Gilardi, R. D., Karle, I. L., & Karle, J. (1972) *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem. B28*, 2605-2612.
Hargrave, P. A., McDowell, J. H., Feldmann, R. J., Atkinson, P. H., Mohana Rao, J. K., & Argos, P. (1984) *Vision Res. 24*, 1487-1499.
Honig, B., & Karplus, M. (1971) *Nature (London) 229*, 558-560.
Hubbard, R., & Wald, G. (1952-1953) *J. Gen. Physiol. 36*, 269-315.
Kawamura, S., Miyatani, S., Matsumoto, H., Yoshizawa, T., & Liu, R. S. H. (1980) *Biochemistry 19*, 1549-1553.
Knudsen, C. G., Carey, S. C., & Okamura, W. H. (1980) *J. Am. Chem. Soc. 102*, 6355-6356.
Köbrich, G., Breckoff, W. E., & Drischel, W. (1967) *Justus Liebigs Ann. Chem. 704*, 51-69.
Kropf, A. (1982) *Vision Res. 22*, 495-497.
Kropf, A., Whittenberger, B. P., Goff, S. P., & Waggoner, A. S. (1973) *Exp. Eye Res. 17*, 591-606.
Liu, R. S. H. (1982) in *Carotenoid Chemistry and Biochemistry* (Britton, G., & Goodwin, T. W., Eds.) pp 253-264, Pergamon, New York.
Liu, R. S. H., & Asato, A. E. (1982) *Methods Enzymol. 88*, 506-516.
Liu, R. S. H., & Asato, A. E. (1984) *Tetrahedron 40*, 1931-1969.
Liu, R. S. H., & Asato, A. E. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 259-263.
Liu, R. S. H., Zingoni, J. P., Kini, A., Trammell, M., Chu, D., Asato, A. E., & Bopp, T. T. (1983) *J. Org. Chem. 48*, 4817-4821.
Liu, R. S. H., Asato, A. E., Denny, M., & Mead, D. (1984a) *J. Am. Chem. Soc. 106*, 8298-8300.
Liu, R. S. H., Matsumoto, H., Kini, A., Asato, A. E., Denny, M., Kropf, A., & DeGrip, W. J. (1984b) *Tetrahedron 40*, 473-482.
Liu, R. S. H., Crescitelli, F., Denny, M., Matsumoto, H., & Asato, A. E. (1986) *Biochemistry* (following paper in this issue).
Matsumoto, H., & Yoshizawa, T. (1978) *Vision Res. 18*, 607-609.
Matsumoto, H., Horiuchi, K., & Yoshizawa, T. (1978) *Biochim. Biophys. Acta 501*, 257-268.
Matsumoto, H., Asato, A. E., Denny, M., Baretz, B., Yen, Y.-P., Tong, D., & Liu, R. S. H. (1980a) *Biochemistry 19*, 4589-4594.
Matsumoto, H., Liu, R. S. H., Simmons, C., & Seff, K. (1980b) *J. Am. Chem. Soc. 102*, 4259-4262.
Oroshnik, W., Wald, G., Brown, P. K., & Hubbard, R. (1955) *Proc. Natl. Acad. Sci. U.S.A. 42*, 578-580.
Tanis, S. P., Brown, R. H., & Nakanishi, K. (1978) *Tetrahedron Lett.*, 869-872.
Wald, G., Brown, P. K., Hubbard, R., & Oroshnik, W. (1955) *Proc. Natl. Acad. Sci. U.S.A. 41*, 438-451.

