LARGE SCALE NONPROTON ION RELEASE AND BACTERIORHODOPSIN'S STATE OF AGGREGATION IN LIPID VESICLES

I. Monomers

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ABSTRACT Light-induced conductivity transients have been observed in preparations of bacteriorhodopsin (bR) in phospholipid vesicles at high lipid/protein molar ratios. Under these conditions, bR is known to be dissolved as monomers in the lipid bilayer. The conductivity transients are due mostly to proton movements, including a trans-membrane component. Kinetic resolution of the conductance change due to proton ionophore-induced leakage through the vesicle membrane provides a novel method to quantitate the number of protons pumped, even in heavily buffered solutions. Some of the transient signal seen on the timescale of the bR photocycle is due to nonproton ions but is smaller than that observed in native purple membranes at pH 7 in low salt. Furthermore, when the pH is raised to 8, the very large transient nonproton ion release seen in purple membranes is not seen in the vesicles. This correlates well with previous results (Marinetti, T., and D. Mauzerall, 1986, *Biophys. J.*, 50:405–415), in which the nonproton ion movements observed with native purple membranes were abolished by solubilization in Triton X-100. Thus, the nonproton ion release appears to be a property of bR in the native aggregated state.

INTRODUCTION

Bacteriorhodopsin (bR) is the sole protein component of the purple membrane (PM) from *Halobacterium halobium* and is known to be a transmembrane proton pump. In intact cells, the PM consists of large aggregates consisting of a two-dimensional crystal of bR molecules; 75% of the mass is protein and the rest is lipid. Recent reviews of the structure, function, and light-dependent properties of bR include those of Stoeckenius and Bogomolni (1) and Dencher (2).

Numerous workers have reported that bR can be incorporated into phospholipid vesicles with a net orientation of the proton pump (for example, references 3–6). As judged by the steady-state light-induced pH changes, bR in the vesicle systems is oriented opposite to the direction in vivo, i.e., the vesicles show a net pumping of protons into the interior aqueous phase. The directionality and the kinetics of the pumping were also examined using pH indicating dyes, and bR in lipid vesicles was shown to behave opposite from cell envelope vesicles (7).

Proton movements after an actinic flash have been observed in PM suspensions by a variety of techniques including pH indicating dyes (e.g., references 7–10), volume changes (11), and conductivity changes (12, 13). The latter experiments also proved that nonproton ions were moving after the flash and that this could be abolished by dissolving the bR in Triton X-100. Since bR is known to be in monomeric form in the detergent (14), a question arises as to whether the disappearance of the large nonproton conductivity signals is due to the state of aggregation of the bR or some perturbation of the bR monomer in the detergent.

The state of aggregation of bR incorporated into phospholipid vesicles has been shown by several groups to vary with the ratio of lipid/protein and with the fluidity of the lipid phase (5, 15, 16). In particular, at high ratios, bR appears to be monomeric regardless of the lipid used, as judged by the disappearance of the circular dichroism band near 600 nm, which is characteristic of the aggregated state. This is true even for saturated lipids such as dipalmitoyl (DPPC) and dimyristoyl phosphatidylcholine (DMPC), which make aggregates visible by electron microscopy at low lipid/bR ratios at temperatures below the phase transition (15, 16).

The experiments presented below were done to see whether incorporation of bR into vesicles under conditions in which it is known to be monomeric would also lead to the loss of the nonproton ion signal seen in PM. In addition, the vesicle preparations offer the opportunity to selectively measure ion movements from bR molecules oriented in the lipid bilayer. At pH 7 in 20 mM NaCl, native PM exhibits nonproton ion transients of magnitude which would correspond to 2–3 Na^+ ions per H^+ (17), i.e., nonproton ions

contribute substantially to the observed signals. In contrast, under the same conditions the bR–vesicle preparations show conductivity transients which are predominantly due to protons as shown by varying the buffer composition of the solution. Transmembrane H^+ influx is also observed, proven by the effect of a proton ionophore, CCCP, which induces a kinetically resolved leakage of the pumped protons. This allows their quantitative determination. The nonproton component of the transient signal occuring during the bR photocycle is considerably smaller in the vesicles than in PM: ~0.6 Na^+ per H^+. Furthermore, when the pH is increased to 8, bR in vesicles does not exhibit the large scale nonproton ion release seen in native PM. This indicates that disruption of the native PM structure leads to loss of the large nonproton ion release.

