Visual Pigments. 9. Fluorescence of Dimers of Retinals[1]

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Abstract: Dry *all-trans-* and 13-*cis*-retinals in dry alkane solvents show no fluorescence at 77 K, $\phi_F < 10^{-4}$, under the experimental conditions of a relatively low concentration, less than 10^{-5} M. However, it is found that if the concentration is high enough, 5×10^{-5} to 10^{-3} M, fluorescence occurs even for dry retinals in dry alkane solutions at 77 K. From experiments regarding the concentration dependence of the absorption and fluorescence, the fluorescence is assigned as being due to a dimer of retinal. The presence of dimers can be an additional cause of the wavelength dependence of the apparent quantum yield of fluorescence in addition to that found involving H bonding. A structure of the dimer is discussed.

Introduction

The luminescence properties of retinals and other substituted polyenes have received much attention in the last few years. Considerable effort has been made regarding the fluorescence properties and excitation wavelength dependence of ϕ_F of the retinals.[1-6] It has been proposed that the ^1A_g (π,π^*) state is lowest in polyenes in general including the retinals.[5,7-11] Others[1] have suggested on the basis of existing evidence for *all-trans*-retinol and 9-*cis*-butylamine Schiff base that the $^1A_g^*$ may be lowest and that it could be true in general for retinols, Schiff bases of retinals, and the protonated Schiff bases, particularly at room temperature (with the possible exception of the 11-*cis* isomers).

Considerable interest has existed in the relative location of the $^1(n,\pi^*)$ state. In recent previous papers we presented evidence that a state of principally $^1(n,\pi^*)$ character was very probably the lowest singlet state for two dry retinals in dry alkane solvents.[12,13] The crucial finding of these works was that no fluorescence was observed in *all-trans*-retinal (ATR) and 13-*cis*-retinal (13-CR) at low concentrations in any aprotic solvents, at any temperatures, under dry conditions. Furthermore, it was shown by studying many solvent systems that most if not all reported fluoresence of these compounds was due to a hydrogen-bonded species. This appeared to be true even of spectra reported in alkanes where water is the proton donor. Thus in the relatively dilute solutions, the wavelength dependence of the quantum yield was explained on the basis of the existence of two absorbing species of which only one fluoresced. In the above papers we showed that one species is the emitting H-bonded species and the other is the nonemitting, non-H-bonded (free) molecules. We assigned a state of principally $^1(n,\pi^*)$ character to be lowest in the free molecule but a state of principally $^1(\pi,\pi^*)$ character to be lowest in the H-bonded complex. Others have assigned the lowest $^1(n,\pi^*)$ state to be considerably higher in energy than the lowest $^1B_u(\pi,\pi^*)$ state in alkane or hydroxylic solvents.[14] Based on current evidence[12,13] as well as other data to be published on homologous polyene aldehydes[15] it is highly probable that a state of principally $^1(n,\pi^*)$ character is lowest in at least two of the retinals. The same is probably true for the other isomers as well (the 11-cis isomer still remains a question because of 12-13 s-cis: s-trans conformers).

The entire picture of the fluorescence of retinal does not end with the discovery of fluorescence for the H-bonded species. We found that if the concentration was high enough, fluorescence occurred even for some dry retinals in dry alkane solutions. The fluorescence behavior of this species was different from that of the H-bonded species, and we believe it to be a dimer (*n*-mers) species.

The origin of the fluorescence of *all-trans*-retinal in ethanol has been previously assigned as the result of dimer emission.[16]

Later results from the same laboratory at relatively lower concentration indicated that the fluorescence was not the result of dimers.[6]

In this paper we would like to report the details of the argument for the existence of dimer fluorescence in *all-trans*-retinal. This is of particular interest because in the case of the retinals, the free monomer does not fluoresce while the dimer does (in dry alkane solvents at 77 K). Commonly, the reverse situation is found. Therefore, consideration of the nature of the dimer needs to be explored. A consideration of the dimer fluorescence is necessary in order to understand all the reported data on the fluorescence of the retinals. Either dimers (*n*-mers) or H-bonded species appear to be the origin of all the fluorescence observed in ATR and 13-CR and whichever fluorescence is dominant depends on the experimental conditions. In particular we find that when dimer fluorescence is present it can be an additional cause for the wavelength dependence of the apparent quantum yield of fluorescence (ϕ_{app}). In terms of state order, we find that dimer formation appears to cause a change in the nature of the lowest excited singlet state in a fashion analogous to that of H-bond formation.

