Linear Dichroism Spectroscopy of Retinal with Picosecond Time Resolution

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For the first time linear dichroism spectroscopy has been extended to the picosecond time regime. 11-*cis* retinal, *all-trans* retinal and 1,8-diphenyl-1,3,5,7-octatetraene (DPOT) are incorporated into polyethylene films and oriented by stretching the films. By measuring picosecond transient absorption spectra polarized parallel and perpendicular to the stretching direction and calculating the dichroic ratio we get informations about the molecular geometry in excited singlet and triplet states. The results may have relevance to the interpretation of primary processes in vision.

Introduction

The visual pigment rhodopsin was the first biological molecule to be investigated by picosecond techniques [1]. In the mean-time, numerous picosecond investigations applying different spectroscopic techniques have been conducted on this topic (for a review, see [2]). Nevertheless the primary photochemical step in vision has not been established beyond doubt. In this context it is surprising that most investigations have been performed on the level of the highly complex intact molecule, without having sufficient knowledge of the picosecond time behavior of the chromophore retinal itself. Only a few papers have appeared reporting picosecond measurements on the isolated chromophore. Hochstrasser *et al.* [3] reported time-resolved absorption measurements on *all-trans* retinal, showing a decay of the first excited state and appearance of the triplet absorption within 34 ± 5 ps. The fluorescence kinetics of *all-trans* retinal was examined by Fugate and Song [4], and later by Doukas *et al.* [5]. A fluorescence life-time of 17 ps at room temperature was measured, lengthening to 190 ps at a temperature of 93 K. No picosecond measurements on *cis* isomers have been reported so far to our knowledge. Also information on geometric properties of the molecules in the various excited states is lacking.

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In this work, picosecond measurements on 11-*cis* and *all-trans* retinal are reported. In addition to measurements of the transient absorption spectra in solution, the technique of linear dichroism spectroscopy [6, 7] was adapted to picosecond spectroscopy to yield information on the directions of the transition dipole moments of the states populated during the relaxation process. To facilitate interpretations of the experimental results, linear dichroism measurements have been extended to a model compound, 1,8-diphenyl-1,3,5,7-octatetraene (DPOT), for which the linear dichroism measurements are easier to interpret.

Materials and Methods

Picosecond apparatus

The picosecond apparatus used was based on a Nd^+ phosphate glass oscillator with single pulse selector, and two amplifier stages. The resulting pulse having an energy of 20 mJ and duration of ~ 6 ps was frequency-tripled yielding ~ 3 mJ at 353 nm. The remaining IR pulse was used to excite a picosecond continuum in a cell filled with D_2O. The useful spectral extension of the continuum was 350−800 nm. The overall continuum energy was ~ 50 µJ. The 353 nm pulse was used to excite the sample, while the continuum was properly delayed by sending the IR pulse along a variable delay line formed by two mirrors. The continuum was split into two paths before reaching the sample, forming a measuring and a reference beam, the reference beam transversing an unexcited sample otherwise identical

with the excited one. The two spectra were focused to the slit of a ISA-200 polychromator and detected by a SIT vidicon tube of a PAR OMA-2 system. The evaluation procedure was the same as given by Greene *et al.* [8], yielding the transient absorption △ OD. The sensitivity of the apparatus was high enough to detect changes in absorption of ~ 0.1 OD. In case of measuring linear dichroism, the exciting pulse was made circulary polarized by a quarterwave plate, while the polarization of the continuum was set parallel or perpendicular to the stretching direction of the sample (see below) by rotating the polarization of the pulse using a halfwave plate before the continuum cell.

Linear dichroism spectroscopy

When non-spherical molecules are incorporated into a polymer matrix, their orientation is determined by the surrounding polymer. When the polymer is stretched, elongated molecules become preferentially orientated with their long axes along the stretching direction. Usually this may be detected by absorption spectroscopy using light polarized parallel and perpendicular to the stretching direction successively. The ratio in optical density for parallel and perpendicular polarization $d_o = OD_{\shortparallel}/OD_{\perp}$ is called the dichroic ratio and is related to the orientation of the transition dipole with respect to the stretching direction.

In the case of planar ring molecules, only the plane can be oriented, the orientation being random in two directions [6]. In the case of a non-planar molecule, the situation is even more complicated. It has been shown by us [9], that in a molecule consisting of a ring and an aliphatic chain tilted with respect to each other, the ring plane is oriented, while the direction of the chain is determined by a cone around the stretching direction.

