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Effect of Stacking Interactions with Poly(riboadenylic acid) on the Triplet State Properties of Tryptophan†

Tiao-te Co and August H. Maki*

ABSTRACT: Optically detected magnetic resonance (ODMR) signals of tryptophan (Trp) have been measured in L-lysyl-L-tryptophyl-L-lysine (Lys-Trp-Lys) and in its complex with poly(riboadenylic acid) [poly(rA)]. Measurements were made with optical narrow band detection through the Trp O–O band. Plots of $|D|$ and $|E|$ vs. λ are distinctly different for Lys-Trp-Lys and its complex with poly(rA). A reduction of $|D|$, in particular, is consistent with stacking of Trp with adenine in the complex, since this effect is expected from charge-transfer contributions in the excited triplet state. Triplet energy transfer from poly(rA) to Lys-Trp-Lys is nearly complete at 77 K, with a Trp:adenine ratio of 0.1. The energy transfer efficiency is considerably reduced at 4.2 K and below, probably resulting from reduction of the triplet mobility in the polymer. Analysis of the phosphorescence decays shows that the triplet states of poly(rA), Lys-Trp-Lys, and their complex decay nonexponentially. Binding of polylysine to poly(rA) has no effect on the phosphorescence spectrum, but the decay kinetics are changed.

Interactions between proteins and nucleic acids play a vital role in the functioning of biological systems. These interactions may involve electrostatic forces between charged groups, hydrogen bonding, and stacking forces (Yarus, 1969; Hwang, 1971). Exposed aromatic side chains—tryptophan (Trp),[1] tyrosine, and phenylalanine—could be important in selective recognition of base sequences through stacking interactions involving π overlap. Quenching of Trp fluorescence as a result of such interactions has been observed in Trp-nucleoside aggregates formed in frozen aqueous solutions (Montenay-Garestier and Hélène, 1968, 1971; Hui Bon Hoa and Douzou, 1970) and from complexation of indole derivatives and small Trp-containing peptides such as Lys-Trp-Lys with DNA and with various RNAs (Wagner, 1969; Smythies and Antun, 1969; Hélène, 1971a; Hélène et al., 1971; Raszka and Mandel, 1971; Brun et al., 1975). Spectroscopic methods also have been used to study stacking interactions in covalently linked indole-base dimer models (Mutai et al., 1975), tRNA-synthe-

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[1] Abbreviations used are: poly(rA), poly(riboadenylic acid); Trp, tryptophan; Lys-Trp-Lys, L-lysyl-L-tryptophyl-L-lysine; poly(Lys), polylysine; CD, circular dichroism; ODMR, optical detection of magnetic resonance; ZFS, zero field splitting; λ, wavelength; ^{1}H NMR, proton magnetic resonance; EPR, electron paramagnetic resonance.

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tase complexes (Hélène et al., 1969; Farrelly et al., 1971; Hélène, 1971b), and complexes between DNA and T4 phage gene 32 protein (Hélène et al., 1976). Specific effects of base stacking with the aromatic amino acids of peptides on the CD of poly(rA) and other helical polyribonucleotides have been reported (Durand et al., 1975) as well as ^{1}H NMR shifts attributed to stacking-induced ring current shifts in these systems (Gabbay et al., 1972; Hélène and Dimicoli, 1972; Dimicoli and Hélène, 1974).

In this communication we report the first study of peptide-nucleic acid interactions using ODMR. In previous work (Zuclich et al., 1974; von Schütz et al., 1974; Maki and Zuclich, 1975), we have shown that the ZFS parameters of Trp are sensitive to environmental perturbations and that heterogeneity in Trp phosphorescence can be detected by a combination of optical wavelength selection and ODMR. Our measurements are made on the system poly(rA) complexed with the tripeptide, Lys-Trp-Lys. The phosphorescence of poly(rA) at 77 K has been shown to be largely quenched upon complexing with Lys-Trp-Lys, and the phosphorescence of Trp is sensitized by triplet-triplet energy transfer from the polynucleotide (Hélène, 1973). It was estimated that the triplet quenching range of a bound stacked Trp is about 70 bases at 77 K. Phosphorescence quenching of poly(rA) by Mn^{2+} has been studied as a function of temperature below 77 K by Szerenyi and Dearman (1973). The triplet mobility and the quenching range were found to be severely reduced at temperatures approaching 4.2 K; this was attributed to a thermally activated detrapping process.

