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Retinal Schiff base position relative to the surfaces of photoreceptor disk

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Surface-enhanced Raman spectra of native and photobleached bovine rod outer segment disks as well as inside-out (inverted) photoreceptor disks adsorbed on silver hydrosol have been analyzed. Surface-enhanced spectra of inverted disks and disk-monoclonal antibody complexes reveal the short-range mechanism of enhancement. The distance between retinal Schiff base and the cytoplasmic side of native disk has been shown to be 5–10 Å.

Rod outer segment disk; Surface-enhanced Raman scattering; Retinal; Schiff base; Rhodopsin

1. INTRODUCTION

Rhodopsin, an intrinsic membrane protein in retinal rod outer segments (ROS), plays a key role in visual transduction [1]. Vision is initiated when a photon strikes rhodopsin and causes isomerization of the 11-*cis*-retinal chromophore to the all-*trans* isomer [2]. Isomerization and charge separation near the aldimine bond ensue conformational changes on the cytoplasmic side of the photoreceptor membrane. The protein structure perturbation accelerates proteolysis of the 12-membered C-terminal fragment [3] and provides high accessibility of a SH-group to modification [4]. As a result rhodopsin activates an enzyme cascade at its cytoplasmic surface [5]. Still the problem of transduction of conformational changes from the chromophore to the membrane surface is unresolved, partly for lack of clear data on spatial location and orientation of the retinal chromophore at different stages of visual reception.

Fluorescence energy transfer measurements of disk membrane vesicles containing Tb^{3+} dipicolinate in the inner and outer aqueous space provided information on the transverse location of the retinal chromophore far from both aqueous surfaces of the membrane [6]. However, nonspherical shape of the vesicles and probable donor interaction with the exposed part of the polypeptide chain can seriously reduce the accuracy of calculations.

This paper presents data on the chromophore location in native rhodopsin obtained by surface-enhanced Raman (SER) spectroscopy.

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2. MATERIALS AND METHODS

Photoreceptor disks were isolated as described in [7]. Apomembranes, free of retinal oxime, were prepared in the following way. Native disks were suspended in 50 mM hydroxylamine and 50 mM NaCl and illuminated with a 800-W lamp KGM-800 (USSR). The light was passed through a GS-18 filter (USSR). The membranes were washed with water by repeated centrifugation. To remove the retinal oxime the sample was lyophilized and washed with hexane. The apomembranes thus obtained were sonicated for 3 min at 15°C using a 22-kHz ultrasonic disintegrator USDN-1 (USSR).

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Inverted photoreceptor disks were formed and tested as described in [8]. Concentration of rhodopsin in native disks, inverted disks, and apomembranes was registered on a Cary-219 spectrophotometer. Corresponding molar extinction coefficients were taken from [7,8].

Silver hydrosols were prepared by reduction of $AgNO_3$ with $NaBH_4$ [9]. All chemical reagents were of analytical grade. Double distilled water was degassed by He for 20 min. Hydrosol-photoreceptor disk complexes were prepared in the dark by adding 10 μl of disk water suspension (rhodopsin concentration was equal to 10^{-5} M) to 1 ml of hydrosol. The mixture was stored in the dark for 10–15 min. The complexes obtained were fairly stable and the Raman spectra were reproducible during 2–3 days.

Electron micrographs of silver colloid-photoreceptor disk complexes were obtained using a Jeol GEM-100-CX-2 electron microscope (Japan). Negative contrasting was performed with 2% water solution of uranyl acetate. The mean number of Ag-particles bound to the disk membrane is about 100 (fig.1). The membrane area per rhodopsin molecule and the average distance between neighbouring protein molecules are known to be approx. 800 Å^2 and 56 Å [10]. The area of interaction of an Ag-micelle (diameter 150–200 Å) with a disk surface is about 10^5 Å^2. So, each metal particle adsorbed on a disk membrane contacts ~10 molecules of rhodopsin.

Resonance Raman (RR) and SER spectra were acquired by exciting with a 514.5 nm line from a Spectra Physics, model 164-03 (USA), Ar^+-laser. For details of the Raman instrumentation see [11,12]. RR and SER spectra were reproduced in independent experiments to control the stability of the experimental conditions.

Monoclonal antibodies to the C-end of the rhodopsin molecule were obtained as described in [13].

3. RESULTS AND DISCUSSION

Upon illumination (λ = 500 nm) of the water suspension of photoreceptor disks the chromophore and its protein environment undergo a number of conformational perturbations leading to rhodopsin photolysis [5]. They may be registered using RR spectroscopy with excitation wavelength within the chromoprotein absorbance band. The RR intensity of rhodopsin diminishes during pigment photolysis, whereas the SER intensity of sol-disk complexes does not change upon laser illumination (line 514.5 nm) for several hours. Thus unlike the native pigments, the adsorbed rhodopsin cannot undergo photoinduced transformations.