The polyethylene film used was Suprathene 200, supplied by Kalle and Co. AG. The technique for incorporating the molecules into the film has been described previously [7]. The preparation was performed in the dark and using oxygen-free solutions to avoid damage of the compounds. The transient dichroic spectra were measured, as described in the previous section, by rotating the polarization of the continuum beam. The transient dichroic ratio was calculated from △ OD$_{\shortparallel}$ and △ OD$_{\perp}$ using previously determined values of OD$_{\shortparallel}$ and OD$_{\perp}$ without excitation. Conventional dichroic spectra were measured

on Cary 118 and UVIKON 810 spectrophotometers, using the previously described PNP method [7]. The picosecond spectra taken in solution were measured at concentrations of 10^{-4} mol/liter in *n*-hexane as a solvent.

Results and Discussion

Fig. 1 shows the conventional c.w. dichroic spectra of 11-*cis* retinal, *all-trans* retinal, and the model compound DPOT. The low dichroic ratio of $d_o \approx 1.8$ for retinals is in accordance with the nonplanar structure of the molecules known from X-ray measurements [11], as has been shown previously [9]. In the case of 11-*cis* retinal, it is not yet quite clear which orientation the molecule could have in the stretched polymer matrix. The dichroic ratio indicates, however, that the orientation resembles that of *all-trans*. In the case of DPOT, the dichroic ratio is high, indicating the fact that the chain forming the chromophore is highly oriented along the stretching direction. Details of the interpretation have been given elsewhere [9].

Fig. 2. shows the transient absorption of 11-*cis* retinal in solution at two different moments (30 ps and 450 ps after excitation), while Fig. 3 gives the change in optical density *vs* time for different wavelengths (360, 400 and 450 nm). The evaluation yields a ground-state depopulation immediately after excitation, documented by a bleaching of the original absorption band. With a time-constant of ~ 30 ps a new absorption centered at ~ 400 nm appears, which partly overlaps the original absorption band, hence simulating a repopulation of the ground state. At short wavelengths ($\lesssim$ 370 nm) the bleaching persists, however, proving a new band to be present at 400 nm. In addition, in favorable cases the new absorption over-compensates the bleaching, thus giving a further proof. We believe the new band to result from an absorption S_m-S_n, m and n being excited states. The life-time of this band is ~ 20 ps. Simultaneously with the decay of this band another band centered around 440 nm appears. This band, once established, remains present over a time long compared to the span of 1.2 ns covered by our experiment. This long-lived state is obviously the triplet also described in ref. [3]. While the strength of the S_m-S_n absorption is comparable to that of the original absorption, the extinction coefficient for the triplet absorption is stronger by a factor ~ 3.

In an unstretched film, 11-*cis* retinal shows essentially the same behavior, only the strength of the

Fig. 2. Transient absorption of 11-*cis* retinal in *n*-hexane, 30 ps and 450 ps after excitation.

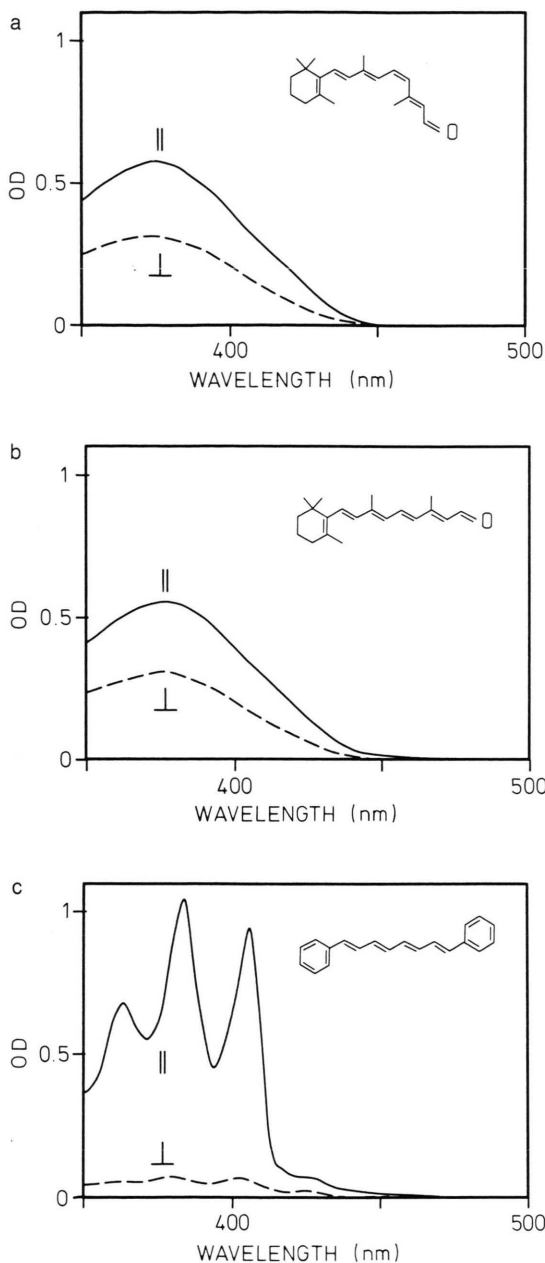Fig. 3. Change in optical density *vs* time after excitation in 11-*cis* retinal, at 360 nm, 400 nm, and 450 nm.