MATERIALS AND METHODS

The 100-kHz differential conductivity apparatus and the calculations used to record and analyze the data presented here have been described previously (12, 13). PM from *H. halobium* strain S-9, from a slant kindly provided by Dr. W. Stoeckenius, was prepared according to Oesterhelt and Stoeckenius (18). Phospholipid vesicles containing bR were prepared by a modification of the procedure of Racker et al. (4). Phospholipid (typically 2–50 mg depending on the desired lipid/bR ratio and the amount of bR used) was dried under nitrogen from $CHCl_3$ or $CHCl_3$/methanol (1:1) in a 15-ml glass centrifuge tube. Buffer was added to the tube (typically 5 ml), and vesicles were prepared by sonication under nitrogen above the phase transition temperature of the lipid using cycles of 15 s on/15 s off with a MSE 100 W probe sonicator (Measuring & Scientific Equip. Ltd., London) at an amplitude of 6 μm peak-to-peak. The cycles were repeated until the suspensions clarified. PM was washed into the desired starting buffer by centrifugation and resuspension; the final resuspension of the pellet was done with the vesicle suspension. To the mixture, sufficient solid octyl-β-glucoside (Calbiochem-Behring Corp., La Jolla, CA) was added to make a final detergent concentration of 1.5% (wt/vol). Normally the suspension became optically clear immediately. The mixture was allowed to incubate for ~20 min and then placed into dialysis tubing and dialyzed overnight against 800-ml buffer.

Undissolved/unincorporated bR was removed by centrifugation. The lipid/bR ratios indicated below are based on the starting bR and lipid added and hence represent a lower estimate on the ratio in the final material. The samples used for conductivity experiments had an optical density of 0.3–0.6 at 570 nm in a 1-cm cell. Soybean phospholipids (asolectin) were from Associated Concentrates (Woodside, NY) and were partially purified by suspension in $CHCl_3$/methanol, removal of insoluble material, then drying to a film and washing twice with ether. The resulting solid was then dissolved in $CHCl_3$/methanol. DMPC and DPPC were synthetic, >99% purity lipids from Avanti Polar Lipids, Inc. (Birmingham, AL) as $CHCl_3$ solutions, stored at −20° C. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was from Aldrich Chemical Co. (Milwaukee, WI). Tetramethylethylenediamine (TEMED; Eastman Kodak Co., Rochester, NY) was redistilled before buffer solutions were made up. Imidazole (Sigma Chemical Co., St. Louis, MO) was twice recrystallized from benzene after treatment with activated charcoal. Glycinamide HCl was from Sigma Chemical Co. and used without further purification.

The average vesicle diameter was determined to be ~100 nm using a light scattering technique (19) in which the frequency spectrum of the scattering intensity from a small volume of sample is recorded. Fluctuations in the scattered light are caused by Brownian motion of the particles and the half width of the power spectrum is directly related to the diffusion coefficient. The spectra of vesicle samples were compared with those of latex spheres of comparable known size (91 ± 6 and 305 ± 9 nm; Sigma Chemical Co.). All vesicles used in the experiments below gave spectra between the two standards, normally very close to the smaller spheres.