SER spectra of photoreceptor disks and apomembranes (fig.2a) revealed that all spectral bands belong to the chromophore vibrations upon adsorption. There is a good correlation between λ_{max} values of both protonated and unprotonated Schiff bases, as well as rhodopsin and bacteriorhodopsin intermediates, and their C=C stretching frequency (fig.2b) [14]. According to fig.2b the ethylenic (C=C) stretching frequency observed in the SER spectrum of photoreceptor disks (1550 cm^{-1}) corresponds to that of chromoprotein with λ_{max} = 500 nm. Therefore ad-

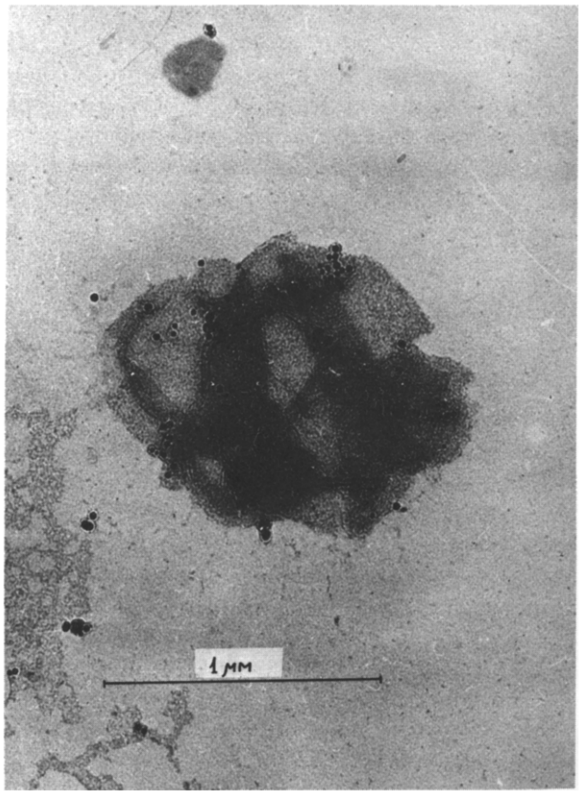

Fig.1. Electron micrograph of silver hydrosol-photoreceptor disk complex.

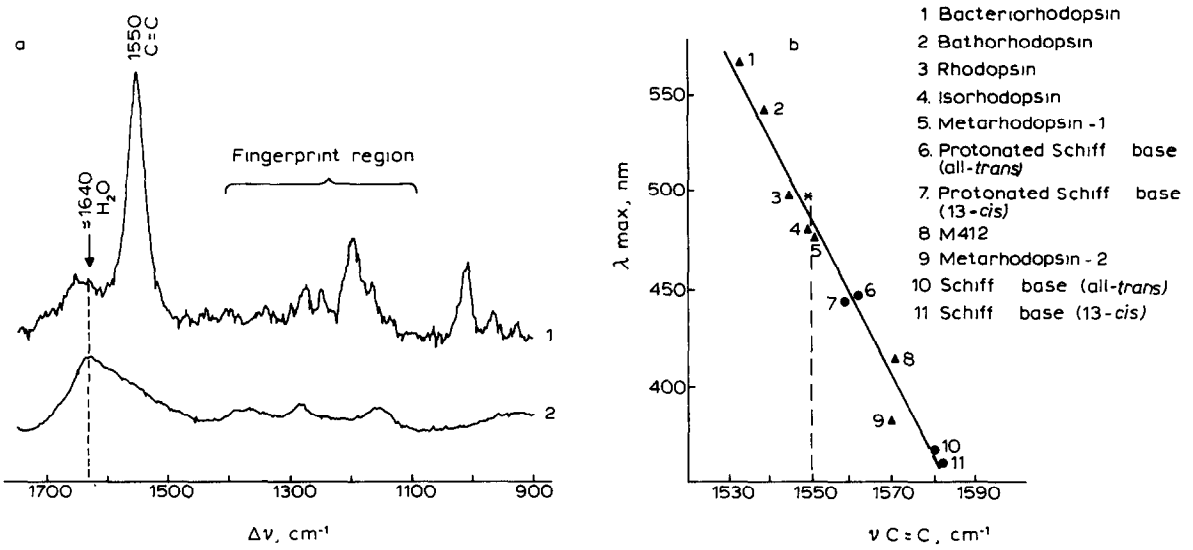

Fig.2. (a) SER spectra of photoreceptor disks (1) and photoreceptor apomembranes, free of retinal oxime (2) adsorbed on silver hydrosol: rhodopsin concentration, 10^{-7} M; λ_{exc}, 514.5 nm. (b) Correlation of ethylenic (C=C) stretching frequency of retinal-based structures with their absorption maxima. All data are from [12], * from this work.