Fig. 1. Dichroic spectra of 11-*cis* retinal (a), *all-trans* retinal (b), and DPOT (c), in stretched polyethylene, obtained by the PNP method.

Fig. 4. Dichroic ratio of 11-*cis* retinal, 30 ps and 450 ps after excitation.

S_n-S_m absorption seems to be higher by a factor of ~ 2 and its appearance a bit faster (within ~ 15 ps).

In stretched films the behavior is about the same. But here we can also follow the change in the dichroic ratio as shown in Fig. 4. During bleaching the
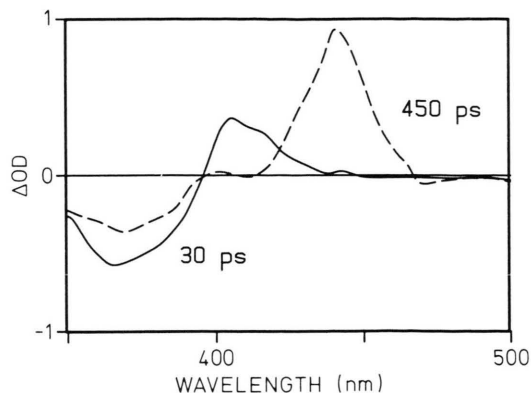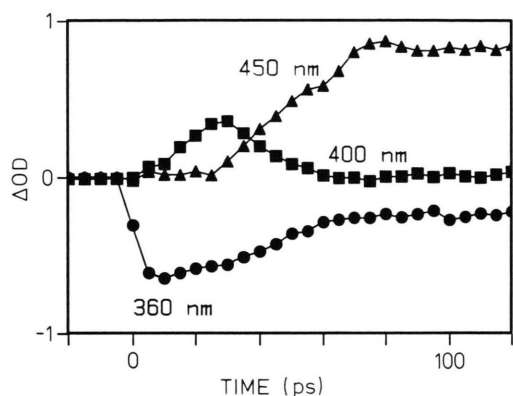
dichroic ratio remains approximately the same as before, while upon appearance of the S_m-S_n band the dichroic ratio within this band raises to $d_o > 5$. The triplet absorption, on the other hand, shows a dichroic ratio $d \approx 0.2$.

All-trans retinal showed a behavior not very different from 11-*cis* retinal (Figs. 5 and 6). The S_m-S_n absorption is masked by bleaching of the overlapping ground-state absorption, but can be restored mathematically. It is found to appear within < 10 ps and to be centered at ~ 390 nm. With a delay of ~ 35 ps the triplet grows in at ~ 440 nm. The joint action of the S_m-S_n and triplet absorption obscure the ground state bleaching, simulating a decay, but once the S_m-S_n absorption has disappeared, the bleaching is partly recovered due to the molecules residing in the triplet for a rather long time ($\gg 1.2$ ns). In the un-

stretched film, we find a dichroic ratio (Fig. 7) for the S_m-S_n transition of $d_o \approx 4$, while the triplet shows $d_o \approx 0.3$.

DPOT as the third sample only was examined in stretched films. The S_m-S_n absorption again is found at ~ 400 nm and appears within < 10 ps. It has a dichroic ratio $d_o \approx 20$. The absorption relaxes within ~ 15 ps, and the triplet appears ~ 485 nm with a dichroic ratio of $d_o \approx 3$ (*cf.* Figs. 8, 9).