Photosensitivity of 10-Substituted Visual Pigment Analogues: Detection of a Specific Secondary Opsin-Retinal Interaction†

Robert S. H. Liu,*,‡ Frederick Crescitelli,*,§ Marlene Denny,‡ Hiroyuki Matsumoto,‡ and Alfred E. Asato‡
Chemistry Department, University of Hawaii, Honolulu, Hawaii 96822, and Biology Department, University of California, Los Angeles, California 90024

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ABSTRACT: The photosensitivities of the bovine rhodopsin and gecko pigment 521 analogues regenerated from C-10-substituted analogues of 11-*cis*- and 9-*cis*-retinals were determined by two different methods. A similar reactivity trend was noted for both pigment systems as revealed in the photosensitivity of the gecko pigments and relative quantum yields of the bovine analogues. The 10-fluoro-11-cis photopigments had a photosensitivity less than, but approaching, that of the native (11-cis) visual pigment while the 10-fluoro-9-cis photopigments had a much lower photosensitivity than the parent 9-cis regenerated pigment. The results are interpreted in terms of recently described models of rhodopsin architecture and of the primary molecular reaction of visual pigments to light. The unusually low photoreactivity of the 10-fluoro-9-cis pigment molecule is viewed as the result of a regiospecific hydrogen-bonding interaction of the electronegative fluorine atom to the opsin.

In the preceding paper (Asato et al., 1986) we described the preparation and spectroscopic properties of a series of 10-substituted bovine rhodopsin analogues. Because of the observation, in a preliminary experiment, of the unusually inefficient photobleaching process of 10-fluoro-9-*cis*-rhodopsin, we decided to carry out a more quantitative study of such processes in this and related analogues. Furthermore, in order to test for the generality of such an observation, a parallel study with the gecko pigment analogues has been carried out. The results will be analyzed in light of the current knowledge of the tertiary structure of rhodopsin (Hargrave et al., 1984) and the postulated models for the primary process of vision.

Experimental Procedures

The photosensitivity of the regenerated photopigments was measured by two different procedures. At UCLA, the chromophore sensitivity of the gecko pigments regenerated with the opsin of pigment 521 was determined by the method of

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‡ University of Hawaii.
§ University of California.

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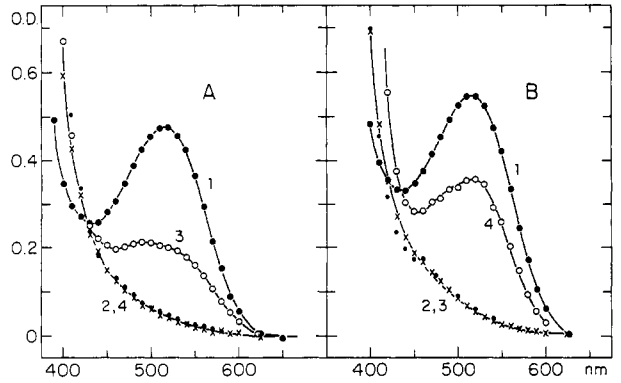

FIGURE 1: Gecko pigment. Responses to 11-cis analogues: 10-F (A) and 10-Cl (B). (Curves 1) Initial spectra; (curves 2) after photic bleach; (curve 3A) after 10-F analogue; (curve 3B) (filled circles) after 10-Cl; (curve 4A) after photic bleach; (curve 4B) (control) after 11-*cis*-retinal (60% regeneration). At 15 °C. NH_2OH added before analogues.

photometric curves (Dartnall, 1977). In Hawaii, the relative quantum yields of photobleaching of the bovine pigment analogues were measured, with rhodopsin as a standard.

Gecko System. The analogue photopigments were prepared basically as described in a previous paper (Crescitelli & Liu, 1985). Briefly, the excised gecko retinas were stored at low temperature (−20 °C) immersed in 2.0 M NaCl solution since chloride is known to stabilize the labile pigment 521 against thermal loss (Crescitelli, 1977). When needed, retinas were thawed out and washed in standard phosphate buffer (pH 7.3) and the visual pigment extracted repeatedly with 0.3 mL of 2% digitonin (Merck) made up in the buffer until most of the pigment was obtained.

