Electronic Relaxation Processes in Retinol and Retinal: Anomalous External Heavy-Atom Effects and Temperature Dependence of Fluorescence[1a]

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Abstract: In contrast to aromatic molecules, diphenylpolyenes, retinol, and retinal show no external heavy-atom effects. The fluorescence of *all-trans*-retinal is enhanced by halide salts. The reverse heavy-atom effect in the latter is attributable to interactions between retinal and cation of halide and thiocynate salts (KI, KBr, KCl, LiBr, LiCl, LiF, and KCNS) and of sodium acetate. The effect of halide salts on retinal has been elucidated in terms of fluorescence quantum yield, lifetime, fluorescence polarization, and triplet–triplet absorption. Consequence of the retinal–cation interaction (i.e., fluorescence enhancement) has been interpreted by a small blue shift of the (n,π^*) state so as to enhance the radiative emission from the lowest excited singlet state. The fluorescence quantum yield of retinal and retinol varies significantly over the temperature range of 14–120 K without accompanying changes in the excitation and emission spectra. It suggests a molecular relaxation of the fluorescent (π,π^*) state even in rigid matrices, competing effectively with the intersystem crossing in the presence and absence of external heavy atoms.

The radiative fluorescence lifetimes of diphenylpolyenes[2] and retinol and retinal derivatives[3] are anomalously longer than the lifetime calculated from the respective absorption band of the strongly allowed $^1B_u \leftarrow {}^1A_g$ electronic transition ($^1B \leftarrow A$ in Platt notation).[3] This anomaly, along with other spectroscopic characteristics of polyene systems, can be explained according to the new assignment that the lowest singlet excited state in polyenes is ^1A_g,[4,5] although objection to the new assignment has been presented on the basis of approximate mirror images of the main absorption band and fluorescence as well as fluorescence polarization results.[6]

In addition to the above mentioned anomalies, retinal shows a marked dependence of the fluorescence quantum yield upon excitation wavelength.[7-9] Several mechanisms for this dependence have been proposed.[7-9] More recently, the excitation wavelength dependence has been explained by preferential $^1(n,\pi^*) \rightsquigarrow {}^3(\pi,\pi^*)$ intersystem crossing.[9]

Intersystem crossing quantum yield for *all-trans*-retinal is 0.6–0.7,[10,11,13] or 0.5 in methylcyclohexane and 0.08 in methanol.[12] Yet direct triplet–triplet absorption has not been detected flash spectroscopically for *all-trans*-retinol, indicating $\Phi_{ISC} \sim 0$. The role of (n,π^*) states in the intersystem crossing and their locations in *all-trans*-retinal are not firmly established at the present. Relation between the excited state assignment and photoisomerizations of retinals[11,13,14] and rhodopsin[15] has been discussed recently.

We initiated a study of the external heavy-atom effects on the excited states of polyenes as to (a) whether such effects are particularly significant in quenching the fluorescence from the anomalously long-lived fluorescent state (relative to the predicted lifetime), and (b) what the intersystem crossing mechanism(s) are in the case of polyenes relative to aromatic systems. Instead, we have unexpectedly observed anomalous heavy-atom effects in that external heavy-atom quenching of fluorescence is absent in polyenes including retinol but fluorescence is actually enhanced in the case of *all-trans*-retinal. Temperature dependence of the fluorescence quantum yield of *all-trans*-retinal was also examined in order to ascertain intermolecular interactions and intramolecular (e.g., conformational) relaxations of the excited state.

Experimental Section

Materials. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1,8-diphenyl-1,3,5,7-octatetraene (DPO) were obtained from Sigma and Aldrich Chemical Companies, respectively, and were recrystallized from benzene. *all-trans*-Retinol and *all-trans*-retinal from Sigma Chemical Co. were purified, and their purity was ascertained by lack of impurity luminescence. Since even the best commercial samples of so-called "pure" retinol and retinal were found to be highly contaminated by luminescent impurities, it was essential to purify each sample prior to its use. Purification procedure used was thin-layer chromatography. Silica gel HR extra pure or silica gel G was used for TLC plates. Cellulose-pulver MN 300 HR was used to check possible decomposition of samples on silica gel, although separation was poor in the former. Solvent systems for TLC were *n*-hexane–ethyl acetate (97.5:2.5, v/v) for separation of cis isomers[16] and *n*-hexane–ethyl acetate (90:10 or 30:5, v/v) for preparative separation of retinol and retinal bands. Benzene (spectrograde) was also used as an alternative TLC developer. TLC bands were eluted with appropriate solvents and aliquots were immediately frozen in liquid nitrogen or in a deep-freeze to prevent oxidative degradation and formation of hemiacetal and diols in the case of retinal.[17] These retinal adducts absorb and fluoresce in the same way as retinol whose fluorescence quantum yield is almost two orders of magnitude higher than that of retinal at 77 K, and spectral overlap between fluorescence bands of retinal and retinol is substantial. *all-trans*-β-Carotene, a gift from Hoffmann-La Roche, was purified by the cellulose-pulver MN 300 HR TLC with solvent system of ethanol–water (200:25, v/v).

