeast $tRNA^{Phe}$ on the ODMR properties of mnm^5s^2U at the wobble position is of great interest. The extremely large variation observed in the zfs of m^1s^2U in various environments (Table I) indicates that it is an extremely sensitive probe of local environmental perturbations. The principal effects that we observe from the ODMR of mnm^5s^2U in *E. coli* $tRNA_2^{Glu}$ is (A) the existence of two major sites with differing zfs, each characterized by a large value of $|D|$ in the uncomplexed tRNA, and (B) the conversion of these sites to one having a smaller value of $|D|$ (comparable to that observed in m^1s^2U in the m^1U host) when complexing with yeast $tRNA^{Phe}$ takes place. The zfs observed for uncomplexed *E. coli* $tRNA_2^{Glu}$ is, in fact, comparable to those found for sites in neat m^1s^2U and for m^1s^2U in ethyl acetate.

We have shown previously (Taherian & Maki, 1981a; Taherian et al., 1982) that the large values of $|D|$ that occur in thiouracils are a consequence of spin–orbit coupling induced by the presence of the sulfur atom. The increase in $|D|$ monotonically in the order $U < s^2U < s^4U < s^2s^4U$, which accompanies the increase in triplet-state decay constant in the same order, indicates that the heavy atom effect from sulfur at the 4-position is larger than that at the 2-position. In order for the heavy atom effect to be produced, the sulfur atom must participate in the delocalized π system that is characteristic of th excited π–π^* state. We expect a more pronounced heavy atom effect from sulfur at position 4, since it is known (Hug & Tinoco, 1979; Nagata et al., 1973) that the lowest excited state of uracils is delocalized over the network O=C(4)—C-

(5)=C(6). A recent investigation (Nishimura & Tsuboi, 1980) of resonance Raman effects in uracils and thiouracils has confirmed this description. In uracil, for instance, they found that the C(4)=O vibration undergoes a resonance enhancement by the 260-nm absorption band, while the C(2)=O vibration does not undergo resonance enhancement. The explanation is that the electronic excitation does not include the C(2)=O group and thus that this chromophore is not conjugated with the one that is responsible for the electronic excitation. In the same work, Nishimura & Tsuboi (1980) show that the C(4)=S group of s^4U is conjugated while the C(2)=O group is not conjugated with the chromophore responsible for the absorption band at 333 nm. On the other hand, they show that the C(4)=O group of s^2U is conjugated with the chromophore absorbing at 300 nm, while the C(2)=S stretching Raman line (717 cm^{-1}) appears weakly on excitation at 300 nm. According to these authors, "the pi-electrons on C(2)=S must migrate into the O=C(4)—C(5)=C(6) system to some extent, in contrast to the uracil C(2)=O, whose pi-electrons do not seem to migrate into the O=C(4)—C(5)=C(6) system at all". These observations are in close accord with our findings from the ODMR measurements on the thiouracil systems. The increase in the value of $|D|$ between U and m^1s^2U in m^1U (Table I) is consistent with some involvement of π electrons of C(2)=S in the delocalized triplet-state wave function. On the other hand, the further increase of $|D|$ when m^1s^2U is placed in the ethyl acetate host or when it is studied as a neat solid is probably best explained in terms of the increased involvement of C(2)=S in the triplet wave function. Such an increased involvement also is characteristic of the mnm^5s^2U site in *E. coli* $tRNA_2^{Glu}$. It is possible that the mnm^5s^2U finds itself in a relatively disordered and solvent-exposed site similar to m^1s^2U in ethyl acetate. Formation of the anticodon–anticodon complex, on the other hand, produces a new environment in which the C(2)=S group is further isolated from the triplet-state chromophore. Judging from the zfs, this environment may be more homogeneous and similar to that of m^1s^2U in the m^1U host crystal. Apparently, the zfs of s^2U are far more sensitive to subtle environmental perturbations than are those of s^4U. Conformational changes in *E. coli* $tRNA_1^{Val}$ induce changes in the zfs parameters of s^4U that are only a few percent (Taherian & Maki, 1981b). This is in contrast to the reduction of $|D|$ by 15–25% that occurs in mnm^5s^2U of *E. coli* $tRNA_2^{Glu}$ upon formation of the anticodon–anticodon complex. The reason for the enhanced sensitivity of s^2U to environmental perturbations appears to stem from the partial involvement of the C=S chromophore in the triplet excited state. Small changes in this involvement induced by environmental effects can produce a relatively large effect on the zfs through the agency of spin–orbit coupling. In s^4U, on the other hand, since the C=S group already is fully involved in the triplet excited state, environmental effects produce relatively small changes in the zfs.

