Interactions of Monomolecular Films of Retinal at Alkaline pH

N. Yckowski and S. S. Brody

Department of Biology, New York University

(Z. Naturforsch. **29 c**, 327–335 [1974]; received April 1, 1974)

Vision, Retinal, Monolayers, Membranes, Model systems

The surface properties of mixed monomolecular films of retinal and phospholipids were studied at a nitrogen/water interface. The subphase was glycine buffer pH 10.5 with an ionic strength of 0.1. Monomolecular films of retinal in the presence of amino acids were also measured. The area per molecule, at a surface pressure of 10 dyn/cm, A_{10}, in the dark for 9-*cis* retinal and 11-*cis* retinal are 42 Å² and 47 Å², respectively. After irradiation A_{10} for 9-*cis* retinal and 11-*cis* retinal dcerease to 40 Å² and 43 Å², respectively. The surface potentials, ΔV, at a surface pressure of 10 dyn/cm, in the dark for 9-*cis* retinal and 11-*cis* retinal are 470 mV and 445 mV, respectively. After irradiation, ΔV for 9-*cis* retinal decreases to 435 mV and 11-*cis* retinal increases to 490 mV. Interaction was observed between retinal and phospholipids and amino acids. The A_{10} and ΔV_{10} of mixed films of retinal and phospholipid were measured as a function of the mole fraction of phospholipid. Maximum interaction is observed at mole ratios of; phosphatidylserine/9-*cis* = 1; phosphatidylethanolamine/9-*cis* = 2, phosphatidylserine/11-*cis* = 3; phosphatidylethanolamine/11-*cis* = 3. It is shown that mixing and interaction between phosphatidylethanolamine and retinal is spontaneous. The A_{10} and ΔV_{10} of films of retinal were measured as a function of the molar concentration of amino acid in the subphase. The nature of the interaction between retinal and phospholipid and amino acid are discussed.

Introduction

From the studies of Morton and Pitt [1], Pitt *et al.* [2], Ball *et al.* [3], and Collins *et al.* [4] dealing with the spectral behavior of model Schiff bases of retinal with simple amines, it was proposed that in rhodopsin, 11-*cis* retinal (11-*cis*) is bound to an amino group by a protonated Schiff base linkage. At pH 13 the Schiff base linkage of the analogue retinylidenemethylamine is stable in the presence of methylamine since it prevents hydrolysis of the linkage. Thus an equilibrium condition is reached where most of the retinal is in the Schiff base form. At pH 1 the Schiff base linkage becomes protonated and is again stable. At pH values between 3 and 10 Schiff base linkages are essentially unstable and are easily hydrolysed.

From their work with Schiff bases of retinal Rosenberg and Kirgas [5] and Toth and Rosenberg [6] hypothesized that a bathochromic shift can be attained by increasing the positive charge on the nitrogen atom by the inductive effect of nearby substituents. Daemen and Bonding [7] proposed that, if, in native rhodopsin, retinal is bound to phosphatidylethanolamine (PE) by a Schiff base, perhaps the phosphoric acid group in PE might cause internal protonation of the Schiff base resulting in a bathochromic shift. Poincelot *et al.* [8, 9], Akhtar and Hirtenstein [10] and Fager *et al.* [11] have presented data showing that PE can bind to 11-*cis* under certain conditions. It would be of interest to examine the nature of the interaction between retinal and charged components of rhodopsin such as phospholipids (PL).

Monolayer studies of retinals at pH 6.0 with amino acids were previously made by Brockman and Brody [12], Brody [13] and with PL's by Puppala [14]. At pH 6.0 Schiff base linkage is unstable so other types of interaction must be considered for retinal. According to Puppala interactions of 9-*cis* retinal (9-*cis*) and 11-*cis* with PE and phosphatidylserine (PS) in mixed films occur at a ratio of retinal/PL of 0.1.

There is also the possibility of forming a charge transfer (Galindo [15] and Akhtar *et al.* [16]) or thiazolidene (Peskin and Love [17] and Mizuno *et al.* [18]) complex between retinal and certain amino acids. For both types of complex the presence of an amino and sulfhydryl groups are required. That interactions occur between retinal films and L-cysteine

Requests for reprints should be sent to Prof. S. S. Brody, Department of Biology, New York University, 1101 Main Building, 100 Washington Square, New York City, *New York 10003*, USA.

Abbrevations: PE, phosphatidylethanolamine; PL, phospholipids; PS, phosphatidylserine; MEA, β-mercaptoethylamine.

or β-mercaptoethylamine (MEA) at pH 6 has been shown by several studies. Brockman and Brody [12] reported complexation between retinal and MEA in the aqueous phase. Brody [13] reported complexation between 9-*cis* and 11-*cis* with cysteine; 11-*cis* also reacted with MEA.

A considerable body of evidence supports the contention that retinal is bound to lysine in native rhodopsin (Bownds and Wald [19], Bownds [20]; Akhtar *et al.* [21]; Heller [22]; Fager *et al.* [11]). An interaction between 11-*cis* and lysine in films has been reported by Brody [13].

In the present work a study of the interaction of 9-*cis* and 11-*cis* with PL, lysine and cystine was carried out at pH 10.5 where Schiff base linkage occurs readily.