Potassium iodide (Baker) was recrystallized, whereas other heavy-atom salts such as LiCl (General Chemical), LiF and LiBr (Fisher), and KCl (Baker) were used without further purification, when these salts showed no significant luminescent impurities. Crown ethers used were dicyclohexyl-18-crown-6 (Aldrich) and 18-crown-6. The former was purified by alumina column chromatography and an ir spectrum of the purified crown ether was identical with the literature.[18] The 18-crown-6 was a gift from Professor Richard Bartsch, prepared according to Gokel and Cram.[19]

Solvents for TLC and spectral measurements were spectrograde, and were further fractionally distilled in some cases. In all cases, luminescent impurities were checked by fluorescence excitation method.

Methods. Fluorescence quantum yields (Φ_F^s) of dilute solutions (OD < 0.05) of *all-trans*-retinol and -retinal were calculated by the following equation

$$\Phi_F^s = \Phi_F^r \left(\frac{\int I_F^s(\bar{\nu})d\bar{\nu}}{\int I_F^r(\bar{\nu})d\bar{\nu}}\right)\left(\frac{OD_\lambda^r}{OD_\lambda^s}\right)\left(\frac{n_r^2}{n_s^2}\right)$$

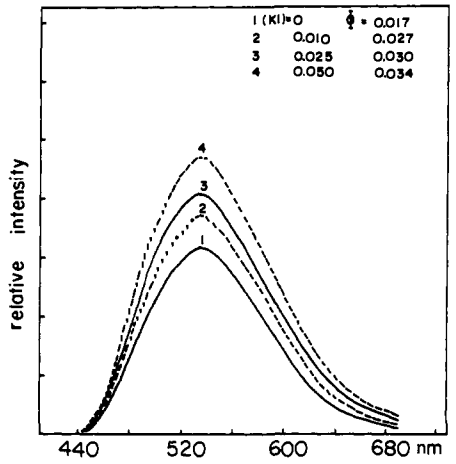

Figure 1. Corrected fluorescence spectra of *all-trans*-retinal (2.7×10^{-6} M) in ethanol at 77 K as a function of KI. Excitation (λ_{ex} ~410 nm) and emission band-passes were 9 and 1.5 nm, respectively.

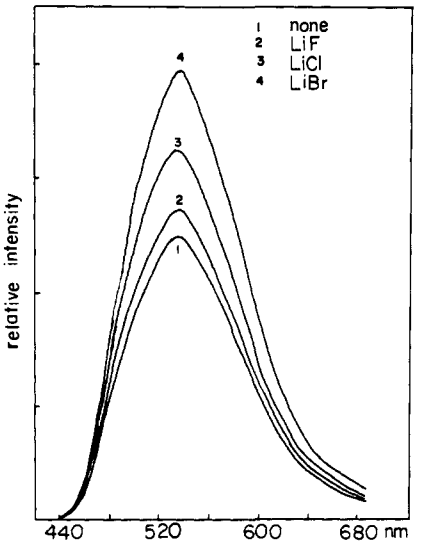

Figure 2. Corrected fluorescence spectra of *all-trans*-retinal (4×10^{-6} M) in ethanol at 77 K in the presence of different lithium halides (0.1 M). Excitation (421 nm) and emission band-passes were 10 and 2 nm, respectively.

where Φ_F^r is the fluorescence quantum yield of a reference compound, riboflavin tetrabutyrate[20] (0.64 after refractive index correction) or 9,10-diphenylanthracene (**1**).[21-24] These references absorb and emit in a convenient wavelength region for quantum yield determinations of retinal and retinol. I_F and n are corrected fluorescence intensity (in arbitrary units) and refractive index of solvent, respectively. Optical densities (OD) of reference and sample compounds were kept identical to minimize errors due to Maclaurin's approximation and band-pass differences between the absorption and fluorescence measurements. The refractive index correction in different solvents and temperatures is not necessary in the above equation since reference and sample fluorescence were measured under identical conditions. Quantum yield of retinal fluorescence at different temperatures was evaluated relative to that at 77 K. In this case, necessary refractive index corrections were made using established procedures and available data.[25-28]

