Reactions of Retinals in a Model Membrane System

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Monomolecular films of 9-*cis*, 11-*cis*, 13-*cis* and *all-trans* retinal were formed at an air-water interface. Area/molecule and surface potential were measured before, during and after illumination. The initial quantum yield of the photoisomerization of 9-*cis* retinal was 0.25. Irradiation of a retinal monolayer resulted in 30 to 60 mVolt changes in surface potential. Complexation of retinals with lysine and cysteine were studied.

Introduction

This study of monomolecular films of retinals is concerned with two aspects. First to increase our knowledge about the type and nature of complex formation between films of retinals and lysine, or cysteine as well as mixed films of retinals and a phospholipid. Second to determine the photoproperties of films of retinals and retinal complexes.

Several possible types of linkages have been suggested for the binding of retinal to opsin: a sulfur linkage (Wald[1]), a charge transfer (Galindo[2]), a thiazolidine (Peskin and Love[3]), or a Schiff base (Morton and Pitt[4]). There have been many studies of the possible role of Schiff base and protonated Schiff base linkages between retinal and amino acids. In rhodopsin, most likely, retinal appears to be linked to a lysyl residue on the opsin molecule (Kimbel *et al.*[6]) and less likely to a phospholipid (Poincelot and Abrahamson[5], Fager *et al.*[16]).

Surface isotherms were previously measured for 9-*cis*, *all-trans* and 13-*cis* at pH 6.0 (Brockman and Brody[7]). Complexation was observed between 9-*cis* and β-mercaptoethylamine over a wide concentration range. It was also reported that 9-*cis* does not complex with lysine (10^{-3}M). Irradiation of 9-*cis* decreased the area/molecule 7 Å^2 and the surface potential about 50 mV. Irradiation of 13-*cis* with L-cysteine (10^{-4}M) in the subphase resulted in a small decrease of the area/molecule.

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Materials and Methods

The equipment and techniques are essentially the the same as those described previously (Aghion *et al.*[8]; Brockman and Brody[7]).

The source of *all-trans* retinal, 9-*cis* retinal, 13-*cis* retinal, lysine, β-mercaptoethylamine (β MEA), L-cysteine and phosphate buffers was Sigma Chemical Co. (St. Louis, Mo.). 11-*cis* retinal was the gracious gift of Hoffman-La Roche, Inc. (Nutley, N. J.); *n*-hexane was reagent grade from Fischer Scientific Co. (Fair Lawn, N. J.); nitrogen was prepurified (99.995%) from Matheson Gas (E. Rutherford, N. J.); phosphatidylethanolamine (PEA) was research grade from Applied Sci. Lab. (State college, Pa.).

In the initial phase of this work, surface potential of the film, ΔV, was measured using two radioactive Ni 63 electrodes and a Ag-AgCl reference electrode. One radioactive electrode measured the potential of the clean water surface, V_{H_2O}, the other measured the film on the aqueous surface, V, so that the potential of the film, ΔV, was $\Delta V = V - V_{H_2O}$. However, this method did not prove entirely satisfactory as the characteristics of the two radioactive electrodes were not the same. Using one electrode and a least squares analysis it was possible to calculate V_{H_2O} and the perpendicular component of the dipole moment of retinal, $\mu_\perp$. It is well known that $\Delta V = 12\pi\mu_\perp / A$ where ΔV is in millivolts (mV, $\mu_\perp$ in milliDebyes (mD) and A is in Å^2/molecule (Gaines[9]). The experimentally measured values of V and A were used to get the best fit with the expression $V = 12\pi\mu_\perp / A + V_{H_2O}$. Using the calculated value of V_{H_2O}, taken to represent the potential of water below the film, ΔV at any π could be determined, π being the surface tension in dyn/cm. The precision for measuring ΔV is $\pm$ 10 mV, for $\mu_\perp$ $\pm$ 35 mD and for A $\pm$ 1 Å^2. For comparison purposes between experiments the accuracy of ΔV is $\pm$ 25 mV.

All experiments were conducted at 15 °C and in a nitrogen or air environment. Experiments with *all-trans* and 13-*cis* gave essentially the same results in air and nitrogen. The aqueous subphase contained phosphate buffer pH 6.0 and sodium chloride to give

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an ionic strength of 0.1. Other additives to the subphase are given in the results section.

