Heyderman, E., & Monaghan, P. (1979) *Invest. Cell Pathol. 2*, 119–122.
Iseki, S., Masaki, S., & Shibasaki, K. (1957) *Proc. Jpn. Acad. 33*, 492–497.
Judd, W. J., Steiner, E. A., Friedman, B. A., & Oberman, H. A. (1978) *Transfusion 18*, 436–440.
Kent, S. P. (1964) *J. Histochem. Cytochem. 12*, 591–599.
Kobata, A. (1975) *Kagaku (Kyoto) 30*, 192–198.
Lemieux, R. U. (1978) *Chem. Soc. Rev. 7*, 423–452.
Lemieux, R. U., Bundle, D. R., & Baker, D. A. (1975) *J. Am. Chem. Soc. 97*, 4076–4083.
Lemieux, R. U., Baker, D. A., & Bundle, D. R. (1977) *Can. J. Biochem. 55*, 507–512.
Lemieux, R. U., Bundle, D. R., & Baker, D. A. (1979) U.S. Patent 4 137 401.
Lemieux, R. U., Bock, K., Delbaere, L. T. J., Koto, S., & Rao, V. S. (1980a) *Can. J. Chem. 58*, 631–653.
Lemieux, R. U., Le Pendu, J., & Hindsgaul, O. (1980b) *J. Antibiot. 32*, S21–31.
Lloyd, K. O., Kabat, E. A., & Licerio, E. (1968) *Biochemistry 7*, 2976–2990.
Marcus, D. M., & Grollman, A. P. (1966) *J. Immunol. 97*, 867–875.
Marr, A. M. S., Donald, A. S. R., Watkins, W. M., & Morgan, W. T. J. (1967) *Nature (London) 215*, 1345–1349.
Mourant, A. E. (1946) *Nature (London) 158*, 237–238.
Perera, D. R., Weinstein, W. M., & Rubin, C. E. (1975) *Hum. Pathol. 6*, 157–215.
Potapov, M. I. (1970) *Probl. Haematol. 11*, 45.
Potapov, M. I. (1976) *Vox Sang. 30*, 211–213.
Prohaska, R., Schenkel-Brunner, H., & Tuppy, H (1978) *Eur. J. Biochem. 84*, 161–166.
Race, R. R., & Sanger, R. (1972) *Haematologia 6*, 63–71.
Rege, V. P., Painter, T. J., Watkins, W. M., & Morgan, W. T. J. (1964) *Nature (London) 204*, 740–742.
Rovis, L., Anderson, B., Kabat, E. A., Greuzo, F., & Liao, J. (1973) *Biochemistry 12*, 5340–5354.
Schenkel-Brunner, H., & Prohaska, R. (1978) *Proc. Int. Congr. Int. Soc. Hematol., 17th–Int. Soc. Blood Transfusion, 15th*, Abstracts p 646.
Schiffman, G., Kabat, E. A., & Thompson, W. (1964) *Biochemistry 3*, 113–120.
Schwarting, G. K., & Marcus, D. M. (1979) *Clin. Immunol. Immunopathol. 14*, 121–129.
Shen, L., Grollman, E. F., & Ginsburg, V. (1968) *Proc. Natl. Acad. Sci. U.S.A. 59*, 224–230.
Smith, E. L., McKibbin, J. M., Karlsson, K.-A., Pascher, I., & Samuelson, B. E. (1975) *J. Biol. Chem. 250*, 6059–6064.
Stellner, K., Watanabe, K., & Hakomori, S. (1973) *Biochemistry 12*, 656–661.
Szulman, A. E. (1962) *J. Exp. Med. 115*, 977–996.
Szulman, A. E., & Marcus, D. M. (1973) *Lab. Invest. 28*, 565–574.
Ueyama, R. (1939) *Hanzaigaku Zasshi 13*, 51–64.
Watkins, W. M., & Morgan, W. T. J. (1957) *Nature (London) 180*, 1038–1040.
Weinstein, W. M., & Lechago, J. (1977) *Gastroenterology 73*, 765–767.
Wilbrant, R., Tung, K. S. K., Deodhar, S. D., Nakamoto, S., & Kolff, W. J. (1969) *Am. J. Clin. Pathol. 51*, 15–23.
Zopf, D. A., & Ginsburg, V. (1975) *Arch. Biochem. Biophys. 167*, 345–350.

