Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 7273–7277, December 1982
Biophysics

Restriction of motion of protein side chains during the photocycle of bacteriorhodopsin

(linear dichroism/photoselection/proton pump/rotational diffusion)

JÓZSEF CZÉGÉ, ANDRÁS DÉR, LÁSZLÓ ZIMÁNYI, AND LAJOS KESZTHELYI

Institute of Biophysics, Biological Research Center, Szeged H-6701, Hungary

Communicated by Hans Frauenfelder, July 6, 1982

ABSTRACT **Linear dichroism was measured during the photocycle of bacteriorhodopsin. The anisotropy of the sample was produced by the photoselection method. The measurements on purple membrane fragments embedded in agar gel were performed at room temperature with 200-μsec time resolution at several wavelengths in the 240- to 550-nm spectral region. The induced anisotropy of the retinal chromophore remained constant after the formation of the photocycle intermediate *M*. The anisotropy was also time independent at the characteristic peaks of the UV absorption change. These experimental data suggest that the direction of the retinal transition dipole moment remains unchanged. Moreover, the affected aromatic protein side chains also do not show any rotational motion when they are in the perturbed or ground states during the photocycle. Our data render it possible to calculate the restricted range of sudden chromophore rotations that might be coupled to the appearance and decay of the *M* intermediate.**

A study of chromophore orientation in bacteriorhodopsin (bR) during its photocycle can provide valuable information about the proton-pumping mechanism of this molecule. Photoselection is a convenient method for this purpose. Photoselection measurements on bR have been reported only in the visible range, however (1–9).

Slow decay of the induced dichroism of the retinal chromophore at different wavelengths caused by the rotational diffusion of purple membranes (PMs) has been observed in aqueous solution (4, 5, 8). Small, fast changes in the polarization anisotropy also have been reported and attributed to some conformational changes of the protein during the photocycle (4, 5). These transients disappeared, however, when PMs were immobilized in polyacrylamide gel, indicating that the fast decay was probably due to rotational diffusion of small membrane fragments (10). Transient dichroism data obtained on bR incorporated into phosphatidylcholine vesicles (9) have shown that the bRs are immobilized below the lipid phase transition temperature and rotate above it. The slow transition of light-adapted bR to the dark-adapted form has been measured by the photoselection method at 530 nm on thin layers containing PM (11). This experiment indicated that both the bR molecules in the PM and the visible retinal chromophore in the ground state are immobilized. Recently, however, changes of the photodichroism in the visible range during the photocycle have been measured and interpreted as a result of rotational displacement of the retinal chromophore (7).

The spectrum of the UV absorption change indicates that the aromatic amino acid residues of the protein participate in the proton-pumping mechanism (12–14). These transients in films containing PM have been found to be dichroic, suggesting that only few residues are involved (14). The formation and decay times have been correlated with the steps of the photocycle (15, 16).

Here we report photoselection measurement on PM performed in a wide wavelength range (240–550 nm). PMs were immobilized in ≈1% agar gel in order to avoid anisotropy changes due to rotational diffusion. We have found that the anisotropy of the visible and UV absorption changes remains constant during the steps of the photocycle after the formation of the intermediate *M*.

MATERIALS AND METHODS

PMs were obtained by a standard procedure from *Halobacterium halobium* strain NRL R_1M_1 (17). They were suspended in pH 6.5 Britton–Robinson buffer (18) or in a 1% (wt/wt) agar-agar gel prepared in the same buffer. Measurements were performed in a UV cell of 1 mm thickness. The temperature of the sample was maintained at 18°C in all experiments. The sample was light-adapted before each measurement and slowly (1 turn per 10 min) rotated during UV measurements to avoid the degradation of bR.