EXPERIMENTAL RESULTS

Fig. 1 shows a schematic drawing of the light-induced proton movements and the resulting conductivity transients expected in bR vesicles near pH 7. There are three time regions of interest: (*a*) <1 ms after the flash. The conductivity changes will be caused by fast proton release only from those bR molecules oriented toward the external phase; this will appear as a positive or negative step depending on whether the buffer ions gain or lose charge upon protonation. Note that bR molecules oriented toward the interior of the vesicle contribute nothing to this instantaneous signal since only ions in the exterior aqueous phase contribute to the conductivity signal. Also there will be a thermal step (always positive) due to the part of the photon energy that is immediately degraded to heat. (*b*) Between 10 and 100 ms after the flash, H^+ uptake by bR will occur and the signals will be due to bR molecules oriented toward the interior. This signal is buffer dependent as well. Thus, ion movements due to bR molecules in each of the two possible orientations in the bilayer can be distinguished kinetically and spatially and quantitated. Note that a high degree of orientation is not required; it only affects the relative amplitudes of the transients due to inward and outward pointed bR. (*c*) Long after the flash (>0.1 s), the photocycle is over and all the energy of the absorbed light has been degraded to heat, giving a positive baseline shift. If there is a net orientation in the bR vesicles (expected to be toward the interior) then this will appear as a baseline shift of variable sign depending on the buffer. A priori, one wouldn't be able to distinguish the pumped proton component from the thermal component since thermal relaxation and the leakage of the pumped protons occur on comparable (long) timescales. However, the two can be separated by adding small amounts of a proton ionophore such as CCCP to speed up the leakage rate.

An example of this is shown in Fig. 2, which shows bR in asolectin vesicles at 16°C in 5 mM potassium phosphate, pH 6.9. The top two traces (*A*) show the response to a flash of light recorded at two different timescales. The conductivity increase resolved in the right-hand trace corresponds to proton uptake by bR which is pointed inward (see middle portion of Fig. 1). The conductivity increases because in phosphate buffer, deprotonation of the buffer results in the conversion of $H_2PO_4^-$ to HPO_4^{2-} which increases the bulk conductivity. The left trace in *A* shows a very slow decay of the signal, which can be attributed to leakage of some of the translocated protons. When 10 μM CCCP is added (*B*), one sees that the conductivity rapidly rises (as in *A*), then relaxes with a time constant (1/*e*) of 0.3 s to a baseline value above that before the flash. CCCP has increased the rate of leakage so that by 2 s after the flash, all protons pumped have returned to the external

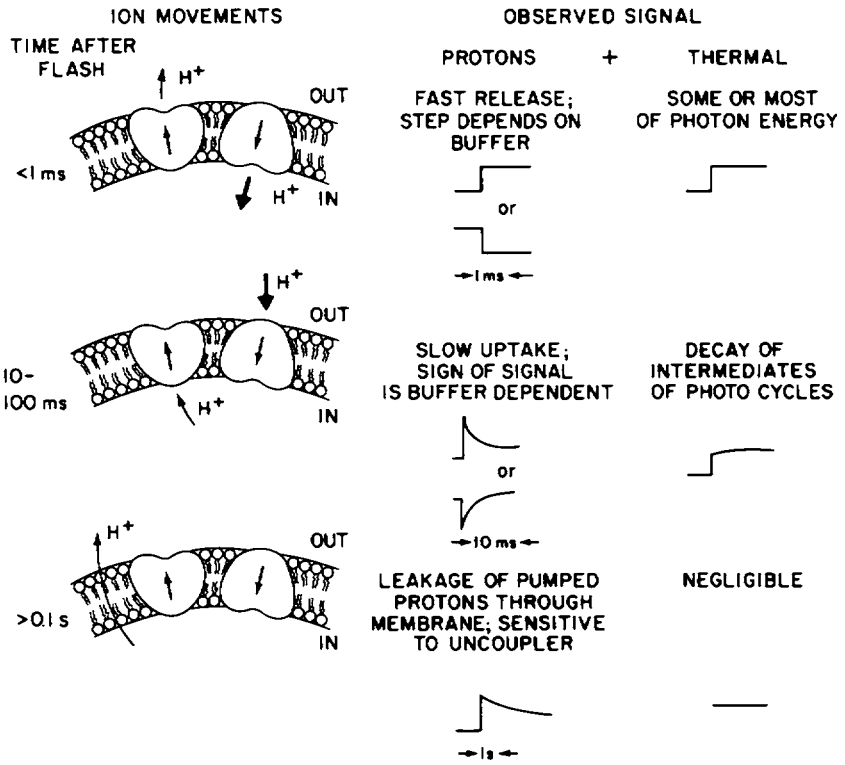

FIGURE 1 Schematic model of conductivity changes expected in suspensions of bR-containing vesicles. The sense of the proton pump is indicated by the arrow on each bR molecule embedded in the lipid bilayer. The net orientation of the vesicles is shown by the inward pointed bR having a larger arrow for H^+ release and uptake. See text for further details.

phase. Note that this signal amplitude is directly proportional to the number of pumped protons, allowing determination of H^+ translocation in heavily buffered solution. The residual baseline shift is the thermal heating effect discussed above.