Photochemistry and Fluorescence of Bacteriorhodopsin Excited in Its 280-nm Absorption Band†

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ABSTRACT: Photochemical and fluorescence studies are carried out, exciting bacteriorhodopsin (BR) in its 280-nm absorption band. The data indicate that energy transfer takes place, with a quantum yield of 0.7–0.8, from excited tyrosines and tryptophans to the retinyl chromophore. All of the tyrosine and five to six tryptophan residues are completely quenched by the transfer process while one tryptophan is unquenched and one is partially (~80%) quenched. Energy transfer to the chromophore leads to a photocycle identical with that triggered in (light adapted) bacteriorhodopsin by excitation within the visible absorption bands of the chromophore. The emissive properties of BR in the intact membrane are found equal to those of a Triton X-100 solubilized BR monomer. The energy transfer data are discussed in terms of the available amino acid sequence and the electron density map of bacteriorhodopsin. Although such data cannot suggest a single fit between the sequence and the density map (one out of the 7! = 5040 possibilities), they do provide a criterion for testing any specific model for the structure of bacteriorhodopsin.

Bacteriorhodopsin, the protein pigment of the purple membrane of *Halobacterium halobium*, is responsible for a light-driven proton pump which leads to ATP synthesis. [For a review, see Stoeckenius et al. (1978).] Analogous to visual pigments [for a review, see Ottolenghi (1980)], the spectrum of bacteriorhodopsin in the visible and in the near-UV is characterized by three absorption bands (Becher et al., 1978). The α band (with an extinction of $\epsilon \approx 63\,000$ M^{-1} cm^{-1}) peaks, in light-adapted (all trans) bacteriorhodopsin (BR^{l}_{570}), at 570 nm. A lower extinction band ($\epsilon \approx 13\,000$ M^{-1} cm^{-1}), lacking a clear maximum, covers the range between 450 and 300 nm (β band). In the UV spectrum, an intense ($\epsilon \approx 75\,000$ M^{-1} cm^{-1}) band is observed, peaking at 280 nm (γ band). Both α and β bands are exclusively due to the retinyl chromophore, which is attached to the protein via a protonated Schiff-base linkage. In keeping with the characteristic protein fluorescence induced by excitation in the near-UV spectrum (Bogomolni

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et al., 1978), the γ band is attributed to the superimposed absorptions of the retinyl moiety and of the aromatic protein residues, tyrosine and tryptophan. Becher et al. (1978) have recently shown that the latter are the major contributors to the absorption at 280 nm, with only ~10% of the total extinction being due to the retinyl polyene.

Extensive investigations have been carried out on the photochemistry of BR'_{570} induced by excitation within the α and β bands [see Stoeckenius et al. (1979) and Ottolenghi (1980) for recent reviews]. In the present work we have studied the photochemical and emissive properties of BR'_{570} following excitation within the 280-nm (γ) band. The results indicate that substantial energy transfer takes place from excited aromatic residues to the polyene moiety, triggering the characteristic photocycle of BR'_{570}. An analysis of the fluorescence data bears on the tertiary structure of the bacteriorhodopsin molecule.

Experimental Procedures

Experiments were carried out at room temperature with aqueous purple-membrane suspensions of M_1 *Halobacterium halobium* prepared according to previously described procedures (Oesterhelt & Stoeckenius, 1974). Work with bacteriorhodopsin solubilized with Triton X-100 at pH 5 (0.1 M acetate buffer) followed the procedures described by Stoeckenius and co-workers (Reynolds & Stoeckenius, 1977; R. Casadio, H. Gutowitz, P. Mowery, M. Taylor, and W. Stoeckenius, unpublished results). At pH 7, we employed the procedure of Becher & Ebrey (1976) using 0.025 M phosphate buffer. Fluorescence measurements in membrane suspensions were performed in the presence of 40% sucrose to minimize light scattering artifacts (Sherman & Caplan, 1975). Solutions of naphthalene, a Fluka (Puriss) product, were prepared in spectrograde cyclohexane. NATA (*N*-acetyltryptophanamide), obtained from Miles-Yeda (Reovoth), and glycyltyrosine, an ICN-National Biochemicals Division product, were used without further purification.

A Laser-Associates Q-switched, frequency quadrupled, Nd laser was employed for pulsed (20 ns) excitation at 265 nm in a previously described experimental setup (Goldschmidt et al., 1976). A conventional fluorometer, including a Spex Minimate double monochromator for excitation and a $f = 4$, 500 nm, focal length Bausch & Lomb monochromator for detection at 90°, was employed for continuous excitation experiments. The emission, detected by a 5256S EMI photomultiplier, was analyzed by a Brookdeal–Ortec photon-counting system (Lasser & Feitelson, 1973).