The solvent used to spread the retinals on the aqueous surface was usually *n*-hexane and in a few cases benzene. The absorption coefficient, ε, of 11-*cis* retinal in hexane is published (BROWN and WALD[10]). To estimate the molar extinction coefficient for 9-*cis*, 13-*cis* and *all-trans* retinal in hexane or benzene these solvents (relative to petroleum ether) the following procedure was adapted. The OD of the absorption maximum at about 360 nm was measured for each retinal in a measured volume of petroleum ether. The petroleum ether was evaporated by slowly bubbling dry nitrogen through the cuvette. When the cuvette was dry a measured volume of benzene or *n*-hexane was added to the cuvette to dissolve the retinal and the OD of the maximum remeasured. The ratio of the latter OD to the OD in petroleum ether was multiplied by the absorption coefficient in petroleum ether, previously determined by HUBBARD *et al.*[11]. The resulting absorption coefficients are given in Table I (see also KROPF and HUBBARD[12]).

Table I. Absorption coefficients of retinals (x 10^{-4} l/mole cm).

	n-hexane	benzene	petroleum ether**
all-trans	4.29	3.97	4.88
9-*cis*	3.36	3.57	3,86
11-*cis*	2.63*	—	—
13-*cis*	3.32	2.80	3.66

* BROWN and WALD[10].
** HUBBARD *et al.*[11].

Irradiation experiments were carried out with blue light (365, 405, and 436 nm) isolated from a 100 W low pressure Hg arc lamp with a blue filter (Corning 7-59, Glass Works N. Y.). The intensity at the film surface is $1.13 \cdot 10^{-10}$ Einstein/cm² sec. In some cases irradiation was carried out using white light from a 500 W slide projector or two 40 W cool white fluorescence lamps.

Results

A. All-trans retinal

The surface properties (A_{10} or ΔV) of a film of *all-trans* retinal are not modified by the presence of L-cysteine in the subphase. The surface potential of *all-trans* retinal in the dark at $\pi = 10$ dyn/cm, ΔV_{10}, is 480 mV; the dipole moment, $\mu_\perp$, is 660 mD; the area/molecule at A_{10}, is 51 Å². See Fig. 1 for surface properties as a function of cysteine concentration in the subphase.

After irradiation the surface potential decreases about 50 mV to 430 mV; $\mu_\perp$ decreases to about 560 mD; A increases about one Å², see Fig. 1.

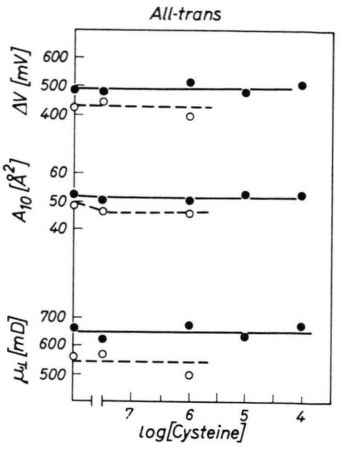


Fig. 1. Surface properties (ΔV_{10}, $\mu_\perp$ and A_{10}) of *all-trans* retinal as a function of the log of the concentration of L-cysteine in the subphase. Before irradiation the data is indicated by solid circles. After irradiation the data is indicated by open circles: Subphase contained phosphate buffer pH 6.0 at a temperature of 15 °C.

Addition of 10^{-6} or 10^{-4}M lysine to the subphase does not modify A_{10} or ΔV. The value of $\mu_\perp$ with 10^{-6}M lysine in the subphase might be slightly lower. After irradiation A_{10}, ΔV_{10}, and $\mu_\perp$ decrease; for the latter two, to about the same values as in the absence of lysine.

Addition of 10^{-4}M lysine to the subphase results in a one Å² decrease of A_{10} and no significant change in ΔV_{10}. Irradiation results in a decrease of A_{10} to 47 Å² and ΔV_{10} to 420 mV. The latter value is just about the same as that obtained with *all-trans* alone.

In summary, *all-trans* retinal does not appear to form a complex with either lysine or L-cysteine at an air-water interface.

The kinetics of the photoreaction in the retinal monolayer are followed by measuring the potential of the surface, V, at constant area. Initially the films are compressed isothermally in the dark to some value of π (*e. g.* $\pi = 14$ dyn/cm). Before irradiation, V is measured in the dark for about 10 min in order to determine whether or not there is any drift in V. In the experiments described in this work, drifts of V in the dark were found to arise from instrumental sources and not from the film. The film was then irradiated until there was no further change in V. At this point the reaction is presumed to be completed or to have reached equilibrium. Such measurements permit an estimate of the quantum yield, Φ, of the initial photoreaction on the surface.