The data were measured by a common flash photolysis system. The actinic light source was a rhodamine 6G dye laser (half-time, ≈5 nsec; energy, ≈0.1 mJ; wavelength, 590 nm) pumped by a N_2 laser built in our laboratory. A stabilized xenon arc lamp was used to produce the continuous measuring light, which was filtered by two monochromators (Oriel model 7240). The monochromator in front of the photomultiplier excluded the disturbing effect of the actinic light. A fixed and an adjustable polarizer were used to polarize the actinic and measuring light, respectively. The changes in light intensity were detected by a photomultiplier (EMI 9526/B) and amplified by a Keithley 604 amplifier. An intelligent on-line measuring and data-processing system (CAMAC) was used to control the measurements, to average and store the signals, to compute absorption changes $\Delta A^{\parallel}(t)$ and $\Delta A^{\perp}(t)$ and the anisotropy factor

$$r(t) = \frac{\Delta A^{\parallel}(t) - \Delta A^{\perp}(t)}{\Delta A^{\parallel}(t) + 2\Delta A^{\perp}(t)}.$$

The anisotropy factor at $t = 0$ is characteristic of the angle between the excited chromophore and the measured one (θ):

$$r(0) = \frac{1}{5}(2 - 3\sin^2\theta). \qquad [1]$$

If θ changes during the lifetime of the species studied, the anisotropy may vary in time. The rotational diffusion in the most general case results in a decay of $r(t)$ consisting of five exponentials (19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: bR, bacteriorhodopsin; PM, purple membrane.

RESULTS

Fig. 1*a* shows the kinetics of the absorption changes $\Delta A^{\parallel}$ and $\Delta A^{\perp}$, and that of the anisotropy factor r of PM in aqueous suspension at 410 nm. The decay of r was caused by the rotational diffusion of the membrane fragments. Decreasing the particle size by lengthier sonication resulted in a more rapid decay of the anisotropy (Fig. 1*b*). Moreover, the decay ceased when the particles were embedded in 1% agar-agar gel, which shows that the membranes were completely immobilized (see Fig. 3).

As saturation of the exitation was approached, the anisotropy decreased. This is shown in Fig. 2, where the $r(0)$ values for 410 nm are plotted as a function of the actinic light intensity.

Fig. 2 demonstrates that very weak actinic flashes were necessary to avoid saturation. On the other hand, measuring the time dependence of the anisotropy required a good signal-to-noise ratio—i.e., large absorption changes due to high-intensity flashes were necessary. Therefore, we measured the kinetics of the anisotropy with higher exciting intensity (Fig. 3). The fraction of excited bR molecules was 4–5% [the maximal possible excitation ≃ 30% (20)].

In Table 1 we collected the values of the anisotropy measured with low excitation at different wavelengths. (The excited fraction was below 1%). These values were determined by averaging the $r(t)$ over a time interval corresponding to one-half of the exponential decay of the absorbance change. That could be done because the anisotropy remained constant.

Measurements of $r(t)$ at 550 and 410 nm showed that the anisotropy caused by the retinal chromophore in the ground state and in the M state did not change with time, in agreement with previous results (1–6, 8, 9) except for the data of Ahl and Cone (7). Similar curves to those in ref. 7 also were observed in the first phase of our studies, which later turned out to be the result of inadequate measurements. The value of r remained constant in the UV range as well, where measurements were performed at the characteristic peaks of the UV difference absorption spectrum.

It was possible to identify the chromophores responsible for the UV absorption change by comparing the transient spectrum of bR at 1 msec after excitation with the difference spectrum derived from spectra corresponding to the deprotonation of tryptophan and tyrosine caused by changing the pH of the solvent over a wide range (Fig. 4). Deprotonation of tryptophan in solution takes place at the amino and carboxyl groups, which are rather far from the aromatic residue responsible for the absorption in the wavelength range of 250–300 nm. Though the protonation state of these groups may not change in proteins, the changing proton environment of the aromatic residue results in a similar absorption variation (15, 16). We assigned the absorption changes at 275 and 295 nm to charge perturbation of tryptophan (a proton moves away from the neighborhood of the aromatic residue). The peak in the absorption change at 240 nm was assigned mainly to the deprotonation of the aromatic residue of tyrosine. The measured absorption spectrum and the assignment of the peaks are in agreement with the results of Bogomolni (14) and Hess and Kuschmitz (15, 16).