The apparatus for determination of the fluorescence lifetimes has been recently described (Barboy & Feitelson, 1978). Briefly, the lifetimes in 2.5×10^{-6} M BR solutions were measured by illumination (at 265 or 292 nm) with a TRW nanosecond light source. The lamp response and the fluorescence were monitored by a DUVP Amperex photomultiplier coupled to a sampling oscilloscope and, hence, to a computer of average transients (CAT). For minimization of errors due to electronic drifts, the signals from the sample and from the scattering solutions were alternately measured for 10-s intervals and the data stored in separate compartments of the CAT. The lifetimes were obtained by convoluting the lamp response curve with a mono- or double-exponential decay function and fitting it to the experimental decay curve.

Results and Discussion

Pulsed Laser Photolysis at 265 nm. Photochemical experiments exciting BR'_{570} within its UV absorption band were carried out by using the 265-nm pulse of the frequency-quadrupled Nd laser. Excitation leads to transient phenomena identical with those previously reported following irradiation within the main (α) or secondary (β) absorption bands [for a review, see Ottolenghi (1980)]. For example, the decay at 420 nm and the corresponding difference spectrum obtained following 265-nm excitation (see Figure 1) are both characteristic of the familiar spectral and kinetic decay patterns associated with the M_{412} phototransient (Goldschmidt et al., 1976). It is therefore suggested that UV irradiation triggers photochemical processes identical with those induced by visible light. A similar conclusion was previously reached for visual (bovine) rhodopsin (Rosenfeld & Ottolenghi, 1977).

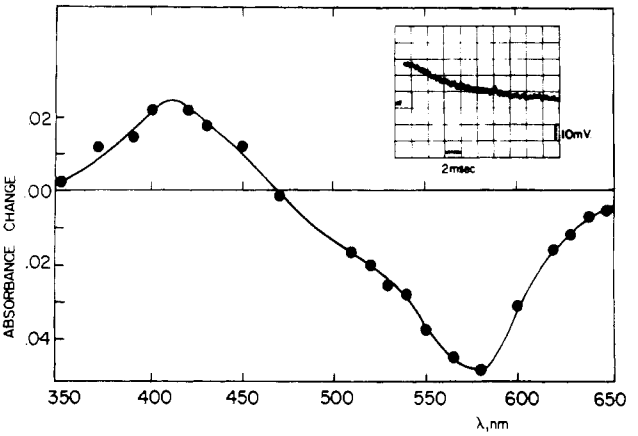

FIGURE 1: Characteristic oscillogram and difference spectrum showing the generation of the M_{410} phototransient of Br'_{570} following excitation with the 265-nm laser pulse (unbuffered purple membrane suspensions, pH 5.5, at 20 °C).

The cycling quantum yield for 265-nm excitation (ϕ_{BR}^{265}) was determined by using the naphthalene triplet state in cyclohexane as the reference. Use was made of the expression (Goldschmidt et al., 1977) $\phi_{BR}^{265} = \phi_R(\Delta\epsilon_R/\Delta\epsilon_{BR})(\Delta D_{BR})/(\Delta D_T)(1 - 10^{-D_R})/1 - 10^{-D_{BR}})$ where $\phi_R = 0.71$ is the reference (naphthalene) triplet yield (Amand & Bensasson, 1975) and $\Delta\epsilon_R$ and $\Delta\epsilon_{BR}$ represent the differences in extinction coefficients between the molecules photolyzed (naphthalene and BR'_{570}) and their respective photoproducts (the triplet and M_{410}). D_R and D_{BR} represent the initial absorbance of the reference and of the BR'_{570} solution at 265 nm while ΔD_T and ΔD_{BR} are the photoinduced changes in absorbance due to the generation of the triplet and the M_{410} intermediate, respectively. Experiments were carried out for two BR'_{570} concentrations ($D_{BR} = 0.45$ and $D_{BR} = 0.81$) by using low intensity pulses so as to assure linearity between ΔD and the pulse intensity (Goldschmidt et al., 1977). ΔD_T was measured at 415 nm and ΔD_{BR} at 600, 580, and 565 nm. The results of a set of ten runs, varying the detection wavelength and the pulse intensity, yielded a consistent value of $\phi_{BR}^{265} = 0.19 \pm 0.02$.

