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SURFACE CHARGE OF PURPLE MEMBRANES MEASURED BY LASER DOPPLER VELOCIMETRY

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SUMMARY: Laser Doppler velocimetry measurements on purple membrane suspensions from Halobacterium halobium showed a linear correlation between electrophoretic mobility and applied electric field, electrokinetic responses could be rapidly monitored. Native membranes are less charged than white membrane preparations (from the R_1mW mutant). Chemical modification of carboxyl residues reduces surface charge, and nitrotyrosine modified membranes are more or less charged than native membranes at pH greater than or less than 6.5, respectively. Changes in surface charge are found upon actinic illumination and are greatest (Ca $5x10^{-4}/Å^2$) under conditions where decay of the M_{412} intermediate of the photoreaction cycle is inhibited, such as at high pH or after chemical modification.

Bacteriorhodopsin in the purple membrane of Halobacterium halobium is a retinal protein which upon illumination undergoes a photocycle resulting in translocation of protons from the inner cytoplasmic surface of the membrane to the external medium building a proton gradient in intact bacteria. In isolated purple membrane preparations, a release of protons into the medium can be observed if the photocycle of bacteriorhodopsin is slowed (for example, by lowering temperature). Under these conditions decay of the M_{412} intermediate of the photocycle is slowed causing its acccumulation. If groups located on the surface of the purple membrane participate in the uptake and release of protons, changes in membrane surface charge density will be observed, under conditions in which protons accumulate in the surrounding medium (1).

Although it is not known which specific groups are the sources of the protons in the medium, it is known that formation of the M_{412} species is

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accompanied by deprotonation of the Schiff base at the point of attachment of the retinal chromophore to lysine-216 on the protein and by reversible deprotonation and reprotonation of tyrosine residues after M decay (2). Chemical modification of carboxyl groups (3) and Fourier transform IR studies (4) have also shown that these residues are important for proton pumping. These observations suggest that a direct and reliable method for measuring the surface electrical charges on purple membranes before and during proton pumping will be important in characterizing the electrokinetic properties of purple membranes and in determining the stoichiometry of the charges exposed at the membrane surfaces and their correlation with accumulation of intermediates of the photoreaction cycle. In the present investigation, laser Doppler velocimetry (electrophoretic light scattering) (5) was used to directly measure exposed charges on the surface of the membrane and changes in surface charge of purple membranes upon illumination.

METHODS

Purple membranes from H. halobium S_9 were isolated as described (6). Membranes were bleached by hydroxylamine plus light (7). White membranes from the R_1mW strain (a gift of Y. Mukohata) were reconstituted with all-trans retinal to form a functional bacteriorhodopsin photocycle (8). White and purple membranes were suspended in 10 mM azide at pH 7 and kept in the cold; before use, suspensions were diluted into test media.

LDV, as described (9), is capable of resolving surface charge in less than two minutes by measuring the electrophoretic light scattering.

Briefly, the electrophoretic velocity of purple membranes can be determined from the power spectrum of the scattered light in the absence or presence of an electric field. In the presence of a uniform electric field, particles move with constant velocity. The power spectrum of the scattered laser light will be modified by a Doppler shift of the wavelength of its power maximum. The velocity of the moving particles is proportional to the electrophoretic mobility and to the electric field. This technique has often been termed laser Doppler velocimetry (LDV). The relationship between the Doppler shift, ($\Delta\nu$), and electrophoretic mobility, (μ), is:

$$\Delta\nu = \frac{K \nu E \cos}{2}$$

where K = wave vector and θ = observation angle. The probe laser used in these studies gave red light at 632.8 nm which lies within the electronic absorption band of bacteriorhodopsin. At low level continuous operation there is, however, no interference with studies using actinic light. Actinic light effects were produced either by raising the intensity of the probe beam or by introduction of white light or high intensity green laser light at 514 nm via

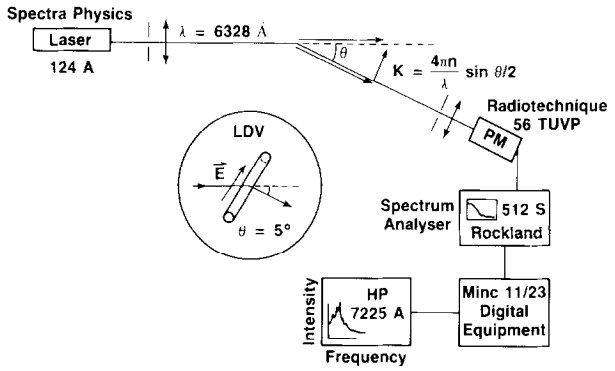

Figure 1. Scheme for LDV measurements.

a light pipe above the sample in a specially designed cell. This latter method minimizes interference with the scattered light from the probe beam. The LDV method (figure 1) employed a low angle for measurement of the scattered intensity.

