Vol. 109, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1982 Pages 402-407

ELECTROPHORETIC MOBILITY OF ISOLATED RETINAL ROD OUTER SEGMENT DISK BY LASER DOPPLER SPECTROSCOPY

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Received October 11, 1982

Laser Doppler Spectroscopy has been used to measure the electrophoretic mobility of isolated bovine retinal rod outer segment disks. The mobility of dark adapted disks is found to be 0.95 μm/sec/v/cm at pH 7.0. The extrapolated isoelectric point is at pH 3.8. To within a two minute time resolution, no change in the mobility is observed upon bleaching of rhodopsin. However, a roughly 12% increase in the mobility occurs upon ultraviolet irradiation.

When an electric field is applied across a suspension of isolated Rod Outer Segment (ROS) disk membrane vesicles, the vesicles are observed to migrate with a pH dependent velocity. As is well known, the migration velocity, or electrophoretic mobility, serves as a characterization of the surface charge of the membrane. Because the surface charge may be expected to change if the number of ionizable groups on the membrane changes or if some of the groups are changed in conformation and exposure to the aqueous phase, mobility may be used as a parameter to detect such membrane changes. The membrane events associated with the rhodopsin photocycle (1) might be an example of processes giving rise to ROS vesicle mobility change. If so, electrophoretic mobility could be used as a convenient parameter to study the processes involved in photoreception.

Heretofore, with one exception, only detergent solubilized ROS rhodopsin has been investigated by electrokinetic methods. Isoelectric focusing has been used to determine the zero mobility or isoelectric point (pI)

0006-291X/82/220402-06$01.00/0

of the purified ROS protein rhodopsin (2 - 8) from several vertebrate species. The electrokinetic properties of sonicated ROS fragments were investigated by Chizhevich and Shukolynkov (5) with again, isoelectric focusing techniques. These investigations, utilizing slow equilibrium techniques, did not address the possibility of ROS vesicle charge changes over a short period of time. Indeed, even the pH dependence of the ROS disk mobility itself may be regarded as not well established to date.

We have utilized a relatively new technique for measuring electrophoretic mobility: laser Doppler spectroscopy (9, 10). This technique permits the measurement of the electrophoretic mobility in a sample in as short a time as 1 minute to 1/2 minute. In this article we report on the use of laser Doppler spectroscopy to determine the electrophoretic mobility of isolated bovine ROS disks at various pH values, both before and shortly after exposure to light of different wavelengths.

MATERIALS AND METHODS

ROS disk vesicles were isolated on ice, in dim red light, from fresh bovine eyes by either the No-Con-A metrizimide or the No-Con-A-Sucrose procedures described in (11). No significant differences in the electrophoretic mobilities of disks prepared by the two methods were observed. The disk vesicles were stored in the dark, on ice, in anaerobic Ringers (115 mM NaCl, 2.7 mM KCl, 0.5 mM, $MgCl_2$, 1.0 mM $CaCl_2$, 0.1 mM ethylenediamine tetraacetic acid, 0.7 mM NaH_2PO_4, 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 0.1 mM dithiothreitol, 25 µg/ml penicillin, 40 µg/ml streptomycin, pH 7.1) until used, generally within the next 24-48 hours. Lower pH values of the suspending medium were achieved by transferring a small droplet of the ROS suspension into 1 mL of acetate-Ringers (as above, but with 10 mM $NaC_2H_3O_2$ and 105 mM NaCl substituted for the 115 mM NaCl. pH 5.0). The pH was adjusted with dilute acetic acid or sodium hydroxide.

The laser Doppler spectrometer used has been described in several recent publications (10,12). Briefly, a crossed beam optics in the reference beam mode of detection was used (13). Parallel plate electrodes inserted into an optical cuvette provided the driving electric field. As particles migrate in the electric field, they scatter the incident laser light with a frequency shift that is proportional to their velocity and to the angle of scattering. The scattered light beam "beats" with the unscattered reference light beam in the photodetector to give a photocurrent that is varying periodically in time at the difference frequency, i.e. the Doppler shift frequency. This modulation of the photocurrent is measured directly with a spectrum analyzer. The plot in Fig. 1 is the direct output from the spectrum analyzer, and gives the photocurrent power as a function of frequency. An averaging time of one minute was used to accumulate each power spectrum.

The photobleaching light source was either the room fluorescent lighting or an Oriel xenon arc lamp, the output of which was filtered through a

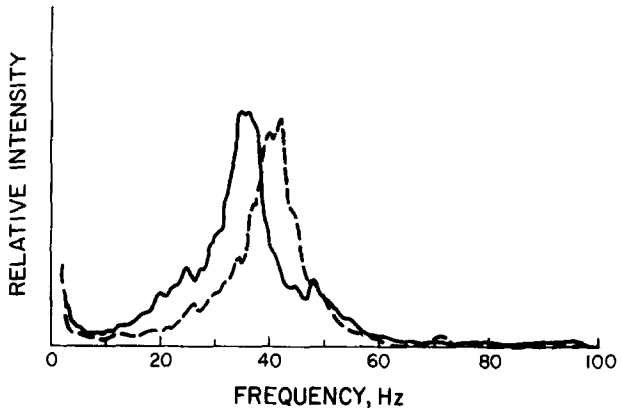

Fig. 1. Doppler spectra from an isolated rod outer segment, ROS, disk vesicle suspension, before and after exposure to fluorescent room lights for identical electric field and temperature conditions — dark adapted-- after exposure to fluorescent lights.

water cuvette to remove the infra-red components and through selected Corning glass color filters (see Results).