The absorbance of BR'_{570} at 265 nm is due to the superimposed contributions, ϵ_{rt}^{265} and ϵ_{op}^{265}, of the retinyl chromophore and of the opsin aromatic amino acids, respectively. The BR'_{570} photocycle may therefore be induced both by direct light absorption by the retinyl polyene and by energy transfer to the latter from the excited amino acids. Denoting the cycling quantum yield by γ, we may write

$$\phi_{BR}^{265} = f\gamma + \gamma(1 - f)\phi_{ET} \qquad (1)$$

where $f = \epsilon_{rt}^{265}/(\epsilon_{rt}^{265} + \epsilon_{op}^{265})$ is the fraction of the 265-nm quanta absorbed by the polyene and ϕ_{ET} is the yield of energy transfer to the latter from the excited aromatic residues.

The application of the above expression is associated with the exact determination of f. On the basis of the spectrum

of a free protonated Schiff base in solution (PRBS), Becher et al. (1978) have recently shown that the contribution of the retinyl moiety to the total BR'_{570} extinction at 280 nm (ϵ_{BR}^{280} = 75 000 M^{-1} cm^{-1}) amounts to ϵ_{rt}^{280} = 8000 M^{-1} cm^{-1}. Since for PRSB the absorbance at 265 nm is essentially identical with that at 280 nm, the procedure of Becher et al. (1978) yields a ϵ_{rt} value close to 8000 M^{-1} cm^{-1} (f = 0.12) also at 265 nm. Using ϕ_{BR}^{265} = 0.19 and γ = 0.25 (see below), we obtain ϕ_{ET} = 0.727. A similar result (ϕ_{ET} = 0.730) is obtained with the value ϵ_{rt}^{265} = 7045 M^{-1} cm^{-1} (f = 0.106), calculated by using the spectrum of the opsin recorded after photobleaching the purple membrane in the presence of hydroxylamine, followed by extraction of the produced oxime (Becher et al., 1978). A third approach involves the use of a NATA/Tyr-Gly solution identical in its tryptophan–tyrosine composition with that of bacteriorhodopsin [11 tyrosines and 7 tryptophans according to Ovchinikov et al. (1979) or 11 tyrosines and 8 tryptophans according to Khorana et al. (1979)]. At 280 nm, such solutions are characterized (Mihalyi, 1968) by $\epsilon \approx$ 55 000 M^{-1} cm^{-1} as compared with ϵ_{BR}^{280} = 75 000 M^{-1} cm^{-1}. The difference between the two values was attributed by Becher et al. (1978) to environmental effects on the absorption of the aromatic amino acids in the opsin. Assuming that the relative contributions of such effects are identical at 280 and 265 nm, we have estimated ϵ_{rt}^{265} by using the respective extinction in the NATA/Tyr-Gly solution and the above value of ϵ_{rt}^{280}. Although the result, $\epsilon_{rt}^{265} \approx$ 15 700 M^{-1} cm^{-1} (f = 0.22-0.23), is substantially different from the values yielded by the previous procedures, the calculated energy transfer quantum yield (ϕ_{ET} = 0.69) is close to those calculated above.

While the first of the three procedures assumes that the relative absorptions at 265 and 280 nm are identical for a free PRSB and for the retinyl chromophore in the opsin, the second overlooks structural changes in the opsin which might be induced by bleaching and the third neglects any wavelength dependence of the environmental effects in the opsin. Nevertheless, in view of the reasonable agreement between the values of ϕ_{ET} yielded by the three methods, we consider ϕ_{ET} = 0.71 ± 0.02 as a good estimate for the yield of energy transfer to the chromophore. We should finally mention that in the above calculations of ϕ_{ET} we have used the value γ = 0.25 which was measured for visible (530 nm) excitation, populating the lowest excited state (S_1) of the chromophore (Goldschmidt et al., 1977). This approximation involves two assumptions: first, as it is almost always the case, the excitation of the chromophore to higher excited states (by direct adsorption at 265 nm or by energy transfer from the protein) results in the quantitative population of the lowest, S_1, excited state, and, second, the quantum yield in S_1 is independent of the particular vibronic levels initially populated. This assumption is justified by the independency of γ on the excitation wavelength within the main 570-nm absorption band, a phenomenon interpreted in terms of the quantitative population of a common minimum along the excited-state potential surface connecting BR'_{570} and its primary photoproduct K_{610} (Rosenfeld et al., 1977; Hurley et al., 1977).

