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CALCIUM-PROTON ANTIPORTS IN PHOTOSYNTHETIC PURPLE BACTERIA

VICTOR L. DAVIDSON and DAVID B. KNAFF

Department of Chemistry, Texas Tech University, Lubbock, TX 79409 (U.S.A.)

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A purple sulfur bacterium, *Chromatium vinosum*, and a purple nonsulfur bacterium, *Rhodospirillum rubrum*, have been shown to contain Ca^{2+}/H^+ antiports. Whole cells of these bacteria show light-dependent calcium efflux that arises from the exchange of internal calcium for protons ejected during light-driven cyclic electron flow. Chromatophores prepared from these bacteria show light-dependent proton efflux coupled to calcium uptake via this antiport. The *C. vinosum* antiport does not respond to Mg^{2+}, Zn^{2+} or monovalent cations but Sr^{2+}, Mn^{2+} and, to a lesser extent, Ba^{2+} can substitute for Ca^{2+}.

Introduction

It has been demonstrated that chromatophores isolated from several species of photosynthetic purple nonsulfur bacteria use the energy released during light-driven electron flow to produce a transmembrane electrochemical proton gradient, $\Delta\bar{\mu}_{H^+}$ (see Refs. 1 and 2 for recent reviews). Considerable evidence exists for the subsequent role of $\Delta\bar{\mu}_{H^+}$ as the direct energy source for endergonic processes such as ATP formation [1–6] and reverse electron flow from succinate to NAD^+ (see Ref. 7 for a recent review). Kinetic measurements have established that the initial electron flow-coupled ion movements that generate $\Delta\bar{\mu}_{H^+}$ in these purple nonsulfur photosynthetic bacteria are those of protons [8–10]. However, in the steady state, $\Delta\bar{\mu}_{H^+}$ consists of a substantial membrane potential ($\Delta\psi$) in addition to the proton gradient itself [1,2,6]. These results imply, as originally suggested by Mitchell [11], that the membranes contain Ca^{2+}/H^+ antiports which exchange cations for the initially translocated protons and thus substitute a $\Delta\psi$ for a portion of the primary ΔpH. Although convincing evidence exists for such Ca^{2+}/H^+ antiports in nonphotosynthetic bacteria [12–16], no evidence has been obtained for such antiports in photosynthetic bacteria.

Chromatophores from the purple sulfur bacterium, *Chromatium vinosum* also exhibit rapid proton uptake [17–19] and are able to maintain, in the steady state, a light-dependent $\Delta\psi$, inside positive [20]. Whole cells of *C. vinosum* are known to have the opposite membrane sidedness of chromatophores [20] and have been shown to generate a membrane potential, outside positive, coupled to light-driven cyclic electron flow [20,21]. This membrane potential, rather than ΔpH, appears to supply the major driving force for the active transport of amino acids, dicarboxylic acids and sugars [21–23] by *C. vinosum* cells. It appeared possible that Ca^{2+}/H^+ antiports were involved in substituting $\Delta\psi$ for a portion of ΔpH in purple sulfur bacteria. We have therefore investigated this possibility in *C. vinosum* and in a representative purple nonsulfur bacterium, *Rhodospirillum rubrum*, and have obtained evidence for Ca^{2+}/H^+ antiports in both bacteria.

Abbreviations: BChl, bacteriochlorophyll; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; HOQNO, 2-(*n*-heptyl)-4-hydroxyquinoline-*N*-oxide; $\Delta\bar{\mu}_{H^+}$, electrochemical proton gradient; $\Delta\psi$, membrane potential; Tricine, *N*-tris(hydroxymethyl)methylglycine.

Methods

C. vinosum was grown as described previously [24]. *R. rubrum*, strain S1, was grown as described by Yoch et al. [25]. Chromatophores were prepared by sonication, essentially as described previously [24], but using a Branson Model 200 sonifier at 50% duty cycle for 4 min at power setting 4.

Light intensities were measured using a Yellow Springs Instruments Model 65A radiometer. BChl was estimated after extraction into acetone/methanol (7 : 2, v/v) as described by Clayton [26]. The $^{45}Ca^{2+}$ content of intact cells was determined after trapping the cells on Millipore cellotate filters (0.45 μm pore size) and counting the filters as described previously for ^{14}C determinations [23]. The cells were loaded with $^{45}Ca^{2+}$ by preincubation with 10 mM $^{45}CaCl_2$ (specific activity = $1.11 \cdot 10^4$ dpm/nmol Ca) for 1.5 h in the dark.

Proton movements across chromatophore membranes were followed by measuring 9-aminoacridine fluorescence [27] in an Aminco-Bowman Spectrofluorometer with the excitation monochromator set at 400 nm and the emission monochromator set at 460 nm. Actinic light was provided, through a Kodak Wratten 88-A filter by a Unitron microscope lamp focused on the reaction cuvette. The actinic intensity was $4 \cdot 10^2$ W/m^2. The gas space above the cuvette was continually flushed with N_2 gas. Ca^{2+} uptake by chromatophores was measured by flow dialysis, using the technique previously described for lipophilic cation uptake [21]. ATP formation was measured as described previously [20].

9-Aminoacridine was obtained from K & K Laboratories. Valinomycin, CCCP, HOQNO, DCCD and phenazine methosulfate were obtained from Sigma Chemical Co. $^{45}CaCl_2$ (specific activity 36.2 mCi/mg Ca) was obtained from ICN Radioisotope Division. Nigericin was a gift from Eli Lilly Co. A23187 was obtained from Calbiochem-Behring.