Aliquots (0.12 mL) of each extract were placed in masked microcuvettes (2-mm width, 10-mm path length) following which the pigment system was analyzed. The gecko pigment was bleached in place by means of a laser beam (632.8 nm). A 30-min exposure sufficed to remove all the pigment 521, leaving the small moiety (8% of the total pigment density) of P-467 that is normally present in these extracts (Crescitelli, 1977). An ethanol solution of the analogue was then added in molar excess. This addition was made under two conditions: NH_2OH (4×10^{-3} to 5×10^{-3} M) added either before (constituting most of the experiments) or after the analogue. No difference was found in the two types of experiments. It was convenient to add the NH_2OH first because it removes the possibility of spontaneous pigment regeneration after a bleach and prevents random Schiff-base formation. Upon completion of pigment formation (≥3 h), the regenerated pigment was then bleached, and the amount of regeneration as a percentage of total original P-521 density was determined by difference spectra. In these calculations, no account was taken of possible changes in molar extinction coefficients of the regenerated pigments since we have no knowledge of such changes. Figures 1 and 2 show the spectral data obtained in such experiments. Table I summarizes results of binding studies.

The specific procedures for determining the photosensitivity and the equipment employed were identical with those employed in three prior studies (Crescitelli & Karvaly, 1983; Crescitelli & Liu, 1985; Crescitelli, 1985). Briefly, aliquots of the regenerated pigments in a cuvette (2-mm width, 10-mm path length) were bleached at successive intervals (1 min) by exposure to the calibrated spectrophotometer light at 520 nm until all photopigment was removed. To monitor the result, the spectral absorbance curve from 380 to 700 nm was determined before and after the bleach. To eliminate the possibility of interference by products of bleaching, NH_2OH was

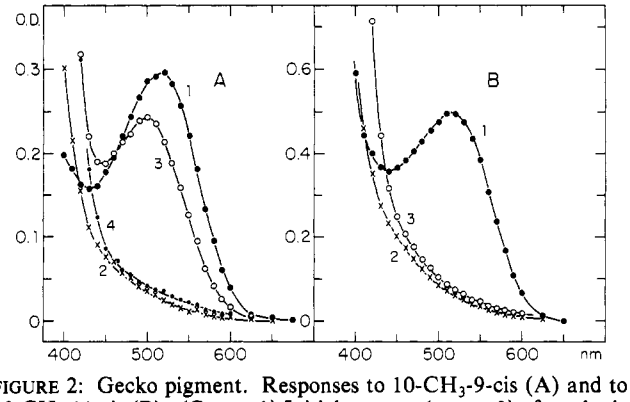

FIGURE 2: Gecko pigment. Responses to 10-CH_3-9-cis (A) and to 10-CH_3-11-cis (B). (Curves 1) Initial spectra; (curves 2) after photic bleach; (curve 3A) after 10-CH_3-9-cis; (curve 3B) after 10-CH_3-11-cis; (curve 4A) after photic bleach. Temperature and NH_2OH as in Figure 1.

Table I: Spectral Maxima (nm) and Yields (%) of Gecko Pigment Analogues Regenerated with 10-Substituted Retinals

pigment	isomer	λ_{max} (nm)[a]	yield (%)[b]
native	11-cis	521	65 ± 18 (20)
	9-cis	488	76 ± 10 (10)
	7-cis		0[c]
	13-cis		0[c]
10-F	11-cis	523	28 ± 10 (13)
	9-cis	487	37 ± 15 (14)
	7-cis		0
	13-cis		0
10-Cl	11-cis		0 (5)
	9-cis	487	28 ± 5 (7)
	7-cis	nt	nt
	13-cis	nt	nt
10-methyl	11-cis		0 (13)
	9-cis	497	51 ± 19 (10)
	7-cis	nt	nt
	13-cis	nt	nt
10-ethyl	11-cis		0 (8)
	9-cis		0 (7)
	7-cis	nt	nt
	13-cis		0

[a] Blank indicates no regeneration; nt, not tested. [b] Standard deviation based on the number of runs shown in parentheses. [c] Crescitelli & Liu (1985).