A typical set of data for the light induced decrease of V for *all-trans* retinal is shown in Fig. 2. The

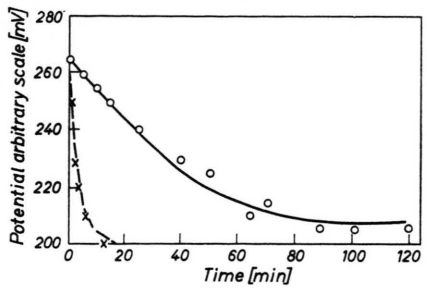


Fig. 2. Potential of a film of retinal at an air-water interface as a function of time of irradiation. Irradiation was carried out at constant film area and an initial surface tension of $\pi = 14$ dyn/cm. Intensity of irradiation $1.13 \cdot 10^{-10}$ Einstein/cm² sec. Subphase contained phosphate buffer pH 6.0 at a temperature of 15 °C. The data for *all-trans* is shown by open circles and for 9-*cis* (plus 10^{-4}M cysteine in the subphase) by crosses. The initial quantum yield for the photoreaction is 0.09 for *all-trans* and 0.26 for 9-*cis*.

presence of cysteine has no effect on the time course of the reaction. A semi-log plot of the data shows that the complete reaction is not first order; this is to be expected as, following the initial photoisomerization, further isomerizations and possibly oxidations are possible.

The quantum yield for the initial photoreaction may be calculated using the following expression:

$$\Phi = \frac{N}{\#h\nu} = \frac{V_o - V_t}{V_o - V_f} \cdot \frac{1}{EI_o t\,(1-r)}$$

where N is number of molecules of photoproduct, $\# h\nu$ is number of photons absorbed, V_0 is surface potential before illumination at $t = 0$, V_t is surface potential after illumination for a time t, V_f is final surface potential reached after prolonged illumination, ε is extinction coefficient of film in cm²/mole (equal to $4.29 \cdot 10^7$), I_o is incident light intensity in Einsteins/cm² sec (equal to $1.13 \cdot 10^{-10}$), t is time of illumination in secs (equal to 600 sec), r is reflection loss from surface and is estimated to be 0.1.

From the data given above and in Fig. 2 it is calculated that the initial phase of the photoreaction has $\Phi \approx 0.09 \pm 0.01$.

B. 9-cis retinal

The values of A_{10}, $\mu_\perp$ and ΔV_{10} for a film of 9-*cis* are 58 Å², 890 mD and 570 mV, respectively. Illumination of a film of 9-*cis* decreases A_{10}, $\mu_\perp$ and ΔV_{10} to 53 Å², 760 mD and 540 mV, respectively. The data for 9-*cis* are summarized in Table II. The quantum yield for the initial photoisomerization of 9-*cis* retinal or 9-*cis* plus 10^{-4}M cysteine (at $\pi = 14$ dyn/cm) is $\Phi \approx 0.26 \pm .09$ calculated from the data in Fig. 2 and using the procedure described above for *all-trans*. In solution KROPF and HUBBARD[12] reported $\Phi = 0.5$.

With cysteine present in the subphase there are significant changes in A_{10}, $\mu_\perp$ and ΔV to show there is an interaction between 9-*cis* and cysteine. With 10^{-4}M cysteine in the subphase, A_{10} increases to 74 Å². The variation of A_{10}, $\mu_\perp$, and ΔV_{10} as a function of cysteine concentration in the subphase is shown in Fig. 3.

As equilibrium constant for complexation, b, may

Table II. Summary of surface properties of retinals.