Materials and Methods

Solutions of 9-*cis* (Sigma Chemical, St. Louis, Mo.), and 11-*cis* (Hoffman La Roche, Nutley, N. J.) retinal were prepared in dim red light by dissolving a few crystals of retinal in 1 ml of *n*-hexane (Fisher Scientific, Fair Lawn, N. J., 99 Mol.-% Pure). This solution was stirred with a miniature magnetic mixer for 1 – 2 min until the retinal was completely dissolved. These stock solutions could be stored for about a week in the dark at 0 °C without any change to the spectral properties of retinal. The exact concentration of the solution was determined with a recording spectrophotometer (Cary Model 14R). PE (Mann Research Laboratory, Division of Schwartz Biochemical, N. Y.) was dissolved in benzene (Analytical Reagent Grade, Mallinckrodt Chemical Works, St. Louis, Mo.) to give a final concentration of 1.4 mg/ml. PS (Applied Science Laboratory, State College, Pa.) was obtained in a chloroform solution and diluted with chloroform (Certified A.C.A., Fisher Scientific Co., Fair Lawn, N. J.) to a concentration of 3.5 mg/ml. The molecular weights used to calculate the number of molecules of PE and PS were 800 and 792, respectively. The PL's were used without further purification so that each probably reprsents a mixture with different types of fatty acid residues. PL concentrations determined by supplier.

Distilled water was prepared by first utilizing an ion-exchange column (Barnstead Standard) then distilling from an all glass still (Corning Model AG-2-Still, Corning, N. Y.) in the presence of permanganate. Glycine buffer, pH 10.5, ionic strength 0.1 was used in the subphase; the temperature was 15 °C.

Monolayer studies were carried out in a Wilhelmy plate film balance, housed in an environmental chamber essentially the same as that described previously (Aghion *et al.* [23]). The latter had provisions for flushing with nitrogen and for evacuation. A Beckman microbalance (Model LM-500) was used to measure surface pressure. The sensitivity of the balance permitted measurement of film pressure (π) with a precision of better than ± 0.05 dyn/cm. The trough, whose dimensions were $68 \times 17 \times 1$ cm, was constructed of aluminum and coated with teflon. The barriers were made of glass and coated with teflon. The Wilhelmy blade was a sand-blasted platinum leaf. Surface potential measurements were made using a radioactive tungsten electrode, about 1 cm above the surface, and a Ag/AgCl electrode in the subphase. After spreading the film, an electrometer (Keithley, Model 610) was used to measured the potential of the film on the aqueous surface (V). The potential of the water beneath the film (V_{H_2O}) was calculated from V using the relationship $V = 2\pi\mu_\perp/A + V_{H_2O}$ and fitting the best straight line to the data, where A = area/molecule in Å^2, $\mu_\perp$ is the perpendicular component of the surface dipole moment in millidebye. The surface potential (ΔV) of the film is equal to $V - V_{H_2O}$. The precision for measuring ΔV is ± 10 mV.

The aqueous surface was cleaned and a measured volume of material was slowly delivered to the surface using a Hamilton microliter syringe. Mixed monomolecular films were prepared by mixing solutions of known concentration of PL and retinal. These operations were done in air. All experiments were carried out in a nitrogen atmosphere using prepurified gas (99.99% purchased from Matheson Gas and Co., E. Rutherford, N. J.). To speed evaporation of the solvent, the film was alternately compressed and expanded while nitrogen was simultaneously pumped into the chamber and drawn out. Approximately 10 – 15 min were allowed for the evaporation of the solvents before measurements commenced.

The experimental $(\pi - A)/\Delta V$ isotherms were measured after the films were spread on the surface and the system flushed with nitrogen. Continuous changes in film properties were observed between repetitively measured isotherms. However, after a period of 20 – 30 min consecutive isotherms showed changes of only 1 – 2%. For photochemical experiments, the light source was a 100 W low pressure mercury arc lamp (General Electric H100-A 4) used without filters. The average energy on the surface, measured with a thermopile (Eppley Laboratories, Newport, R. I.) was 2×10^3 ergs/cm^2 sec. Sur-

face isotherms ($\pi - A$) and ΔV were measured before and after irradiation as a function of area (A) where π is the surface pressure in dyn/cm. To determine the effect of light, ΔV and A were measured in the dark at $\pi = 10$ dyn/cm, *i. e.* ΔV_{10} and A_{10}. The film was irradiated in this compressed state. After irradiation the film was expanded and recompressed and ΔV_{10} and A_{10} were measured again. The differences in ΔV_{10}'s before and after irradiation is given as (light − ark) ΔV_{10} and (light − dark) A_{10}.

A theoretical (Theor) area was calculated for each mixed film using the equation: Eq 1 (Theor) $A = n_a A_a + n_b A_b$ where A_a and A_b are the areas for retinal and PL, in the dark, respectively and n_a and n_b are the mole fractions of retinal and PL, respectively. To determine if there is any interaction or complexation between retinal and PL the experimentally measured areas (Exper) were subtracted from the (Theor) areas, *i. e.* (Exper − Theor). The Theor value of ΔV for mixed films was calculated from the equation Eq 2 (Theor) $\Delta V = n_a \Delta V_a + n_b \Delta V_b$. The difference in (Exper − Theor) ΔV_{10} was also used to assay for interaction between PL and retinal.

Results

Retinal: The $\pi - A$ curves of 9-cis and 11-*cis* before and after irradiation are shown in Fig. 1. The A_{10}'s of 9-*cis* and 11-*cis* in the dark are 42 Å² and 47 Å², respectively. These areas are smaller than those measured by Brody [13] and Puppala [14] at pH 6.0. From a comparison of these measurements it appears that increasing the pH from 6.0 to 10.5 may result in a different angular orientation for retinal on the surface which might result in an A_{10} and, perhaps, a ΔV_{10} that is different from those reported at pH 6.0. (See Table I for a comparison of the various A's and ΔV's reported at pH 6.0 and 10.5.) A mechanism for the orientation of the polar retinal molecule depending on pH is not apparent. The angular orientation on the aqueous surface is, in part, related to the polar nature of the molecule which might be modified by solvent environment. Differences in A_{10} might also arise from differences in experimental technique (*e. g.* time allowed for the film to stabilize) or purity of the retinal preparation.

