Conformational Changes in Rhodopsin Probed by Surface Plasmon Resonance Spectroscopy†

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Received June 15, 1994; Revised Manuscript Received August 31, 1994⊗

ABSTRACT: Surface plasmon resonance (SPR) spectroscopy has been used to follow incorporation and light-induced conformational changes in bovine rhodopsin reconstituted into an egg phosphatidylcholine bilayer deposited on a thin silver film. The magnitude of the SPR spectral changes caused by light varies with pH in a manner paralleling that in flash photolysis experiments, which monitor formation of metarhodopsin II. Irradiation produces an increase of approximately 4 Å in the average thickness of the proteolipid layer, consistent with exposure of recognition sites for the G protein. The results demonstrate that the SPR technology described herein may be used to monitor conformational events in membrane-associated receptors such as rhodopsin.

The visual photoreceptor pigment rhodopsin is found in the disk membranes of the rod outer segments (ROS) of the retina and functions as a signal transducer by converting light energy to an intracellular, G protein-mediated enzyme cascade (Fung et al., 1981; Stryer, 1987, 1991; Chabre & Deterre, 1989; McNaughton, 1990; Pugh & Lamb, 1990). Absorption of a photon of yellow light (λ = 498 nm) by this chromoprotein triggers a sequence of thermal decay steps characterized by distinct absorption maxima. Two important intermediates are metarhodopsin I and II (MI and MII), which are present in a pH-dependent equilibrium on the millisecond time scale in native ROS membranes (Wald, 1968; Applebury et al., 1974). Rhodopsin is thought to undergo a conformational change during the MI−MII transition, which facilitates binding of the G protein (Hargrave, 1982; Franke et al., 1988, 1990; Kibelbek et al., 1991; Dratz et al., 1993), and subsequent activation of phosphodiesterase, signal amplification, and ultimately transmission of a nerve impulse. The ratio MII:MI which is present following an actinic flash is therefore indicative of the ability of rhodopsin in a membrane lipid recombinant to function properly *in vitro*. Interaction of rhodopsin with the surrounding lipid appears to be important for its conformation and function (Applebury et al., 1974; Lamola et al., 1974; O'Brien et al., 1977; Beach et al., 1984; Baldwin & Hubbell, 1985a,b; Wiedmann et al., 1988; Farahbakhsh et al., 1992; Gibson & Brown, 1991, 1993; Brown & Gibson, 1992). In order to further investigate the interaction between rhodopsin and the solvating lipid, as well as to study the conformational transitions of the protein and binding of the G protein to MII and subsequent G protein activation (Hargrave, 1982; Franke et al., 1988, 1990; Kibelbek et al., 1991; Dratz et al., 1993), we have utilized a novel experimental approach, surface plasmon resonance (SPR) spectroscopy. A protrusion of rhodopsin from the membrane at the MII stage of photolysis has been detected, consistent with exposure of recognition sites for the signal-transducing G protein. The results demonstrate the potential of this new methodology to provide important insights into the structural biology of integral membrane proteins in general, and especially of the large family of membrane receptors which interact with G proteins, of which rhodopsin is an important paradigm (Stryer & Bourne, 1986; Gilman, 1987; Taylor, 1990; Hargrave & McDowell, 1992).