pH 6.0	A_{10}[Å²]		μ[mD]		ΔV10[mV]		A_{calcd}*
	dark	$h\nu$	dark	$h\nu$	dark	$h\nu$	64
all *trans*	51	52	660	560	480	430	
all trans + 10^{-4}M lysine	50	47	580	480	460	420	
11-*cis*	54	51	590	555	430	420	80
11-*cis* + $4 \cdot 10^{-5}$M lysine	48	45	525	560	410	420	
11-*cis* + $4 \cdot 10^{-5}$M β-MEA	61	58	730	600	460	390	
11-*cis* + $3 \cdot 10^{-6}$M cysteine	62	58	600	540	420	390	
11-*cis* + PEA	–	–	–	–	460	350	
9-*cis*	58	53	890	760	570	540	76
9-*cis* + 10^{-4}M cysteine	74	65	960	830	320	250	
9-*cis* + 10^{-4}M lysine	56	52	777	712	560	560	
13-*cis*	44	41	560	455	440	490	70
13-*cis* + 10^{-4}M cysteine	40	38	540	570	440	490	
13-*cis* + 10^{-4}M lysine	40	39	520	477	435	420	

* A_{calcd} is the area estimated from the structure of retinal (see Fig. 7).

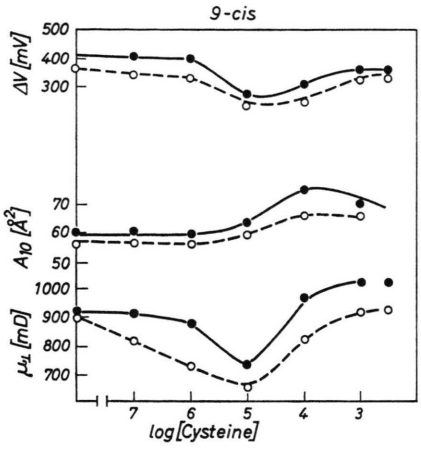


Fig. 3. Surface properties (ΔV_{10}, $\mu_\perp$ and A_{10}) of 9-*cis* retinal as a function of the log of the concentration of L-cysteine in the subphase. Before irradiation the data is indicated by solid circles. After irradiation the data is indicated by open circles: Subphase contained phosphate buffer pH 6.0 at a temperature of 15 °C.

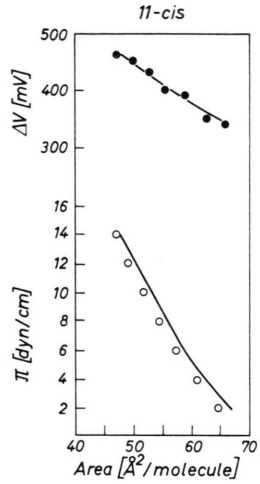


Fig. 4. Surface isotherm (π-A) of 11-*cis* retinal at an air-water interface. Isotherm before irradiation is shown by solid circles and solid lines. After irradiation by open circles.

be calculated frm the data shown in Fig. 3 for A_{10} using the expression $b = [A_{c,\,c\cdot s} - A_c] / ([A_{c.s} - A_c] \cdot [s])$ where A_c is the area of 9-*cis* alone (58 Å²), $A_{c.s}$ is the maximum area for the complex (74 Å²), and $A_{c,\,c.s}$ is the area (63 Å²) at a cysteine concentation, [*S*], of 10-⁵M (see AGHION *et al.*[8]). The value of *b* is found to be equal to $3 \cdot 10^4$M-¹. This value of *b* is considerably smaller than that obtained from complexation between 9-*cis* retinal and β-MEA (*i. e.* $b = 7 \cdot 10^6$ M-¹) (BROCKMAN and BRODY[7]).

In either nitrogen or air environment, irradiation of 9-*cis* retinal with cysteine in the subphase results in significant decreases of A_{10}, $\mu_\perp$ and ΔV_{10} (see Figs 3 and 2). With 10^{-4}M cysteine in the subphase, irradiation with an Hg arc lamp results in a 70 mV decrease of ΔV. The large change in ΔV_{10} could originate in the breaking up of a 9-*cis* cysteine complex.

C. 11-cis retinal

The values of A_{10}, $\mu_\perp$ and ΔV_{10} for a film of 11-*cis* retinal are 54 Å², 590 mD and 430 mV, respectively. Illumination of such a film results in a decrease of A_{10}, $\mu_\perp$ and ΔV_{10} to 51 Å², 555 mD and 420 mV, respectively (see Fig. 4). See Table II for the summary of data.

11-*cis* retinal interacts with $4 \cdot 10^{-5}$M β-MEA as evidenced by the significant increases in A_{10} and $\mu_\perp$ (see Table II). Irradiation results in a decrease of A_{10}, $\mu_\perp$ and ΔV_{10}.