Absorption spectra were measured on a Cary 118C spectrophotometer equipped with a special cell holder for low-temperature Dewar cells. Corrected fluorescence spectra (emission and excitation) for quantum yield measurements were recorded on a Hitachi Perkin-Elmer spectrofluorometer (MPF3) with correction capable to 600 nm. Above 600 nm, the correction was made by applying correction factors obtained by using the reference spectrum of rose bengal.[29]

Temperature dependence of the fluorescence spectra of retinal was measured on a high resolution, single photon counting spectrofluorometer equipped with an EMI PM tube (9659, S-20 response, −40°C) and a closed-cycle cryogenic cooler (12–300 K range, Cryogenic Technology) as described previously.[30] Fluorescence polarization (photoselection) was also measured on this spectrofluorometer, employing Polacoat uv sensitive polarizing and analyzing sheets and was corrected by the Azumi–McGlynn procedure.[31]

Fluorescence lifetimes at 77 K were measured using a tungsten–air gap discharge pulse generator–synchroscope unit[32] and N_2-laser system assembled at Hokkaido for room-temperature samples. In addition, the single photon counting method using a time-to-amplitude converter[33] was also employed for measuring short lifetimes of retinol at room temperature.

Because the excitation pulse width affects a fluorescence decay curve, a number of points were read from the oscilloscope tracing and were fed to the computer–*XY* plotter, yielding best fit decay curve expressed in counts vs. channel number. The resulting decay curve was then subjected to the lifetime determination by the method of simulated convolution curve.[34]

Triplet–triplet absorption and decay were followed on a Q-switched ruby pulse-laser photolysis apparatus (flash duration ~30 ns) constructed at Hokkaido. For pulse laser photolysis experiments, the frequency doubling to 347.2 nm was accomplished with ADP crystal. Optical density of solutions for fluorescence and triplet lifetime measurements was kept in the 0.5–0.9 range, and solutions were degassed by six to eight freeze–pump cycles, unless stated otherwise.

Table I. Effects of LiCl and KI on the Fluorescence Quantum Yield of *all-trans*-Retinal (~2×10^{-6} M) in ethanol at 77 K. The Excitation Wavelength Was 410 nm

[Salt], M		Φ_{total}[a]	Φ_F°[b]	$\Phi_{total}/\Phi_F^{\circ}$	f[c]
LiCl	0.00	0.017	0.017	1.00	0.00
	0.10	0.030	0.017	1.75	0.41
	0.20	0.034	0.017	2.00	0.55
	0.30	0.037	0.017	2.20	0.66
	0.40	0.041	0.017	2.40	0.78
	0.50	0.046	0.017	2.70	0.90
	0.60	0.048	0.017	2.80	1.00
	0.70	0.048	0.017	2.80	1.00
	0.80	0.048	0.017	2.80	1.00
KI	0.01	0.027	0.017	1.59	0.25
	0.025	0.030	0.017	1.75	0.33
	0.05	0.034	0.017	2.00	0.43
KCNS	0.05	0.037	0.017	2.20	

[a] Fluorescence quantum yield in the presence of salt. [b] Fluorescence quantum yield in the absence of salt. [c] Fraction of the fluorescence due to retinal–K^+ or –Li^+ complexes calculated from $\Phi_{total} = f\Phi_F^X + (1 - f)\Phi_F^O$, where Φ_F^X is the quantum yield due to the complex.

Results

1. Heavy-Atom Effects. Heavy-atom salts such as KI and alkyl halides strongly quench fluorescence of aromatic and some heterocyclic molecules.[35-38] The fluorescence quenching by heavy atoms is general and is largely due to enhanced $S_1 \rightsquigarrow T_1$ intersystem crossing, and it is particularly effective if the excited states involved are of (π,π^*) type and that $S_1 \rightarrow S_0$ decay is slow.[36] Thus, diphenylpolyenes and retinol are expected to show strong external heavy-atom effects in view of their anomalously long fluorescence lifetimes (radiative) and high quantum yields (slow radiationless decay).[2-4] However, our results are completely in contrast to the above expectations.