In other laboratories, the SPR technique has been applied to investigate the optical properties of thin film coatings (Rothenhauser et al., 1988; Lloyd et al., 1988), to metal−electrolyte interfaces (Rothenhauser et al., 1988; Abeles, 1976), and to specific recognition reactions at self-assembled monolayers on gold (Pockrand et al., 1977; Hickel & Knoll, 1990; Haussling et al., 1991; Morgan et al., 1992; Schuster et al., 1993; Terretaz et al., 1993; Swanson et al., 1993). The first biological application of this method involved analysis of arachidate monolayer assemblies on silver films (Pockrand et al., 1977). Since that time SPR has been used to optically characterize lipid monolayers, chemisorbed protein multilayers, and a variety of protein−protein interactions (Swanson et al., 1993; Bondeson et al., 1993). In previous work from this laboratory involving SPR measurements (Salamon et al., 1994), we have characterized the structural properties of self-assembled solid-supported planar lipid membranes composed of egg phosphatidylcholine (PC), which we have previously used in electrochemical measurements (Salamon & Tollin, 1991, 1992; Salamon et al., 1992, 1993). The results were consistent with the formation of durable and homogeneous lipid bilayers. Here we apply this technique to bovine rhodopsin molecules recombined into a solid-supported planar lipid bilayer membrane composed of PC. We report experiments which monitor (i) the process of rhodopsin reconstitution into a thin silver film supported lipid bilayer membrane composed of egg PC, (ii) the occurrence of conformational alterations of the protein and the lipid membrane as a result of rhodopsin photoexcitation, and (iii) the effect of hydroxylamine on the rhodopsin MII

† This work was supported in part by a grant from the Vice-President for Research, University of Arizona, and by NIH Grant EY03754 to M.F.B.

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⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1994.

0006-2960/94/0433-13706$04.50/0

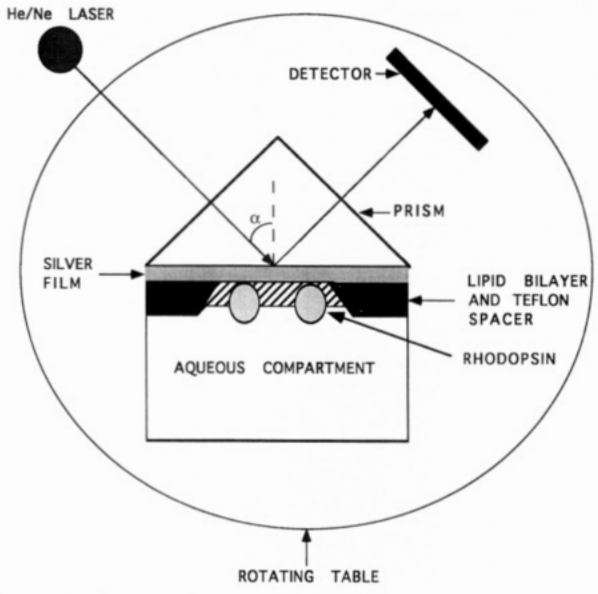

FIGURE 1: Cross-sectional top view of the experimental geometry employed for surface plasmon resonance studies of self-assembled lipid bilayer membranes containing rhodopsin.

state. The SPR spectral changes are interpreted within the context of the optical parameters which describe the structure of the lipid−protein layer, and they demonstrate the occurrence of protein−lipid conformational changes associated with the formation of the MII state of rhodopsin and the conversion of MII to opsin plus retinal by hydroxylamine treatment. The pH dependence of the SPR effects is similar to that measured for MII formation by conventional flash spectroscopy methods (Gibson & Brown, 1991, 1993; Brown & Gibson, 1992).

MATERIALS AND METHODS

Bovine ROS membranes containing rhodopsin were prepared and characterized as previously described (Gibson & Brown, 1993; Salamon et al., 1993). All rhodopsin samples were handled in dim red light (Kodak safelight filter no. 1, 15-W bulb) and kept under an argon atmosphere when possible; all measurements were done at ambient temperature (23 ± 1 °C).

SPR measurements of membranes deposited on a silver film were performed using the attenuated total reflection technique, which involves rotation of the prism using a rotating table with a programmable controller [Model 855C, Newport Corp.; angular resolution, better than 0.01°] and a laser light source (helium−neon CW laser; wavelength, 6328 Å) mounted on the fixed arm of a goniometer. The configuration of the optical elements together with the aqueous sample compartment [volume, 1.5 mL; cf. Salamon and Tollin (1993) and Salamon et al. (1994) for more detailed descriptions of the cell] is shown in Figure 1. The photocurrent output from the light detector (silicon solar cell) was digitized by a Heath-Zenith SD 4850 digital oscilloscope, and the data were transferred to the hard disk of a computer. The optical arrangement allows an SPR spectrum to be measured over a 20° rotation in approximately 10 s.