After irradiation, the value of A_{10} in the presence of β-MEA is still larger than for 11-*cis* alone, indicating that β-MEA is still interacting with the photoproducts and/or that the photoproducts are different. A similar result was shown by BROCKMAN and BRODY[7] for 9-*cis* and β-MEA. Perhaps the sulfur group of β-MEA is involved in mediating the photoreaction of 11-*cis* and its isomeric forms.

There is an interaction between 11-*cis* and cysteine at pH 6.0. With $3 \cdot 10^{-6}$ M cysteine in the subphase the value of A_{10} increases to 62 Å² while $\mu_\perp$ and ΔV_{10} remain unchanged (within experimental error). The variation of A_{10}, $\mu_\perp$ and ΔV_{10} as a function of cysteine concentration are shown in Fig. 5. After

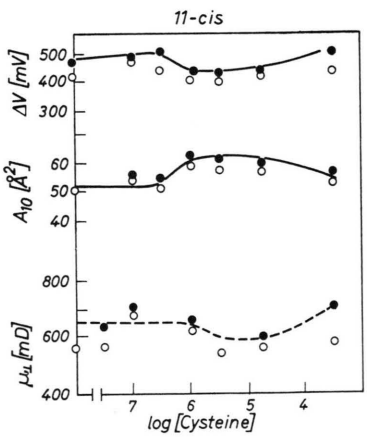


Fig. 5. Surface properties (ΔV_{10}, $\mu_\perp$ and A_{10}) of 11-*cis* retinal as a function of the log of the concentration of L-cysteine in the subphase. Before irradiation the data is indicated by solid circles. After irradiation the data is indicated by open circles.

irradiation A_{10}, $\mu_\perp$ and ΔV_{10} consistently show a decrease in value. Upon irradiation ΔV_{10} decreases in potential from 10 to 60 mV (see Fig. 5). [Part of the variation observed in the light induced change in ΔV_{10} arises from the use of different methods of irradiation (cool white fluorescent, tungsten, or monochromatic lights). These changes in potential probably reflect, in part, photoisomerization of 11-*cis*.]

There appears to be an interaction between 11-*cis* and $4 \cdot 10^{-5}$M lysine. Interaction between 11-*cis* and lysine results in a smaller value of A_{10} (see Table II). The change in A_{10} induced by lysine is opposite to that induced by the sulfur containing compounds (cysteine or β-MEA).

After irradiating 11-*cis* with no additives in the subphase, there is a decrease in A_{10}, $\mu_\perp$ and ΔV_{10}. On the other hand, after irradiating the 11-*cis* with $4 \cdot 10^{-5}$M lysine in the subphase there is an increase in both $\mu_\perp$ and ΔV_{10}, and a decrease in A_{10}. After irradiation, $\mu_\perp$ and ΔV_{10} are the same for both 11-*cis* and the complex. However, the value of A_{10} after irradiation is different from those obtained for 11-*cis* alone, indicating that the complex might favor either the formation of different photoproducts, or that lysine is interacting with the photoproducts. A distinguishing characteristic of the irradiated 11-*cis* with lysine in the subphase is that the surface potential increases in value after irradiation; in almost all other cases ΔV_{10} decreases.

The interaction between mixed films of 11-*cis* retinal and PEA was examined. The technique is the same as that previously published for mixed films of chlorophyll and ferredoxin (BRODY[13]). The experimentally measured isotherm is compared with a theoretical isotherm calculated from the individual isotherms and known amounts of 11-*cis* and PEA added to the surface. The theoretical area of the mixed film is significantly smaller than the experimentally measured film. See Fig. 6 where the mole ratio of [PEA][11-*cis*] equals 1.4. Interaction reorients the 11-*cis* and/or PEA so that the area is larger than measured for the individual materials in pure monomolecular films. While irradiation does not significantly alter the area of the mixed film it does result in a — 110 mV change in ΔV_{10}.

D. 13-cis Retinal

The values for A_{10}, $\mu_\perp$ and ΔA_{10} for 13-*cis* are 44 Å², 560 mD and 440 mV, respectively.