routinely added to the aliquots. The photosensitivity was computed by plotting the function log $[I_t/(I_f - I_t)]$ against the successive integrated exposure times, yielding a straight line whose slope (S) was the measured rate of the light reaction. Here, I_t is the light transmission after each exposure (1 min), and I_f is the transmission after complete bleaching. The slope (S) was used to calculate the chromophore sensitivity ($\alpha\gamma$), where α is the absorption cross-section (cm^2) for a single chromophore and γ is the quantum efficiency of photic bleaching. The photosensitivity was calculated by use of (Dartnall et al., 1936; Dartnall, 1958)

$$\alpha\gamma = 2.303S/(\varphi I)$$

Here, I is the light intensity (photons cm^{-2} s^{-1}) and φ is a slope-correcting factor that corrects for the reduction in rate by light-stable pigment impurities and by products of bleaching that absorb at the measuring (520 nm) wavelength. The value of φ was close to 1 since the effects of both impurities and products are minimal. The incident light intensity (I) was measured with a calibrated bolometer (checked against photosensitivity of frog rhodopsin; Dartnall et al., 1936), its output read off by use of a Yellow Springs Radiometer (Model 65). The light source for measuring and bleaching was a 6-V tungsten lamp powered by a current-stabilized supply (Gilford, Model 205).

Table II: Chromophore Photosensitivities of Gecko Pigment Analogues

pigment analogue	photosensitivity, $\alpha\gamma$ ($\times 10^{-17}$ cm^2)[a]	means	relative %
native	12.31		
	8.61		
	9.33		
11-cis regenerated	8.48	10.26	
	11.70		
	8.56		
	10.70		
	12.42		100[b]
9-cis regenerated	5.41	3.59	
	3.49		
	2.64		
	2.80		35
10-F-11-cis regenerated	5.17	4.65	
	4.34		
	3.46		
	7.67		
	3.55		
	3.71		45
10-F-9-cis regenerated	0.348	0.243	
	0.274		
	0.318		
	0.153		
	0.065		
	0.296		2.4
10-Cl-9-cis regenerated	0.402	0.426	
	0.450		4.2
10-CH_3-9-cis regenerated	0.96	0.80	
	0.64		7.8

[a] Defined as a product of extinction coefficient (α) and quantum efficiency (γ) $\times 10^{-17}$ cm. The photosensitivity is the value at maximum absorption of the respective pigments. [b] Standard, based on average values for both the native pigment and the 11-cis regenerated pigment.

Bovine System. The pigment analogues were prepared as described in a preceding paper (Asato et al., 1986). The preparation of 14-fluororhodopsin was described in a separate paper (Asato et al., 1978), while 8-fluororhodopsin was formed with 8-fluororetinal. The latter was synthesized in a manner similar to that reported for 12-fluororetinal (Liu et al., 1981; Pawson et al., 1979).

Quantum yields of photobleaching of pigment analogues were measured relative to that of rhodopsin. The following simplified procedure was followed. The collimated beam from a Schoeffel 100-W Hg–Xe arc lamp was passed through a Heathkit monochromator with the slit width set at 1.68 mm. The window of a small low-temperature merry-go-round apparatus (Applied Photophysics) was then placed in the path of the broad-band monochromatic beam. Sample temperature was maintained at 20-22 °C. The irradiating wavelength was chosen to center at 480 nm, an averaged value for absorption maxima of all pigment analogues. A cuvette of 1-cm path length was used. Sample absorbance was adjusted close to 0.2–0.3 in all cases. The photobleaching process conducted after the addition of an excess of NH_2OH was followed by determination of the decrease of absorbance maxima as a function of time. The initial slope in a plot of absorbance vs. irradiation time was used for calculation of relative quantum yields. A bovine rhodopsin solution of equal absorbance was used as the reference in each separate quantum yield determination.

For calculation of quantum yields, the extinction coefficients of the 11-cis isomers of the 10-substituted pigment analogues were assumed to be 75% of those of rhodopsin (Asato et al., 1986) while those of the more planar 9-cis isomers to be the same as that of 9-*cis*-rhodopsin. Absorbance measurements were conducted on a Perkin-Elmer λ-5 spectrometer.