In the present experiments, effective heavy-atom salts (KI) and solvents (iodopropane and ethyl iodide) did not quench the fluorescence of DPH, DPO, and *all-trans*-retinol. LiBr (1 M) and other halide salts (0.01–1 M) also did not quench the fluorescence. In the case of *all-trans*-retinal, KI actually enhances the fluorescence emission (Figure 1). However, the fluorescence enhancement is not limited to KI since other halide salts were also found to increase the fluorescence quantum yield of *all-trans*-retinal. In fact, the

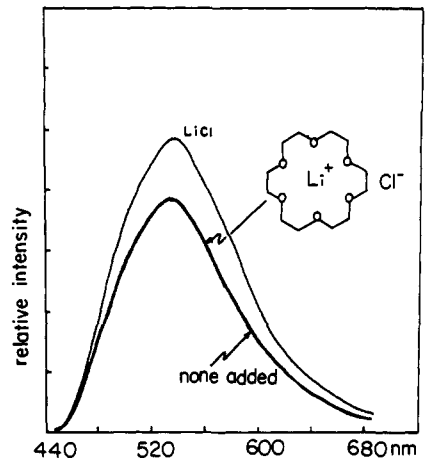

Figure 3. Effect of crown ether (18-crown-6, 0.1 M) on the LiCl-enhanced fluorescence of *all-trans*-retinal (6.3×10^{-6} M) in ethanol at 77 K. [LiCl] = 0.1 M. Excitation (410 nm) and emission band-passes were 8 and 2 nm, respectively.

fluorescence enhancement increases with atomic number of the halogen atom (Figure 2) and with its ionic radius from 1.33 (F^-) to 2.19 Å (I^-). Table I shows fluorescence dependence on the LiCl concentration. In all cases, there are no significant changes in the spectral shape and wavelength maxima in the excitation and fluorescence spectra upon addition of halide salts.[39]

We propose that the enhanced fluorescence described above is due to a complexation between retinal and monocation. Evidence for the complexation will be presented below. Monocations such as K^+, Li^+, and Na^+ [Φ_F(0.1 *M* NaAc)/$\Phi_F^\circ \sim 3.3$, sodium acetate in ethanolic solution, as sodium halide salts are not very soluble] are effective for the enhancement. The observed effect is similar to protonation (HCl added to ethanolic solution) which enhances fluorescence of *all-trans*-retinal. In the case of protonation, an equilibrium for the retinal hemiacetal formation is rapidly established to yield an isosbestic point at 342 nm and isoexcitation point at 364 nm. The apparent difference between isosbestic and isoexcitation points arises from the wavelength dependence of the fluorescence quantum yield of retinal and the higher fluorescence quantum yield of the hemiacetal (similar to retinol in yield).

The fact that retinol, DPH, and DPO do not show similar fluorescence enhancement is suggestive of the complexation between retinal carbonyl and monocations. More conclusive evidence for the complexation effect is presented in Figure 3. Thus, it can be seen from Figure 3 that the LiCl-enhanced fluorescence is completely abolished by addition of crown ether which specifically traps monocations, thus disallowing retinal-Li^+ complexation.

The effect of crown ether was also demonstrated for other systems (retinal-KI, retinal-KCNS, retinal-KCl) with either dicyclohexyl-18-crown-6 or 18-crown-6 ether. The effects of monocations and results of crown ether experiments further suggest that the fluorescence of *all-trans*-retinal is not due to intermolecular mechanisms such as the dimeric exciton model[8] since fluorescence excitation and emission spectra are identical with and without crown ethers. Further, fluorescence excitation, emission, and polarization spectra of *all-trans*-retinal are not affected by the complex formation with K^+ (cf., Figure 4), Li^+, or Na^+ ions. Thus, there is no indication that the enhanced fluorescence is from an electronic state origin drastically perturbed or different from the original fluorescent state of retinal.

LiCl is readily soluble in ethanol, and quantum yield determinations over a wide concentration range were made for this salt (Table I). From data in Table I, an apparent equi-

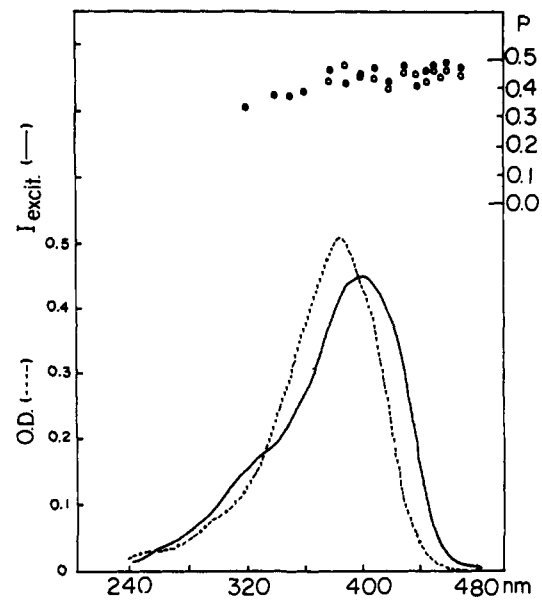

Figure 4. Corrected fluorescence excitation (—) and polarization (P with (○) and without (●) 0.05 M KI) of *all-trans*-retinal (2×10^{-6} M) in ethanol at 77 K with respect to emission at 540 nm. Absorption (1×10^{-5} M) spectrum is also shown. Polarization was measured at 0.2-nm band-pass on a single photon counting spectrofluorometer.