Experimental Section

All experimental data pertinent to the retinals, solvents, procedures for determining spectra, lifetimes, and quantum yields as well as the inner filter effect can be found in the preceding paper.[13]

Results

As mentioned in the Introduction, one of the unusual features of the fluorescence of the retinals is that the quantum yield did not remain constant as the wavelength of excitation was changed. This is illustrated quite dramatically by the fact that the fluorescence excitation spectra of these compounds do not coincide with the absorption spectra. Part of this effect has been explained by us in terms of fluorescence of a H-bonded species.[12,13] However, this picture is further complicated by a concentration dependence of the fluorescence.

Some of the complications can be seen by investigating the results in Figure 1. The figure shows the fluorescence spectrum at 77 K for 5×10^{-5} M ATR especially dried in 3MeP (a) and the fluorescence of ATR in 3MeP including a small amount of water (b). Both the spectral shape and position of the fluorescence (a) are independent of the concentration in the range 10^{-3} to 5×10^{-5} M. On the other hand, the spectrum (b) depends on the concentration in the range 10^{-3}–10^{-4} M and approaches the spectrum as the concentration of ATR increases. For relatively low concentrations of ATR ($<10^{-4}$ M), the fluorescence spectrum with λ_F^{max} 520 nm has been shown to be due to a hydrogen-bonded species between ATR and water.[13]

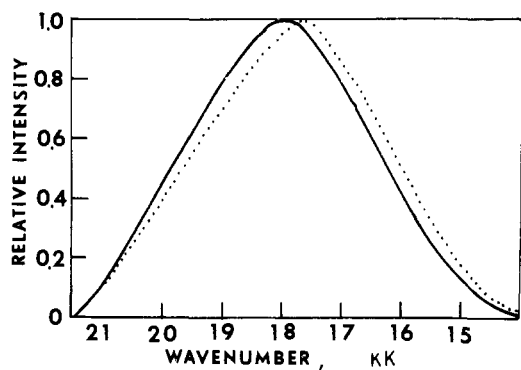Other effects of the concentration on the fluorescence

Figure 1. Fluorescence spectra of dried ATR in especially dried 3MeP (—) and in 3MeP including a small amount of water (- - - -).
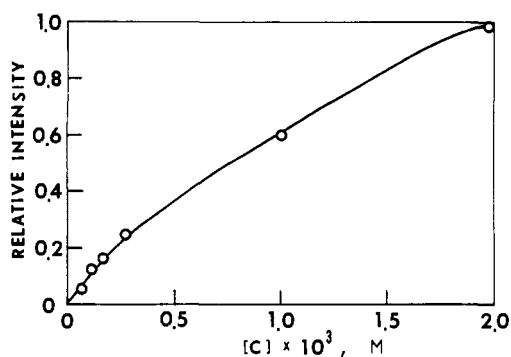

Figure 3. The absorption spectrum of the emitting species as derived from the excitation spectrum by eq 5 of ref 13 (—) and the *difference* absorption spectra (- - - -) of dried ATR in especially dried 3MeP.

Figure 2. Concentration dependence of the apparent relative quantum yield with 420-nm excitation.

spectra of ATR in hydrocarbon solvents can be seen in other experiments. Figure 2 contains the results of a set of experiments that gave the relative values of ϕ_{app} with an excitation at 420 nm, ϕ_{app} (λ_{ex} 420), vs. the concentration of especially dried ATR in 3MeP at 77 K. It is seen that ϕ_{app} (λ_{ex} 420) increases with an increase of the concentration of retinal. Similar behavior can be observed with excitations at 380, 400, and 440 nm. This concentration dependence indicates that the fluorescence may be attributed to changes in the photophysical properties of the molecule due to the molecular interaction between ATR molecules.

In order to clarify whether the interaction occurs in the ground or in the excited state, we measured the *difference* absorption spectrum. In this case, the *difference* absorption spectrum is the difference between the absorption spectra (per molecule) of 10^{-5} and 10^{-4} M ATR in 3MeP at 77 K. In Figure 3 the dotted line represents the *difference* absorption spectrum which later will be assigned to the dimer, and the solid line is the excitation spectrum of the fluorescence monitored at 530 nm. After correction for the lamp intensity and for the inner filter effect[13] the shape and maximum of the excitation spectrum, corrected for inner filter effect, are independent of the concentration in the range 10^{-3} to 5×10^{-5} M and independent of the fluorescence wavelength monitored. Using the same concentrations, we could not detect a difference in absorption at room temperature, inferring that essentially no dimers exist at room temperature.