Knowing the size of the heat signal, the step in conductivity immediately after the flash can be broken into its component parts. Referring to the top of Fig. 1, this should be the sum of the thermal heating plus fast proton release by bR pointed outward. Using the long-time baseline with CCCP as the thermal signal, we can calculate the fast proton signal by difference. Note that this ignores any energy storage by the bR, but introduces only a small error.

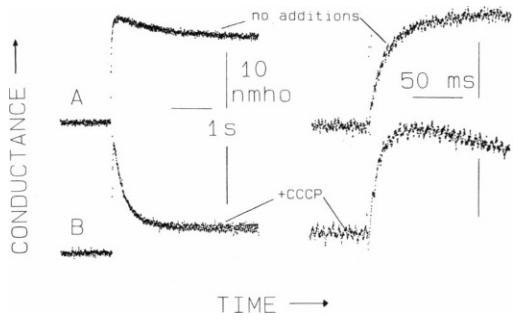

FIGURE 2 Conductivity transients observed in bR-asolectin vesicles. Starting bR/lipid is 1:1000 (mol/mol). The sample is in 5 mM potassium phosphate pH 6.95 at 16.0°C. The upper traces are recorded on two different timebases, total sweep time 5 s (*left*) and 200 ms (*right*). The lower traces were recorded after addition of 12 μM CCCP. All traces are the average of 64 flashes. The vertical bar by each trace represents a conductivity change of 10 nmho.

Birge and Cooper (20) have measured the enthalpy difference between light-adapted bR and its primary photoproduct K. Even this early in the photocycle, 70% of the photon energy is degraded and more would presumably be lost in proceeding to the M412 species which is of concern here. Extrapolation of the transients on the right of Fig. 2 to time zero shows that they are very close to the pre-flash baseline, i.e., the net size of the fast step is small. Since the thermal step can only be positive, there must also be a fast negative transient that is adding to it, resulting in a net step close to zero. A negative step is exactly what is expected for the rapid proton release into this buffer by the fraction of bR molecules that are oriented outward.

This allows a quantitative test: the fast negative step measures bR pointed outward, while the 10-ms transient measures those pointed in. The difference should be the same as the observed amplitude of the CCCP-induced leakage independently resolved in *B*. Estimating the error in the <1-ms and 10-ms amplitudes as ¼ the peak-to-peak noise in each trace and including a 10% correction on the thermal step for energy storage, the pumped protons are calculated to represent 16.2 ± 2 nmho. The observed CCCP leakage amplitude is 15.5 ± 0.3 and within experimental error, these are the same. It can easily be shown with reference to Fig. 1 that as long as there is no net translocation of nonproton ions, their contributions to the fast and slow transients will exactly cancel out when the difference is taken. The quantitative agreement between the kinetically resolved proton transients from bR in the two orientations and the amplitude of the slower CCCP-induced leakage independently measured shows conclu-

sively that there are no "Bohr" protons released by bR under these conditions.

Fig. 3 shows the results of similar experiments using bR incorporated in DMPC vesicles. (See legend for sample details.) These samples are buffered with TEMED, a diamine that gives positive signals upon protonation, opposite to phosphate. TEMED was chosen since both forms of the buffer are charged and hence nonpermeant. Toth-Boconadi et al. (21) have reported a supposed reversal of the proton pump in low concentrations of TEMED, but there are reasons to question these authors' interpretation (see reference 17). Moreover, the effect of CCCP on these vesicles shows no pump reversal. In the left series of Fig. 3, the effect of CCCP is to cause an apparent increase in the long time baseline, as shown clearly in the difference trace. In *A*, after the photocycle is over, there are protons pumped into the vesicles that cannot get out. This gives a negative baseline shift that algebraically sums with the positive thermal step. When CCCP is added, the leakage becomes fast (see difference trace) and the pumped protons return to the external phase. The difference is proportional to the net number of protons pumped. When a calculation is done using the amplitudes of the transients as was done for Fig. 2, the results were similar. The right side of Fig. 3 shows an analogous experiment at 12°C, a temperature below the phase transition temperature of the lipid. Here, one observes that CCCP has no apparent effect; evidently the ionophore traverses the bilayer very slowly when the lipid phase is frozen. This is in agreement with other workers (5).