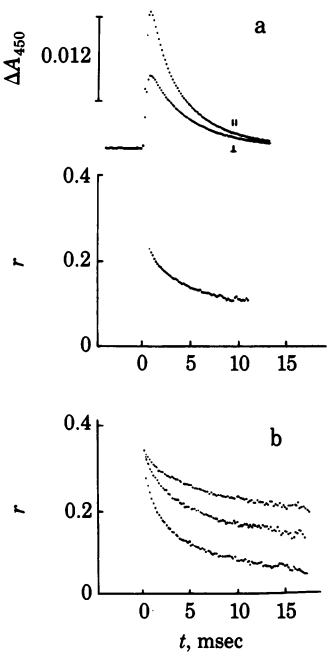

FIG. 1. (*a*) The absorption changes with light polarized parallel and perpendicular to the polarization of the exciting light, and the corresponding anisotropy factor, measured at 410 nm in an aqueous suspension of PM. (*b*) Time dependence of the anisotropy factor at 410 nm in an aqueous suspension of PM after different durations of sonication. Curves from top to bottom: 10-sec, 20-sec, and 40-sec sonication. Number of flashes: 2000.

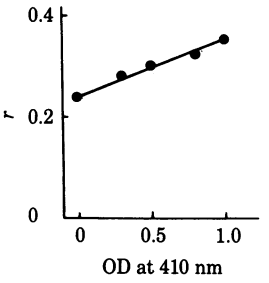

FIG. 2. The value of the anisotropy factor as a function of the exciting light intensity, measured at 410 nm. OD, optical density of neutral filters placed in the path of the exciting light.

The chromophores responsible for the UV absorption changes assigned to tryptophan and tyrosine side chains will be called amino acid chromophores.

DISCUSSION

The question arises whether the time-independent value of $r(t)$ argues for constant retinal and amino acid chromophore directions in the bR molecule during the photocycle. The measured absorption changes are related to the altered state of the chromophores appearing and decaying (or at 550 nm decaying and reappearing) during the photocycle. We call the new state of the chromophore the perturbed state and the state before and after the perturbation the ground state. (For example, the absorption signal at 410 nm is caused by the retinal chromophores of those bR molecules that are in the M state. The perturbed state now is M, the ground state is bR 570). The constancy of $r(t)$ means, to a first approximation, that the direction of the chromophores does not change when they are in the perturbed state (i.e., either the bR molecule does not rotate as a whole or there are no large intramolecular conformational changes), independent of the appearance and decay of the chromophores. Apparently we cannot know whether or not sudden directional changes of the chromophores are coupled with the transitions themselves. However, a closer investigation of $r(t)$ at various wavelengths does allow one to conclude more.

The argument is rather simple in the case of the retinal chromophore. The $r_{(I\simeq 0)}$ value in Table 1 for 550 and 410 nm is very near to $r_{(I\simeq 0)} = 0.4$, which is characteristic for $\theta = 0$ (Eq. 1). θ is the angle between the chromophore excited by the laser flash (the retinal chromophore in the ground state) and the measured chromophore [the retinal chromophore in M ($\lambda = 410$ nm) or the reappearing chromophore ($\lambda = 550$ nm)]. Because $\theta \simeq 0$ in both cases—i.e., the same chromophore appears in the same direction at a slightly different wavelength—we may state that the direction of the retinal chromophore (and consequently the direction of the retinal itself) does not change even during the transitions in the photocycle. Recently El Sayed *et al.* (23) reported that $\theta \simeq 0$ for retinal chromophore of K.