ΔV_{10}'s for 9-*cis* and 11-*cis* in the dark at pH 10.5 are 470 mV and 445 mV, respectively. ΔV's for 9-*cis* and 11-*cis* as a function of $1/A$ in Å²/molecule are shown in Fig. 2 a. At pH 6.0 a considerably higher ΔV_{10} of 570 mV is given for 9-*cis*, whereas a smaller ΔV_{10} of 420 mV is given for 11-*cis* (Table I, Brody [13]).

Irradiation of retinal films at pH 10.5 results in a decrease in A. After illumination, A_{10} decreases to 40 Å² for 9-*cis* and to 43 Å² for 11-*cis*. The ΔV_{10} for 9-*cis* decreases to 435 mV and increases to 490

Table I. Summary of surface properties of retinals.

Reference	Material *	A_{10} [Å²] Dark	A_{10} [Å²] (Light-Dark)	ΔV_{10} [mV] Dark	ΔV_{10} [mV] (Light-Dark)
ref. 12					
pH 6.0	9-*cis*	48	−7	485	−45
ref. 14	9-*cis*	48	−2	500	+20
pH 6.0	11-*cis*	42	−4	510	+70
ref. 13	9-*cis*	58	−5	570	−30
pH 6.0	11-*cis*	54	−3	430	−10
	9-*cis* + L-cysteine (10^{-4} M)	74	−9	320	−70
	11-*cis* + L-lysine (10^{-5} M)	48	−3	410	+10
	11-*cis* + L-cysteine (10^{-6} M)	62	−4	420	−30
	11-*cis* + Pe (0.7)	—	—	—	−110
	9-*cis*	42	−2	470	−35
pH 10.5	11-*cis*	47	−4	445	+45
	PE	71	—	190	—
	PS	73	—	135	—
	9-*cis* + PE (0.3)	81	−7	415	−32
	9-*cis* + PS (0.7)	40	−6	335	−90
	9-*cis* + L-lysine (10^{-4} M)	42	−4	479	−35
	9-*cis* + L-cysteine (10^{-4} M)	43	−5	557	−25
	11-*cis* + PE (0.7)	68	−3	221	−45
	11-*cis* + PS (0.3)	27	−1	378	−40
	11-*cis* + L-lysine (10^{-6} M)	47	−4	434	−70
	11-*cis* + L-cysteine (10^{-4} M)	46	−5	407	−80

* Mole fractions of P. L. or concentration of amino-acid which show the largest effect of light are given in parenthesis.

mV for 11-*cis*. The light induced change in ΔV observed for 9-*cis* is opposite in sign to that for 11-*cis*. This observation might be of interest with regard to the visual system. No differences in biological function have been assigned to isorhodopsin which contains 9-*cis* and rhodopsin which contains 11-*cis*. Since illumination of 9-*cis* and 11-*cis* produce opposite changes in ΔV perhaps the two forms of rhodopsin could illicit different types of visual excitation *in vivo*.

During each experiment there is a small slow time dependent decrease of π and ΔV in the dark in the compressed state. The change in π may amount to as much as 1 dyn/cm in an hour and in ΔV as much as 140 mV in an hour. These decreases occur with both retinal films and mixed films. The origin of this decrease is not clear, it may simply reflect evaporation of the last traces of spreading solvent or a dark reaction of retinal on the surface. To test the latter possibility retinal films were collected from the surface after several hours and examined spectrophotometrically. No significant change in absorption spectrum was observed. Irradiation speeds up the rate of decrease to 2 dyn/cm in 20 min and 50 – 80 mV in 15 min. With some compressed films small increases in ΔV are observed upon irradiation.

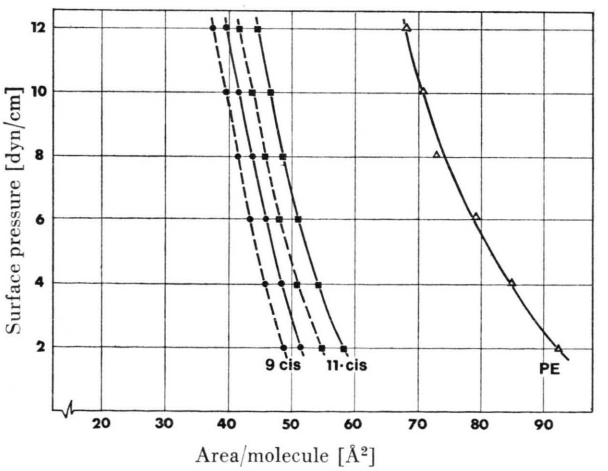


Fig. 1. Surface pressure-area (π-A) isotherms of 9-*cis* (●), 11-*cis* (■) and PE (△). Area per molecule in Å² is measured as a function of surface pressure (π), in dyn/cm. Area before irradiation is shown by a solid line (—) and after irradiation by a broken line (- - -). Area at 10 dyn/cm, A_{10} for 9-*cis* before and after irradiation is 42 Å² and 40 Å², respectively. A_{10} for 11-*cis* before and after irradiations is 47 Å² and 43 Å² respectively. A_{10} for P6 is 71 Å². The subphase is glycine buffer, pH 10.5, ionic strength 0.1 and the temperature 15 °C.