Fluorescence Measurements. The emission of BR'_{570}, excited in the UV band, was studied by using continuous and pulsed excitation to gain further insight into the details of the energy transfer process. Emission spectra (λ_{max} = 320 nm) obtained by continuous irradiation at 265 and 292 nm agreed with that reported by Bogomolni et al. (1978) for 280-nm excitation. There are several points indicating that only light absorbed by tryptophan residues is responsible for the observed emission. First, identical emission spectra are obtained for excitation at 292 nm, where only tryphotopan absorbs, and for 265-nm excitation where there is a considerable tyrosine contribution to the absorption. Second, the relative emission intensities due to 265- and 292-nm excitation in a dilute NATA solution are the same as in a comparable BR'_{570} solution. This not only indicates that the emission is solely due to tryptophan residues but also that the excitation of tyrosines in BR'_{570} does not lead to a tryptophan fluorescence via energy transfer. An additional argument favoring the same conclusions may be made on the basis of the measured emission decay kinetics in BR'_{570} which were the same, within experimental error, for 265- and 292-nm excitation. Thus, the deconvolution analysis yielded in both cases a double-exponential decay with half-lives of τ_1 = 3.5–5.0 ns and τ_2 = 0.5–0.9 ns.

For the determination of the fluorescence quantum yield, ϕ_f, we used the values for the extinction coefficients of the aromatic residues and of the nonemissive retinyl chromophore, estimated as discussed above. In calculating the value for 265-nm excitation, we assumed, as previously concluded, that light absorption by tyrosine residues does not lead to emission. The value obtained, ϕ_f = 0.023 ± 0.002, was essentially the same for both excitation wavelengths, in agreement with the assumption that only tryptophan residues emit.

For N tryptophan residues present in bacteriorhodopsin, we have $\phi_f = (\sum n_i\phi_i)/N$, where n_i is the number of molecules characterized by the same emission lifetime, $\tau_i = \phi_i\tau_0$, τ_0 is the radiative lifetime, and ϕ_i is the emission quantum yield of a tryptophan residue belonging to the ith group. The previously reported lifetime data indicate that the emitting molecules ($\tau_i > 0.1$ ns) can be divided into two major groups with τ_1 = 3.5–5.0 ns and τ_2 = 0.5–0.9 ns, respectively. In applying the above equation, we first substituted N = 7 (Ovchinikov et al., 1979) and ϕ_f = 0.023. For τ_0 we have used the value (τ_0 = 22.3 ns) characteristic of aqueous tryptophan (NATA) solutions (Chen et al., 1969; Grinvald et al., 1975). We are left with the two unknown parameters, n_1 and n_2. Since it can readily be verified that any value of n_1 which is greater than 1 will lead to the violation of the elementary relation $n_1\phi_1 \leq \phi_f N$, we conclude that n_1 = 1. This conclusion is consistent only with n_2 = 1, implying that only one tryptophan is responsible for the long lifetime emission and a second one for the subnanosecond component, while the remaining five residues are undetectable by the present experiments. This conclusion is independent on the specific choice of τ_1 and of τ_2 in the 3.5–5.0-ns and 0.5–0.9-ns ranges, respectively. An identical picture is obtained by using N = 8 (Khorana et al., 1979) except that, in this case, the possibility of n_2 = 2 cannot be absolutely ruled out.

At 265 nm the contribution to the total protein absorbance by the 7 tryptophan and the 11 tyrosine residues can be approximated from the data of Mihalyi (1968) in water as 0.77 and 0.23, respectively. With 11 fully quenched tyrosines, five (Ovchinikov et al., 1979) or six (Khorana et al., 1979) fully quenched tryptophans, one partially quenched (~80%) tryptophan, and one unquenched tryptophan, we obtain 0.86–0.88 as the yield of all quenching pathways of the excited aromatic amino acids in bacteriorhodopsin. This value constitutes an upper limit to ϕ_{ET}. In view of the value ϕ_{ET} = 0.71 ± 0.02, obtained above from the photochemical data, it is thus implied that about 80% of the quenching is due to energy transfer to the chromophore.