The purpose of this communication is to determine whether ODMR can be used to detect Trp-nucleic base stacking through environmental perturbations of the ZFS parameters, $|D|$ and $|E|$. Shifts in these parameters which depend upon the relative distribution of the triplet electron pair (McGlynn et al., 1969) are to be expected if a stacked triplet state involves significant charge transfer character (McGlynn et al., 1960). The model system was chosen in light of the compelling evidence from previous work for the existence of nucleic base-Trp stacking.

Materials and Methods

Lys-Trp-Lys was obtained from Research Plus Laboratories, Inc., while poly(rA) and poly(Lys) (average mol wt 3400) were products of Sigma Chemical Co. All were used without further treatment. A solution of 5×10^{-4} M Lys-Trp-Lys was prepared by mixing equal volumes of ethylene glycol and an aqueous buffer solution containing 10^{-3} M Lys-Trp-Lys, 5×10^{-3} M sodium cacodylate, 10^{-3} M NaCl and adjusted to pH 7 with HCl. A solution of 5×10^{-4} M poly(rA), one containing 10^{-3} M poly(rA) and 10^{-4} M Lys-Trp-Lys, and one containing 10^{-3} M poly(rA) and 2×10^{-4} M polylysine were prepared in the same manner. All concentrations refer to the monomer. Phosphorescence and ODMR measurements were made with the sample contained in a 1-mm quartz tube.

The apparatus for measurement of phosphorescence spectra and slow passage ODMR spectra at pumped liquid He temperature (ca. 1.15 K) has been described previously, as has the procedure for deconvolution of signal-averaged phosphorescence decays (Maki and Co, 1976). An EMI, Inc., Model 6256S photomultiplier was used to detect the phosphorescence, and the exciting light was passed through a Bausch and Lomb, Inc., 0.25-m grating monochromator with a band pass of 6.5 nm.

Phosphorescence decays were followed over a dynamic range of approximately three decades in intensity and signal averaging was employed for 16 decays. Linearity of the instrumentation was verified by measurement of the phosphorescence decay of tryptophan over the same dynamic range. The tryptophan decay is observed to be a single exponential at 77 K and 4.2 K over a three-decade dynamic range.

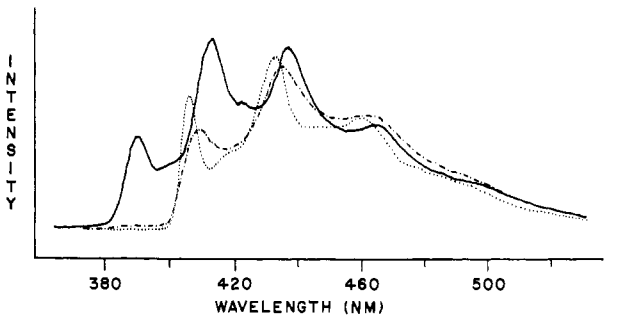

FIGURE 1: Phosphorescence spectra at 77 K of 5×10^{-4} M poly(rA) (—, excitation = 280 nm); 5×10^{-4} M Lys-Trp-Lys (· · · · ·, excitation 290 nm); and 10^{-3} M poly(rA) with 10^{-4} M Lys-Trp-Lys (- - - -, excitation = 280 nm). The solvent systems for these samples are described in the Experimental Section.

Samples typically were cooled to ca. 100 K over a period of 2–3 h, whereupon they were subsequently chilled to either 77 K or 4.2 K over a period of a few minutes by addition of either liquid nitrogen or liquid helium.

All slow-passage ODMR measurements were made with the monitoring monochromator set for 1-nm band pass. Two measurements were made at each detection wavelength; one with increasing and the other with decreasing microwave frequency, and the peak microwave frequencies averaged. The same sweep rate of 34 MHz/s was used for all measurements. This method of measurement compensates for displacement of the magnetic resonance peaks resulting from residual fast-passage effects. All spectra were signal averaged to improve the signal-to-noise ratio. ODMR measurements were made in the $|D| - |E|$ and $2|E|$ zero-field transition regions of Trp and adenine.

Experimental Results

The 77 K phosphorescence spectra of poly(rA), Lys-Trp-Lys, and poly(rA) complexed with Lys-Trp-Lys are shown in Figure 1. The quenching of the poly(rA) phosphorescence is nearly complete as a result of complexing with Lys-Trp-Lys, and the resulting phosphorescence spectrum resembles that of Lys-Trp-Lys, although the band maxima are broader and shifted to the red by about 4 nm. Residual phosphorescence remaining at ca. 390 nm confirms a small amount of unquenched adenine emission. These results are in agreement with previous measurements (Hélène, 1973) where the quenching of the poly(rA) phosphorescence has been attributed to triplet-triplet energy transfer from adenine to the indole chromophore.