To understand the motion of the amino acid chromophores during the transitions, a more general treatment is needed. Let us assume that at a given wavelength the absorption change

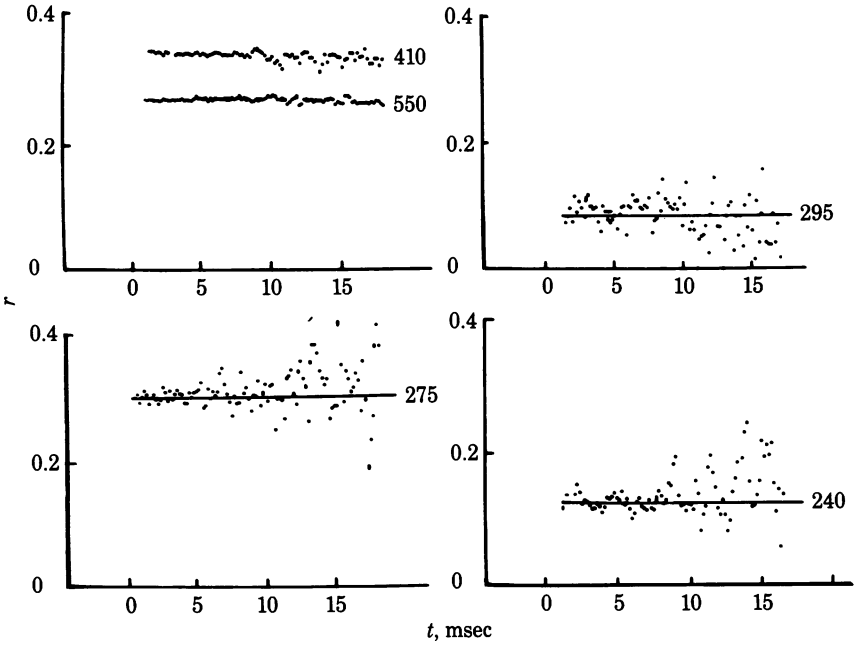

FIG. 3. The anisotropy factors measured at different wavelengths (shown in nm) on PM immobilized in agar gel. Number of flashes: 2,000 at 410 nm and 4,000 otherwise.

arises from only one chromophore. The chromophore in the ground state and in the perturbed state may have overlapping absorption bands. Let the angle between the transition dipole moment of the retinal chromophore in the ground state and that of the considered chromophore be β_g in the ground state and β_p in the perturbed state, and let μ_g and μ_p be the absolute values of the transition dipole moment of the chromophore in the ground and perturbed states, respectively, multiplied by a wavelength-dependent factor. This means that μ_g^2 and μ_p^2 are proportional to the absorption coefficient of the chromophore in the two different states at a given wavelength. Note that β_p may be different from β_g for two reasons: (*i*) the orientation of the transition dipole moment relative to the atoms of the amino acid may be different in the ground and perturbed states and (*ii*) the orientation of the amino acid may change.

As a result of a simple calculation (see *Appendix*) the absorption changes with the measuring light polarized parallel and perpendicular to the polarization of the actinic light are:

$$\Delta A^{\parallel}(t) = C[\mu_p^2(3 - 2\sin^2\beta_p) - \mu_g^2(3 - 2\sin^2\beta_g)]\, k(t)$$

and

$$\Delta A^{\perp}(t) = C[\mu_p^2(1 + \sin^2\beta_p) - \mu_g^2(1 + \sin^2\beta_g)]\, k(t), \quad [2]$$

where $k(t)$ describes the fraction of chromophores in the perturbed state as a function of time. Using the above formulae, we can obtain the antisotropy factor:

$$r(t) = \frac{\Delta A^{\parallel}(t) - \Delta A^{\perp}(t)}{\Delta A^{\parallel}(t) + 2\Delta A^{\perp}(t)} = \frac{\mu_p^2(2 - 3\sin^2\beta_p) - \mu_g^2(2 - 3\sin^2\beta_g)}{5(\mu_p^2 - \mu_g^2)}. \quad [3]$$

Table 1. The values of the antisotropy at different wavelengths with low exciting intensity

λ, nm	$r_{(t=0)}$
550	0.35
410	0.38
295	0.16
275	0.37
240	0.22

r could have any value between $-\infty$ and $+\infty$ if two states of the chromophore (perturbed and ground states) are taken into account in contrast with the limited range expressed in Eq. 1.