From Figure 3, it is clear that within our experimental error, the absorption spectrum of the emitting species derived from the excitation spectrum based on eq 6 of ref 13 is in good agreement with the *difference* absorption spectrum. This indicates that the fluorescence originates from species formed by the interaction between ATR molecules in the ground state. We assume this species to be a dimer. From inspection of the full absorption and the *difference* absorption or excitation spectrum in the range 10^{-3} to 5×10^{-5} M, the absorption at
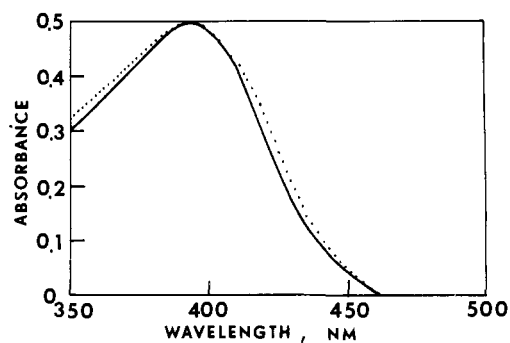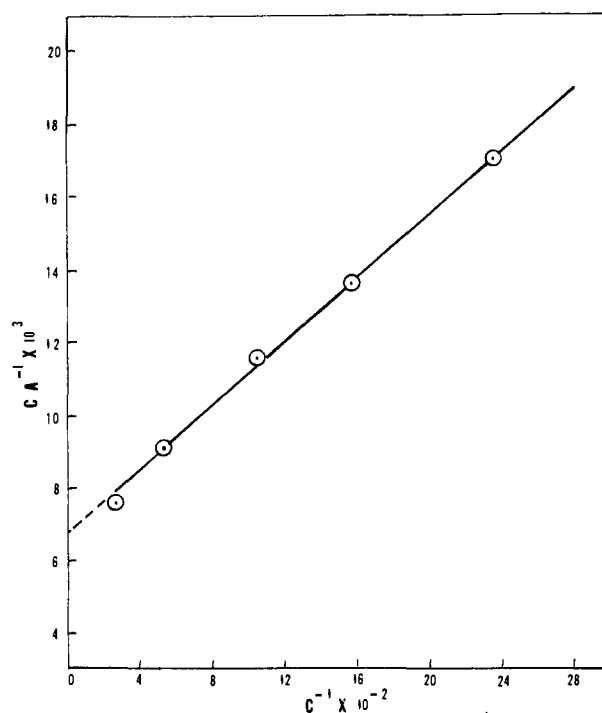

Figure 4. A plot of c/A vs. $1/c$ where c is the concentration of ATR and A is the absorbance at 460 nm and is essentially that only of the dimer (see text and ref 17) in 3MeP, 77 K.

wavelength longer than 450 nm seems to be due mostly to the dimer.

Figure 4 comes from a modified Benesi–Hildebrand relationship from which the equilibrium constant for the dimer formation, K_D, can be obtained.[17] The linearity of the plot is excellent over the concentration range studied. From the plot where A is the absorbance at 460 nm, K_D is found to be 3.9×10^2 (77 K).

We also obtained K_D from an approach parallel to that above based on the corrected relative intensity of fluorescence, $I' = I \, ^{A_T}/(1 - 10^{A_T})$, monitored at 540 nm (excitation at 460 nm). The plot is one of c/I' vs. $1/c$ giving a $K_D = 3.7 \times 10^2$ (77 K). Note the excellent agreement between the K_D values from the two different methods. With these values of K_D, approximately 6–7% of the ATR exists as dimers at 10^{-4} M in 3MeP at 77 K.