Lipid membrane films were formed by spreading 4 μL of a freshly prepared lipid solution containing 10 mg/mL egg PC (Sigma Chemical Co.) in squalene (Fluka Chemie AG)/butanol (0.5:10, v/v) across an orifice in a Teflon spacer sheet (4 mm in diameter), which separated the silver film from the aqueous phase. Subsequently, 5 mM sodium phosphate buffer at the desired pH was added. After 10−15 min the SPR spectrum of the self-assembled lipid bilayer membrane was measured. Such films were found to be stable for many hours under the conditions of the experiment [cf. Salamon and Tollin (1993) and Salamon et al. (1994)]. Small aliquots of a concentrated solution of solubilized bovine ROS membranes in 200 mM octyl glucoside were added to the aqueous compartment of the SPR cell, thereby diluting the rhodopsin and detergent by a factor of about 100 to a final concentration below the critical micelle concentration of the detergent (25 mM). The SPR spectrum was then recorded over a 20−30-min interval (i.e., until the spectrum stabilized).

In order to photolyse rhodopsin, the SPR cell was illuminated from the back side through a fiber optic light guide using a Sunpak AP-52 flash unit, fitted either with a Schott OG 515 filter (wavelength > 500 nm, i.e., yellow light, which converts rhodopsin to MII) or with a blue filter (transmission band between 300 and 430 nm, which photoregenerates rhodopsin plus isorhodopsin from MII). After a saturating number of yellow light flashes, aliquots of a stock solution of hydroxylamine were added to the aqueous compartment of the SPR cell to a final concentration of 0.5 mM. The hydroxylamine reacted with any photolyzed rhodopsin produced as a result of the flashes, to hydrolyze the retinal−lysine Schiff base linkage and produce the apoprotein (opsin) plus retinal oxime (absorption wavelength, 365 nm). The optical parameters of the proteolipid membrane adsorbed on the silver film were obtained by fitting a theoretical curve to the experimental SPR curve, using a nonlinear least squares technique (Liddel, 1981; Tang & Zheng, 1982; Zhang et al., 1987). The standard deviation of such fits varied between 0.010 and 0.025.

RESULTS AND DISCUSSION

Lipid−Protein Film Structure Determination by SPR Spectroscopy. A surface plasmon can be described as a fluctuation of electron density propagating along a metal surface (Figure 1). Plasmons can be excited by photons, inasmuch as light passing from a higher to a lower refractive index medium under total reflection conditions creates an evanescent electromagnetic field of about 100-nm depth in the latter material. Within a metallic film, the field polarized parallel to the incident plane excites collective oscillations of the free electrons within the metal (i.e., surface plasmons). These in turn generate an electromagnetic field which propagates along the interface between the two media with an amplitude that decays exponentially on either side of the interface, a situation readily described theoretically using Maxwell's equations (Macleod, 1986, 1992; Salamon et al., 1994). A resonance condition exists for the excitation of surface plasmons which is fulfilled by varying the incident angle (labeled α in Figure 1) at a fixed excitation wavelength; the phenomenon is exceedingly sensitive to the optical conditions at the surface. Inasmuch as resonance causes the incident photons to be absorbed by the plasmon wave, less energy is thereby totally internally reflected and the reflected energy reaches its minimum; thus measurement of light reflectance as a function of incident angle produces the SPR curve. Any surface modification in the immediate vicinity of the metal−electrolyte interface, such as deposition of a lipid bilayer or adsorption of a protein, will change the resonance condition by shifting the position of the minimum

and altering the shape of the resonance curve. Electromagnetic theory (Macleod, 1986, 1992) shows that the properties of the SPR curve depend on three optical parameters: the refractive index (n), the extinction coefficient (k), and the thickness (t) of the metal film plus the dielectric medium with which it is in close contact.