RESULTS

Purple membranes suspended in a low salt solution show a linear relationship between the applied electric field and electrophoretic mobility (figure 2)

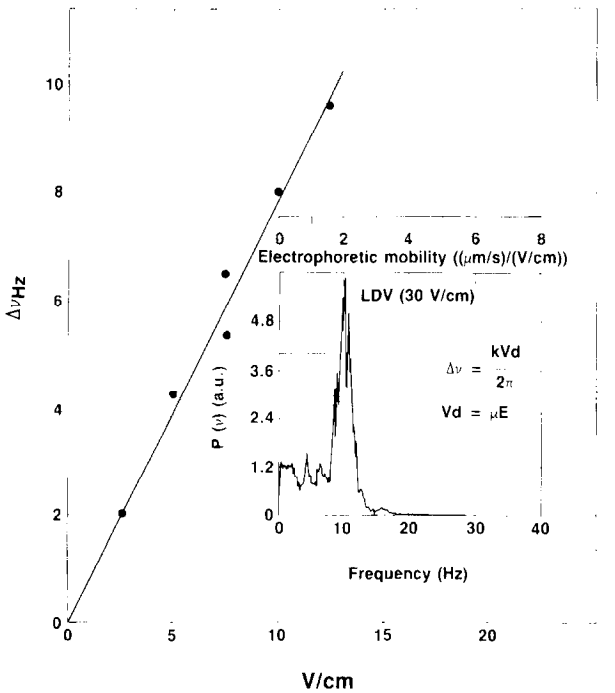

Figure 2. Relation of purple membrane mobility to applied electric field. Purple membranes (0.1 mg/ml) were in 10 mm HEPES-Na buffer pH 7.2, ionic strength 4.7 mM at 21°C. The LDV scattering angle was 4.8°. Insert show results where an electric field of 30 V/cm (frequency 10.3 Hz.) was applied for about 30 sec.

TABLE I. LIGHT-INDUCED SURFACE CHARGE CHANGES IN PURPLE MEMBRANES CALCULATED FROM LDV MEASUREMENTS

Purple Membrane Preparation	pH	Condition Laser Intensity	White Light	LDV Values Hz	Charge Density $x10^{-4}$ e/Å^2
Native, Boiled	11	Low	-	9.5	
Native, Boiled	11	High	-	9.5	
Native	11	Low	-	10.5	
Native	11	High	-	13.0	
Native	11	High	+	15.5	
Nitrated	7	Low	-	10.5	3.6
Nitrated	7	High	-	12.5	4.75

indicating that these membrane preparations are ideally suited for surface charge measurements. Illumination of the sample with white light by means of the light pipe reveals increases in surface charge density this is readily reversible in the dark. Table I shows that surface charge changes are observed at room temperature in samples at pH 11, where it is known that the M_{412} intermediate accumulates and that the photocycle is slowed. At pH 7, no detectable light-induced surface charge changes are observed unless the purple membrane preparations are chemically modified, as by nitration. Such samples show an inhibition in the decay of M_{412} even at pH 7 (10). Chemically bleached or white membrane preparations or native purple membranes boiled at 100° C for 10 minutes show no light-induced surface charge changes (figure 3).