Phospholipids: A fresh sample of PE has an A_{10} of 71 Å² (Fig. 1). The A_{10} increases progressively to 109 Å² when PE is stored in the dark for six months at about 2 °C. The A_{10} for PS increases from 73 Å² to 86 Å² over a period of three months. These changes in A_{10} arise primarily from evaporation of solvent from stock solutions which increases the concentration of the solution, and in part from oxidation of PL. The area for PL is measured for each experiment in order to correct for the increase in concentration arising from evaporation. The A_{10}'s for PE and PS reported by Puppala [14] at pH 6.0 are 76 Å² and 43 Å² respectively. Differences in A for PL as a function of pH were previously reported (Paoletti and Kletchev-

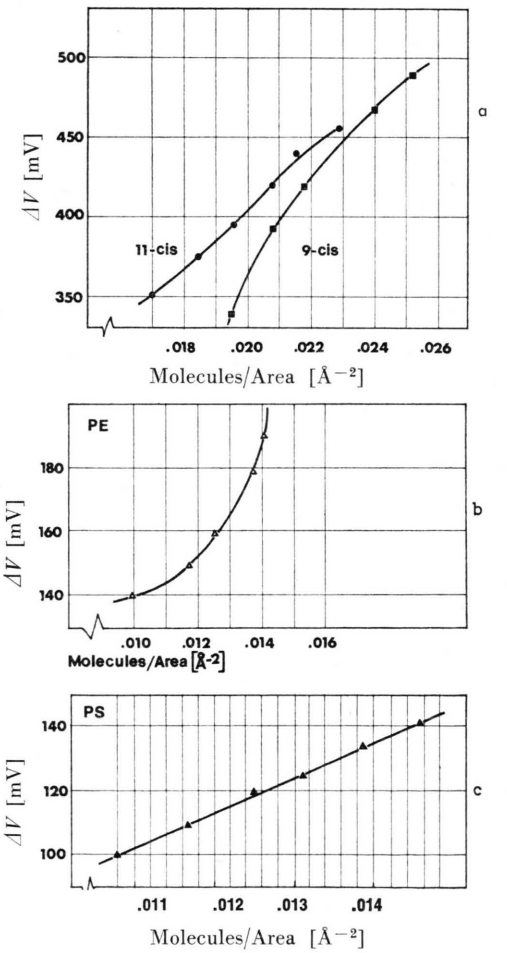


Fig. 2. The surface potential (ΔV), in mV, of 9-*cis* and 11-*cis*, in the dark as a function of retinal concentration on the surface in $Å^{-2}$ is shown in Fig. 2 a. ΔV for 9-*cis* at $\pi = 10$ dyn/cm is 470 mV, ΔV_{10} for 11-*cis* is 445 mV. The ΔV_{10} for PS and PE are shown in Figs 2 b and 2 c, respectively. The ΔV_{10} for PS is 135 mV and PE, 190 mV.

sky[24]), *e.g.* for PE, A increases with pH. The ΔV's for PE and PS at pH 10.5 are 190 mV and 135 mV. respectively. The ΔV's for PE and PS as a function of $1/A$ are shown in Figs 2 b and 2 c. Neither the $\pi - A$ nor ΔV isotherms for PE and PS are affected by irradiation.

9-*cis* and PE: The $\pi - A$ and ΔV isotherms for mixed films of 9-*cis* and PE, in the dark and light are measured as a function of the mole fraction of PE. The difference between Exper and Theor A's at $\pi = 10$ dyn/cm, *i.e.* (Exper − Theor) A_{10}, is shown in Fig. 3. A minimum value for (Exper − Theor) A_{10} occurs at a mole ratio of PE/9-*cis* ≅ 2.

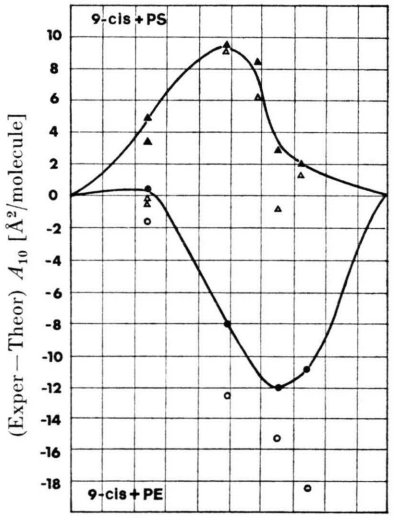


Fig. 3. The difference between experimentally measured (Exper) and theoretically calculated (Theor) isotherms for area of mixed films of 9-*cis* and PS (▲) and 9-*cis* and PE (●) in the dark as a function of the mole fraction of PL. The difference at $\pi = 10$ dyn/cm is given as (Exper − Theor) A_{10}. The (Exper − Theor) A_{10} for 9-*cis* and PS and 9-*cis* and PE after irradiation are shown as (△) and (○), respectively. A maximum for (Exper − Theor) A_{10} occurs at a mole ratio of PS/9-*cis* of 1:1; a minimum value occurs at a ratio of PE/9-*cis* of 2:1. Subphase and temperature same as in Fig. 1.

The differences between the (Exper − Theor) ΔV's for mixed films of 9-*cis* and PE as a function of the mole fraction of PE are shown in Fig. 4. A minimum value for (Exper − Theor) ΔV_{10} occurs at a mole ratio of PE/9-*cis* ≅ 2. After irradiation both ΔV_{10} and A_{10} decrease. In determining (Exper − Theor) after irradiation, the data measured in the dark is used to evaluate Theor. The largest decrease observed for ΔV_{10} is 32 mV and for A_{10}, 7 Å².