Table III: Quantum Yields of Photobleaching and Relative Photosensitivities of Bovine Rhodopsin Analogues

rhodopsin analogue	quantum yield (%)[a] 11-cis	quantum yield (%)[a] 9-cis	relative photosensitivity (%)[b] 11-cis	relative photosensitivity (%)[b] 9-cis
rhodopsin	0.67[c]	0.2;[d] 0.24	100	24
10-F-Rdp	0.65	0.09	73	13
10-Cl-Rdp	0.33	0.07	37	10
10-CH_3-Rdp	0.32	0.08	48	12
8-F-Rdp	0.6	0.22	90	33
14-F-Rdp	0.51	0.40	76	60

[a] For the quantum yield values determined in this work, we estimate an error limit of ±20%. This is based on observed error spread for results obtained from irradiation of 14 samples of rhodopsin conducted under identical experimental conditions (see text). [b] From 100 × (quantum yield/0.67) × (ratio of extinction coefficients of analogues vs. rhodopsin), the latter being 0.75 for 10-substituted 11-cis isomers and 1.00 for all others (see text). [c] Dartnall (1972); Hurley et al. (1977). This value was used as standard for quantum yield calculations. [d] From Hubbard & Kropf (1958). They reported a value of 0.3 relative to that of rhodopsin. Our measurements led to a value of 0.24 ± 0.05 for 9-*cis*-rhodopsin.

Results and Discussion

Results for relative photosensitivities of 10-substituted gecko and bovine visual pigment analogues are listed in Tables II and III. For comparison, the corresponding data for the parent pigments and 9-cis pigments are also listed. Table III also includes data for bleaching of the related 8-fluororhodopsin and 14-fluororhodopsin (Asato et al., 1978). The data in the two tables reveal several interesting trends. Before commenting on these trends, it should be mentioned that the data in both tables should be viewed as qualitatively suggestive of their photoreactivities because they suffer from lack of knowledge of accurate extinction coefficients for the pigment analogues. For the gecko data (Table II), the use of photosensitivity units, which are directly proportional to the product of the extinction coefficient (ϵ) and the quantum efficiency (γ) of the pigment analogue, eliminated the need to determine the extinction coefficient of each analogue. For the bovine system, the data for quantum yields of photobleaching were determined relative to that of rhodopsin. For comparison with the gecko data, they were further converted to relative photosensitivities. In spite of the different approaches, there are noticeable trends of the pigment response toward light.

The quantum efficiencies of photobleaching of the C-10 analogue photopigments with the 11-cis geometry approach those of rhodopsin and the gecko pigment 521 (Tables II and III). For the 9-cis analogues, a different trend is revealed by these data. Particularly noticeable is the low photosensitivity of the 10-fluoro gecko pigment, which was 6.8% of the parent 9-cis regenerated pigment and 2.4% of the native 11-cis pigment. A low reactivity of the corresponding bovine fluoro analogue was also noted although the result was less dramatic, being less than $^1/_2$[1] that of the parent 9-cis compound. The 9-cis isomer with Cl and methyl substituents gave the same relationship with the bovine and gecko systems, i.e., lowered reactivities, with the gecko pigments somewhat lower than the comparable bovine analogues.

It is instructive to speculate on the possible causes for these variations in photosensitivities, particularly in light of recent knowledge of the structure of rhodopsin and the nature of the primary process. In Hargrave's three-dimensional model of

[1] This could be a higher limit because judging from the low extinction (about half of that of all-trans) of the product oxime from photobleaching of the pigment, one suspects a more facile and different photochemical process might have taken place.