In our experiments, the incident medium was a 90° glass prism ($n = 1.515$) upon which a thin layer of metallic silver was vacuum deposited, as shown in Figure 1. Coating the silver film with a lipid membrane and incorporating proteins into this lipid layer introduces a new interface, which influences the resonance properties of the plasmon wave and thus allows the properties of these materials to be monitored. As demonstrated by previous studies (Salamon et al., 1993, 1994), the properties of the lipid film are consistent with those expected of a bilayer structure. With regard to the SPR parameters, three sources of experimental error are important. First, there is the error in the determination of the incident angle ($\Delta\alpha$). In our case, $\Delta\alpha \approx 0.01°$, which translates into thickness and refractive index errors of 0.5 Å and 0.005, respectively. Second, there is the error which is related to the precision of the theoretical fitting (the standard deviation is between 0.010 and 0.025), which is relatively small. Probably the largest error in the absolute values of the parameters derives from variations of both the lipid bilayer and the amount of rhodopsin incorporated into the bilayer. This is reflected in the scattering of the experimental shifts and the shape changes of the SPR spectra between different samples. Such scattering produced errors in thickness of $\Delta t = \pm 1$ Å, in refractive index of $\Delta n = \pm 0.02$, and in extinction coefficient of $\Delta k = \pm 0.02$. Consequently, SPR spectroscopy allows the determination of the spatial dimensions and the optical properties of thin membrane films with high accuracy.

SPR Spectral Changes Observed upon Incorporation of Rhodopsin into a Solid-Supported Planar Lipid Bilayer Membrane. The SPR spectral changes which occurred upon addition of rhodopsin involved a shift of the resonance minimum toward larger values of the incident angle, an increase in the depth of the resonance, and an increase of the half-width of the spectrum. These changes were observed to saturate as the rhodopsin concentration increased; i.e., beyond this point further additions of rhodopsin did not alter the spectrum (data not shown). Typical SPR spectra (obtained at pH 4.5) are presented in panel a of Figure 2. It should be noted that, prior to illumination of the sample, neither the spectra nor the response to rhodopsin concentration change with pH over the pH range 4.5−7.0 (not shown). Curve 1 is the SPR spectrum for a silver film in contact with buffer solution, curve 2 is the resonance curve after deposition of an egg PC bilayer, and curves 3 and 4 represent the SPR spectra after two incremental additions of detergent solutions of bovine ROS membranes to the aqueous compartment. By fitting theoretical SPR curves to these experimental data, the three optical parameters that describe the physical properties of the metal−water interface region, i.e., the average thickness and refractive index (t, n) of the proteolipid layer and the extinction coefficient (k), can be evaluated. In panel b of Figure 2 (curve 1) an example of such a fit is depicted, using curve 4 of Figure 2a plotted on an expanded incident angle scale to demonstrate the precision of the fitting procedure (for this fit the least squares standard deviation is 0.01). Also shown in panel b of Figure 2 are

