

Figure 4. Fluorescence titration of calf thymus DNA with BMSp. The concentration of DNA was  $1.726 \times 10^{-6}$  M in base pairs.

ethidium/two BP)<sup>31</sup> and in accordance with the nearest neighbor exclusion model.<sup>9</sup>

The calculation of the binding affinity of bisintercalated BMSp from the fluorescence and spectrophotometric titration data requires a knowledge of the dependence of the spectral and fluorescence properties of the bisintercalated species as a function of the degree of saturation and the effect of other bound BMSp species on the quantum yield of the bisintercalated species. These uncertainties plus the inability to detect sufficient unbound BMSp renders the traditional Scatchard analysis unreliable. Nevertheless, a *minimum* binding constant can be estimated which is compatible with both the fluorescence and spectrophotometric titration data. We find that the binding constant of bisintercalated BMSp is  $\geq 4 \times 10^9$  M<sup>-1</sup>,<sup>32</sup> which can be compared with  $3 \times 10^5$  M<sup>-1</sup><sup>35</sup> for ethidium (EB) under similar conditions.

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- The observation of a sharp break in ΔA<sub>490</sub> was found to be independent of wavelength over the range monitored (360–700 nm).
- The A<sub>490</sub> for unbound BMSp as a function of [BMSp] is reexpressed in terms of [BMSp]/[BP] by dividing [BMSp] by the [BP] used in determining A<sub>490</sub> for bound BMSp.
- Fluorescence titrations were conducted in D<sub>2</sub>O (>99%)–phosphate buffer (0.01 mM EDTA, 0.025 M KHPO<sub>4</sub>, and 0.025 M Na<sub>2</sub>PO<sub>4</sub>) at 25 °C on a Perkin-Elmer MPF-4 fluorimeter. Excitation was at 482 nm and emission monitored at 640 nm.
- We have observed that the quenching of fluorescence which takes place for BMSp/BP > 0.25 at these low ionic strengths is substantially reduced when the ionic strength is increased. Similar behavior has been observed for ethidium suggesting that at least one of the additional bound BMSp species is an electrostatically bound nonintercalated species.<sup>23</sup>
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- Alfred P. Sloan Research Fellow, 1977–1979.
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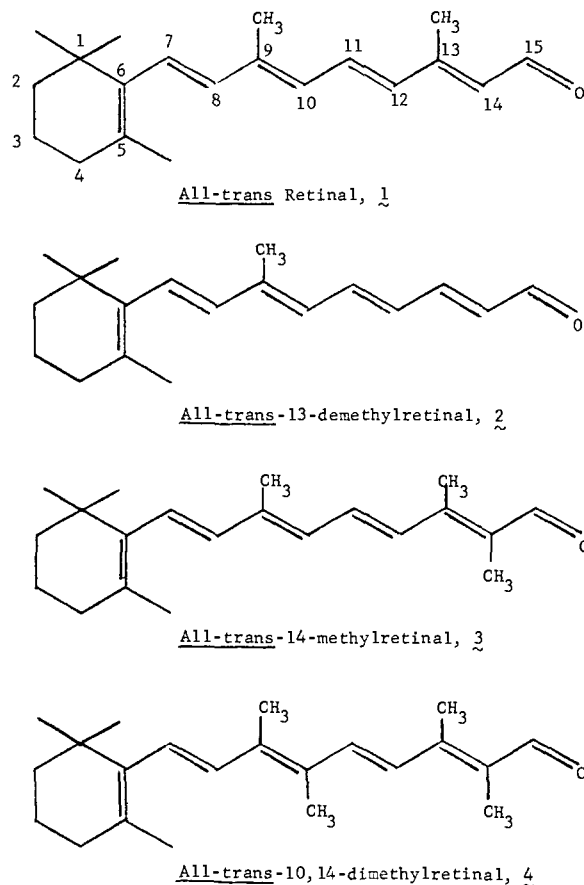
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## Influence of Alkyl Substitution on the Trans → Cis Photoisomerization of all-trans-Retinal and Related Polyenes