Continuous excitation fluorescence experiments were also carried out in dark-adapted BR solutions solubilized by Triton X-100. Solubilization was carried out at either pH 5 or pH 7 (see Experimental Procedures). We used a 1:32 (w/w)

protein to detergent ratio and performed the experiments 50 h after adding the detergent so as to allow almost complete solubilization to take place (Reynolds & Stoeckenius, 1977; Dencher & Heyn, 1978a,b). Since Triton X-100 absorbs a substantial fraction of the exciting light and is also highly fluorescent when illuminated near 280 nm, we have used excitation at 300 nm where the detergent absorption is relatively low. The emission intensity of a BR-solubilized solution at 340 nm was compared to that containing only the detergent and to an aqueous sucrose solution of purple membranes. After correcting for the large detergent background emission (~90%) and evaluating the exact absorbance at 300 nm (correcting for scattering artifacts), we obtained the values 0.8 ± 0.2 and 1.1 ± 0.2 for the ratio between the BR emission intensity in the detergent micelle and in the intact membrane fragments at pH 5 and 7, respectively. Experiments carried out in a NATA solution in the prescence of the same amount of Triton X-100 indicated a small (~25%) increase in the emission in the presence of the detergent, implying that no quenching of tryptophan fluorescence by Triton X-100 takes place. It should be pointed out that we preferred the use of Triton X-100 in spite of its strong fluorescence to that of the nonfluorescent octyl glucoside. Solubilization with the latter has been reported to induce an increase in the BR fluorescence which is not restored upon reconstitution of the membrane (Dencher & Heyn, 1978a,b). It is likely that this effect is due to an irreversible damage to the bacteriorhodopsin (e.g., bleaching).

Structural Implications. It is worthwhile examining the above conclusions in terms of the available information on the structure of bacteriorhodopsin in the purple membrane. On the basis of electron microscopy data, Henderson & Unwin (1975) have shown that each BR molecule is composed of seven α-helical segments spanning the membrane. The molecules are arranged in the form of trimers in a two-dimensional hexagonal lattice (Figure 2). The amino acid sequence in bacteriorhodopsin has recently been obtained by Ovchinikov et al. (1979) and by Khorana et al. (1979) who presented a detailed model describing the composition of each α-helical segment, including the location of the retinyl chromophore. An attempt has recently been made to correlate the α-helical segments in the electron density map with those suggested on the basis of the primary structural analysis (Engelman et al., 1980).

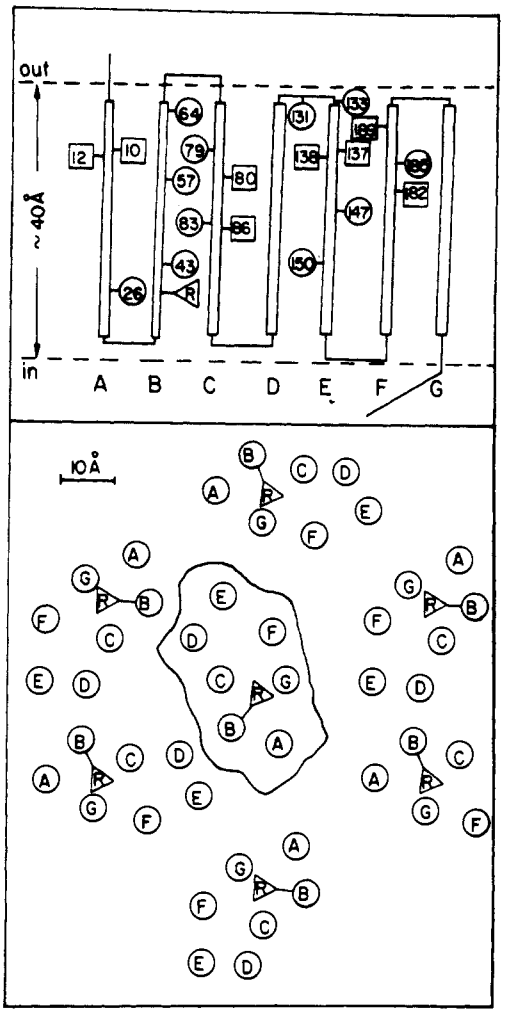

FIGURE 2: (Top) Schematic representation of the seven α-helical segments of bacteriorhodopsin in the purple membrane according to Ovchinikov et al. (1979). The circles and squares represent tyrosine and tryptophan residues, respectively, numbered according to the sequence of Khorana et al. (1979). R represents the retinyl moiety attached to lysine-41. (Bottom) Schematic representation of the electron density map of bacteriorhodopsin according to Henderson & Unwin (1975). The circles represent the helical segments, and the specific fit of the amino acid sequence with the density map is that suggested as most probable by Engelman et al. (1980). The orientation of the retinyl moiety is suggested by the present energy transfer data.