librium constant was estimated to be 6.53 M^{-1}, assuming that the enhanced fluorescence is due to retinal-Li^+ complex.[40] The equilibrium constant for retinal-K^+ complex is about 18 M^{-1}.

Fluorescence lifetimes of *all-trans*-retinol in the presence and absence of KI are essentially identical (Table II), thus confirming the peculiar lack of external heavy-atom effects on the basis of fluorescence intensity data. Increase in fluorescence quantum yield of *all-trans*-retinal with KI is accompanied by a roughly proportional rise in the lifetime.

In contrast to the fluorescence lifetime measured at 77 K, the triplet decay of retinal at room temperature is significantly enhanced by KI, although the effect is not as efficient as the quenching by dissolved oxygen at much lower concentration (Table III and Figure 5). Since the anthracene triplet shows similar shortening of the lifetime in the presence of KI, the retinal triplet is responding to the external heavy atom in an expected manner, unlike the retinal singlet. This peculiar behavior is consistent with the lack of fluorescence quenching by KI. Namely, the lack of fluorescence quenching must be due to relative invariance of the intersystem crossing rate with respect to KI, as illustrated by the following comparison. For retinal (at zero time in the decay process)

$$\frac{\Phi_{ISC}^\circ}{\Phi_{ISC}^{KI}}\Bigg|_{\substack{\lambda_{T\text{-}T} \sim 450\text{ nm} \\ t = 0}} \sim \frac{\Phi_T^\circ}{\Phi_T^{KI}} \sim \frac{OD_{T\text{-}T}^\circ\ (= 0.57 \pm 0.06)}{OD_{T\text{-}T}^{KL}\ (= 0.57 \pm 0.05)} = \frac{[T]^\circ = 7.6 \times 10^{-6}\text{ M}}{[T]^{KI} = 7.6 \times 10^{-6}\text{ M}} = 1 \quad (1)$$

but

$$\tau_T^{KI}/\tau_T^\circ = 0.42 \text{ at } [KI] = 0.085\text{ M} \quad (2)$$

and for anthracene

$$\frac{\Phi_{ISC}^\circ}{\Phi_{ISC}^{KI}}\Bigg|_{\substack{\lambda_{T\text{-}T} \sim 425\text{ nm} \\ t = 0}} \sim \frac{OD_{T\text{-}T}^\circ\ (= 0.203)}{OD_{T\text{-}T}^{KI}\ (= 0.382)} = 0.53 \quad (3)$$

and

$$\tau_T^{KI}/\tau_T^\circ = 0.66 \text{ at } [KI] \sim 0.08\text{ M} \quad (4)$$

Table II. Fluorescence Lifetime (τ_F) of *all-trans*-Retinol (1×10^{-5} M) and *all-trans*-Retinal (2×10^{-5} M) with Excitation Wavelength Maxima (Using Interference Filters) of 297 nm and 335 nm for the Former and 410 nm for the Latter. Values Listed Are an Average of at least Five or More Scans of Exponential Decay

Compd	Solvent	Temp, K	Φ_F	τ_F, ns[g]	Remark
Retinol	Ethanol–methanol (5:1, v/v)	77		7.6 ± 0.5	No degas
	Ethanol–methanol (5:1, v/v)	77	0.454 (0.008)[c]	8.4	Degassed[a]
	Ethanol–methanol (1:1, v/v)	77		8.0 (1.6)[c]	Degassed
	Ethanol–methanol (5:1, v/v)	77	0.45 (0.008)[c]	8.0	Degassed and [KI] = 0.104 M added
	Isopentane–methylcyclohexane (1:1, v/v)	77	0.62[d] (0.025)[d]	7.8 (4.0)[e]	No degas
	Ethanol, absolute	77	0.459 (0.011)[c]	7.2	No degas
Retinol	Ethanol, absolute	77	~0.04	1.1 ± 0.03[b]	Degassed [KI] = 0.08 M
	Ethanol, absolute	77	0.017[f]	~0.6[b]	Degassed no KI

[a] Degassing by freeze–thaw cycles. [b] From the best convoluted curves. [c] At room temperature. [d] In isopentane. [e] 4.7 ns in methycyclohexane–isohexane at room temperature. [3] [f] $\Phi_F = 0.005$ in methylcyclohexane with λ_{ex} ~340–350 nm, where excitation is less efficient than 410 nm. [g] The lifetimes of the first-order decay without correction for convolution errors range from 23 to 24 ns for retinol at 77 K.