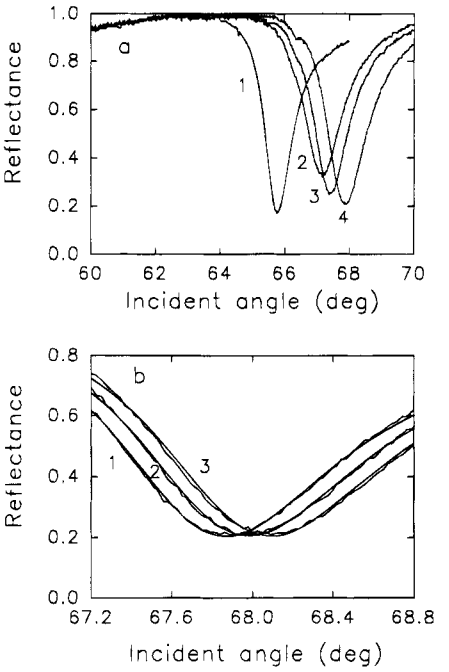

FIGURE 2: Typical SPR spectra obtained with a 48 nm thick silver film in contact with 5 mM sodium phosphate buffer solution (pH 4.5; $T = 23 \pm 1$ °C) in the aqueous compartment (see Figure 1). Panel a: Bare metal in contact with buffer solution (curve 1), coated with a lipid (PC) bilayer membrane (curve 2), and after two incremental additions of rhodopsin to the aqueous compartment (curves 3 and 4; final total concentrations in the aqueous phase were 3 and 7 μM, respectively). Panel b: Curve 1 corresponds to curve 4 from panel a; curves 2 and 3 were obtained after saturating flashes of yellow light (curve 2) and after addition of 0.5 mM (final concentration) hydroxylamine to the aqueous compartment (curve 3). Smooth curves represent theoretical fits (see text).

SPR spectra (along with theoretical fits) obtained after multiple sequential flashes of yellow light (curve 2) and after addition to the illuminated sample of 0.5 mM (final concentration) hydroxylamine (curve 3). The shifts of the resonance minima to larger values caused by these treatments are clearly apparent, as is the excellent quality of the theoretical fits. These results will be further discussed below.

The optical parameters obtained from theoretical fits to the SPR spectra as a function of rhodopsin concentration at all pH values allow several important quantitative conclusions to be drawn. First, the values of all three parameters saturate with rhodopsin concentration (between 2 and 6 μM rhodopsin in the total aqueous phase), indicating that incorporation of rhodopsin into the lipid bilayer leads to the formation of a well-defined final structure. Second, the average thickness of the lipid film increases from 7.1 nm for a pure lipid layer to 8.8 nm at the saturating concentration of rhodopsin (Table 1). This t value for the pure lipid bilayer includes not only the hydrocarbon region of the lipid but also the fully hydrated lipid polar headgroups (Nagle & Wiener, 1988; Wiener & White, 1992; Salamon et al., 1994), and it is consistent with values determined by X-ray and neutron scattering and by NMR measurements (Nagle & Wiener, 1988; Wiener & White, 1992; Blaurock, 1982) for bilayer films made of lipid molecules of comparable sizes. The refractive index also increases from the pure lipid bilayer value of 1.62 [which again agrees very well with an average value of 1.61 measured with freely suspended bilayer membranes (Tien & Diana, 1968)] to 1.70 at saturation. Finally, the extinction coefficient, which represents losses

Table 1: Calculated SPR Parameters for Rhodopsin Proteolipid Layer

sample[a]	t (Å)	n	k
lipid alone	71	1.62	0.063
+ rhodopsin	88	1.70	0.051
+ light (yellow)	92	1.69	0.049
+ light (blue)	89	1.70	0.047
+ light (yellow)	92	1.70	0.050
+ hydroxylamine	94	1.71	0.052

[a] See text and figure captions for experimental details. Measurements were made at pH 5.5 and 23 ± 1 °C.

of light energy due to processes other than SPR itself (Macleod, 1986, 1992), decreases with increasing rhodopsin concentration to a limiting value of 0.049. This latter parameter cannot be due to the absorption of SPR excitation light by either PC or rhodopsin molecules, because the wavelength of the helium–neon laser (632.8 nm) is too far removed from the absorption bands of both of these materials. Therefore, the most likely possibility is scattering of light by the proteolipid matrix structure. A similar effect has been observed in SPR studies of Langmuir–Blodgett films of long-chain fatty acid derivatives (Wijekoon et al., 1992).