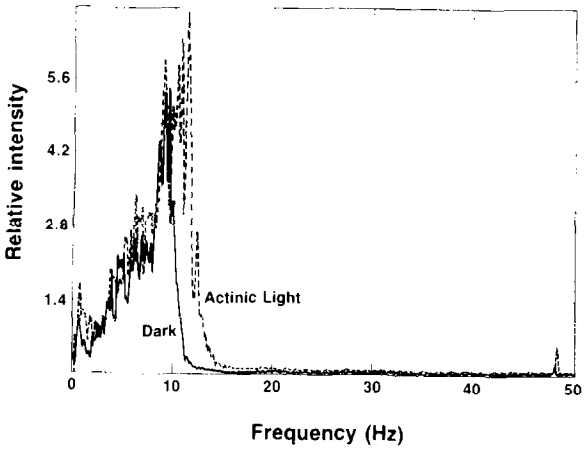

Figure 3. Light-induced changes in purple membrane surface charge density measured by LDV. Conditions as in figure 2, insert.

TABLE II. SURFACE CHARGE DENSITY OF PURPLE AND WHITE MEMBRANE PREPARATIONS AT pH 7.2

Membrane Preparation	Charges/Å^2 x 10^{-4}	Charge Density[a] x 10^{-3}	Mean. ± Standard Deviation x10^{-5}
Native Purple	4.55	7.29	1.3
Bleached Purple	5.0	8.01	2.7
Bleached Purple, + Retinal	5.0	8.01	2.7
Nitrated Purple	3.4	5.415	6.0
White	3.7	5.193	1.9
White + Retinal	3.9	6.25	3.0

[a] 1 charge/A^2 x 16.02 = coulombs/m^2

Investigation of the pH-dependence of the electrophoretic mobility of white and native purple membranes and chemically modified purple membranes (Table II) gave the following results: purple membranes are less charged than white membranes, carboxyl modified purple membranes modified with a water soluble carbodiimide (EDC) are less charged than purple membranes, tyrosine-modified purple membranes nitrated with tetranitromethane are found to be more or less charged than purple membranes at pH greater or less than 6.5 respectively (figure 4).

These electrokinetic results compare favorably with light induced surface charge measurements on purple membrane determined by at least two other techniques: an ESR method which employs an amphipathic spin probe, has demonstrated light-induced accumulation of negative charge at the purple membrane surfaces during the photoreaction cycle, under conditions of M_{412} accumulation (1,11); and the aggregation of a flourescent probe, responsive to surface potential changes, measured by resonance Raman spectroscopy (12). These methods reveal that the negative surface charge density of purple membranes increases by 10-20% upon illumination. Raman spectra of the dye at various concentrations of electrolyte led to determination of the surface potential as 18 mV and the surface charge density at 6.3 x 10^{-4} charges/angstrom (12), which is consistent with the direct determination in the present investigation of 4-6 x 10^{-4} charges/Å^2.

It is of interest to note that the LDV method has also been used by Gruner and Uzgiris (15) to show that rod outer segment disk membranes also show increases in negative surface charge density after illumination with

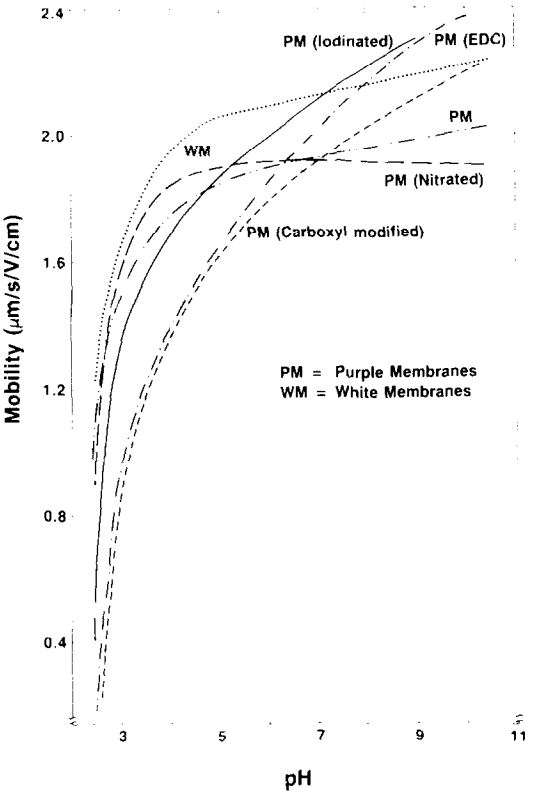

Figure 4. pH dependence of the surface charge density of purple and white membrane preparations. Conditions as in figure 2 at pH indicated.

ultraviolet light, but the significance of these light induced surface charge changes to photoreceptor function warrants clarification.

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