Measurement of the phosphorescence decay of the various samples was made at 77 K, and the results are given in Table I.

The phosphorescence spectra of the complex of poly(rA) with Lys-Trp-Lys are compared at 4.2 K and 77 K in Figure 2. Although the phosphorescence peak of poly(rA) at 390 nm is nearly completely absent at $T = 77$ K, cooling the sample to 4.2 K results in the emergence of a peak at ca. 387 nm which we attribute to the adenine triplet state. ODMR signals observed at 1.15 K monitoring the emission at 387 nm occur at frequencies characteristic of the adenine triplet state (vide infra). The emergence of adenine phosphorescence when the poly(rA) complex of Lys-Trp-Lys is cooled to liquid helium

TABLE I: Phosphorescence Decays at 77 K of Poly(rA), Lys-Trp-Lys, and Their Complexes.

Sample[a]	Excitation λ (nm)	Monitoring λ (nm)	α_i[b]	τ_i[b] (s)
Poly(rA), 5×10^{-4} M	280	390	25	2.71
			7.3	1.49
			1	0.42
	280	410	25	2.67
			7.0	1.34
			1	0.30
Lys-Trp-Lys, 5×10^{-4} M	290	410	3.8	6.92
			1	4.38
Poly(rA), 10^{-3} M; Lys-Trp-Lys, 10^{-4} M	280	410	11.8	6.67
			3.8	3.78
			1	1.11
Poly(rA), 10^{-3} M;[c] poly(Lys), 2×10^{-4} M	280	390	5.7	2.84
			4.4	1.52
			1	0.35
	280	410	4.5	2.82
			3.6	1.58
			1	0.42

[a] Samples were measured in 1:1 ethylene glycol-aqueous buffer containing 5×10^{-3} M cacodylate, 10^{-3} M NaCl, pH 7.0. [b] Decay was fitted to the equation $I(t) = \Sigma\alpha_i \exp(-t/\tau_i)$. Values are the average of several independent determinations. Errors in τ_i and α_i of the dominant component estimated as ±0.1 s, and ±10%, respectively. Weaker components are less accurate. [c] Sample of the poly(Lys) bromide dialyzed for 24 h against two 16-fold excess volumes of buffer. Results are the same as for undialyzed sample within experimental error.

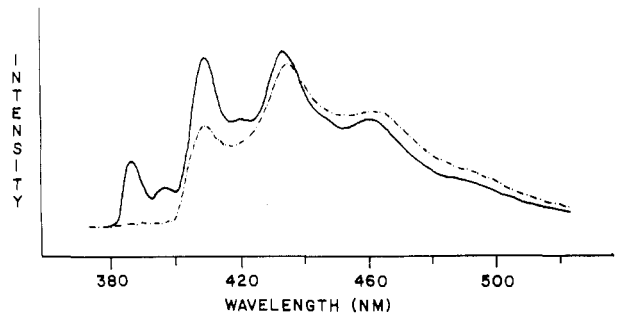

FIGURE 2: Phosphorescence spectra of 10^{-3} M poly(rA) with 10^{-4} M Lys-Trp-Lys (excitation = 280 nm) at 4.2 K (—) and at 77 K (- - - -).

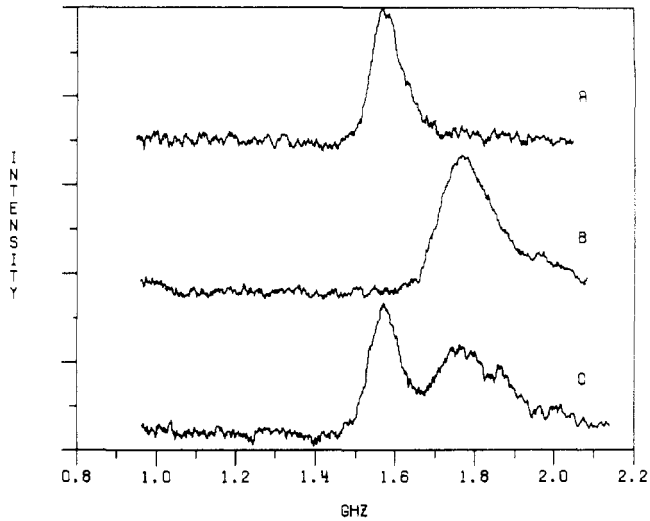

FIGURE 3: Slow-passage ODMR signals of (A) 5×10^{-4} M poly(rA), excitation = 280 nm; (B) 5×10^{-4} M Lys-Trp-Lys, excitation = 290 nm; and (C) 10^{-3} M poly(rA) with 10^{-4} M Lys-Trp-Lys, excitation = 280 nm. Monitored wavelength = 407 nm and $T \approx 1.15$ K for all measurements.