In addition to the evidence presented above for the existence of dimer fluorescence of ATR in 3MeP solutions, there is evidence that dimers exist and can be the source of fluorescence in EPA. However, in EPA at 77 K, we previously found that ATR has a fluorescence spectrum with λ_F^{max} 540 nm with excitation at $\leqslant$420 nm which is the result of an H-bonded complex between ATR and ethanol.[9,13] This makes the study

Table I. Lifetimes and Quantum Yields for Retinyl Derivatives

| | ATR | | | ATA | |
	H$_2$O	PhOH	Dimer	3MeP– 10% ether	Dimer (3MeP)
τ_{obsd},[a] ns	1.0	1.6	1.0	3.7	1.2
ϕ_F[b]	0.05	0.07	0.05	0.45	0.30
τ_0,[c] ns	20	23	20	8.2	4.0

[a] $\pm$0.2–0.4 ns. [b] Determined from exciting on the long-wavelength band leading edge where essentially only the complex absorbs (generally λ_{ex} 450 nm, >420 nm for PhOH case). [c] τ_0 is the natural radiative lifetime.

of the dimer fluorescence somewhat less direct than a study of dimer fluorescence in a dry solution of ATR in 3MeP. The consequences of the presence of two different fluorescing complexes can be seen by exploring the changes in the fluorescence spectra as the concentration and wavelength of excitation are changed. For example, upon excitation of solutions of ATR with $\lambda_{ex} <$ 420 nm, the shape, position, and ϕ_{app} of the fluorescence spectrum are apparently independent of the concentration. However, with excitation of ATR at $\lambda_{ex} >$ 440 nm, the shape, position, and the ϕ_{app} of the fluorescence spectrum vary with the change of the exciting wavelength and with a change in the concentration.

The excitation wavelength dependence of the fluorescence spectra in EPA is seen by examining the results presented in Figure 5. Here the system under study is a 10^{-4} M solution of ATR in EPA at 77 K. By exciting this system at 420 nm, we obtain a fluorescence spectrum with λ_F^{max} 540 nm. However, as the exciting wavelength increases the fluorescence spectrum changes gradually to the spectrum with λ_F^{max} 550 nm.

The results of the concentration dependence of the fluorescence spectra in EPA at 77 K are as follows: the ϕ_{app} with the excitation at 400 and 420 nm are almost independent of the concentration in contrast to the parallel excitation of ATR especially dried in 3MeP. Excitation at 440 nm results in an increase in ϕ_{app} with an increase of concentration similar to that for ATR especially dried in 3MeP.

From the data described above and the comparison with those in 3MeP, the fluorescence spectrum reported above, λ_F^{max} 550 nm in EPA, which appears when exciting at wavelengths longer than 440 nm is assigned as originating from dimers formed in the ground state.

Discussion

From the data given in the Results sections, it is clear that dimer formation of ATR does occur and excitation of the dimer results in observable fluorescence in 3MeP at 77 K. Thus the general discussion of the wavelength dependence of ϕ_{app} must be expanded to include the fluorescence of retinal dimers as well as the fluorescence of H-bonded monomers. In dry solutions of ATR in 3MeP at any concentration or in not especially dried solutions at concentrations of 10^{-4} M and higher, the fluorescence observed by excitation at $\lambda \geqslant$440 nm is mainly from the dimer. We can thus study the dimer fluorescence directly and arrive at the absorption spectrum of the dimer via the fluorescence excitation spectrum based on eq 6 of ref 13; $E_{\lambda'}(\lambda)$ corresponds to the dimer absorption spectrum. Since the absorption spectrum of the dimer turns out to be red shifted from that of the monomer and only the dimer fluoresces, the ϕ_{app} is wavelength dependent. Thus, the excitation wavelength dependence of ϕ_{app} originates from the varying magnitude of the ratio of the absorbance of the emitting species (dimer) and that of all absorbing species; see eq 7, ref 13. Also it can be noted that this result verifies that the true quantum yield of fluorescence, ϕ_F, is essentially constant.

One of the two previous structures suggested for a retinal
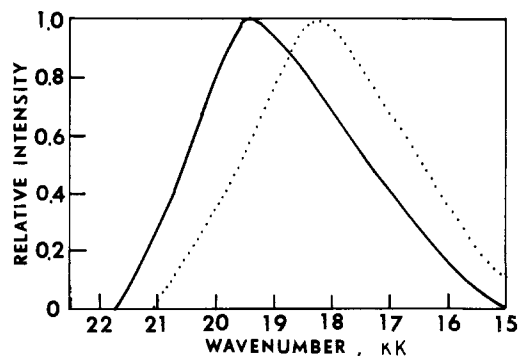
Figure 5. Excitation wavelength dependence of fluorescence spectra in EPA: the spectrum upon 420-nm excitation (—) and that upon 450-nm excitation (- - - -).