In order to understand the changes in optical parameters induced by rhodopsin incorporation, one can use a Lorentz–Lorenz relationship between mass, refractive index, and thickness of an adsorbed layer (Born & Wolf, 1965; Salamon et al., 1994), as follows:

$$(n^2 - 1)/(n^2 + 2) = A_1N_1 + A_2N_2 + A_3N_3 + \ldots \quad (1)$$

where A_i and N_i are the molar refractivity of substance i and the number of moles of substance i per unit volume, respectively, and n is the refractive index. For a pure substance we may write

$$\sigma = MN = (M/A)[(n^2 - 1)(n^2 + 2)] \quad (2)$$

where σ is the density in mass per unit volume and M is the molar mass. Therefore, for an adsorbed layer of thickness t, the adsorbed mass (m) of a pure substance will be given by

$$m = \sigma t = 0.1(M/A)t[(n^2 - 1)/(n^2 + 2)] \quad (3)$$

where the thickness t is expressed in nanometers and the adsorbed mass m is expressed in micrograms per square centimeter. Equation 3 allows the calculation of an adsorbed mass of lipid or protein from the refractive index and thickness of an adsorbed layer, knowing the molar mass and the molar refractivity. In general, the molar refractivity of any mixture of molecular species can be obtained from the known values of its constituents. We can, therefore, estimate the mass of a PC bilayer, assuming (Cuypers et al., 1983) $M/A = 3.6$ and using the values of t and n obtained for a pure lipid bilayer membrane as above discussed. Such a calculation yields not only the total lipid mass adsorbed on the silver surface but also a mass sensitivity ($\Delta\alpha_s$) for our SPR system. This latter parameter is defined as an amount of mass per unit surface area per unit incident angle. On the basis of the value of $\Delta\alpha_s$, we can then estimate the mass of rhodopsin incorporated into the lipid bilayer, using the incident angle change from the SPR spectra ($\Delta\alpha_{rho}$) caused

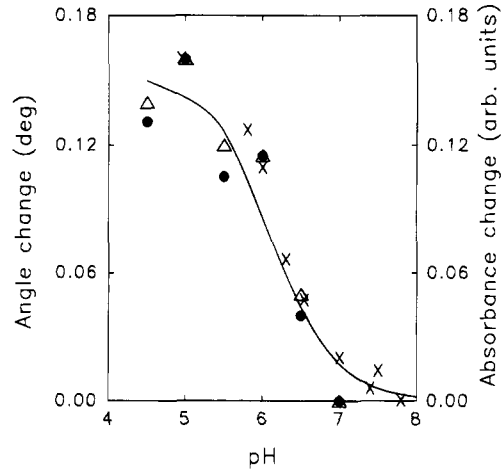

FIGURE 3: Dependence on pH of the changes in the SPR resonance angle observed in recombinant rhodopsin/egg PC films upon yellow light irradiation and hydroxylamine addition. All other conditions were as in Figure 2. Also shown are values (normalized at pH 5.0) of the absorbance changes obtained (Gibson & Brown, 1993) with recombinant rhodopsin/egg PC vesicles in flash photolysis experiments. The closed circles, open triangles, and crosses represent the yellow light, hydroxylamine, and flash photolysis results, respectively. The solid curve through the data points corresponds to the Henderson–Hasselbalch equation with an apparent pK value of 6.1. The pK value obtained from the flash photolysis data (Gibson & Brown, 1993) is 6.3.

by rhodopsin reconstitution. From measurements carried out at pH 4.5, the mass sensitivity is 0.59 ng/mm^2/0.1°, and the concentrations of PC and rhodopsin present on the deposited silver film are 1.67 and 0.0149 M, respectively. Note that the molar ratio of PC to rhodopsin is 112, which is very similar to that obtained with the lipid vesicles used in flash spectroscopy (Gibson & Brown, 1993).