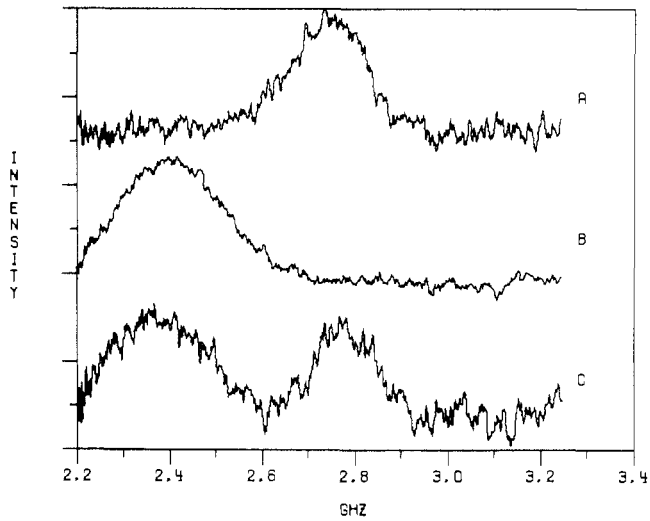

FIGURE 4: Slow-passage ODMR signals. Samples A, B, and C correspond to identically labeled samples of Figure 3. Conditions of measurement are the same as those in Figure 3 except for the microwave frequency range.

temperature conforms to the models of Hélène and Longworth (1972), and Szerenyi and Dearman (1973) in which the mobility of the triplet excitation in poly(rA) is limited by a thermally activated detrapping process.

ODMR measurements were carried out at 1.15 K using narrow optical band-width detection within the 410-nm phosphorescence band of the complex of poly(rA) with Lys-Trp-Lys. See Figure 2, solid curve. For comparison, similar measurements were made on Lys-Trp-Lys within the origin band (Figure 1, dotted curve), and measurements were made on poly(rA) at a few selected detection wavelengths. Examples of slow-passage ODMR signals observed in two frequency ranges are shown in Figures 3 and 4. The triplet states of both adenine and Trp have a zero-field ODMR signal within the microwave frequency ranges covered in Figures 3 and 4. In each frequency range only one resonance signal is observed from poly(rA) or from Lys-Trp-Lys. Each range contains two resonance signals from the complex of poly(rA) and Lys-Trp-Lys, however; one was attributed to the adenine triplet, and the other to that of Trp. Since the signals in each range are well resolved, we were able to measure the microwave fast-passage transient phosphorescence response for each signal individually (Winscom and Maki, 1971) and to compare their decay lifetimes with those of the signals of poly(rA) and Lys-Trp-Lys samples. This set of measurements served to verify that the lower frequency magnetic resonance signal in Figure 3C, and the higher frequency one in Figure 4C originate from adenine, while the other signals are due to Trp. The peak frequencies of the Trp and the adenine signals were found to be sensitive to monitoring wavelength. Monitoring the emission of the complex at 387 nm produced ODMR signals at frequencies very close to those of Figures 3A and 4A, verifying that the short wavelength peak is due to adenine phosphorescence.

The tryptophan signals of Figures 3 and 4 are due to the $|D|$

$-|E|$ and $2|E|$ transitions, respectively (Zuclich, 1970). We have plotted $|D|$ and $|E|$ calculated from the observed peak frequencies vs. monitored λ for Lys-Trp-Lys, and for its complex with poly(rA) in Figure 5. The wavelength dependence of $|D|$ and $|E|$ observed for Lys-Trp-Lys through the O-O band is very similar to that observed previously for Trp and for the solvent-exposed Trp of horse liver alcohol dehydrogenase in ethylene glycol–buffer (von Schutz et al., 1974). The break in the upward trend of $|E|$, and the downward trend of $|D|$ with increasing λ which occurs at λ = 411–412 nm, is a result of extending these measurements into the second vibronic emission band of Lys-Trp-Lys (Figure 1).