For an analysis of our energy-transfer data in terms of the above structural information, we apply Förster's mechanism (Förster, 1966), which leads to the expression $\phi_{ET}^{i} = R_i^{-6}/(R_i^{-6} + R_0^{-6})$ for the yield of energy transfer from the ith aromatic residue to the chromophore. R_i is the donor–acceptor distance, and R_0, the value of R for which $\phi_{ET}^{i} = 0.5$, is given by R_0 (Å) $= (JK^2\phi_f^0 n^{-4})^{1/6}(9.79 \times 10^3)$. The parameter J (cm^3 M^{-1}), the overlap function between the emission of the donor and the (β band) absorption of the acceptor, is given by $J = \int F(\lambda)\epsilon(\lambda)\lambda^4\, d\lambda / \int F(\lambda)d\lambda$ where $F(\lambda)$ represents the wavelength-dependent fluorescence intensity and $\epsilon(\lambda)$ represents the extinction coefficient of the acceptor. For all residues we assumed a value of $n = 1.4$ for the refractive index (Cheng-Wen & Stryer, 1972) and of $K^2 = {}^2/_3$ for the square of the orientation factor K. The latter assumption, which is strictly valid only when the donor and acceptor rotation rates are fast compared to those of energy transfer, is considered a fair approximation in proteins (Cheng-Wen & Stryer, 1972; Gafni, 1978). In all cases we assume that ϕ_f^0, the fluorescence yield of each tryptophan residue in the absence of the chromophore acceptor, is identical with that of the long-lived emitting tryptophan ($\tau_1 = 3.5$–5.0 ns). This assumption is in keeping with the models of Ovchinikov et al. (1979) and of Khorana et al. (1979) which place all tryptophans in the interior of the membrane (see Figure 2). By using the above approximations, the value of R_0 for transfer from tryptophans to the chromophore is calculated as $R_0 = 25$ Å.

The complete set of R_i for all aromatic residues is determined by the overall protein structure. In other words, the values of R_i may be derived from the exact correlation (one out of 7! = 5040 possibilites) between the α-helical segments in the electron density diagram (see Figure 2) and those described in the molecular model (Ovchinikov et al., 1979; Khorana et al., 1979). Although an examination of such correlations in terms of our energy-transfer data shows that most structures are inconsistent with the experimental results, it is impossible to point out a specific configuration on the basis of the fluorescence data alone. It is evident, however, that any specific model proposed must be compatible with the main conclusions of the present work, namely, (a) the complete quenching of all tyrosine residues, (b) the complete quenching of all tryptophan residues, with the exception of one un-

quenched and one (or two) partially (~80%) quenched, and (c) the identity, within our experimental limits, between the quenching (energy transfer) phenomena in the intact lattice and those observed after treatment with Triton X-100. The latter process involves the formation of a monomeric BR micelle, free of intermolecular interactions (Becher & Ebrey, 1976; Reynolds & Stoeckenius, 1977). It is thus implied that all energy-transfer phenomena from tyrosines and tryptophans are solely accounted for by the chromophore from the same BR molecule.

Figure 2 shows a specific model recently selected as most probable by Engelman et al. (1980). The suggestion was based on criteria of connectively of the nonhelical link regions, charge neutralization, and total scattering density per helix. It can be verified that the model meets the three requirements mentioned above if the chromophore, attached to lysine 41 on helix B, is placed close to helix G as shown in Figure 2. Further structural restrictions are imposed by the fact that the complete quenching of five to six tryptophan residues implies that their respective distances from the chromophore cannot exceed ~20 Å, a value which corresponds to ~90% energy transfer. As shown in Figure 2, the model of Ovchinikov et al. (1979) places seven tryptophan residues in a plane which is ~10 Å from the external surface of the membrane. Since the total membrane width is ~40 Å, it is thus implied that the chromophore plane lies at least 10 Å from the cytoplasmic surface.