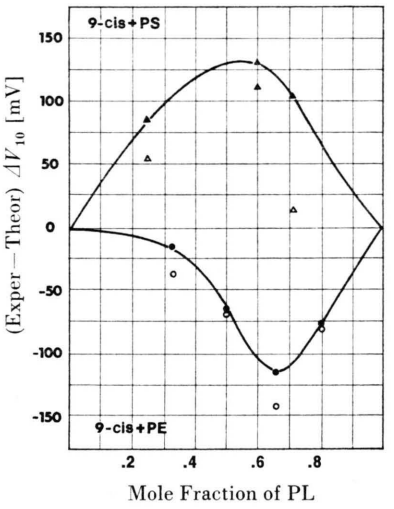


Fig. 4. The difference of (Exper − Theor) ΔV_{10} for mixed films of 9-*cis* and PS (▲) and 9-*cis* and PE (●) in the dark as a function of the mole fraction of PL. The (Exper − Theor) ΔV_{10} of 9-*cis* and PS and 9-*cis* and PE after irradiation are shown as (△) and (○), respectively. A maximum for (Exper − Theor) ΔV_{10} occurs at a ratio of PS/9-*cis* of 1.5:1, a minimum value occurs at a ratio of PE/9-*cis* of 2:1. Subphase and temperature same as in Fig. 1.

11-*cis* and PE: The (Exper − Theor) A_{10} of mixed films of 11-*cis* and PE as a function of the mole fraction of PE, before and after irradiation is

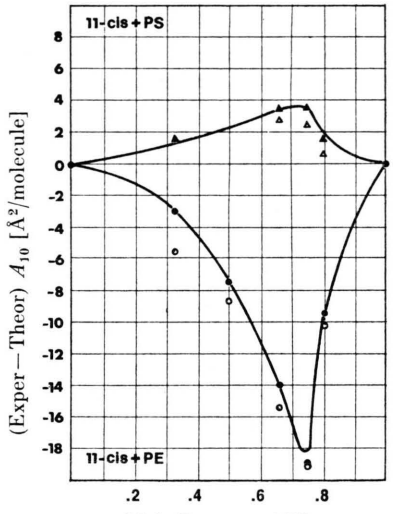


Fig. 5. The difference of (Exper − Theor) A_{10} of mixed films of 11-*cis* and PS (▲) and 11-*cis* and PE (●) in the dark as a function of the mole fraction of PL. The (Exper − Theor) A_{10} for 11-*cis* and PS and 11-*cis* and PE after irradiation are shown as (△) and (○), respectively. A maximum for (Exper − Theor) A_{10} occurs at a ratio of PS/11-*cis* of 3:1; a minimum value occurs at a ratio of PE/11-*cis* of 3:1. Subphase and temperature same as in Fig. 1.

shown in Fig. 5. The minimum value for (Exper – Theror) A_{10} occurs at a mole ratio of PE/11-*cis* ≅ 3.

The (Exper – Theor) ΔV_{10} as a function of the mole fraction of PE is shown in Fig. 6. In this case there is no well defined minimum. In general, after irradiation both A_{10} and ΔV_{10} decrease. The largest light induced decrease observed for ΔV_{10} is 45 mV and for A_{10}, 3 Å².

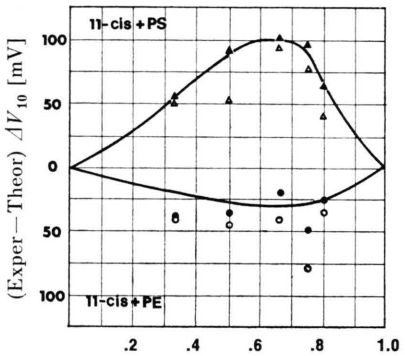


Fig. 6. The difference of (Exper – Theor) ΔV_{10} for mixed films of 11-*cis* and PS (▲) and 11-*cis* and PE (●) in the dark as a function of the mole fraction of PL. The (Exper – Theor) ΔV_{10} for 11-*cis* and PS and 11-*cis* and PE are shown as (△) and (○), respectively. A maximum for (Exper – Theor) ΔV_{10} occurs at a ratio of PS/ 11-*cis* of 2:1. Subphase and temperature same as in Fig. 1.

9-*cis* and PS: The (Exper – Theor) A_{10} for mixed films of 9-*cis* PS as a function of the mole fraction of PS, before and after irradiation, is shown in Fig. 3. As opposed to the mixed films of PE where (Exper – Theor) A_{10} always give negative values, in the case of PS (Exper – Theor) A_{10} is always positive. A maximum value for (Exper – Theor) A_{10} occurs at a ratio of PS/9-*cis* = 1.

The (Exper – Theor) ΔV_{10} as a function of the mole fraction of PS is shown in Fig. 4. A maximum value for (Exper – Theor) ΔV_{10} occurs at a ratio of PS/9-*cis* ≅ 1.5. After irradiation A_{10} and ΔV_{10} decrease. The largest light induced decrease observed for ΔV_{10} is 90 mV and for A_{10}, 6 Å².

11-*cis* and PS: The (Exper – Theor) A_{10} of mixed films of 11-*cis* and PS as a function of the mole fraction of PS, before and after irradiation is shown in Fig. 5. A maximum value for (Exper – Theor) A_{10} is obtained at a mole ratio of PS/ 11-*cis* = 3.