Sir:

Although the primary photochemical step and subsequent sequence of chemical events following absorption of a photon of light by the visual protein rhodopsin is uncertain,<sup>1–8</sup> it is known that the 11-*cis*-retinyl chromophore<sup>1</sup> undergoes a cis → trans isomerization<sup>2,3</sup> forming *all-trans*-retinal (structure **1**) and the protein opsin as the final products<sup>4</sup> of the rhodopsin bleaching process. To better understand the nature of the factors that may influence the photochemically initiated transformation of the chromophore in rhodopsin, we have examined the solution photochemical properties of the isomeric retinals<sup>9,10</sup> and related synthetic polyenes. The photochemical



properties of *all-trans*-13-demethylretinal (**2**), *all-trans*-14-methylretinal (**3**), and *all-trans*-10,14-dimethylretinal (**4**) have been investigated, including measurement of the quantum efficiencies of the *trans* → *cis* photoisomerization reactions and determination of the primary product ratios by using high pressure liquid chromatographic methods. An interesting structure/photochemical reactivity relationship is observed for compounds **1–4**. The photochemistry of polyenes **1–4** is consistent with the theory of photochemical isomerization of linear polyenes developed by Kushick and Rice.<sup>11</sup>

*all-trans*-Retinal was purchased from the Sigma Chemical Co. The *all-trans* isomers of 13-demethylretinal,<sup>12</sup> 14-methylretinal,<sup>13,14</sup> and 10,14-dimethylretinal were synthesized according to literature methods, isolated on a Waters Prep LC/System 500 liquid chromatograph, and identified using NMR spectra recorded on a 250-MHz NMR spectrometer. Polyenes **1–4** were then purified to >99.5% on a Waters Model ALC/GPC 204 liquid chromatograph. Absorption spectra were recorded on a Cary 14 spectrophotometer. Aerated samples were irradiated in quartz cuvettes using the 150-W Xenon lamp and 1/4-m monochromator of an Aminco-Bowman spectrofluorimeter. Photoproducts were identified by HPLC retention times<sup>15</sup> and product ratios from peak areas obtained by multiple planimeter tracings. Quantum yields of photoisomerization ( $\phi_{PI}$ ) were determined by using ferrioxalate actinometry,<sup>16</sup> percentage conversions from HPLC curves, and the equation used in our previous studies.<sup>9</sup>

The photochemical properties of retinals **1–4** have been examined in hexane solutions at room temperature. Table I is a summary. Upon 350-nm excitation of polyenes **1–4** the following results are obtained: (a) the 9-*cis* and 13-*cis* isomers of retinals **1**, **3**, and **4** are formed as primary photoproducts, but the 9-*cis* to 13-*cis* product ratios differ substantially; (b) the absolute quantum yields of the *trans* → *cis* photoisomerization processes ( $\phi_{PI}$ ) also are substantially different; (c) the quantum yields for the *trans* → 9-*cis* photoisomerization processes of

**Table I.** Photoisomerization of Synthetic Retinals

Compd <sup>a</sup>	$\phi_{PI}$ <sup>b</sup>		
	<i>trans</i> → <i>cis</i>	<i>trans</i> → 9- <i>cis</i>	<i>trans</i> → 13- <i>cis</i>
<i>all-trans</i> -Retinal <sup>c</sup>	0.08 ± 0.02	0.015	0.065
<i>all-trans</i> -13-Demethylretinal	0.022 ± 0.003	0.01	0.00
<i>all-trans</i> -14-Methylretinal	0.26 ± 0.035	0.01	0.25
<i>all-trans</i> -10,14-Dimethylretinal	0.40 ± 0.04	0.10	0.30