Discussion

Extensive work by Hélène and co-workers (Hélène and Dimicoli, 1972; Dimicoli and Hélène, 1974; Toulme et al., 1974; Brun et al., 1975; Durand et al., 1975) on the binding of Lys-Trp-Lys to nucleic acids has led them to propose a two step binding mechanism:

$$\text{Lys-Trp-Lys} + \text{polynucleotide} \underset{}{\overset{K_1}{\rightleftharpoons}} \text{complex I} \overset{K_2}{\rightleftharpoons} \text{complex II} \quad (1)$$

Complex I is stabilized by electrostatic interactions between phosphate anions and the lysyl side chain cations and does not involve interactions between the aromatic side chain and the nucleic acid. Complex II is stabilized by aromatic stacking interactions as well as the electrostatic interactions of the charged groups. Both complexes dissociate at high ionic strength. K_1 and K_2 have been obtained by Brun et al. (1975). K_1 increases with decreasing T in 1 mM NaCl at pH 7 and at 2 °C has a value of 1.9×10^4 M^{-1}. $K_2 = 2.2$ at this temperature. We do not know the association constant K_1 at the glassing temperature of aqueous ethylene glycol. We assume that, at a ratio of 1:10, effectively all the Lys-Trp-Lys is bound to poly(rA). About 70% of the phosphorescence intensity monitored at 410 nm decays with a lifetime of 6.7 s at 77 K, while the poly(rA) band at 390 nm is effectively quenched. Shorter lifetime components are definitely present in the decay, however. The phosphorescence decay of Lys-Trp-Lys at 77 K is itself noticeably nonexponential (Table I). Its decay can be fitted to two exponentials: a dominant component of 6.9 s, and a minor one (20%) of 4.4 s. This behavior is unusual for Trp which is usually found to decay exponentially at 77 K (Longworth, 1971; Maki and Zuclich, 1975). The charged lysine side chains in Lys-Trp-Lys may well be responsible for partial quenching of the Trp triplet state (Longworth, 1971). Nonexponential decay of the poly(rA) phosphorescence also is observed with a dominant component of 2.7 s at 77 K. Nonexponential decay of adenine has been previously reported in aqueous glasses (Szerenyi and Dearman, 1973).

We observe nonexponential decay of AMP phosphorescence at 77 K at a concentration of 1 mM in our 50:50 ethylene glycol–pH 7 cacodylate buffer containing 10 mM EDTA. The decay is fit well to two exponential components of 3.05 (± 0.1) and 1.90 (± 0.1) s with closely equal intensity. Although the decay of poly(rA) phosphorescence has previously been reported to be exponential (Hélène and Longworth, 1972) with a lifetime of 2.60 s, our decay data cannot be fit to a simple exponential decay; rather, shorter lifetime decay components appear to be present. It is unlikely that paramagnetic ion impurities are responsible for the nonexponential decay behavior since it is known from the work of Eisinger and Shulman (1966) that paramagnetic ions quench poly(rA) phosphorescence without affecting the lifetime of the emission. The

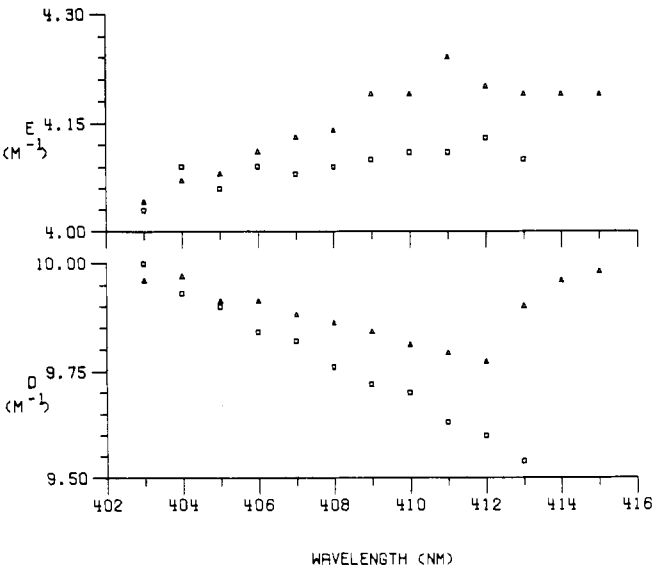

FIGURE 5: The zero-field splitting parameters $|D|$ and $|E|$ of the tryptophan triplet state vs. monitored wavelength in ethylene glycol–buffer glasses of Lys-Trp-Lys (△) and Lys-Trp-Lys-poly(rA) complex (□).