To determine whether nonproton ions are involved in the individual fast and slow transients, the buffer composition must be varied. One such experiment is shown in Fig. 4,

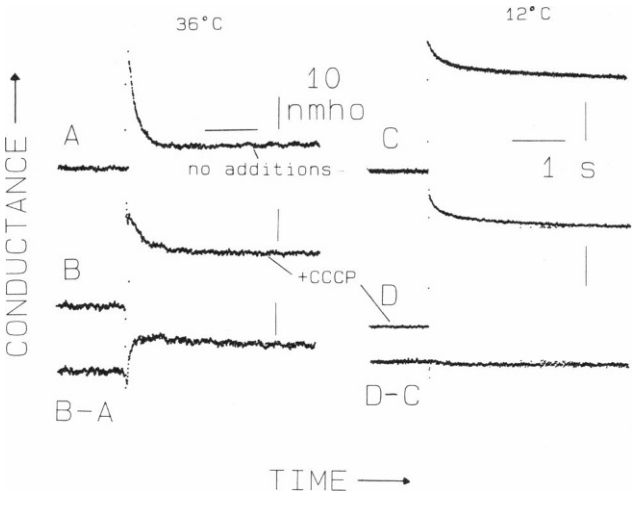

FIGURE 3 Conductivity transients in bR-DMPC vesicles. Starting bR/lipid is 1:800 (*left*) and 1:100 (*right*) mol/mol, respectively. Buffer is 2 mM TEMED, 20 mM NaCl, pH 6.5 (*left*) and 6.85 (*right*). *A* and *B* are before and after addition of 24 μM CCCP. Total sweep time was 5 s in all cases; each trace is the average of 64 transients. The vertical bar by each trace represents a conductivity change of 10 nmho.

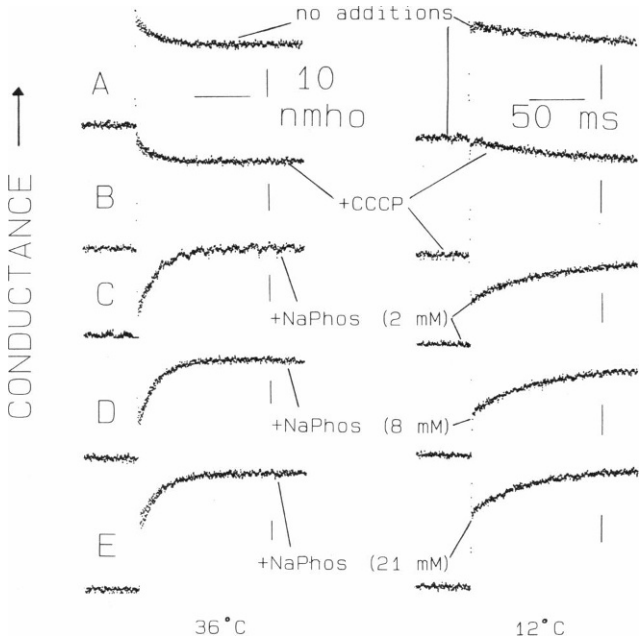

FIGURE 4 Effect of buffer variation on conductivity transients in bR-DMPC vesicles. Starting bR/lipid is 1:100 mol/mol. The starting buffer is 2 mM TEMED, 20 mM NaCl, pH 6.9. The left traces were recorded at 36°C; the right at 12°C. (*A*) As is. (*B*) After addition of CCCP (*left*, 24 μM; *right*, 19 μM). (*C–E*) After successive addition of sodium phosphate to the concentrations indicated. All traces are the average of 64 transients recorded on a total sweep time of 200 ms. The vertical bar by each trace represents a conductivity change of 10 nmho.