It follows that if the chromophore has two different orientations in its two states and it jumps from one orientation to the other (i.e., there are no intermediate states with a different transition dipole moment direction), the anisotropy is constant in time. If more than two states existed, new terms would appear in the expressions for $\Delta A^{\parallel}(t)$ and $\Delta A^{\perp}(t)$ with time-dependent factors different from $k(t)$, resulting in an explicit time-dependence of $r(t)$. However, our measurements did not indicate a change of the anisotropy in time.

We now use Eq. 3 to evaluate $r(t)$ data at different wavelengths. When the retinal chromophore in the ground state is studied at $\lambda = 550$ nm, only the ground state is observed at this wavelength (i.e., $\mu_p^2 \simeq 0$). In this way Eq. 1 follows from Eq.

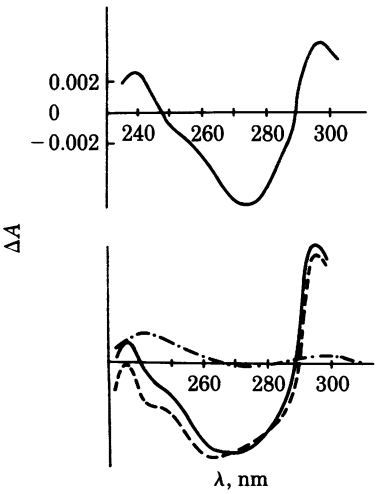

FIG. 4. Comparison of the spectrum of the light-induced UV absorption change of bR (*Upper*) with the difference spectrum (——) derived from spectra of tyrosine and tryptophan in solution (*Lower*), with changing pH of the solvent. (*Lower*) Spectra of tyrosine at pH 13–6 from ref. 21 (–·–·), tryptophan at pH 10.3–1.2 from ref. 22 (– – –), and the sum of the two spectra with arbitrary weighting factors (——). The spectrum of bR has been recorded in 5-nm steps with a band-width of 3 nm.

3. The measured anisotropy is close to 0.4, corresponding to $\beta_g = 0°$.

At 410 nm we can observe the retinal chromophore in the *M* state without significant overlap with the ground state absorption, implying that $\mu_g^2 \simeq 0$; $\theta \simeq 0$ is obtained from the anisotropy value of ≈0.4. Thus, we see that the more general Eq. 3 includes the special case of Eq. 1. The general treatment confirms the simple argument outlined above.

The situation is more complicated in the UV range because the absorption bands of the ground and perturbed states of the amino acid chromophores significantly overlap each other. In this case we are not able to determine the angle between the transition dipole moment of the perturbed or ground state amino acid chromophores and that of the retinal chromophore. However, the relationship between these angles can be calculated by putting the measured value of the anisotropy ($r_{(t\sim 0)}$) and the estimated value of μ_p^2/μ_g^2 into Eq. 3. In Fig. 5 this relationship is shown for the chromophores at 295 nm and 275 nm. The ratio of the absorption in the perturbed and ground state (i.e., μ_p^2/μ_g^2) was determined from the measured absorption and the absorption change of the sample, assuming that only one tryptophan side chain per excited bR is involved in the absorption change [probably its two different transition moments, being perpendicular to each other, are detected at 275 and 295 nm (24)]. At 240 nm the signal may be a mixture of the absorption changes of one or more side chains (tyrosine and tryptophan), so we did not estimate the angles at this wavelength.