The (Exper – Theor) ΔV_{10} as a function of the mole fraction of PS is shown in Fig. 6. A maximum value for ΔV_{10} occurs at a ratio of PS/11-*cis* ≅ 2. Irradiation causes a decrease of both A_{10} and ΔV_{10}. The largest decreases observed for ΔV_{10} is 40 mV and A_{10}, 1 Å².

9-*cis* and L-cysteine: In Fig. 7 is shown ΔV_{10} and A_{10} for 9-*cis* as a function of the molar concentration of L-cysteine in the subphase, before and after irradiation. The A_{10} of 9-*cis* increases in the

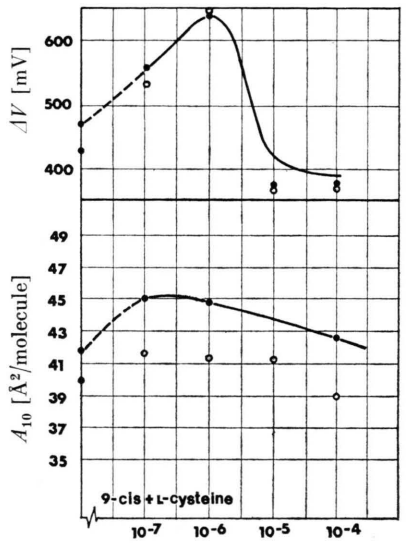
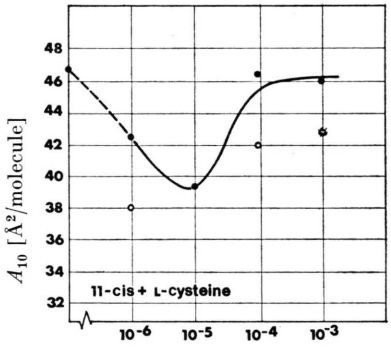


Fig. 7. The A_{10} and ΔV_{10} of 9-*cis* in dark (●) and light (○) is measured as a function of the molar concentration of L-cysteine in the subphase. The subphase and temperature same as in Fig. 1.

presence of L-cysteine and appears to reach a maximum value at a concentration of between 10^{-7} and 10^{-6} M L-cysteine. Such a concentration dependence has been observed previously for the interactions of films with materials in the subphase that are not surface active (Brockman and Brody [12] and Brody [25]).

The ΔV_{10} seems to reach a maximum at about 10^{-6} M L-cysteine then decreases sharply to a value less than that for 9-*cis* alone. Generally, illumination of the film results in a decrease in A_{10}. At subphase concentrations of 10^{-6} M L-cysteine or greater there is no effect of light on ΔV_{10}. The largest light induced decrease observed for A_{10} is 5 Å² and for ΔV_{10}, 25 mV.

11-*cis* and L-cysteine: In Fig. 8 is shown A_{10} for 11-*cis* as a function of the molar concentration of L-cysteine in the subphase, before and after irradiation. The A_{10} of 11-*cis* decreases in the presence of L-cysteine and reaches a minimum value

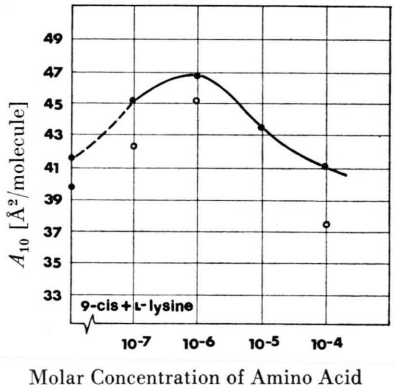


Molar Concentration of Amino Acid

Fig. 8. The A_{10} of 11-*cis* in the dark (●) and light (○) as a function of the molar concentration of L-cysteine in the subphase. Subphase and temperature same as in Fig. 1.

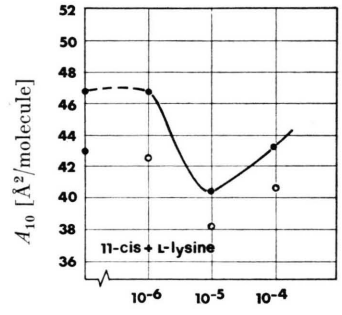
at a concentration of about 10^{-5} M L-cysteine. The ΔV_{10} shows no definite trend as a function of cysteine concentration. Irradiation of the film results in a decrease of both A_{10} and ΔV_{10}. The largest light induced decrease observed for ΔV_{10} is 80 mV and for A_{10}, 5 Å².

9-*cis* and L-lysine: In Fig. 9 is shown A_{10} for 9-*cis* as a function of the molar concentration of L-lysine in the subphase, before and after irradiation. The A_{10} of 9-*cis* increases in the presence of L-lysine and reaches a maximum value at a concentration of about 10^{-6} M L-lysine. The ΔV_{10} increases slightly as a function of the lysine concentration in the subphase. Illumination of the film results in a decrease of both A_{10} and ΔV_{10}. The largest light induced decrease observed for A_{10} is 4 Å² and for ΔV_{10}, 35 mV.



Molar Concentration of Amino Acid

Fig. 9. The A_{10} of 9-*cis* in the dark (●) and light (○) as a function of the molar concentration of L-lysine in the subphase. Subphase and temperature same as in Fig. 1.



Molar Concentration of Amino Acid

Fig. 10. The A_{10} of 11-*cis* in the dark (●) and light (○) is measured as a function of the molar concentrations of L-lysine in the subphase. Subphase and temperature same as in Fig. 1.