SPR Changes Induced by Light and Hydroxylamine. As can be seen from Figure 2, both yellow light (Schott OG 515 filter with a wavelength cutoff of 515 nm) flash irradiation and hydroxylamine addition induce further shifts of the resonance curve toward larger incident angles. Although we cannot directly determine the amount of rhodopsin bleached by these treatments, the SPR changes saturate with increasing irradiation and/or hydroxylamine addition (not shown). Figure 3 shows the experimental maximal shifts (obtained under saturation conditions) of the incident angle resonance position caused by rhodopsin irradiation as a function of pH. Superimposed on these data are previously obtained flash photolysis results (Gibson & Brown, 1991, 1993; Brown & Gibson, 1992), normalized at pH 5.0, which represent the MII:MI ratio produced in recombinant egg PC vesicles as a function of pH. It is clear that the SPR changes upon yellow light irradiation have the same pH dependence as the flash photolysis absorbance changes, strongly indicating that these two techniques are measuring the same phenomenon, i.e., the formation of the MII state of rhodopsin. Further evidence supporting this conclusion is found in the effect of hydroxylamine, which is known to interact only with the MII form to produce opsin plus retinal (Falk & Fatt, 1968). Therefore, the hydroxylamine effects should correlate with the yellow light changes, as indeed they do (Figure 3). Table 1 presents the theoretical values of the optical parameters obtained from fitting the SPR curves obtained at pH 5.5 in these experiments. It is evident from these data that both yellow light and hydroxylamine cause increases of the average thickness of the

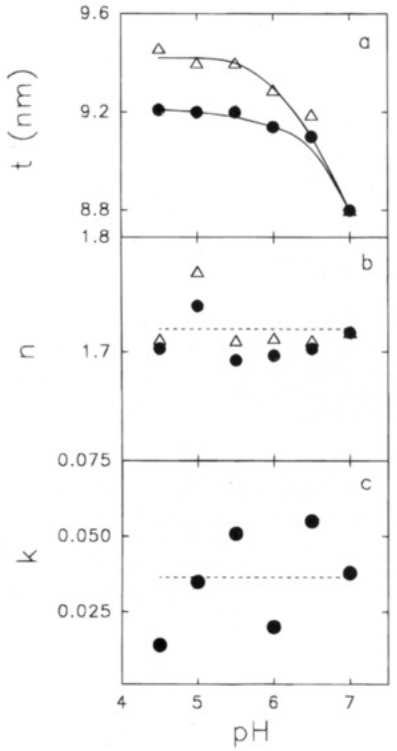

FIGURE 4: Optical parameters (t, n, and k) obtained from theoretical fits to the SPR curves obtained in the experiments of Figure 3. The closed circles and open triangles represent the yellow light and hydroxylamine results, respectively. In panel c only a single symbol (closed circles) is shown, inasmuch as the values of the parameters in all experiments were the same within experimental error. All conditions were as in Figures 2 and 3.

proteolipid layer, without appreciable changes in the other two parameters (see below for further discussion).

Additional support for the conclusion that the SPR results are due to MII formation is found in the effect of flash irradiation with blue light (transmission band of filter between 300 and 430 nm), which is known to reverse the effect of yellow light, thus causing back reaction of MII to yield rhodopsin plus isorhodopsin (9-*cis*-retinol chromophore). As can be seen in Table 1, blue light irradiation subsequent to yellow light produces a decrease of the average thickness of the proteolipid membrane to 8.9 ± 0.1 nm, which is almost its initial value prior to irradiation. It should also be noted that further yellow light irradiation of this sample restores the original thickness (Table 1), clearly demonstrating the photoreversibility of the rhodopsin changes observed in these experiments.

Theoretical fitting of the experimental SPR spectra for both light and hydroxylamine produces a set of optical parameters, which are shown in Figure 4 (panels a−c). There are several conclusions which can be drawn from these results. As noted, for both treatments the effect on the SPR spectrum is mainly due to increases in the average thickness of the proteolipid membrane (Figure 4, panel a). The small changes in the refractive index value (Figure 4, panel b) are within the range of our experimental error (±0.02). It is also difficult to discern any pattern in the small changes in k (Figure 4, panel c).