The maximal change of the transition dipole moment direction at 295 nm is less than 18°, but it is only as small as a few degrees at almost all orientations of the ground state amino acid chromophore (Fig. 5). The $\beta_{p_1} = \beta_{g_1}$ case, which means no jump of the chromophore direction during the photocycle, corresponds to $\beta_{g_1} \simeq 40°$. At 275 nm the allowed change of the transition dipole moment orientation is larger; at $\beta_{g_2} \simeq 50°$ it reaches a maximum of 40°. The ground state chromophore may be directed only in the range from 10° to 50°. $\beta_{p_2} = \beta_{g_2}$ (i.e., the case of no jump) corresponds to $\beta_{g_2} \simeq 12°$. It is reasonable to assume that the small perturbation leading to the absorption change does not alter the direction of the transition dipole moments relative to the tryptophan axis (i.e., to consider $\beta_{pg} = \beta_p - \beta_g$ as the consequence of the rotational motion of this axis). In this way the data restrict the space allowed for sudden rotation of the tryptophan side chain during the transition to $\beta_{p_1g_1} < 18°$ and $\beta_{p_2g_2} < 40°$. It should be mentioned, however, that the value of the anisotropy is not sensitive to the rotation around an axis parallel to the transition dipole moment of the retinal.

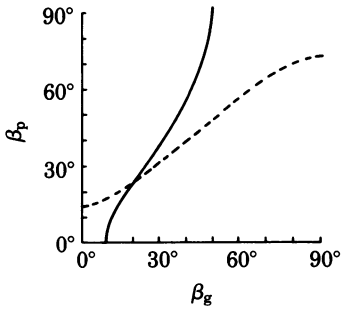

FIG. 5. The angle between $\vec{\mu}_p$ and $\vec{\mu}_R$ (β_p) as a function of the angle between $\vec{\mu}_g$ and $\vec{\mu}_R$ (β_g) calculated from Eq. 3 for $\lambda = 275$ nm (——) and $\lambda = 295$ nm (---). μ_p, μ_g, and μ_R are the transition dipole moments of the UV amino acid chromophores in the perturbed state and the ground state and that of the retinal chromophore. Values are $\mu_{p_1}^2/\mu_{g_1}^2 = 1.17$ at 295 nm and $\mu_{p_2}^2/\mu_{g_2}^2 = 0.55$ at 275 nm. $r_{(t\sim 0)}$ values from Table 1 were used.

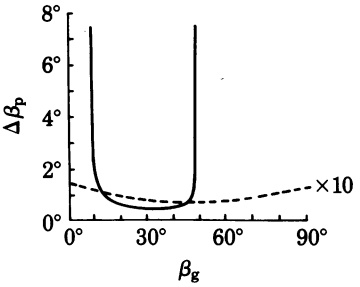

FIG. 6. The allowed changes of β_p estimated from the standard deviation of a linear fitting on the $r(t)$ curves at 275 nm (——) and 295 nm (---).

Such motions cannot be observed by the photoselection method.

As stated above, the time independence of r indicates that the chromophores are fixed when they exist in one of their states. From the standard deviation of a linear fitting on the $r(t)$ functions shown in Fig. 3, an upper limit for the rotation angles of the different dipole moments can be estimated in the ground or perturbed state of the chromophores. $\Delta\beta_g < 0.3°$ at 550 nm and $\Delta\beta_p < 2°$ at 410 nm have been determined. In the UV range, curves similar to those shown in Fig. 5 can be obtained by using the value of the anisotropy increased and decreased by its standard deviation. $\Delta\beta_p$ is shown in Fig. 6 as a function of β_g. It is apparent that, at almost all orientations of the ground state chromophore, the rotation of the transition dipole moments is highly restricted.

The analysis of the data leads to two important conclusions: (*i*) The chromophores do not move during the photocycle in their ground and perturbed states, and (*ii*) a limit for sudden rotations of chromophores corresponding to the transitions can be given. The angle is rather small (≈0°) for the retinal chromophore; for amino acid chromophores (tryptophan), the angle depends on the angle between the ground state directions of both the amino acid chromophore itself and the retinal chromophore. These conclusions are consistent with (though do not prove) the idea that the proton transfer in bR is independent of the side chain motions (25, 26).