nonexponential decay kinetics of both poly(rA) and Lys-Trp-Lys phosphorescence eliminates the possibility of the complete interpretation of the decay of the complex. We note, though, that the decay of the complex is rather similar to that of Lys-Trp-Lys. Since the 390-nm emission band is nearly absent, we think that effectively all of the phosphorescence at 77 K originates from Trp. In order to be sure that there is no perturbation of the adenine phosphorescence spectrum resulting from the binding of lysine-containing peptides, we examined poly(rA) complexed with poly(Lys). There is no observable effect of poly(Lys) binding on the phosphorescence spectrum of poly(rA). We do observe a change in the decay kinetics of the adenine triplet state, however. Although the decay lifetimes obtained from the deconvolution of the emission remain effectively the same, there is a significant change in the relative preexponential terms of the decay components which accompanies poly(Lys) binding (Table I). The absence of an observable contribution of poly(rA) to the phosphorescence of the Lys-Trp-Lys-poly(rA) complex supports previous conclusions (Hélène, 1973) that the Trp excited state is populated by triplet–triplet transfer from poly(rA). The presence of mobile triplets at 77 K is strongly suggested by the delayed fluorescence measurements of Hélène and Longworth (1972). At 4.2 K, however, the contribution of adenine triplet states to the phosphorescence increases markedly due to the decreased triplet mobility in the polymer (Szerenyi and Dearman, 1973) which reduces their quenching by Trp. Even at 1.15 K, however, the ODMR data reveal a significant contribution of Trp to the phosphorescence emission (Figures 3 and 4). The best evidence that the Trp phosphorescence at 4.2 K and below originates from sites which are stacked with adenine (and therefore populated by energy transfer, since the fluorescence of Trp is known to be quenched by staking interactions with nucleic bases) comes from the ZFS determined by ODMR (Figure 5). The phosphorescence spectrum of Trp in aqueous glass results from a distribution of sites and thus the bands are inhomogeneously broadened. It has been observed previously (von Schütz et al., 1974), using optical narrow-band detection, that the Trp ODMR frequencies vary with detection wavelength. There is a correlation between the phosphorescence Stokes shift and the ZFS resulting from the heterogeneity in

the microenvironments. This is not surprising since the distribution of the triplet electron pair which determines the ZFS through the magnetic dipole-dipole interaction is influenced by local electric fields from the microenvironment. The Stark effect of these same local fields is largely responsible for the phosphorescence Stokes' shift. Therefore, it is important to measure the wavelength-dependent ZFS when the phosphorescence is inhomogeneously broadened if effects such as stacking on the ZFS are to be assessed. The decrease in $|D|$ of the complex with increasing λ over the band is 5% compared with a 2% decrease for Lys-Trp-Lys. $|E|$ increases only by about 1.5% with increasing λ over the O-O band of the complex, whereas it increases by 5% in Lys-Trp-Lys. A significant fraction of emission from Trp which is not stacked with adenine would be expected to contribute to ODMR signals in the vicinity of those observed for free Lys-Trp-Lys and would be readily resolved from the signals observed from stacked Trp near the long wavelength edge of the O-O band.

Using an average of the Trp $|D|$ and $|E|$ values over the O-O band, we calculate $D^* = 12.19\ m^{-1}$ for Lys-Trp-Lys, and $12.03\ m^{-1}$ for its complex with poly(rA).[2] These values should be directly comparable with EPR measurements of the $\Delta M = 2$ transitions, which yield D^* directly. Such measurements at 77 K have been reported on these systems by Hélène. At this higher temperature the adenine triplet state is largely quenched in the complex and the triplet state observed is that of Trp. Values of D^* estimated from the EPR data (Hélène, 1973, Figure 6) are in good agreement with the ODMR values reported above. A reduced value of $|D|$, in particular, is consistent with stacking of Trp with an aromatic chromophore. Charge transfer in the triplet state accompanying the stacking would result in an increase of $\langle z_{12}^2 \rangle$, where z_{12} is the interelectron distance of the triplet electron pair along the z (stacking) axis. Since $D \propto \langle (r_{12}^2 - 3z_{12}^2)/r_{12}^5 \rangle$, a reduction in $|D|$ (since D certainly is positive) is the expected result.

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[2] $D^* = (D^2 + 3E^2)^{1/2}$.