11-*cis* and L-lysine: In Fig. 10 is shown A_{10} for 11-*cis* as a function of the concentration of L-lysine in the subphase, before and after irradiation. The area of 11-*cis* decreases in the presence of L-lysine and reaches a minimum value with about 10^{-5} M L-lysine in the subphase. The ΔV_{10} decreases slightly in the presence of L-lysine and a minimum value of 400 mV occurs at about 10^{-5} M L-lysine. Generally, irradiation causes a decrease in both A_{10} and ΔV_{10}. The largest light induced decrease observed for A_{10} is 4 Å² and ΔV_{10}, 70 mV.

All results are summarized in Table I.

Discussion

One of the objectives of this study was to test for interactions between retinal and PL's or amino acids at pH 10.5. (At this pH Schiff base linkage should be stable.) The effect of light on the interactions between retinal and PL's or amino acids is also of interest as it may relate to the visual process.

That retinal and PL interact with one another in mixed films is shown by the difference in (Exper − Theor) both for A_{10} and ΔV_{10}, Figs 3 − 6. Thermodynamically, the value of (Exper − Theor) A_{10} is proportional to the free energy of mixing and reaction on the surface (Davis and Rideal [26]). The negative values for (Exper − Theor) A_{10} in Figs 3 and 5 shows a spontaneous reaction between retinal and PE. On the other hand, the positive values for (Exper − Theor) A_{10} in Figs 3 and 5 for retinal and PS show the reaction is not spontaneous. These findings are in agreement with those of Puppala [14] at pH 6.0, and Jacobs [27] (with chlorophyll) at pH

7.8. They also reported that PE results in a spontaneous reaction while PS does not, indicating that PE is much more reactive than PS. The positive or negative value for (Exper – Theor) A_{10} might reflect a change in orientation or interdigitation of the complex on the surface.

If interdigitation between PL's or between PL and retinal is promoted by retinal than (Exper – Theor) A_{10} would be expected to give a negative value. If, however, interdigitation between PL's is inhibited by retinal then the (Exper – Theor) A_{10} would be expected to give a positive value. The linear change of ΔV of PS indicates the "condensed" film character of PS (Fig. 2 c) and there is probably extensive interdigitation; the non-linear change of ΔV for PE (Fig. 2 b) is consistent with the "expanded" type film indicating little interdigitation (Gaines [28]). The retinal may prevent or inhibit interdigitation either by passive steric hinderance or by the presence of Schiff base linkage.

The difference in the interaction between retinal and the two PL's probably depends on the charge, polarity and the structure of the PL's and retinals. At pH 10.5 PE has a single negative charge while PS has a double negative charge (Garvin and Karnovsky [29]). That there is a difference in the charge distribution between 9-*cis* and 11-*cis* is indicated by the difference in ΔV's and also by the great difference in the light induced change in ΔV (see Table I). That there are differences in the charge distribution for the various retinals was shown theoretically by Galindo [15].

A Schiff base linkage might be considered for the interaction between retinal and PL. An optimum interaction between retinal and PL is expected at a ratio of 1:1 since Schiff base linkage involves one molecule each of retinal and PL. From the data for 9-*cis* and PS in which (Exper – Theor) A_{10} is a maximum at a mole ratio of 1:1, it is possible that Schiff base linkage occurs. Furthermore, since (Exper – Theor) A_{10} is positive there is probably inhibition of the extensive interdigitation that occurs in PS.

Experiments are planned to obtain spectral evidence testing whether or not there is formation of Schiff base linkage in the monolayer.

Interaction between 9-*cis* and PE occurs at a ratio of PE/9-*cis* = 2. One possible model for this interaction might be that 9-*cis* and one PE form a Schiff base while the second PE molecule interdigitates with either PE or 9-*cis*. Such interdigitation would result in a negative value for (Exper – Theor) A_{10}.

A more complicated type of interaction appears to occur in the other mixed films studied. A maximum interaction occurs between 11-*cis* and PE and PS at a ratio of PL/11-*cis* $\cong$ 3. Without additional information, the ratio of PL/11-*cis* $\cong$ 3 is difficult to incorporate into a simple pictorial model. Morton and Pitt [1] have shown that at alkaline pH's the Schiff base linkage of the analogue retinylidenemethylamine is stable in the presence of excess methylamine. In an analogous manner perhaps, in the reactions between 11-*cis* and PL excess PL is required to stabilize the complex between 11-*cis* and PL.

That there is an interaction between retinal and L-cysteine and L-lysine is indicated by the change in A_{10} and ΔV_{10} of retinal when these amino acids are present in the subphase. A positive (or negative) change in A_{10} of retinal might be accounted for by a change in orientation of retinal at the nitrogen/water interface upon complexation. The two retinal isomers studied interact in very different manners with amino acids in the subphase. The interaction may by a property of the conformational state, charge distribution of the retinal or orientation of the retinal on the surface.

The two amino acids used in this study were selected because lysine can only form a linkage to retinal *via* its amine while cysteine can link *via* its amine or sulfhydryl group or both. In the case of lysine only Schiff base linkage appears possible.

A charge transfer complex might also form in the case of retinal and L-cysteine. This complex would depend on the distance, between C_9 and/or C_{11} and the carbonyl group of retinal, being the same as the distance between the S and N of L-cysteine (Galindo [15]). Also, the C_9 and C_{11} and the carbonyl must be close to the aqueous surface so they are readily available for complexation. Of course, there is no possibility of such a charge transfer complex with lysine because of the absence of a sulfhydryl.

The most likely possibility for the interaction of 9-*cis* and 11-*cis* with L-lysine is a Schiff base complex. 9-*cis* and 11-*cis* react with lysine differently, *i. e.* with 9-*cis* A_{10} decreases and ΔV_{10} increases while with 11-*cis* A_{10} increases and ΔV_{10} decreases.