Table III. Triplet Lifetime Data for *all-trans*-Retinal (1.0×10^{-5} M) at Room Temperature. Values Listed Represent an Average of 3–6 Synchroscope Tracings. Data for Anthracene (~1×10^{-4} M) Are also Included

Solvent	[KI], M	O_2	λ_{T-T}^{obsd}, nm	τ_T
Retinal				
Ethanol–methanol (1:1)	0	Degassed	450	10 ± 0.5 μs[a]
Ethanol–methanol (1:1)	0	Aerated	450	0.112 ± 0.005
Ethanol–methanol (1:1)	0.085	Degassed	450	4.2 ± 0.3
Ethanol–methanol (1:1)	0.085	Aerated	450	0.11 ± 0.01
Anthracene				
Ethanol–ethanol (1:1)	0	Degassed	425	~11
Ethanol–ethanol (1:1)	~0.08	Degassed	425	~7.3[b]

[a] Similar to results in nonpolar solvents. [10–12] [b] Nonexponential decay.

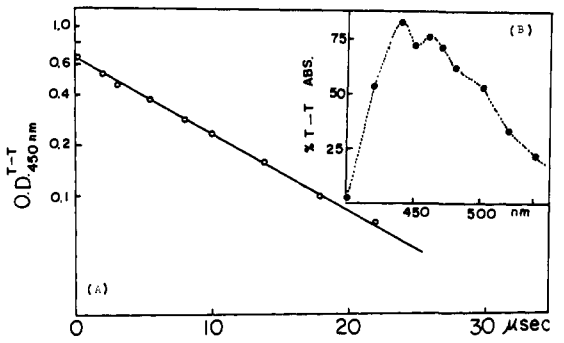

Figure 5. (A) Decay of the triplet state (triplet-triplet absorption at 450 nm) of *all-trans*-retinal (1.8×10^{-5} M) in ethanol–methanol (1:1) at room temperature. (B) The triplet–triplet absorption spectrum of *all-trans*-retinal (uncorrected for the ground state absorption in the 400–420 nm region).

where concentration of the triplet retinal was calculated using the triplet molar extinction coefficient of 7.5×10^4 l. mol^{-1} cm^{-1}.[11]

2. Temperature Dependence of Fluorescence. Figure 6 shows the temperature dependence of fluorescence of *all-trans*-retinal. Although the fluorescence quantum yield decreases sharply with temperature, emission as well as excitation maxima remain essentially unchanged. The fact that fluorescence and excitation spectra at low temperature are independent of excitation and monitoring wavelengths

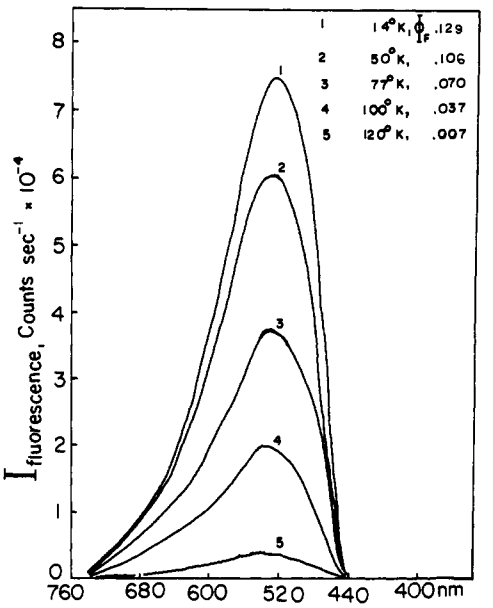

Figure 6. Temperature dependence of technical fluorescence spectra of *all-trans*-retinal (2×10^{-6} M) in isopentane, measured on a single photon counting spectrofluorometer. Excitation (420 nm) and emission band-passes were 3.2 and 0.6 nm, respectively. Quantum yield at each temperature is also included.

suggests a lack of substantial intermolecular interaction such as dimerization of retinal (dilute solution) in the radiative process.

At 120 K the fluorescence quantum yield of *all-trans*-retinal is less than 1/40 of the yield at 14 K. On the basis of our single photon counting capability, we estimate the fluorescence quantum yield at room temperature to be $<10^{-4}$ if fluorescent at all. The wavelength dependence of the fluorescence quantum yield also persists over the temperature range examined.