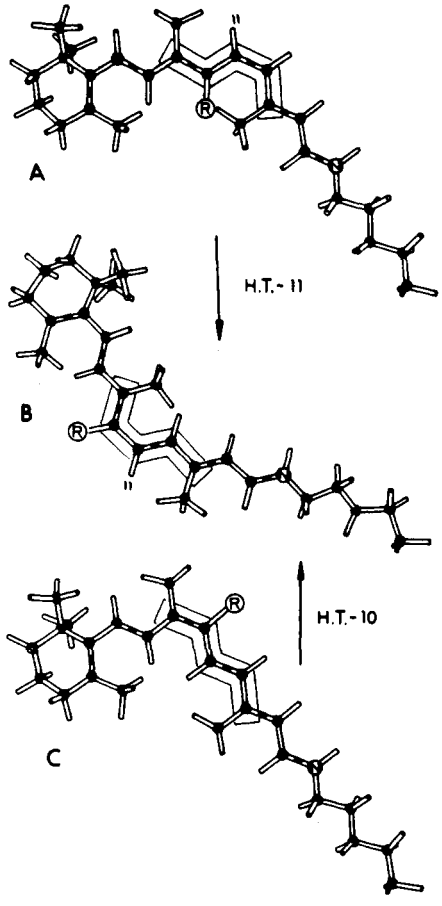

FIGURE 3: The H.T.-11 and H.T.-10 processes based on Liu and Asato (1985). (A) "Anchored" 11-cis chromophore of 10-substituted (®) rhodopsin analogue. (B) Proposed 10-s-cis,all-trans chromophore for bathorhodopsin. The H.T.-11 process of which H-11 transposes from one side of the polyene chromophore to the other while the 10-substituent remains on the same side, transforms A to B. The H.T.-10 process of which the 10-substituent transposes from one side of the polyene chromophore to the other, transforms C to B. (C) The 10-substituted 9-cis pigment analogue.

bovine rhodopsin [Dratz & Hargrave, 1983; Hargrave et al., 1984; see also Liu and Browne (1986)], the chromophore is interpreted to be sandwiched between two layers of protein helices with its long axis perpendicular to the axes of the helices. Substituents at different positions of the chromophore could therefore be in juxtaposition to side chains from different helices. The exact shape of the binding site cavity must be determined by and subject to variation of the orientation of the side chains on the amino acid residues nearby the binding site. This model was recently incorporated into a two-dimensional binding site map of rhodopsin (Liu et al., 1984). The map has been discussed in detail in the preceding paper (Asato et al., 1986). In the recently proposed specific H.T.-*n* model for the primary process, the photobleaching of rhodopsin (11-cis) and of the 9-*cis*-rhodopsin and their interconversion at liquid nitrogen temperature by way of bathorhodopsin are believed to involve a 180° rotational motion of a single atom at C-11 or C-10 as shown in Figure 3 (Liu & Asato, 1985).

The present data are compatible with these views. That the 10-substituent in 11-cis analogues has relatively little effect on the photochemistry of the 10-F pigment is consistent with the view that in these isomers the C-10 position, where structural perturbation has been introduced, does not directly coincide with the reaction center in the H.T.-11 process (Figure 3). But for isomerization of the 9-cis isomers the substituent at the 10-position is at the reaction center of the H.T.-10 process. Hence, the quantum yield data are expected to be sensitive to the nature of the 10-substituent. Most dramatic is the decrease in quantum yield of reaction upon replacement of 10-H by 10-F in either the bovine or the gecko analogue. Since fluorine is relatively a small atom with its van der Waal radius only ~20% larger than that of a hydrogen atom, it appears that a reason other than steric inhibition must be responsible for the low photoreactivity.

The high electron density around the highly electronegative fluorine atom is likely to induce electrostatic interaction with a nearby protein moiety. Early searches, however, failed to detect a substantial interaction of this nature [see, e.g., Gerig (1978)]. But ample recent literature evidence suggests that organic fluorides are capable of undergoing weak intermolecular or intramolecular hydrogen bonding with organic acids. For example, the ΔH for hydrogen-bonding interaction between fluorohexane and phenol was determined to be 2.5 kcal/mol (Joesten & Schaad, 1974). And the doublet for the OH bond in the infrared spectrum of *o*-fluorophenol was attributed to the presence of both the free and intramolecularly hydrogen-bonded hydroxyl groups (Carlson et al., 1972). Such interactions are also suggested by recent X-ray crystal structural data (Murray-Rust et al., 1983; Karipides & Miller, 1984) and results of a more extensive search (71 cases) of the Cambridge Crystallographic Data File (Murray-Rust et al., 1983). Such a weak interaction should be sufficient to affect the course of deactivation of a short-lived singly excited molecule through torsional relaxation (Liu et al., 1983). Therefore, we wish to suggest that the low photoreactivity of such analogues could be due to the presence of protein–substrate H–F hydrogen bonding. Furthermore, this interaction appears to be highly regiospecific, observable only upon substitution at C-10 because the data in Table III and results reported earlier (Liu et al., 1981) indicate the absence of such an interaction when the fluorine atom is located at C-8, -12, or -14 of the chromophore (Liu, 1982).