dimer was a tail-to-tail structure.[16] Also, an inclined dimer involving stacking of the C=O groups was suggested.[16] In light of the new evidence, we believe that a partial sandwich dimer is a more credible structure, vide infra. The red shift in absorption was at first thought to favor a tail-to-tail structure, but red shifts are also observed in many symmetrical dimers because the van der Waals shift is usually greater than the intermolecular excitation exchange energy.[18] Thus the red shift in absorption does not eliminate either structure. However, the following points tend to weaken the plausibility of the tail-to-tail dimer. (1) In EPA solution, the carbonyl group of ATR is surrounded by ethanol molecules so that it would appear unlikely that ATR would form a carbonyl-to-carbonyl (tail-to-tail) dimer. (2) The radiative lifetime calculated from the data on the quantum yield and the observed lifetime for dimers are very similar to that of the H-bonded species (corresponding to the monomer) with proton donors such as water and phenol, Table I. If we assume that the nature of the fluorescing state of the H-bonded molecules is the same as the parent state of the fluorescing exciton state in the dimer, then point (2) seems contrary to the result expected if the structure of the dimer were tail-to-tail. The foregoing can be further clarified by the following considerations.

Partial sandwich dimers refer to dimers with centers of symmetry. As the molecules slide along each other from configuration $\rightarrow_{\leftarrow}$ to configuration $\rightleftarrows$, the lowest exciton state is allowed and the upper one is forbidden; also see below. The Davydov splitting varies with the angle of orientation between the transition dipoles and the line connecting their centers as well as with the distance between the centers. Through all these positions the lower state has all the intensity, namely, twice the monomer intensity. As the molecules slide from configuration $\rightleftarrows$ to configuration $\rightleftharpoons$, the lower state is now forbidden and the upper state is allowed with twice the intensity of the monomer.

μ_1 — ⟩θ r — μ_2

$$V_{12} = \frac{\vec{\mu}_1 \cdot \vec{\mu}_2}{r^3} - \frac{3(\vec{\mu}_1 \cdot \vec{r})(\vec{\mu}_2 \cdot \vec{r})}{r^5}$$
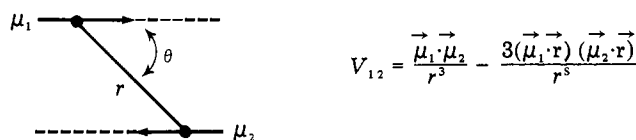
for our case $\mu_1 = \mu_2 = \mu$, $\vec{\mu}_1 \cdot \vec{r} = \mu r \cos\theta$, $\vec{\mu}_2 \cdot \vec{r} = -\mu r \cos\theta$, and

$$V_{12} = \frac{-\mu^2}{r^3} + \frac{3\mu^2 \cos^2\theta}{r^3} = \frac{-\mu^2}{r^3}(3\sin^2\theta - 2)$$

When θ is between 0 and arc sin ($\sqrt{2/3}$), θ approximately 55°, V_{12} is positive and the allowed state is lowest, but between arc sin ($\sqrt{2/3}$) and $\pi/2$, V_{12} is negative and the forbidden state is lowest. For all θ the allowed state has twice the intensity of the monomer and in the case of tail-to-tail, the allowed state would be lowest. For the partial sandwich dimer of interest where 0
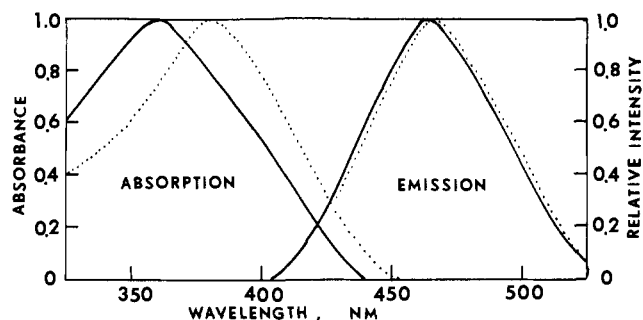
Figure 6. Absorption and fluorescence spectra of *all-trans*-retinoic acid in 3MeP (- - - -), 3MeP-10% ethyl ether (—).

$\leqslant \theta \leqslant$ arc sin ($\sqrt{2/3}$), the exciton model gives that the allowed state (it is a "u" state) is lowest with τ_0(dimer) = $\frac{1}{2}\tau_0$(monomer).