Structural Interpretation of SPR Changes Induced by Light And Hydroxylamine. The changes in the optical parameters induced either by light or by addition of hydroxylamine can be discussed in the context of eq 3. An increase of t with a

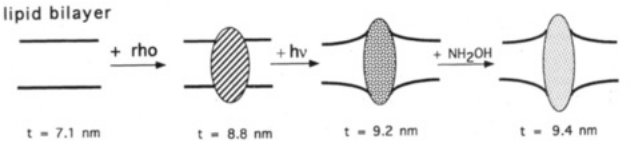

FIGURE 5: Schematic interpretation of the changes induced in the dimensions of the surface proteolipid bilayer upon rhodopsin incorporation, yellow light irradiation, and hydroxylamine addition. Upon rhodopsin incorporation, the average thickness of the film deposited on the silver surface increases from 7 to 8.8 nm. In response to light and hydroxylamine, structural changes occur in both the lipid and protein components, resulting in further increases in the average thickness of the proteolipid layer.

practically unchanged value of n indicates strongly that the yellow light irradiation induces an alteration of the shape of the rhodopsin molecule, probably involving an elongation. Such an alteration of the protein shape must in turn generate changes in the mass distribution within the proteolipid membrane and aqueous buffer interface regions. The "empty" space which is produced by elongation of the protein has to be filled either by buffer solution or by changes in the proteolipid matrix itself. The constant value of n during this conformational change strongly indicates that the average water contribution to the lipid−protein layer does not change significantly, and that "spillover" of lipid and protein mass, rather than aqueous solution, which has much lower value of n (≈1.33), seals the empty space produced in the matrix by elongation of the protein. In a more general model, one can suppose that lipid mass not only seals the space after elongation of the protein but also relocates together with protein, thereby changing the shape of the lipid−aqueous interface, as represented schematically in Figure 5. We conclude, therefore, that the change of the average proteolipid layer thickness upon yellow light irradiation represents both lipid bilayer and protein alterations. On the basis of these results, one can propose that intrahelical loops become more exposed during the MI−MII transition, yielding a protrusion from the membrane and increased exposure of the chromophore and of recognition sites for G protein binding. This is consistent with other evidence indicating that the chromophore is more accessible in the MII state (Dratz et al., 1993; Gilman, 1987; Hargave & McDowell, 1992).

It is noteworthy that a somewhat different situation exists for the changes in the optical parameters t and n caused by hydroxylamine addition (again no significant changes in k are obtained; Figure 4, panel c). The SPR resonance angle shift due to hydroxylamine is very similar to that caused by yellow light (see Figure 3), indicating that the mass changes due to both effects are similar (see eq 3). However, the thickness changes (Figure 4, panel a) are smaller, whereas the refractive index values appear to be slightly larger (Figure 4, panel b), than the corresponding values obtained with yellow light. These results indicate further alterations in the protein dimensions, which are similar in direction to those described above for the light effect, with an additional small average n value increase, perhaps due to the removal of the chromophore from opsin as a result of hydroxylamine interaction. These changes are consistent with a further opening of the protein, increasing its protrusion from the membrane. Figure 5 depicts a schematic interpretation of the structural changes observed here upon rhodopsin incorporation into the lipid bilayer, upon yellow light irradiation, and upon hydroxylamine treatment.

The results described above clearly illustrate the applicability of SPR spectroscopy to the investigation of rhodopsin structure−function relations, and by implication to G-coupled membrane-bound receptor systems in general. This technique provides information which is complementary to that obtained using flash photolysis spectrophotometry, in that it can directly monitor proteolipid conformational alterations which accompany biochemical function. Surface plasmon resonance spectroscopy is also readily applicable to measurements of the kinetics and thermodynamics of the larger effects observed upon protein−ligand binding (Terrettaz et al., 1993), a facet of this technique which we intend to pursue in future studies of the MII−G protein interaction.

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