Examination of Hargrave's tertiary molecular model indeed suggests the possibility of such a protein–substrate hydrogen-bonding interaction. Only three carboxyl-bearing amino acid residues are believed to be present in the seven helices comprising the binding site. Asp-83 in helix 4 is generally considered to be the counterion for the iminium nitrogen (Dratz & Hargrave, 1983). This leaves two other carboxyl-bearing residues in helix 3: Glu-122 and Glu-134. Hargrave's model further suggests possible proximal alignment of the side chains in helix 3 to the 9,10 double bond. Therefore, we suggest that the low photosensitivity of the 10-fluoro pigment analogues is due to interaction of the fluorine atom to one of the two glutamic acids or its partner in the ion pair (such as Arg-135).[2] In Figure 4, one possible mode of hydrogen-bonding interaction and the orientation of the substrate is depicted.

In agreement with the current interpretation of the presence of a specific protein–substrate interaction to account for the low photosensitivity of the fluorinated pigment analogue is the reported photoreactivity of the corresponding fluorinated retinals (Liu et al., 1979). 10-Fluororetinal was found to possess similar photochemical properties in nonpolar solvents as 14-fluororetinal and retinal. Therefore, the effect reported in this paper is clearly not an intrinsic property of the fluorine substituent.

[2] In a recent FT-IR study, participation of hydrogen bonding among protein side chains including the buried glutamic acids was considered (deGrip et al., 1985). It will be of interest to examine the effect of the 10-F substituent on the vibrational frequencies of the carboxyl groups.

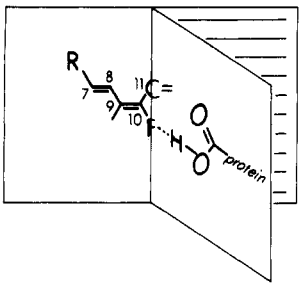

FIGURE 4: A possible mode of hydrogen bonding based on Hargrave's model of rhodopsin. The proton for hydrogen bonding could come from either Glu-122 or Glu-134 or the associated ion pair (see text). A carboxyl group extending from an α-helix surrounding the binding site should orient perpendicularly to the chromophore.

While these interpretations are obviously speculative in nature, they nevertheless suggest the exciting possibility of a new form of specific protein–substrate interaction made possible by the use of a specifically modified retinal. This interaction is potentially useful for elucidating three-dimensional structural information of the binding site. It is our hope that the observations reported here will encourage detailed spectroscopic studies leading to detection of such interactions.

The results of other substituted analogues appear to be somewhat less conclusive. The sterically bulkier 10-Cl or 10-CH_3 substituent indeed reduces the quantum efficiency of isomerization of the 9-cis bovine pigment to less than one-third of the original value. This is consistent with the notion of steric inhibition of the H.T.-10 process. However, one should note that there is a corresponding decrease (by one-half) of the 11-cis pigment where the 10-substituent is not directly located at the reaction center. The cause for this smaller drop of quantum efficiency of isomerization is not immediately obvious no matter whether the isomerization takes place in the form of the H.T.-*n* or the conventional one-bond rotational process. It is possible that the changed conformation of the 11-cis chromophore upon introduction of a bulkier substituent at the C-10 position (Asato et al., 1986) has a detrimental effect on the quantum yield of isomerization.