which shows bR in DMPC vesicles. The top two traces are in 2 mM TEMED pH 7 before and after addition of CCCP. These preparations had a relatively low degree of orientation, as judged by the CCCP-induced shift of the long-time baseline recorded separately (data not shown). Huang et al. (6) also reported much lower net orientation of bR in DMPC as compared with soybean lipid vesicles. However, this will not affect any conclusions below. There is clearly a transient resolved on the 50-ms timescale (the $1/e$ time is 20 ms at 36°C and 50 ms at 12°C). This corresponds to the uptake of protons by bR molecules pointed inward (see Fig. 1), which results in a loss of conductivity in TEMED. *C–E* show the result of successive additions of sodium phosphate buffer at constant pH. In the mixed buffer, protons taken up by bR will come partially from TEMED (giving a loss of conductivity) and partially from phosphate (giving a gain in conductivity). The net signal will be the weighted sum of the two and the effective conductance for each buffer mixture can be calculated from the known concentrations, equivalent conductances and pKs of each buffer (see reference 13). Qualitatively the results are quite clear: addition of phosphate buffer in excess causes the transient to change sign, as expected if the bulk of the transient was due to protons.

Quantitative analysis of an analogous experiment is shown in Fig. 5. Here, the bR-DMPC vesicles started in potassium phosphate buffer (left-most point) and TEMED

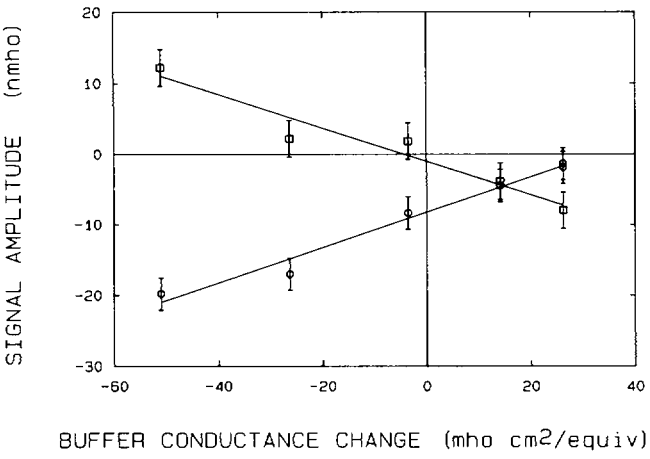

FIGURE 5 Analysis of buffer variation data. bR-DMPC vesicles, starting bR/lipid is 1:800 mol/mol. The titration proceeds from left to right by successive additions of TEMED (0, 6.2, 18.3, 41.9, and 87 mM, respectively) to a sample in 5 mM potassium phosphate, 20 mM NaCl, pH 7 at 36°C. The squares are the amplitudes of the proton pumping, as determined by the CCCP-induced baseline shift (see text). The circles are the amplitudes of the transient corresponding to the slow H^+ uptake phase of the bR photocycle. The horizontal axis is the effective conductance change for protonation of each buffer mixture, calculated from the known concentrations, pKs, and measured equivalent conductances of each buffer ion (see reference 13).

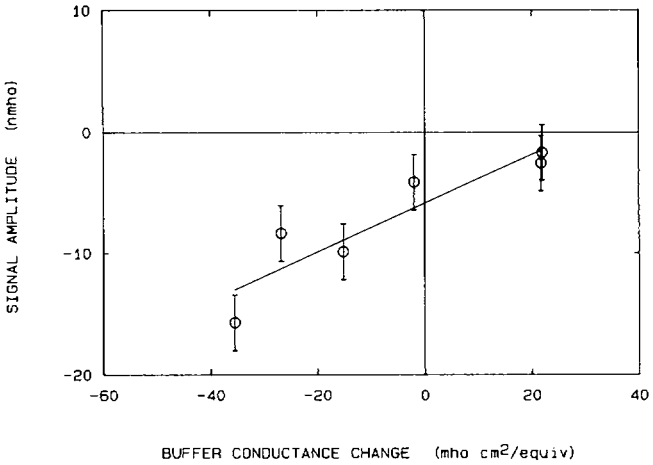

FIGURE 6 Analysis of buffer variation data. bR-DMPC vesicles, starting bR/DMPC is 1:800 mol/mol. The sample started in 2 mM TEMED, 2.5 mM glycinamide, 20 mM NaCl pH 8 (right-most points, with and without 18 μM CCCP). Then phosphate was added successively, proceeding right to left at concentrations of 6.1, 12.3, 24.4, and 42.2 mM, respectively. The horizontal axis is the same as in Fig. 5.