<sup>a</sup>  $5 \times 10^{-5}$  to  $2 \times 10^{-4}$  M retinal in hexane. <sup>b</sup> 350-nm excitation, 7-nm band pass; 150-W Xe lamp; average of at least eight determinations; 2–5% conversions. <sup>c</sup> Data from ref 10.

compounds **1–3** are equal within our experimental error; and (d) polyene **2** yields 7-*cis*-,<sup>17</sup> 9-*cis*-, and 11-*cis*-13-demethylretinal as primary photoproducts in the approximate ratio of 2:2:1, respectively.<sup>18</sup>

In polyenes **1–3** there are no substitutional changes at the 9,10 carbon-carbon double bond and the  $\phi_{PI}$  values for the *trans* → 9-*cis* processes are unaffected. Substitution at the 13,14 carbon-carbon double bond of compounds **1–3** differs in that the carbon atoms of the double bond have two, one, and zero carbon-hydrogen bonds in polyenes **2**, **1**, and **3**, respectively, and  $\phi_{PI}$  values for the *trans* → 13-*cis* processes are 0.00, 0.065, and 0.25, respectively. *all-trans*-10,14-Dimethylretinal (**4**) possesses substitutional differences at both the 9,10 and 13,14 carbon-carbon double bonds, whereby methyl groups have replaced the C-10 and C-14 hydrogen atoms of *all-trans*-retinal (**1**). We find that the  $\phi_{PI}$  values for the *trans* → 9-*cis* and the *trans* → 13-*cis* processes of **4** are much greater than for comparable processes in **1**. Furthermore, we find that  $\phi_{PI}$  values for the *trans* → 13-*cis* processes in polyenes **3** and **4** are identical within our experimental reproducibility.

Kushick and Rice<sup>11</sup> have recently developed a model for the photochemical isomerization of linear polyenes. Using butadiene as an example, they conclude that the torsional modes of conjugated polyenes are poor acceptors of electronic excitation energy, which is preferentially directed into the vibrational modes of the molecule. Based upon the theoretical approach of Robinson and Frosch,<sup>20</sup> it is generally believed that the carbon-hydrogen stretching vibrations are the most important modes for radiationless processes in aromatic hydrocarbons. Froehlich and Morrison<sup>21</sup> have reported that the quantum yields of fluorescence for a series of monosubstituted alkylbenzenes decreased linearly as the number of  $\beta$ -hydrogen atoms of the substituent increased—thought to be a result of enhancements in the nonradiative rate constants. To the extent that the extrapolation that carbon-hydrogen stretching vibrations are important radiationless modes in linear polyenes is valid, then the results of our quantum yield measurements are understandable. As the number of available olefinic carbon-hydrogen stretching vibrations is *reduced*, torsional modes become important and the quantum yield of *trans* → *cis* photoisomerization about that carbon-carbon double bond is *markedly increased*. Thus, our experimental results are consistent with the theory of photochemical isomerization of linear polyenes developed by Kushick and Rice.<sup>11</sup>

Owing to the importance of the 11-*cis*-retinyl chromophore to the visual process,<sup>1–5</sup> the presence of the retinyl chromophore in *Halobacterium halobium*,<sup>22</sup> and the employment of vitamin A derivatives for medicinal purposes, we are continuing to investigate the photochemical properties of isomers of polyenes **1–4** and related compounds. We hope to obtain additional information on the structure/photochemical reactivity relationships for this class of linear polyenes.