The interpretation of the transient dichroic ratios has to take into account two different causes for the experimentally determined values. The first is the intrinsic dichroic ratio given by the ratio of transition dipole moments along the polyene chain and perpendicular to it. The second aspect is the orientation of the polyene chain with respect to the stretching direction. The experimental determination of the dichroic ratio is not sufficient to distinguish between these two causes.

The most simple case in this respect is DPOT. From previous investigations [9] it is known that DPOT is highly oriented in the stretched film, the measured value of $d_o = 14.5$ thus representing essentially the intrinsic dichroic ratio of the molecule. The S_m-S_n absorption with its high value $d_o \approx 20$ then shows that the intrinsic dichroic ratio is higher in the excited state than in the ground state, which can be imagined to be due to the higher π electron delocalization. The ratio $d_o(S_0)/d_o(S_1) \approx 1.4$ may be considered to be representative also for other polyene molecules of similar chain length. In the case of retinals, due to the terminal groups one would expect the electron delocalization to be rather smaller than

Fig. 5. Transient absorption of *all-trans* retinal in polyethylene film, 10 ps and 450 ps after excitation.

Fig. 6. Change in optical density *vs* time after excitation in *all-trans* retinal, at 365 nm, 390 nm and 450 nm.
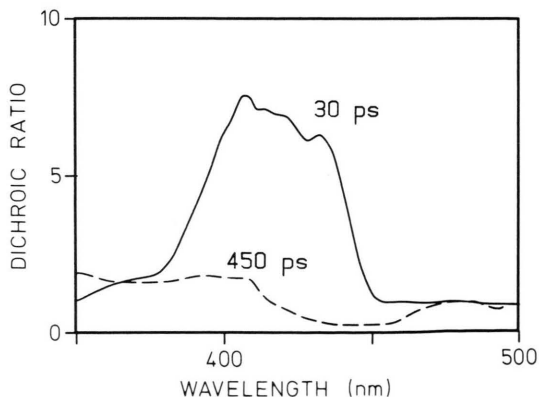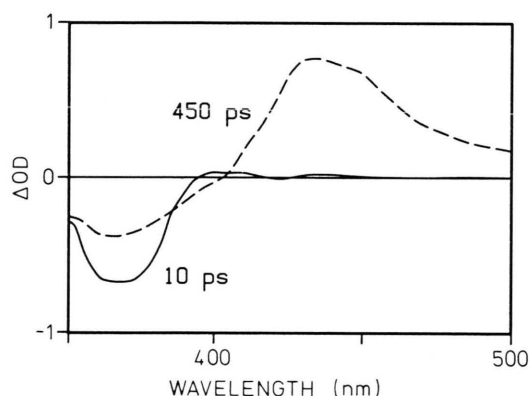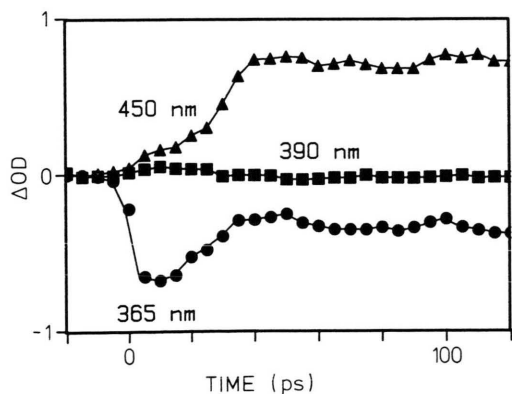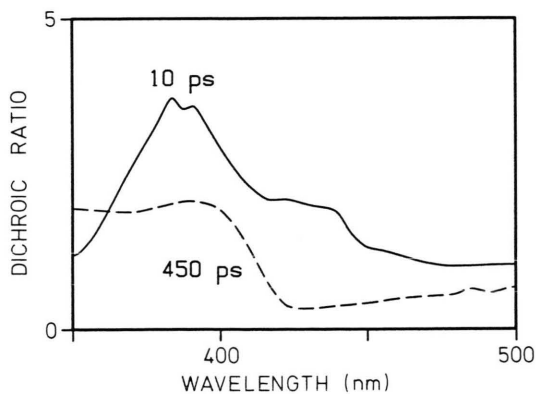