APPENDIX

A brief derivation of Eq. 2 is given. The light absorption is described by the transition dipole moment, μ (26). Suppose that this μ stands at an angle θ with respect to the polarization of the light. Then the absorbance $A(\theta)$ of the sample is

$$A(\theta) \approx \mu^2 \cos^2 \theta. \qquad [A.1]$$

If the transition dipole moment of a chromophore, μ, makes an angle β with the retinal chromophore, and the angle between the retinal chromophore and the light polarization is $\bar{\theta}$ (Fig. 7), then—as it is easily seen from Eq. A.1—the absorbance corresponding to μ is

$$A(\bar{\theta},\beta) = K'\mu^2 \left[\frac{1}{2}\sin^2 \bar{\theta} \sin^2 \beta + \cos^2 \bar{\theta} \cos^2 \beta\right]. \qquad [A.2]$$

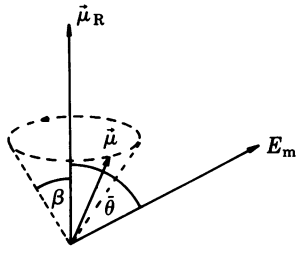

FIG. 7. Geometry of the transition dipole moments and the polarization of the measuring light. $\vec{\mu}_R$, The retinal chromophore; $\vec{\mu}$, any chromophore; $\vec{E}_m$, polarization of the measuring light.

[K' contains the necessary quantum mechanical factors (26).]

If a polarized actinic flash triggers the photocycle in an optically thin bR sample, then, according to Eq. **A.1**, the number of the excited molecules in a solid angle $d\Omega$ characterized by the angles θ and ϕ (Fig. 8) will be

$$\frac{R}{4\pi} K\mu_R^2 \cos^2\theta \, d\Omega, \qquad [A.3]$$

and that of unexcited ones will be

$$\frac{R}{4\pi}(1 - K\mu_R^2 \cos^2\theta)\, d\Omega. \qquad [A.4]$$

Here R means the total number of retinal chromophores and K contains all the necessary quantum mechanical factors (27) and the intensity of the actinic flash.

The absorption after flash excitation should be calculated for chromophores in the ground and perturbed states characterized by transition dipole moments μ_g and μ_p and by angles β_g and β_p with respect to the retinal chromophores. We relate the absorbance of plane polarized light to the polarization direction of the actinic light; therefore, Eqs. **A.3**, **A.4**, and **A.2** must be combined and integrated for the angles θ and ϕ.

Elementary calculations yield the following equations,

$$A^{\parallel} = \frac{1}{3} R\mu_g^2 K' + C[\mu_p^2(3 - 2\sin^2\beta_p) - \mu_g^2(3 - 2\sin^2\beta_g)] \qquad [A.5]$$

$$A^{\perp} = \frac{1}{3} R\mu_g^2 K' + C[\mu_p^2(1 + \sin^2\beta_p) - \mu_g^2(1 + \sin^2\beta_g)], \qquad [A.6]$$

where $C = 1/15\, KK'\, R\mu_R^2$.

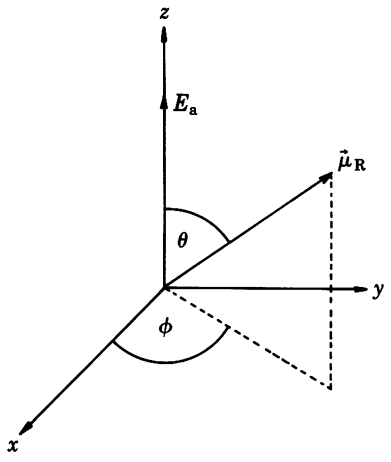

FIG. 8. Geometry of the transition dipole moment of the retinal chromophore, μ_R and the polarization of the actinic light, $\vec{E}_a$.