There is a slight indication of interaction between

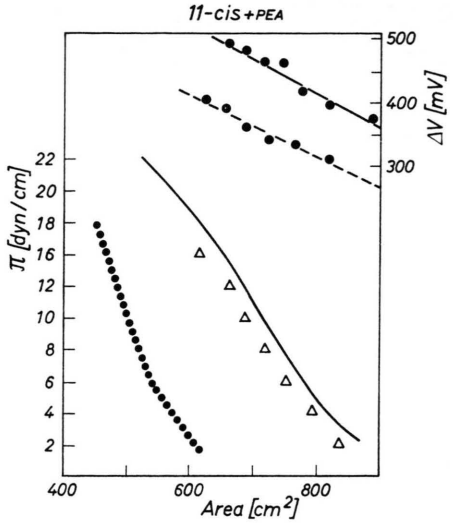


Fig. 6. Theoretical and experimental isotherm of a mixed film of 11-*cis* retinal and phosphatidylethanolamine (PEA) in a mole ratio of 1 to 1.4, respectively. The theoretical and experimental isotherms are indicated by open triangles and solid line, respectively. The values of ΔV before and after irradiation are indicated by solid and open circles, respectively. The isotherm of PEA is shown by dotted line.

13-*cis* and 10^{-4}M cysteine as well as between 13-*cis* and 10^{-4}M lysine. In both cases A_{10} and ΔV_{10} decrease so there appears to be some sort of interaction. In the case of *all-trans*, 11-*cis* and 9-*cis*, the nature of the interaction with lysine is different from that with cysteine or β-MEA primarily because A_{10} decreases instead of increases upon interaction (see Table II).

During the illumination of 13-*cis* at π = 12 dyn/cm with 10^{-4} M cysteine in the subphase ΔV_{10} is observed to decrease about 40 mV; however, after the film is expanded and recompressed ΔV_{10} is observed to have increased about 50 mV. See Table II for a summary of data.

Discussion

From a comparison of the area of a molecule projected on the aqueous surface and its theoretical size, the angle the plane of the molecule makes with the surface may be determined. The estimated area for 13-*cis* is 64 Å², for *all trans* 50 Å², for 11-*cis* 80 Å², and for 9-*cis* 76 Å². These estimated sizes were made using the dimensions shown in Fig. 7. Thus at π = 10 dyn/cm the angles for 13-*cis*, *all-trans*, 11-*cis* and 9-*cis* are 51°, 37°, 48° and 40°, respectively.

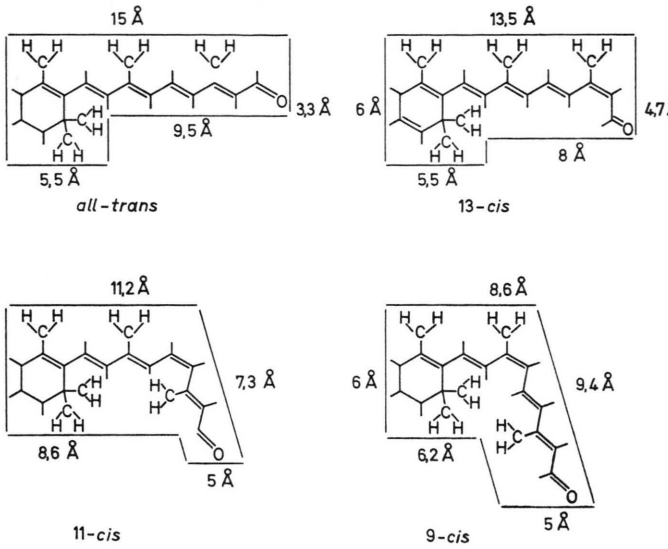
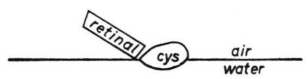
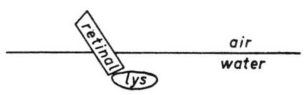


Fig. 7. Dimensions used to calculate molecular areas for retinals are shown above.

The increase in A can be accounted for if the aqueous phase molecule, upon complexation, assumed a position at the air/water interface as indicated in the sketch or reorientation of retinal into a more horizontal position.



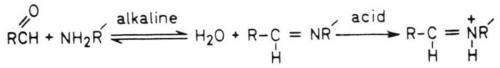
A decrease in A upon complexation could result from a reorientation of retinal into a more vertical position, for example if part of the retinal were pulled into the subphase as indicated in the sketch.



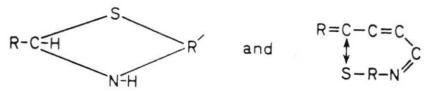
It was shown above that there is an interaction between certain forms of retinal and amino acids. This interaction is probably in the nature of a complex between retinal and the amino acid. Each of the various forms of retinal interact in a different specific manner with the amino acids added to the subphase. Furthermore, each of the various forms of retinal and their complexes have a different specific photosensitivity.