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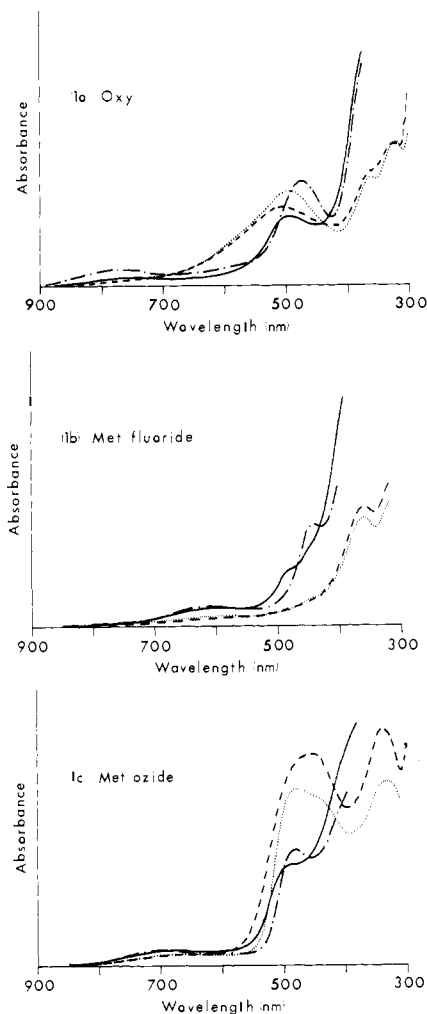
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## Polarized Single-Crystal Spectroscopic Studies of Oxyhemerythrin

Sir:

We report the room and low temperature polarized single-crystal electronic spectra of oxyhemerythrin and methemerythrin fluoride and azide. A number of the absorption bands are found to be extremely polarized, and a comparison between the oxy and met forms allows the oxygen to iron charge-transfer transition to be determined to be dominantly polarized perpendicular to the Fe-Fe axis. Using a transition dipole vector coupling model, the observed polarizations require that the Fe-O axis make only a small projection on the Fe-Fe axis, thereby placing strong restrictions on the proposed modes of oxygen binding to the active site.

The data available on hemerythrin, the binuclear iron oxygen binding protein, is rapidly evolving to a point where a detailed picture of the active site can be obtained. In particular, resonance Raman studies<sup>1</sup> have shown that O<sub>2</sub> binds asymmetrically as peroxide to the binuclear iron site, which has further been shown to be antiferromagnetically coupled high-spin Fe(III)'s.<sup>2</sup> Recent x-ray crystal structure determi-



**Figure 1.** Polarized single-crystal absorption spectra of hemerythrin: (a) oxyhemerythrin; (b) methemerythrin fluoride; (c) methemerythrin azide. Polarizations and temperatures are indicated as follows: (—) 273 K, parallel; (---) 77 K, parallel; (-·-) 273 K, perpendicular; (···) 77 K, perpendicular. Polarizations refer to the orientation of the E vector relative to the needle axis.

nations<sup>3</sup> have shown this site to be approximately a trigonal antiprism with the Fe-Fe axes for all eight of the subunits in the octamer approximately parallel.<sup>3a</sup> This structure is ideal for polarized single-crystal spectroscopic studies which should provide some extremely detailed insight into this active site. Further, the dilute yet oriented nature of the active site of protein crystals makes them ideally suited for obtaining polarized spectra<sup>4</sup> of charge-transfer and other intense absorption processes.

Hemerythrin crystals suitable for spectroscopic study were prepared by dialysis of the purified protein<sup>5</sup> (from *Golfingia gouldii*) against 15% ethanol. The crystals obtained were of the approximate dimensions  $2 \times 0.6 \times 0.1$  mm. These small crystals required modification of our McPherson RS-10 spectrometer for low temperature polarized studies, as described elsewhere.<sup>6</sup> The orientation of the Fe-Fe axis in these crystals was determined by comparison with those forms used for x-ray analysis. The colors observed under polarized light for the crystal form of the met azide derivative used in the structural studies<sup>3b</sup> require that the intense visible absorption<sup>7</sup> be dominantly polarized perpendicular to the crystallographically defined Fe-Fe axis. As shown in Figure 1c, the intense 480-nm band in our met azide crystals is strongly polarized perpendicular to the needle axis, defining the Fe-Fe vector to be oriented along the needle axis.