sorption on silver colloids prevents photoinduced photochemical transformations at the basic stage with λ_{max} = 500 nm. The polyene chain configuration of the chromophore [15] was established by analysis of fingerprint regions in SER spectra of the purple membranes of *Halobacterium halobium* and the visual pigments. The ratio of the high-frequency (1197 cm^{-1}) band area to that of low frequency (1168 cm^{-1}) is a sensitive indicator of the retinal isomeric state. The fingerprint regions in SER spectra of native and photobleached disks, purple membrane of *H. halobium* as well as the protonated Schiff base of all-*trans*-retinal are shown in fig.3. It is noteworthy that for photobleached rhodopsin, bacteriorhodopsin, and protonated retinal Schiff base of all-*trans*-retinal the spectral ranges from 1100 to 1400 cm^{-1} (fingerprint regions) are quite similar (fig.3, curves 2–4).

Adsorption on silver hydrosol was found to preclude photoinduced transformations of light-adapted bacteriorhodopsin in the state with the all-*trans* conformation of the chromophore and λ_{max} = 570 nm [12]. Thus the retinal polyene chains of the compounds shown in fig.3 maintain the all-*trans* conformation upon adsorption on silver colloids. The SER spectrum of native disks is quite different from that in fig.3 (curve 1). Ad-

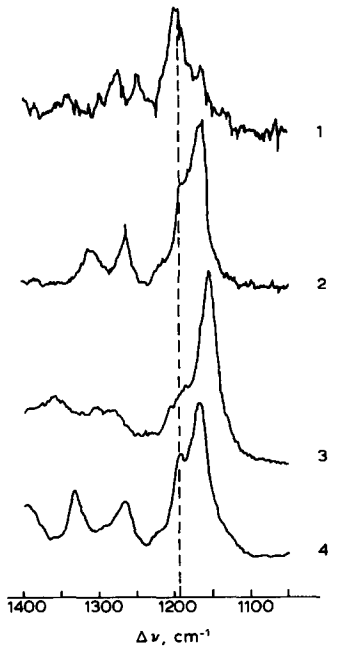

Fig.3. Fingerprint regions in SER spectra of rhodopsin (1), photobleached rhodopsin (2), bacteriorhodopsin (3) and all-*trans*-retinal (4). All compounds are adsorbed on silver hydrosol. Conditions as in fig.2a.

sorption on the metal surface causes a heterogeneous broadening of the Raman bands [16]. That is why we did not succeed in precise comparison of all spectra in fig.3. However, the high intensity of the high-frequency band (1197 cm^{-1}) as well as the unshifted C=C vibrational band position in the SER spectrum of native photoreceptor disks in comparison with those in spectra in solution allow us to conclude that upon adsorption the retinal polyene chain maintains the 11-*cis* conformation as in the native Rh500 form in the dark.

Complexes of the metal particles and the molecule in question are formed basically via π- and σ-bonds and only occasionally via the electrostatic molecule-metal interaction [17,18]. Adsorption of biomolecules on the metal surface in our experimental conditions seems to occur without significant altering of its essential conformational and chemical properties [11,12,17–19]. It should be noted that for the adsorbed molecules an enhancement mechanism has a short-range character: moving the molecule away by 5 Å [20,21] results in the complete disappearance of the SER signal. Thus the presence of the chromophore-assigned bands in the SER spectrum of photoreceptor disks supports the partial penetration of the π-electron system of the retinal into the metal double electrical layer of about 5 Å in depth [20].

Our conclusion about the proximity of retinal Schiff base to the cytoplasmic side of the photoreceptor disk membrane was confirmed by the analysis of SER spectra of photoreceptor disks, disks partially digested by papain, inverted disks prepared as in [8] and disk-monoclonal antibody complexes. In SER spectra of papain-treated disks the signal-to-noise ratio is 3–5-times as high as that for SER spectra of native disks adsorbed by silver hydrosol (curves 1 and 2 in fig.4). At the same time there are no enhanced chromophore signals in SER spectra of inside-out disks (curve 3, fig.4).

Probably, the papain digestion of the C-terminal part of the rhodopsin molecule results in a decrease of the average distance between hydrosol micelles and the membrane surface. It induces an increase in the SER signal of the chromophore which is in proximity to the membrane cytoplasmic surface. At the same time disk inversion removes the chromophore π-electron system from the metal surface inducing the disappearance of the SER signal.