is added successively. The horizontal axis shows the calculated conductance change for each buffer mixture per equivalent of protons added. The ordinate is the observed signal amplitude from nonlinear least squares fits to the slow uptake phase of the photocycle (*circles*) and the calculated baseline shift due to proton pumping (*squares*). Note that the negative amplitude for the uptake phase actually corresponds to a conductivity increase: the fit is to an instantaneous negative step which relaxes in a positive direction. If the signals were due to protons only, the points should fall on a straight line with an intercept of 0. If nonproton ions contribute a constant amplitude to the signal, then the result will still be a straight line, but one with an offset, which will give a non-zero y-intercept. The lines drawn in the figure are the result of linear regression to the points; the error bars indicate the standard deviation of the fit. Within experimental error, the signal amplitude for the proton pumping has no nonproton component, whereas that of the slow uptake phase has a component that would correspond to ion release concomitant with proton uptake by the bR. The ratio of the intercept to the slope, divided by the equivalent conductance of the nonproton ion, gives the number of nonproton ions per H^+. If this was a typical cation such as Na^+, the result is 0.8 ions per H^+. This is on the high end of the range observed in other experiments. For example, the same analysis applied to the data of Fig. 4 gives ratios of 0.5 and 0.4 for the traces at 36° and 12°C, respectively.

Fig. 6 presents the results of a buffer variation, beginning in TEMED/glycinamide at pH 8 with successive addtions of phosphate. The amplitude of a transient corresponding to the 15 ms H^+ uptake phase of the bR photocycle is plotted as in Fig. 5. Although nonproton ions contribute to the signal, as evidenced by the non-zero y-intercept, protons still account for the bulk of the transient. The linear least squares fit yields a value of 0.6 nonproton ions per H^+. Note that the sign of the y-intercept indicates ion release occurring alongside H^+ uptake, as observed in the experiments at pH 7.

The same qualitative results were obtained when the ionic strength was raised. For example, when NaCl was added to 0.1 M to the sample of Fig. 2, the major effect was a large increase in the thermal signal, as expected from the increase in bulk conductivity. The transient associated with the uptake phase of the bR photocycle remained an increase in conductivity as in Fig. 2 *B*. Addition of imidazole (18 mM) sharply reduced the magnitude of this transient, but did not change its sign as would have been expected for this buffer mixture. Quantitative analysis was not attempted due to the small size of the signals in this case.

DISCUSSION

The experiments described above were undertaken to examine the light-induced conductivity transients under conditions where the signals from the two sides of the bR molecules could be resolved. They were also done to investigate whether the large nonproton ion transients seen in PM (12, 13, 17) would still be present when bR was dissolved in lipid vesicles. The data clearly show that the conductivity transients arise from two sources. The largest component of the signal is due to rapid proton release followed by uptake as expected from the bR photocycle near neutral pH, in agreement with the experiments using

indicating dyes (7–9). In vesicles containing bR with a net orientation, this results in transmembrane proton pumping as proven by the experiments in Figs. 2 and 3 in which the proton ionophore CCCP caused conductivity changes, the sign of which could be altered by the choice of buffer ions. In addition, the difference in the amplitudes of the fast H^+ release by outward oriented bR and the slow H^+ uptake by inward oriented bR quantitatively equaled the CCCP-induced leakage. This shows that within experimental error, all proton transients are due to pumped ions, as opposed to H^+ transiently released and bound from the same side of the membrane. Furthermore, the number of protons translocated can be determined even in the presence of buffers, a unique feature of the conductivity method. This is important for two reasons: (*a*) the physiological environment in which bR operates is strongly buffered, and (*b*) Drachev et al. (9) observed that small amounts of buffers were sufficient to bring the observed apparent kinetics of H^+ release into coincidence with those of M412 formation. Hence, the absence of buffering species, required by other techniques to measure proton movement, might well lead to systematic artifacts.