As $C = 0$ whenever the intensity of the actinic flash is zero, the absorbance changes caused by the excitation will be

$$\Delta A^{\parallel} = C[\mu_p^2(3 - 2\sin^2\beta_p) - \mu_g^2(3 - 2\sin^2\beta_g)] \qquad [A.7]$$

and

$$\Delta A^{\perp} = C[\mu_p^2(1 + \sin^2\beta_p) - \mu_g^2(1 + \sin^2\beta_g)]. \qquad [A.8]$$

The authors are indebted to Professors M. P. Heyn and P. Läuger and to Dr. Cs. Fajszi for helpful discussions. This work was supported in part by National Science Foundation Grant INT 78-27606.

1. Razi Naqvi, K., Gonzales-Rodriguez, J., Cherry, R. J. & Chapman, D. (1973) *Nature (London) New Biol.* **245**, 249–251.
2. Sherman, W. V., Slifkin, M. A. & Caplan, S. R. (1976) *Biochim. Biophys. Acta* **423**, 238–248.
3. Cherry, R. J., Heyn, M. P. & Oesterhelt, D. (1977) *FEBS Lett.* **78**, 25–30.
4. Lozier, R. H. & Niederberger, W. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 1805–1809.
5. Sherman, W. V. & Caplan, S. R. (1977) *Nature (London)* **265**, 273–274.
6. Gillbro, T. (1978) *Biochim. Biophys. Acta* **504**, 175–186.
7. Ahl, P. L. & Cone, R. A. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1847.
8. Kouyama, T., Kimura, Y., Kinosita, K., Jr., & Ikegami, A. (1981) *FEBS Lett.* **124**, 100–104.
9. Heyn, M. P., Cherry, R. J. & Muller, U. (1977) *J. Mol. Biol.* **117**, 607–620.
10. Stoeckenius, W., Lozier, R. H. & Bogomolni, R. A. (1979) *Biochim. Biophys. Acta* **505**, 215–278.
11. Korenstein, R. & Hess, B. (1978) *FEBS Lett.* **89**, 15–20.
12. Bogomolni, R. A., Stubbs, L. & Lanyi, J. K. (1978) *Biochemistry* **17**, 1037–1041.
13. Becher, B., Tokunaga, F. & Ebrey, T. G. (1978) *Biochemistry* **17**, 2293–2300.
14. Bogomolni, R. A. (1980) in *Bioelectrochemistry*, eds. Keyzer, H. & Guttman, F. (Plenum, New York), pp. 83–95.
15. Hess, B. & Kuschmitz, D. (1979) *FEBS Lett.* **100**, 334–340.
16. Kuschmitz, D. & Hess, B. (1982) *FEBS Lett.* **138**, 137–140.
17. Oesterhelt, D. & Stoeckenius, W. (1974) *Methods Enzymol.* **31**, 667–668.
18. Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1969) *Data for Biochemical Research* (Clarendon, Oxford), p. 485.
19. Belford, G. G., Belford, R. L. & Weber, G. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1392–1393.
20. Becher, B. & Ebrey, T. G. (1977) *Biophys. J.* **17**, 185–191.
21. Wetlaufer, D. B. (1962) *Adv. Protein Chem.* **17**, 303–390.
22. Donovan, J. W., Laskowski, M., Jr., & Scheraga, H. A. (1961) *J. Am. Chem. Soc.* **83**, 2686–2694.
23. El-Sayed, M. A., Karvaly, B. & Fukumoto, J. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7512–7516.
24. Hirakawa, A. Y., Nishimura, Y. N., Matsumoto, T., Nakanishi, M. & Touboi, M. (1978) *J. Raman Spectrosc.* **7**, 282–287.
25. Stoeckenius, W. (1979) in *Membrane Transduction Mechanisms*, eds. Cone, R. A. & Dowling, J. E. (Raven, New York), p. 39.
26. Keszthelyi, L. & Ormos, P. (1980) *FEBS Lett.* **109**, 189–193.
27. Fredericq, E. & Houssier, C. (1973) *Electric Dichroism and Electric Birefringence* (Clarendon, Oxford), p. 16.