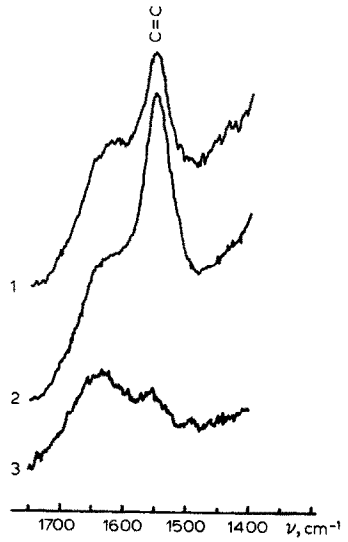

Fig.4. SER spectra of photoreceptor disks (1), papain-treated photoreceptor disks (2) and inverted (inside-out) disks (3) adsorbed on silver hydrosol. Conditions as in fig.2a.

To verify once again the short-range mechanism underlying the SER spectra, we recorded spectra of disk-monoclonal antibody complexes at the C-end of the rhodopsin molecule for different values of the rhodopsin/antibody ratio. It appeared that at the 1/1 molar ratio SER spectra of the chromophore are not observed, with a lack of increase in antibodies' SER signals (fig.5). Antibodies to the N-end of the rhodopsin molecule do not affect the SER signal of the chromophore. The results obtained are in support of the SER effect being short-range: screening of the chromophore adjacent to the cytoplasmic disk surface with the C-terminal antibodies induces signal decrease, whereas the N-terminal antibodies not shielding the chromophore from the metal surface do not influence the spectrum.

Several research groups [22–24] found the angle to be equal to 11–18° between the dipole moment of the rhodopsin chromophore and the membrane plane. Using the geometrical considerations we calculated the distance between the aldimine bond in rhodopsin and the cytoplasmic side of the photoreceptor membrane to be 5–10 Å.

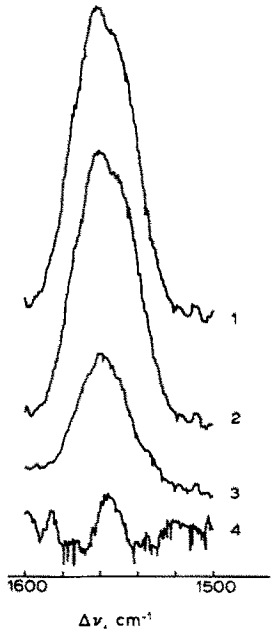

Fig.5. Surface-enhanced Raman spectra of photoreceptor disks (1) and complexes of photoreceptor disk-monoclonal antibodies to the N-end of the rhodopsin molecule (2) and disk-monoclonal antibodies to the C-end of rhodopsin (3,4). Molar ratio rhodopsin/antibody was 1/1 for (2) and (4), and 5/1 for (3). Rhodopsin concentration, 10^{-7} M. All compounds were adsorbed on silver hydrosol.

Upon isomerization the angle between the dipole moment of the retinal and the membrane plane seems to be unchanged since isomerization occurs in the membrane plane [24]. Comparison of spectra 1 and 2 (fig.3) demonstrates that the signal-to-noise ratio in the SER spectrum of photobleached disks is higher than that of native disks. For photobleached rhodopsin the average distance between the π-electron system of the chromophore and the metal surface is less than that for the native pigment. Apparently the conformational perturbations occurring on the cytoplasmic surface of photoreceptor disks during photobleaching result in a decrease of the average distance between hydrosol micelles and the membrane surface.

Using aromatic retinal analogs of varying chain lengths we have previously found that in light-adapted bacteriorhodopsin from purple membranes of *H. halobium* the distance between the aldimine bond of the chromophore and the external side of the membrane is equal to 6–9 Å [25].

It should be noted that the results obtained contradict the experimental data of Thomas and Stryer [6] who employed the fluorescence energy transfer technique to determine the transmembrane location of the retinal chromophore in the photoreceptor membrane. We succeeded in computing the transfer efficiency (E) on distance of closest approach from Tb^{3+} donors and chromophore acceptors for ellipsoidal vesicles with the axial ratio < 1 (compressed ellipsoid) and trapped volume equal to 0.6 V (V, the volume of spherical vesicles employed in [6]) [26]. Taking into account the real shape of the vesicles we found the rhodopsin chromophore to be located near the cytoplasmic side of a photoreceptor disk.

Such a near-surface position of the chromophore may play a principal role in rhodopsin functioning because isomerization of retinal induces a charge separation near the aldimine bond and, consequently, near the external membrane surface. In this case rhodopsin may become a substrate for enzymes from the cytoplasmic space of the cell.

We realize clearly that the results obtained are only the first step to establish the structural organization of rhodopsin in the membrane.

We believe that additional structural research on rhodopsin with the application of monoclonal antibodies for the study of the topographic arrangement of rhodopsin in the membrane will undoubtedly shed light on the visual pigment function.

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