Specificity of interaction of the various retinal forms probably is a property of their conformational state. The latter may determine the preferred type of bonding with an amino acid at an air/water interface and presumably at the opsin surface.

Most of these experiments were conducted at pH 6.0 where there is little possibility for the formation of a Schiff base linkage. In order to have a Schiff base linkage at acid pH's the linkage must first be formed in alkaline conditions, then protonated by lowering the pH (MORTON and PITT[4]). Only in the protonated form (in acid conditions up to pH 3.0) is the Schiff base stable, *i. e.*



At pH 6.0 two likely possibilities for complexation are charge transfer (GALINDO[2]) and thiazolidine (PESKIN and LOVE[3]). In both these complexes a sulfur and nitrogen linkages are involved, *i. e.*



If a charge transfer is involved, as suggested by GALINDO[2], the specificity for complexation would depend upon the distance in retinal between the carbonyl and the C_{-9} or the C_{-11} being the same as the distance between the S and N in the cysteine. In addition, the C_{-9} or C_{-11} and carbonyl in the *cis* forms should be in close proximity to the aqueous surface so they are more readily available for complexation than in the case of the *trans* form where no interaction occurs. One of these linkages is probably involved in the interaction between 11-*cis* retinal and crysteine or β-MEA. Both of these subphase materials contain S and N, their interaction gives rise to a significant increase in A_{10}.

In the case of 9-*cis* and 11-*cis*, interaction is signaled by an increase in A_{10} while in the case of lysine or 13-*cis*, there is a small decrease in A_{10}. The latter interaction apparently changes the orientation of retinal into a more vertical position so as to project a smaller area on the surface. Since a thiazolidine or charge transfer linkage is not possible between lysine and 11-*cis* or 13-*cis* because of the absence of S, the exact nature of the interaction is not apparent. Perhaps there is a small amount of Schiff base linkage formed even at pH 6.0 to account for the interaction between 11-*cis* and lysine. It is to be noted that in every case complexation resulted in a decrease of ΔV. This decrease could result from an amino acid with a low $\mu_\perp$ being brought into the film so as to dilute the contribution of the retinal's dipole or the reorientation of μ into a more horizontal thereby decreasing $\mu_\perp$.

The values of A_0 (at $\pi = 0$) previously reported for 9-*cis*, *all-trans*, and 13-*cis* at pH 6.0 by BROCKMAN and BRODY[7] were 56, 54, and 45 Å², respectively; A_{10} for 13-*cis* shown in their isotherm is 36 Å². In the present work, 13-*cis* has an A_{10} of 44 Å²; this is considerably larger than the 36 Å² reported by BROCKMAN and BRODY[7]. In the present work benzene was used as a spreading solvent while Brockman and Brody used hexane. The absorption coefficient used by Brockman and Brody for 13-*cis* in hexane of $3.56 \cdot 10^4$ (BROCKMAN[14]) is similar to that in Table I. The origin of the discrepancy is not apparent unless the purity of the material was different in the two studies.

Regardless of the starting isomer, long irradiation of a solution of isomer would be expected to lead to the same equilibrium mixture of isomers. However, in monolayers the A_{10}'s of irradiated 13-*cis*, 9-*cis*, 11-*cis* and *all-trans* are all different. Therefore, it is apparent that photoreactions in films do not lead to the same equilibrium mixture of isomers. (A summary of the light induced changes in A_{10} and ΔV_{10} are given in Table III). This apparent difference in photochemistry of retinals in solution and in oriented monomolecular films should be considered. The relatively high value of π used during irradiation may favor formation of the smaller size isomers rather than the larger ones. When starting with small isomers (13-*cis* and *all-trans*) there is little room on the surface for the formation of the larger isomers. However, when starting with the larger isomers (9-*cis* and 11-*cis*) smaller isomers may form without hindrance; as smaller isomers are formed, space is left on the surface to photochemically reform some larger isomers from the smaller ones.

Table III. Summary of light induced changes in A and in ΔV.