Within the limits imposed by the approximation $K^2 = {}^2/_3$, adoption of the model of Engelman et al. (1980) (Figure 2) suggests that the unquenched tryptophan residue lies on helix E (Trp-137 or -138) which is the most distant from the chromophore. The partially (~80%) quenched residue may be the second tryptophan of helix E (with an unsuitable orientation) or one of those on helix F (Trp-189). Total quenching is predicted for at least five tryptophan residues and also for all tyrosine residues, in agreement with the experimental data. Quenching of the tyrosines is accounted for by energy transfer to neighboring tryptophans, a process characterized by $R_0 \approx 17$ Å (Berlman, 1973). Thus, tryptophans will act as efficient mediators for the transfer of tyrosine excitation energy to the chromophore, even from residues which are too distant for direct transfer to the retinyl moiety. An examination of Figure 2 shows that the model does not imply any solubilization effects on the energy transfer phenomena.

References

Amand, B., & Bensasson, R. (1975) *Chem. Phys. Lett. 34*, 44–48.

Barboy, N., & Feitelson, J. (1978) *Biochemistry 17*, 4923–4926.

Becher, B., & Ebrey, T. G. (1976) *Biochem. Biophys. Res. Commun. 69*, 1–6.

Becher, B., Tokunaga, F., & Ebrey, T. G. (1978) *Biochemistry 17*, 2293–2300.

Berlman, I. B. (1973) *Energy Transfer Parameters of Aromatic Compounds*, Academic Press, New York.

Bogomolni, R. A., Stubbs, L., & Lanyi, J. K. (1978) *Biochemistry 17*, 1037–1041.

Chen, R. F., Edelhoch, H., & Steiner, R. F. (1969) in *Physical Principles and Techniques of Protein*, Part A (Leach, S. J., Ed.) pp 171–244, Academic Press, New York, NY.

Cheng-Wen, W., & Stryer, L. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1104–1108.

Dencher, N. A., & Heyn, P. M. (1978a) in *Energetics and Structure of Halophilic Microorganisms* (Caplan, S. R., & Ginzburg, M., Eds.) pp 233–238, Elsevier/North-Holland Biochemical Press.

Dencher, N. A., & Heyn, P. M. (1978b) *FEBS Lett. 96*, 322–326.

Engelman, D. M., Henderson, R., McLachlan, A. D., & Wallace, B. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2023.

Förster, Th. (1966) in *Modern Quantum Chemistry, Istanbul Lectures* (Sinnanoglu, O., Ed.) Section III-B, pp 93–137, Academic Press, New York.

Gafni, A. (1978) *Annu. Rep. Prog. Chem., Sect. A 75*, 5–24.

Goldschmidt, C. R., Ottolenghi, M., & Korenstein, R. (1976) *Biophys. J. 16*, 838–843.

Goldschmidt, C. R., Kalisky, O., Rosenfeld, T., & Ottolenghi, M. (1977) *Biophys. J. 17*, 179–183.

Grinvald, A., Schlessinger, J., Pecht, I., & Steinberg, I. Z. (1975) *Biochemistry 14*, 1921–1928.

Henderson, R., & Unwin, P. N. T. (1975) *Nature (London) 257*, 28–32.

Hurley, J. B., Ebrey, T. G., Honig, B., & Ottolenghi, M. (1977) *Nature (London) 270*, 540–542.

Khorana, H. G., Gerber, G. E., Herlihy, W. C., Gray, C. P., Anderegg, R. J., Nihel, K., & Biemann, K. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5046–5050.

Lasser, N., & Feitelson, J. (1973) *J. Phys. Chem. 77*, 1011–1016.

Mihalyi, E. (1968) *J. Chem. Eng. Data 13*, 179–182.

Oesterhelt, D., & Stoechenius, W. (1974) *Methods Enzymol. 31*, 667–678.

Ottolenghi, M. (1980) *Adv. Photochem. 12*, 97–200.

Ovchinikov, A. Yu., Abdulaev, N. G., Feigina, M. Yu., Kiselev, A. V., & Labanov, N. A. (1979) *FEBS Lett. 100*, 219–224.

Reynolds, J. A., & Stoeckenius, W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2803–2804.

Rosenfeld, T., & Ottolenghi, M. (1977) in *Research in Photobiology* (Castellani, A., Ed.) pp 667–675, Plenum Press, New York.

Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J., & Ebrey, T. G. (1977) *Pure Appl. Chem. 49*, 341–351.

Sherman, W. V., & Caplan, S. R. (1975) *Nature (London) 258*, 766–768.

Stoeckenius, W., Lozier, R. H., & Bogomolni, R. A. (1979) *Biochim. Biophys. Acta 505*, 215–278.