The second component of the transients is due to ions other than protons, as shown by the non-zero intercepts of the plots in Figs. 5 and 6. Under all conditions used here, this signal must be due to rapid ion uptake followed by release, occurring at the same time as proton pumping by bR. This is qualitatively similar to the behavior of PM suspensions at pH 7 and low ionic strength; however, the nonproton uptake and subsequent release is three to four times smaller in magnitude in the vesicle preparations than in native PM.

The most striking difference between PM and the bR-containing vesicles is at pH 8 (Fig. 6). Native PM exhibits an abrupt transition when the pH is increased from 7 to 8 at low salt concentrations or if the salt is increased at pH 7 (17). The conductivity transient becomes very large—a 10-fold increase maximally—and is dominated by nonproton ion release followed by uptake. No such behavior is shown by the bR-vesicle preparations: the nonproton component of the signal at pH 8 is very similar to that at pH 7 both in magnitude and direction. In particular, large scale nonproton ion release is not observed in the vesicles.

Increasing the ionic strength at pH 7 also had little effect on the conductivity transients observed in the vesicle preparations. When NaCl was added to the asolectin vesicles of Fig. 2, there was no dramatic increase in the transient signal amplitude and again, the nonproton component corresponded to ion uptake followed by release, opposite to the behavior of PM. The data of Fig. 5 also illustrate the lack of an ionic strength effect: rather large amounts of TEMED buffer (about 90 mM) had to be added to dominate the phosphate buffer in this particular titration. Had the corresponding increase in ionic strength induced even a part of the large nonproton ion release seen in PM, the right-most points of Fig. 5 would have been significantly off the straight line. No such deviation is observed.

These data lead to an unambiguous conclusion: disruption of the highly ordered aggregated structure of the PM leads to loss of the large nonproton ion release. Other workers (15, 16) have determined by circular dichroism and electron microscopy that at high lipid/bR ratios, such as used in the experiments reported here, bR is dispersed into monomers dissolved in the lipid bilayer. The absence of large nonproton ion transients in the vesicles correlates well with the fact that bR solubilized in Triton X-100 shows no such transients (13).

One possible objection is that since only the external aqueous phase is observed, nonproton ions are being released, but only in the interior of the vesicle. This is unlikely since the CCCP-induced leakage showed the degree of net orientation in the DMPC vesicles was not very great. Hence there are substantial amounts of bR in each of the two possible orientations. If all the ions were coming from only one side of the bR, there would still be enough of those pointed toward the external phase to give an observable ion release signal.

Interpretation of the large nonproton ion release signals in terms of ion condensation (22) or accumulation (23) near the charged PM surface has been given in references 13 and 17. When bR is dispersed in detergent micelles or in lipid vesicles, the extended surface charge is also reduced. Hence there are few trapped counterions to be transiently released during the photocycle. The observed ion uptake in the vesicles, equivalent to <1 Na^+ per H^+, can be understood as partial charge compensation for the additional negative charge that appears on the bR surface when the proton is released.

Since the bR monomers pump protons, the native aggregated structure is not an absolute requirement for activity. But, it may enhance the efficiency of net pumping: at pH 4 where we observe H^+ uptake before release, bR in Triton X-100 (13) gives a quantum yield considerably lower than native PM (12). For bR in vesicles, analysis of the data of Fig. 2 gives a quantum yield of proton pumping of 0.05 from the CCCP-induced leakage. This value is a lower bound, and correction for the misoriented fraction of bR and for light saturation would increase the quantum yield by a factor on the order of 2. This would bring it close to the value estimated for bR in Triton X-100 at pH 7 (13), and both are considerably less than the 0.43 quantum yield for H^+ release reported for PM (24).

A remaining question is what is the minimum size of the bR aggregate necessary for the appearance of rapid nonproton ion release. The experiments carried out so far have dealt with the extreme cases: native PM and bR monomers. Since two groups (15, 16) report that bR aggregates are visible in vesicles at low lipid/protein at temperatures

below the lipid phase transition, such preparations could offer an experimentally achievable intermediate case. Such experiments are in progress.

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