Discussion

There appears to be no straightforward explanation for the lack of external heavy-atom effects (heavy-atom solvents and salts) in diphenylpolyenes and retinol (Table II). Since most of the studies on the heavy-atom effect have been on aromatic systems, our observation clearly suggests

that the external heavy-atom effect is not a general phenomenon in the case of conjugated polyenes. In view of the long fluorescence lifetime, high fluorescence quantum yield, and inefficient intersystem crossing, we had expected a strong external heavy-atom effect in diphenylhexatriene, diphenyloctatetraene, and *all-trans*-retinol. This effect was sought specifically to ascertain the long-lived, forbidden state (^1A_g) as the lowest singlet state[4] which would be more sensitive to external heavy-atom quenching than the short-lived, strongly allowed state (^1B_u) on the basis of kinetic considerations.[36] However, lack of the expected external heavy-atom quenching of the singlet state does not rule out the former as the lowest singlet since the mechanism of external heavy-atom effect (or lack of it) on polyenes remains to be further investigated experimentally and theoretically.

Results shown in Tables I and II and Figures 1-3 suggest that the heavy-atom salt enhancement of fluorescence from *all-trans*-retinal is due to specific effects of cations rather than due to halide ions. Thus, heavy-atom solvents do not quench fluorescence of *all-trans*-retinal at low temperature and do not enhance the singlet-triplet absorption.[41] The specific interaction between *all-trans*-retinal (most likely carbonyl) and cation is supported by the crown ether experiment shown in Figure 3. While the crown ether experiment rules out the dimeric exciton model for wavelength dependent Φ_F[8] and the complexation between cation and carbonyl group of retinal, the interactions are probably more complicated than the 1:1 stoichiometric complex alone since salt concentrations used in rigid matrix are relatively high. The formation of aggregates of retinal and ions which form a spectrum of microscopically distinguishable arrays or complexes is one such likely complication. However, two different rates of cooling for the low temperature sample preparation did not show significant difference in the fluorescence enhancement by high LiCl concentrations (>0.5 M); rapid cooling was achieved by immersing the optical Dewar in liquid N_2, whereas relatively slow cooling of ca. 4°C/min was accomplished by using a closed-cycle cryocooler. The apparent equilibrium constant determinations at different concentrations were done at the slow rate of cooling.

Dependence of the fluorescence enhancement upon the atomic number of the halogen atom (Figure 2) can be readily understood on the basis of the complexation model proposed. Thus, the enhancement increases with increasing atomic number of the halogen and the ionic polarizability (from 1.2 Å^3 for F^- to 4.5 Å^3 for Br^-) and bond length.[42] The higher polarizability and increasing effective charge on cation in going from F^- to Br^- salts (Figure 2) are consistent with the relation between the complex formation and fluorescence enhancement in ethanol.

The fact that the triplet yields are essentially identical in the presence and absence of 0.085 M KI is indicative of a reduction in the internal conversion rate from S_1 to the ground state in the presence of KI. However, lack of the KI effect on Φ_{ISC} at room temperature (eq 1) requires that the complexing of retinal with K^+ affects not only rate constants for fluorescence and internal conversion but also k_{ISC}. An illustration of this suggestion is shown in Figure 7, in which modified rate constants are shown without superscript "°". The main perturbation of K^+ effects on the fluorescence quantum yield and lifetime (Table III) is ascribed to either a blue shift of hidden $^1(n,\pi^*)$ and $^3(n,\pi^*)$ states or a red shift of $^1(\pi,\pi^*)$ state.[39] Increase in the energy gap between the two low-lying singlet states (Figure 7) would then lead to a retardation in the internal conversion rate, whereas the blue shift of $^3(n,\pi^*)$ can compensate for the weakening of $^1(n,\pi^*)$-$^1(\pi,\pi^*)$ interactions (e.g., pseudo-Jahn-Teller distortion).[47] The overall consequence of these perturbations then is the present observation (eq 1), analogous to the coumarin system.[44] Further experimental and theoretical studies are clearly warranted in order to ascertain the validity of the proposed mechanism. It should be mentioned that the observation noted in eq 1 has been reproduced using other wavelengths covering the T-T absorption spectrum (Figure 5). Based on the absence of an effect by KI on the intersystem crossing yield at room temperature and the lack of fluorescence quenching by KI at all temperatures below 150 K, the assumption that Φ_{ISC} is unaffected by KI at low temperatures is reasonable.