RESULTS AND DISCUSSION

Figure 1 shows the Doppler frequency spectrum typical of unbleached disk vesicles. Also shown is the frequency spectrum after the mobility had stabilized following a 15 minute irradiation by room fluorescent lighting.

The mean electrophoretic mobilities of the dark adapted and room light irradiated disk vesicles at pH 7.0 were 0.95 ± 0.05 and 1.06 ± 0.05 um/sec/v/cm, respectively. The 12% frequency increase was small but reproducible for different ROS vesicle preparations. Hydrodynamic size was difficult to characterize precisely by light scattering spectroscopy (14) due to polydispersity of ROS vesicle samples. However, the time delay correlation curves of the fluctuations from dark adapted ROS vesicle suspensions were not altered by irradiation with light. Hence, in spite of the polydispersity, we conclude that there is no significant vesicle expansion or contraction due to bleaching in these solution conditions. The electrophoretic mobilities of both dark adapted and irradiated disk vesicles were observed to decrease as the pH of the suspending Ringers was lowered from pH 8.5 to pH 4.2 (Fig. 2). At each pH, the mobility of the dark adapted material was less than that observed after exposure to the room lighting. The extrapolated isoelectric

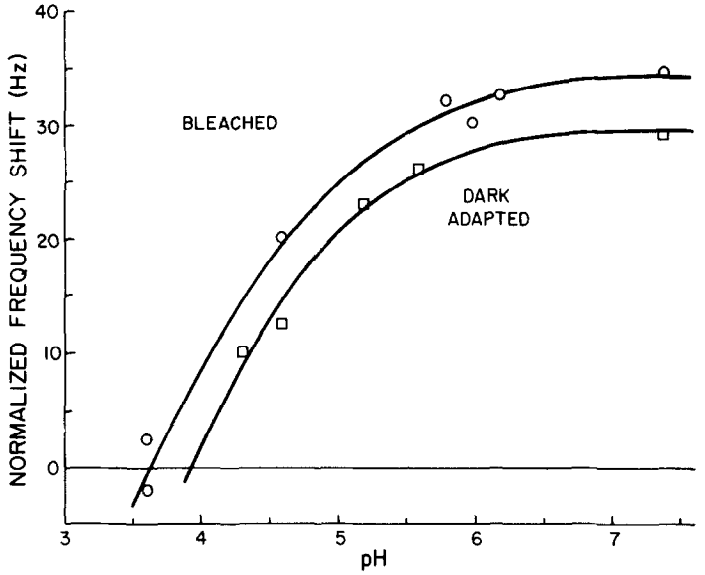

Fig. 2. Doppler frequency shifts normalized to constant electric field and temperature conditions as a function of pH.

points (pI) were at 3.8 and 3.6 ± 0.2 pH for the dark adapted and room light irradiated samples, respectively.

These results differ from the results of Chizhevich and Shukolynkov(5),who found a pI in the range of 4.4 to 4.6 for sonicated bull ROS. We cannot account for this difference other than noting that they dealt with ROS fragments, and not isolated disks.

An investigation of the wavelength dependence of the mobility shift indicated, surprisingly, that the exciting wavelengths causing mobility shifts were not under the peak of the rhodopsin absorption spectrum. Aliquots were irradiated with water filtered xenon arc white light through Corning glass filters. After a 2 minute irradiation, the Doppler frequency shift was measured. Figure 3 illustrates that the half response position of the mobility increase occurs at approximately 375 nm (the high frequency ½ transmission point of Corning colored filters). The width of the spectral response cannot be easily determined from these data because the glass filters are broad band cut off filters.

We conclude that initiation of the rhodopsin photocycle with 500 nm light has no long term effect upon the disk electrophoretic mobility. However,

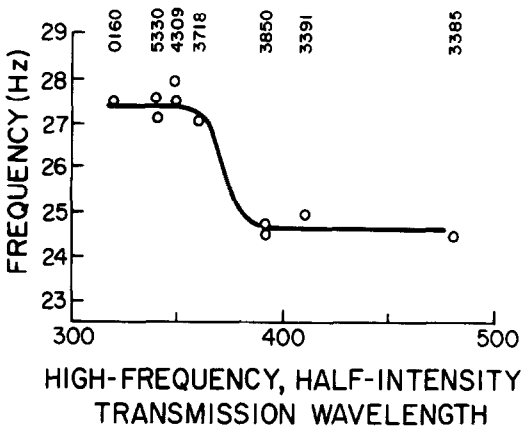

Fig. 3. Effects of UV irradiation on the mobility of ROS vesicles. Each ROS vesicle sample was irradiated for 2 minutes through a Corning colored filter, the number of which is indicated along the top of the graph. The ordinate is the wavelength of the ½ intensity transmission point of the high frequency side of the filter transmission curve. The Doppler frequency shifts are normalized for a constant electric field and temperature.

due to the roughly 2 minute time resolution of the experiment, we cannot exclude the existence of transient mobility shifts associated with the more rapidly decaying intermediates of the rhodopsin cycle. Irradiation with broadband UV light caused an increase in the negative surface charge of the disk vesicles. The cause of the UV induced mobility shift has not been determined. However, in this regard, it is noted that a number of insect retinal polypeptides exhibit reversible, light-induced shifts in their isoelectric points (15). Perhaps a similar mechanism is responsible for the shifts we have observed.

ACKNOWLEDGMENTS

We wish to thank Geo. T. Reynolds and M. L. Applebury for helpful discussions. This work was supported, in part, by DOE grant #EY-76-S-02-3120.

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