Film	Subphase	A [Å²]	ΔV [mV]
all-trans		+1	−50
all-trans	lysine	−3	−40
9-*cis*	———	−5	−30
9-*cis*	10^{-4} M cysteine	−9	−70
9-*cis*	lysine	−4	0
11-*cis*	———	−3	−10
11-*cis*	$4 \cdot 10^{-5}$ M β-MEA	−3	−70
11-*cis*	$3 \cdot 10^{-6}$ M cysteine	−4	−30
11-*cis*	$4 \cdot 10^{-5}$ M lysine	−3	+10
11-*cis* + PEA	———	0	−110
13-*cis*	———	−3	+50
13-*cis*	10^{-4} M cysteine	−2	+50
13-*cis*	10^{-4} M lysine	−1	−15

It was frequently observed that the light induced change in π or ΔV observed in the compressed state is opposite in sign to that which obtains after the film is expanded and recompressed. This observation may simply reflect the inability of the photoproduct to reorient while in a compressed state. Similar observations of this type were reported previously (AGHION *et al.*[8]).

Irradiation almost always results in a decrease in A_{10} and ΔV_{10}. The largest decrease in A_{10} is observed with 9-*cis* and 10^{-4} M cysteine. While no change in A_{10} is observed in the case of mixed films of 11-*cis* and PEA, it gave the largest decrease of ΔV_{10} (*i. e.* -110 mV). In two cases an increase of ΔV_{10} is observed after irradiation, *i. e.* 11-*cis* complexed with $4 \cdot 10^{-5}$ M lysine and 13-*cis* with and without cysteine.

The origin of the light-induced change in ΔV (or other surface properties) might arise from either of two sources: the breaking up of the complex and/or photoisomerization. For example, the photoisomerization of 9-*cis* results in only a decrease in potential of about 30 mV whereas in the presence of 10^{-4} M cysteine the decrease is 70 mV (Table III). The -70 and -40 mV probably arises from cysteine interacting differently with 9-*cis* and and the photoproducts of 9-*cis*. If, after irradiation, the surface properties of 9-*cis* over cysteine were the same as in the absence of cysteine then two points would be apparent. First, that the complex with cysteine does not alter the photoreactions of 9-*cis*. Second, that there is no significant concentration of cysteine complexed with the photoproducts of the reaction. After irradiating 9-*cis* with cysteine in the subphase, A_{10} is not the same as that obtained in the absence of cysteine (see Table II); in addition, the value of A_{10} after irradiation is larger than those measured for any of the pure isomers. Since the surface properties after irradiation are different with and without cysteine, either the equilibrium mixture of photoproducts is different and/or cysteine also interacts with the photoproducts and/or acts to stabilize specific photoprodutcs. This same interpretation may be applied to all the photoeffects, reported in the study of the retinals in the presence of subphase additives.

If in visual excitation, as in other neural excitations, a minimum threshold voltage is required to trigger the action potential then the polarity and magnitude of the light-induced changes in ΔV

(Table III) are critical in determining the possible activity of the retinal complexes in the visual process. Depending upon the orientation of retinal in the disc membrane, a large negative change in potential could either reinforce an existing membrane potential or completely alter membrane permeability. With this view one could argue that the small positive increase in ΔV_{10}, observed upon irradiating 11-*cis* with lysine, distinguishes this complex from most of the others studied and marks it as a possibility for the photoactive complex in rhodopsin. The possibility of the existence of such a complex *in vivo* has been discussed previously (BONTING[15]). However, the light induced change in ΔV_{10} of 10 mV may not be large enough to trigger a neutral excitation. Perhaps under other pH conditions a more decisive increase in ΔV might be observed. A large light-induced change in ΔV could readily trigger a neutral excitation by changing membrane permeablility or directly transmitting an electrical signal (BROCKMAN and BRODY[7]).

While irradiation of all forms of retinal result in a change in ΔV_{10} the unique characteristic of the 11-*cis*-PEA complex for the visual process might be the large negative change in ΔV_{10}. The retinals 13-*cis* and *all-trans* might be unable to produce a visible excitation because the largest values measured for a light-induced change in ΔV were only +50 and — 50 mV, respectively, while 9-*cis* (plus 10^{-4} M cysteine), which can form *iso*-rhodopsin, gives a larger light-induced change of —70 mV (see Table III).

The area and potential changes associated with the absorption of light by retinals could alter the conformational state of the opsin to which they are attached *in vivo*. If neural excitation does indeed originate at the primary photoisomerization act then the various forms of rhodopsin (lumirhodopsin, metarhodopsins) might be merely stages in the regeneration of rhodopsin.

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