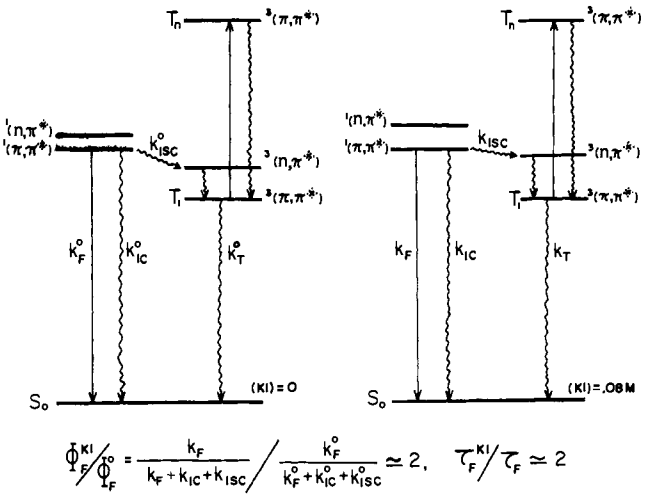

Figure 7. The Jablonski diagrams for *all-trans*-retinal in ethanol at 77 K. The shaded area represents band broadening (e.g., pseudo-Jahn-Teller distortions;[52] also see ref 53 on conformation changes such as torsion[54]). In these diagrams, no assignment is made as to the symmetry (^1A_g or ^1B_u) of the $^1(\pi,\pi^*)$ state. T_n is tentatively identified as T_6[43a] or T_7.[48]

In order to elucidate the role of monocations on the carbonyl n,π^* state of retinal, we offer coumarin as a model example since its fluorescence band broadening and polarization characteristics indicate a strong perturbation of the S_1 (π,π^*) state by the nearby $^1(n,\pi^*)$ state.[30] Addition of alkali halides to coumarin brings about a dramatic fluorescence enhancement as well as vibrational resolution.[44] A number of authors put the $^1(n,\pi^*)$ state close to the $^1(\pi,\pi^*)$ state of retinal and related model carbonyls, either slightly above[9,41,46] or below[47,48] the $^1(\pi,\pi^*)$ state. The most recent suggestion[7-9] for the location of $^1(n,\pi^*)$ state in retinal is to place it above $^1(\pi,\pi^*)$ state (^1A_g) in order to explain the excitation wavelength dependence of the retinal fluorescence yield (e.g., Figure 5).[49]

The fact that the difference between experimental radiative lifetime (τ_F°) and that from the integrated absorption band is reduced substantially at lower temperatures[3,44,45] is indicative of temperature-dependent relaxation which possibly involves conformational flexibility in the ground and excited states. A strong dependence of the fluorescence quantum yield of retinal (Figure 6) and retinol (Table II) on temperature in a rigid matrix is suggestive of a molecular torsional relaxation. The fluorescence quantum yield also changes slightly below 77 K [Φ_F(50 K)/Φ_F(14 K) ~ 0.82, Figure 6]. The importance of the conformational or torsional contribution to the relaxation process of retinol and retinal is perhaps best illustrated by a tight, specifically bound *all-trans*-retinol-β-lactoglobulin complex which is strongly fluorescent and shows a remarkable vibrational resolution in the absorption[50] and CD bands.[51] These characteristics reflect "freezing" of conformational or torsional degrees of freedom by the protein, not achieved by the temperature lowering (down to 14 K) of the glassy solution. Nonetheless, the fluorescence is virtually structureless. This is inter-

preted in terms of conformational relaxations (faster than nanosecond scale) in the excited state.[50]

Conclusions

Diphenylhexatriene, diphenyloctatetraene, and *all-trans*-retinol do not show external heavy-atom effects in that fluorescence quantum yields and lifetimes are not affected by heavy-atom solvents and halide salts. This contrasts with the generally expected external heavy-atom effects in aromatic systems.

On the other hand, *all-trans*-retinal shows an inverse heavy-atom effect; i.e., the fluorescence quantum yield is enhanced without apparent overall reduction in the intersystem crossing yield. A possible mechanism for the reverse heavy-atom effect has been proposed by invoking a blue shift of the (n,π^*) state as a result of carbonyl cation coordination complexes, thus widening the gap between the lowest $^1(\pi,\pi^*)$ and imbedded $^1(n,\pi^*)$ states (Figure 7). The reverse heavy-atom effect observed is particularly interesting, because heavy-atom salts do quench the retinal triplet as expected.

The fluorescence quantum yield of *all-trans*-retinal and retinol depends significantly on temperature in the range even below 77 K for the former. This suggests the possibility that the fluorescent state is able to relax in rigid matrices.

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