With interactions of retinal and L-cysteine there is the possibility of Schiff base linkage plus charge transfer and thiazolidene complexation. These various possibilities are not distinguishable from these experiments except that the interactions of 9-*cis* and L-cysteine are similar to those of 9-*cis* and L-lysine since both result in an increase in A_{10}. Also, the interactions of 11-*cis* and L-cysteine appear similar to those of 11-*cis* and L-lysine (both result in a decrease in A_{10}) so that Schiff base linkage may also be the main reaction for cysteine as well as lysine at pH 10.5. It should be possible to resolve these possibilities by measuring the spectrum of the films.

The light induced changes in ΔV are of particular interest. Such changes in potential could rapidly modify membrane permeability. With such a mechanism it might be possible to trigger the visual process by the primary photoreaction rather than after some biochemical steps. Light induced changes in both A and ΔV of retinal may also result in large conformational changes in rhodopsin.

From Table I it can be seen that at pH 10.5 where Schiff base linkage can occur readily the largest light induced changes in ΔV occur with 9-*cis* and PS (-90 mV), 11-*cis* and L-cysteine (-80 mV) and 11-*cis* and L-lysine (-70 mV).

On the basis of the light induced change of ΔV, interaction with L-lysine, L-cysteine and PE does not appear to modify the photoproperties of 9-*cis*. In all cases irradiation changes ΔV about -30 mV (Table I). Only with 9-*cis* and PS, where a maximum interaction occurs at PS/9-*cis* $\cong 1$, is there a significantly different effect of light on ΔV (-90 mV).

With 11-*cis*, on the other hand, interactions result in a significant difference in the light induced change in ΔV. Irradiation of films of only 11-*cis* results in an increase in ΔV, however, upon interaction with PL and amino acids irradiation results in a decrease in ΔV. The effect of light on complexes of 11-*cis* and amino acids is larger than those of 11-*cis* and PL's. The significance of these observations to the *in vivo* case cannot be fully evaluated until the binding of retinal in rhodopsin and the moieties surrounding retinal are determined. In any event there is a wide range of light induced voltage change possible for visual excitation from the various retinal complexes. It might be of interest to consider these differences with respect to color vision and in other visual systems. It remains to be seen how the specific interactions between retinal and PL's or amino acids at alkaline pH relate to the *in vivo* situation.

This work was supported, in part, by a research grant from the National Institut of Health (ROI EY-00173).

[1] R. A. Morton and A. J. Pitt, Biochem. J. **59**, 128 [1955].
[2] G. A. Pitt, F. D. Collins, R. A. Morton, and P. Stok, Biochem. J. **59**, 122 [1955].
[3] S. Ball, F. D. Collins, P. D. Dalvi, and R. A. Morton, Biochem. J. **45**, 304 [1949].
[4] F. D. Collins, R. M. Love, and R. A. Morton, Biochem. J. **51**, 292, 669 [1952].
[5] B. Rosenberg and T. M. Krigas, Photochem. Photobiol. **6**, 769 [1967].
[6] T. Toth and B. Rosenberg, Vis. Res. **8**, 1471 [1968].
[7] F. J. Daemen and S. L. Bonting, Nature **222**, 879 [1969].
[8] R. P. Poincelot, P. G. Millar, R. L. Kimbel, and E. W. Abrahamson, Nature **221**, 256 [1967].
[9] R. P. Poincelot, P. G. Millar, R. L. Kimbel, and E. W. Abrahamson, Biochem. **9**, 1809 [1970].
[10] M. Akhtar and M. D. Hirtenstein, Biochem. J. **115**, 607 [1969].
[11] R. S. Fager, P. Sejnowski, and E. W. Abrahamson, Biochem. Biophys. Res. Comm. **47**, 1244 [1972].
[12] R. E. Brockman and S. S. Brody, Z. Naturforsch. **26 b**, 119 [1971].
[13] S. S. Brody, Z. Naturforsch. **28 c**, 97 [1973].
[14] N. Puppala, Master's Thesis in Department of Biology, New York University, 1973.
[15] I. G. Galindo, Bull. Math. Biophys. **29**, 677 [1967].
[16] M. Akhtar, P. T. Blosse, and P. B. Dewhurst, Biochem. J. **110**, 693 [1968].
[17] J. C. Peskin and B. B. Love, Biochim. Biophys. Acta **78**, 751 [1963].
[18] K. Mizuno, K. Ozawa, and Y. Kuno, Exp. Eye Res. **5**, 276 [1966].
[19] D. Bownds and G. Wald, Nature **205**, 254 [1965].
[20] D. Bownds, Nature **216**, 1178 [1967].
[21] M. Akhtar, P. T. Blosse, and P. B. Dewhurst, Chem. Comm. **1967**, 631.
[22] J. Heller, Biochem. **7**, 2914 [1968].
[23] J. Aghion, S. Broyde, and S. S. Brody, Biochem. **8**, 3120 [1969].
[24] R. Paoletti and D. Kletchevsky, Adv. Lipid Res. **8**, 348 [1970].
[25] S. S. Brody, Z. Naturforsch. **26 b**, 134, 922 [1971].
[26] J. T. Davis and E. K. Rideal, Interfacial Phenomena, Academic Press, New York 1963.
[27] R. Jacobs, Ph. D. Thesis in Department of Biology, New York University 1972.
[28] G. L. Gaines, Insoluble Monolayers at a Liquid-Gas Interface, Interscience Pub., 1966.
[29] J. E. Garvin and M. L. Karnovsky, J. Biochem. **221**, 211 [1955].