The validity of the objection to the tail-to-tail dimers raised in point (2) rests on an expectation that quantitative results can be expected from the excitation-resonance theory. That this is a justifiable expectation can be illustrated with the following example of a polyene that forms a tail-to-tail dimer. Although more details will be published elsewhere,[19] let us consider the absorption and fluorescence spectra of *all-trans*-retinoic acid (ATA) in 3MeP and 3MeP-10% ethyl ether at 77 K as shown in Figure 6. Note that there is a blue shift when going from pure 3MeP to 3MeP-10% ether. Even at room temperature, there is a ~10-nm blue shift when going from 3MeP to 10% ether-3MeP or 10% EPA-3MeP. This is what would be expected based on a dimer in 3MeP of the type
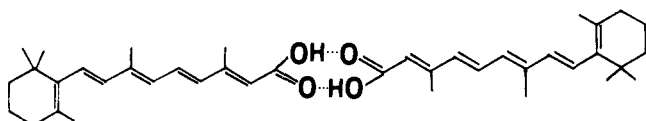
where the dominant interaction is of the exciton or excitation resonance type. Furthermore, it would be expected that the radiative lifetime of the dimer would ideally be one-half of that of the monomer. This is essentially what is found experimentally; see Table I. This dimer type is in harmony with that known to exist for other acids such as benzoic,[20] naphthoic,[21] and acetic acids.

The above features of tail-to-tail dimers are not seen in the dimers of retinal. Thus, although it is not possible to give the precise structure for the dimer of ATR, a partial sandwich structure seems more compatible with the existence of dimers in EPA solution (and with the similarity of the τ_0 data of the dimer and H-bonded monomers; also see below). One problem with assigning the dimer as having a partial sandwich structure is that based on a rough calculation of the Davydov splitting, $2V_{12}$, it does not appear that the $^1(\pi,\pi^*)$ exciton state can be lowered below the $^1(\eta,\pi^*)$ state. However, a partial sandwich dimer of the type
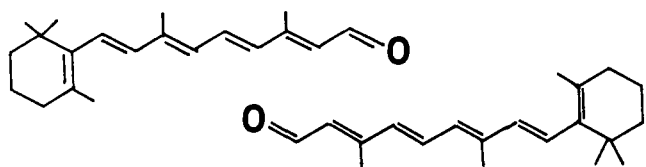
would allow for significant overlap of the π clouds. This overlap of the π clouds would be favorable for the existence of lower energy charge resonance ($R^+R^- \leftrightarrow R^-R^+$) states which can

interact with the exciton states. In general excimer formation and other phenomena are often explained by introducing configuration interaction between charge transfer and exciton states. This configuration interaction can often lower the energy of a low-lying exciton state sufficiently to allow excimer formation.[21,22] Therefore, the proposition is that configuration interaction of the zero-order exciton state with charge transfer states lowers the allowed $^1(\pi,\pi^*)$ state below the $^1(n,\pi^*)$ state. The CT states in tail-to-tail dimers would probably have energies too high to make a significant contribution to configuration interaction.

In addition to the foregoing consideration of the zero-order state from exciton theory not being low enough in energy, recall that it is predicted to have twice the intensity. That is experimentally observed; vide supra. A decrease in its intensity can be obtained by mixing in an allowed charge transfer state CT_u ("u" symmetry) through CI. Even though the CT state is allowed, it is not expected to have nearly as large an oscillator strength as the allowed exciton state. If this configuration interaction is strong enough, the large mixing will increase the lifetime of the lowest exciton state.

References and Notes

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(17) The appropriate equations for obtaining K_D are

$$2ATR \underset{c - 2x}{\overset{K_D}{\rightleftharpoons}} \overset{D}{x}$$
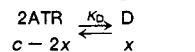
$$K_D = \frac{x}{(c - 2x)^2} \sim \frac{x}{c^2 - 4cx}$$

where x is not large and

$$x = \frac{K_D c^2}{1 + 4K_D c}$$

At 460 nm, $A \sim$ absorbance dimer only = $\epsilon_D x$

$$\frac{1}{A} = \frac{1}{\epsilon_D \ell} \frac{[1 + 4K_D c]}{K_D c^2}$$

$$\frac{c}{A} = \frac{1}{\epsilon_D \ell} \left[4 + \frac{1}{K_D c} \right]$$

where the ratio intercept/slope = $4K_D$.
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