REFERENCES

Asato, A. E., Matsumoto, H., Denny, M., & Liu, R. S. H. (1978) *J. Am. Chem. Soc. 100*, 5957–5960.

Asato, A. E., Denny, M., Matsumoto, H., Mirzadegan, T., Ripka, W., Crescitelli, F., & Liu, R. S. H. (1986) *Biochemistry* (preceding paper in this issue).

Carlson, G. L., Fateley, W. G., Manocha, A. J., & Bentley, F. F. (1972) *J. Phys. Chem. 76*, 1553–1557.

Crescitelli, F. (1977) *Handbook Sensory Physiology* (Crescitelli, F., Ed.) Vol. 7, pp 391–449, Springer-Verlag, Heidelberg.

Crescitelli, F. (1985) *Exp. Eye Res. 40*, 521–535.

Crescitelli, F., & Karvaly, B. (1983) *Proc. R. Soc. London, Ser. B 220*, 69–87.

Crescitelli, F., & Liu, R. S. H. (1985) *Photochem. Photobiol. 41*, 309–316.

Dartnall, H. J. A. (1958) in *Visual Problems of Colour*, Vol. 1, pp 123–149, Her Majesty's Stationary Office, London.

Dartnall, H. J. A. (1972) *Handbook for Sensory Physiology* (Dartnall, H. J. A., Ed.) Vol. 7, Part 1, pp 122–145, Springer, Berlin.

Dartnall, H. J. A., Goodeve, C. F., & Lythgoe, R. J. (1936) *Proc. R. Soc. London, Ser. A 156*, 158–170.

deGrip, W. J., Gillespie, J., & Rothschild, K. J. (1985) *Biochim. Biophys. Acta 809*, 97–106.

Dratz, E. A., & Hargrave, P. A. (1983) *Trends Biochem. Sci. (Pers. Ed.) 8*, 128–131.

Gerig, J. T. (1978) *Biol. Magn. Reson. 1*, 139–203.

Hargrave, P. A., McDowell, J. H., Feldmann, R. J., Atkinson, P. H., Mohana Rao, J. K., & Argos, P. (1984) *Vision Res. 24*, 1487–1499.

Hubbard, R., & Kropf, A. (1958) *Proc. Natl. Acad. Sci. U.S.A. 44*, 130–139.

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

Joesten, M. O., & Schaad, L. J. (1974) *Hydrogen Bonding*, p 325, Marcel Dekker, New York.

Karipides, A., & Miller, C. (1984) *J. Am. Chem. Soc. 106*, 1494–1495.

Liu, R. S. H. (1982) in *Carotenoid Chemistry and Biochemistry* (Britton, G., & Goodwin, T. W., Eds.) pp 253–264, Pergamon, New York.

Liu, R. S. H., & Asato, A. E. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 259–263.

Liu, R. S. H., & Browne, D. (1986) *Acc. Chem. Res. 19*, 42–48.

Liu, R. S. H., Denny, M., Asato, A. E., & Grodowski, M. (1979) *Nouv. J. Chim. 3*, 503–505.

Liu, R. S. H., Matsumoto, H., Asato, A. E., Denny, M., Shichida, Y., Yoshizawa, T., & Dahlquist, F. (1981) *J. Am. Chem. Soc. 103*, 7195–7201.

Liu, R. S. H., Asato, A. E., & Denny, M. (1983) *J. Am. Chem. Soc. 105*, 4829–4830.

Liu, R. S. H., Asato, A. E., Denny, M., & Mead, D. (1984) *J. Am. Chem. Soc. 106*, 8298–8300.

Murray-Rust, P., Stallings, W. C., Monti, C. T., Preston, R. K., & Glusker, J. P. (1983) *J. Am. Chem. Soc. 105*, 3206–3214.

Pawson, B. A., Chan, K.-K., DeNoble, J., Han, R.-J., Piermattie, V., Speciau, A. C., Srisethnil, S., Trown, P. W., Bohoslawec, O., Machlin, L. J., & Gabriel, E. (1979) *J. Med. Chem. 22*, 1059–1067.