Fig. 7. Dichroic ratio of *all-trans* retinal, 10 ps and 450 ps after excitation.
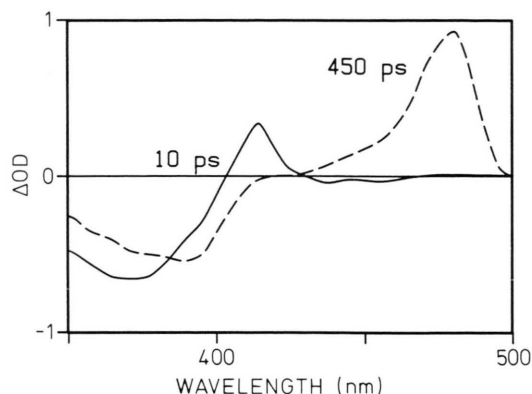
Fig. 8. Transient absorption of DPOT in polyethylene film, 10 ps and 450 ps after excitation.
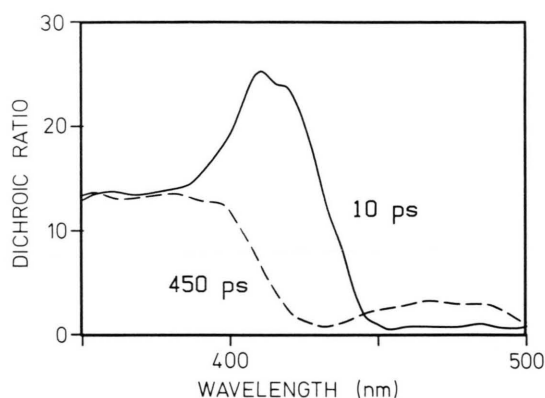

Fig. 9. Dichroic ratio of DPOT, 10 ps and 450 ps after excitation.

in DPOT. The low dichroic ratio of the triplet-triplet absorption suggests that the direction of this transition moment forms an angle with respect to the stretching direction and hence with respect to the direction of the polyene chain.

A definite proof of this assumption cannot be given, however, since the intrinsic dichroic ratio for the triplet-triplet transition is unknown.

In the case of retinals the interpretation is more difficult. As mentioned above, in these molecules the plane of the ring is aligned by stretching, the polyene chain being tilted by a rather large angle due to steric hindrance between the 1,1' methyl groups and the 7-hydrogen atom, as reflected by the low dichroic ratio $d_0 = 1.9$ for both 11-*cis* and *all-trans* retinal. In both compounds the S_m-S_n transition has a dichroic ratio

2–3 times larger than the S_0-S_1 transition. From the comparison with DPOT we expect only a factor up to 1.4 for the change in the intrinsic value. So we have to assume, in addition to a higher intrinsic dichroic ratio, a higher alignment of the transition dipoles and hence the polyene chains in the excited state than in the ground state. It cannot be decided, however, if this alignment is caused by the stress exerted on the molecule by the stretched film, or if also without any influence from the surroundings the excited state conformation of retinals is more planar than in the ground state. Because of the complex steric situation, no quantitative estimations on the alignment can be given for 11-*cis* retinal. For *all-trans* retinal we estimate an angle between stretching direction and transition dipole direction of $\varphi \approx 40-50°$ for the S_0-S_1 transition, assuming the intrinsic dichroic ratio to be equal to that of DPOT. This has to be compared with an angle of 54° between the polyene chain and the plane of the aromatic ring, as known from X-ray studies [10]. For the S_m-S_n absorption we estimate from $d_0 \approx 4$ a value of $\varphi \approx 20-35°$. The triplet-triplet transition has a dichroic ratio $d_0 < 1$, so that in this case it is certain that the main direction of the transition dipole lies at a large angle with respect to the stretching direction. We estimate a value $\varphi \approx 70-90°$ for this angle, with only small dependence on the intrinsic dichroism.

Summarizing the results we can state that our measurements with respect to the spectral and temporal evolution of the transient absorption in retinal are in excellent agreement with earlier measurements reported by Hochstrasser *et al.* [3].

In addition, however, we could show that incorporation of the molecules into a stretched film does not alter these properties significantly. This fact opens up the possibility to make use of the advantages of linear dichroism spectroscopy also in the picosecond regime. The values of the dichroic ratio for retinals in the excited state measured for the first time in these experiments, prove that singlet excited state transition moments are more oriented than ground-state transition moments. Hence it may be concluded that retinals are more planar in the excited state, at least in anisotropic environments. This finding is in agreement with theoretical results, but has not been shown experimentally before. The triplet excited state moment has a quite different direction, on the other hand. Possibly this is responsible for the fact, that, while intersystem crossing is important in isolated

retinal, in the more restrictive environment provided by the protein it plays no major role. These results should be considered in theoretical models of the primary reactions in vision.

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