Registry No. m^1s^2U, 615-78-1; mnm^5s^2U, 21263-85-4.

References

Agris, P. F., Soell, D., & Seno, T. (1973) *Biochemistry 12*, 4331–4337.

Berman, H. M., Marcu, D., Narayanan, P., Fissekis, J. D., & Lipnick, R. L. (1978) *Nucleic Acids Res. 5*, 893–903.

Clarke, R. H., Ed. (1982) *Triplet State ODMR Spectroscopy: Techniques and Applications to Biophysical Systems*, Wiley, New York.

Crothers, D. M., Cole, P. E., Hilbers, C. W., & Shulman, R. G. (1974) *J. Mol. Biol. 87*, 63–88.

Davis, J. M., & Maki, A. H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 4313–4316.

Eisinger, J. (1971a) *Biochem. Biophys. Res. Commun. 43*, 854–861.

Eisinger, J. (1971b) *Biochem. Biophys. Res. Commun. 44*, 1135–1142.

ElSayed, M. A., Owens, D. V., & Tinti, D. S. (1970) *Chem. Phys. Lett. 3*, 395–399.

Hélène, C., & Yaniv, M. (1970) *Eur. J. Biochem. 15*, 500–504.

Hélène, C., Yaniv, M., & Elder, J. W. (1968) *Biochem. Biophys. Res. Commun. 31*, 660–664.

Hillen, W., Egert, E., Lindner, H. J., & Gassen, H. G. (1978) *Biochemistry 17*, 5314–5320.

Hoover, R. J., Luk, K. F. S., & Maki, A. H. (1974) *J. Mol. Biol. 89*, 363–378.

Hug, W., & Tinoco, I., Jr. (1979) *J. Am. Chem. Soc. 95*, 2803–2813.

Ishikura, H., Yamada, Y., & Nishimura, S. (1971) *Biochim. Biophys. Acta 228*, 471–481.

Luk, K. F. S. (1975) Thesis, University of California, Riverside.

Maki, A. H., & Co, T. (1976) *Biochemistry 15*, 1229–1235.

Maki, A. H., Svejda, P., & Huber, J. R. (1978) *Chem. Phys. 32*, 369–380.

McCloskey, J. A., & Nishimura, S. (1977) *Acc. Chem. Res. 10*, 403–410.

Nagata, C., Imamura, A., & Fujita, H. (1973) *Adv. Biophys. 4*, 1–69.

Nishimura, S. (1972) *Prog. Nucleic Acid Res. Mol. Biol. 12*, 49–85.

Nishimura, Y., & Tsuboi, M. (1980) *Science (Washington, D.C.) 210*, 1358–1360.

Olmstead, J., & ElSayed, M. A. (1974) in *Creation and Detection of the Excited State* (Ware, W. R., Ed.) Vol. 2, pp 2–63, Marcel Dekker, New York.

Pownall, H. J., Schaffer, A. M., Becker, R. S., & Mantulin, W. W. (1978) *Photochem. Photobiol. 27*, 625–628.

Sekyia, T., Takeishi, K., & Ukita, T. (1969) *Biochim. Biophys. Acta 182*, 411–426.

Sen, G. C., & Ghosh, H. P. (1976) *Nucleic Acids Res. 3*, 523–535.

Singhal, R. P., & Fallis, P. A. M. (1979) *Prog. Nucleic Acid Res. Mol. Biol. 23*, 227–290.

Taherian, M. R. (1981) Thesis, University of California, Davis.

Taherian, M. R., & Maki, A. H. (1981a) *Chem. Phys. 55*, 85–96.

Taherian, M. R., & Maki, A. H. (1981b) *Biochemistry 20*, 7295–7301.

Taherian, M. R., Fink, W. H., & Maki, A. H. (1982) *J. Phys. Chem. 86*, 2586–2591.

Vorbrueggen, H., & Strehlke, P. (1973) *Chem. Ber. 106*, 3039–3061.

Yoshida, M., Takeishi, K., & Ukita, T. (1970) *Biochem. Biophys. Res. Commun. 39*, 852–857.