Results

As discussed above, chromatophores from both purple nonsulfur and purple sulfur photosynthetic bacteria show rapid proton uptake coupled to light-driven electron flow. Whole cells of representative species of both classes of bacteria, having the opposite membrane sidedness of chromatophores [20,28–30], show light-dependent proton ejection [29,30]. Thus, if these bacteria contain Ca^{2+}/H^+ antiports, internal Ca^{2+} should be ejected from the cells in exchange for external H^+ initially ejected during light-driven electron flow. Fig. 1 shows that cells of both

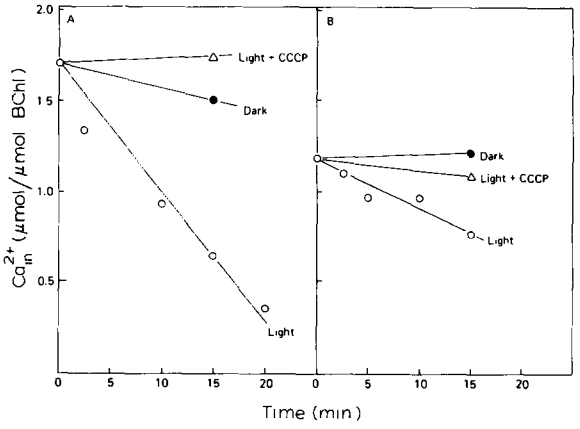

Fig. 1. Light-dependent Ca^{2+} efflux in photosynthetic purple bacteria. *R. rubrum* (A) or *C. vinosum* (B) cells were loaded with $^{45}CaCl_2$ as described in Methods. The reaction mixtures (100 μl volume) contained cells equivalent to 340 μM BChl in 50 mM potassium phosphate buffer (pH 6.5) and 10 mM $^{45}CaCl_2$. 30 μM CCCP was present where indicated. The reaction was terminated by addition of 2 ml of 1 M LiCl.

TABLE I

THE EFFECT OF INHIBITORS AND UNCOUPLER ON LIGHT-DEPENDENT Ca^{2+} EFFLUX BY *C. VINOSUM* CELLS

The reaction mixture (100 μl) contained *C. vinosum* cells equivalent to 340 μM BChl suspended in 50 mM potassium phosphate buffer (pH 6.5). $^{45}CaCl_2$ was present at a concentration of 10 mM with a specific activity of $1.11 \cdot 10^4$ dpm/nmol Ca. The light intensity was $7.5 \cdot 10^4$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1}. All samples were maintained under an atmosphere of water-saturated N_2. 250 μM DCCD, 100 μM HOQNO or 30 μM CCCP were present where indicated.

Sample	Ca^{2+} content (μmol Ca^{2+} per μmol BChl)	Percent Inhibition of Light-Dependent Efflux
(1) Zero Time	1.50	–
(2) 15 Min, Dark	1.53	–
(3) 15 Min, Light	0.68	0.0
(4) (3) + DCCD	0.84	19.7
(5) (3) + HOQNO	1.21	64.2
(6) (3) + CCCP	1.38	85.0

R. rubrum and *C. vinosum* exhibit a light-dependent loss of internal Ca^{2+}. Little Ca^{2+} is lost in the dark. If the Ca^{2+} movements are catalyzed by a Ca^{2+}/H^+ antiport, proton ionophoric uncouplers such as CCCP and gramicidin that prevent formation of any proton gradient [31] would be expected to abolish the Ca^{2+} efflux. Fig. 1 shows that CCCP does, in fact, inhibit light-dependent Ca^{2+} efflux in both *C. vinosum* and *R. rubrum* cells. Gramicidin also inhibited light-dependent Ca^{2+} efflux.

Table I shows, in addition to the effect of CCCP, the effects of additional specific inhibitors on light-dependent Ca^{2+} efflux by *C. vinosum* cells. DCCD is known to inhibit the membrane-bound enzyme (ATPase) that couples electron transport to ATP synthesis in *C. vinosum* chromatophores [32]. DCCD has also been shown to be an effective inhibitor of active transport by *C. vinosum* cells in the dark, but to have no effect on light-dependent transport in *C. vinosum* [22,23]. Presumably, DCCD-treated *C. vinosum* cells are unable to utilize ATP hydrolysis to generate the $\Delta\bar{\mu}_{H^+}$ required for active transport [21,22]. Table I shows that, compared to CCCP, DCCD has little effect on light-dependent Ca^{2+} efflux by *C. vinosum* cells, suggesting that the DCCD-sensitive ATPase plays no role in the Ca^{2+} efflux. HOQNO, an inhibitor of cyclic electron flow in *C. vinosum* [21–24,33], substantially inhibits Ca^{2+} efflux. Previous studies in our laboratory on the effect of HOQNO on alanine and malate transport [22] suggest that, in *C. vinosum*, HOQNO acts specifically as an inhibitor of electron flow and not as an uncoupler. The results in Table I are strikingly similar to those obtained previously for light-dependent alanine uptake by *C. vinosum* [22] with respect to the percent inhibition caused by DCCD, HOQNO and CCCP. These results are consistent with a driving force for Ca^{2+} efflux (and active transport) in *C. vinosum* that arises from proton efflux coupled to light-dependent cyclic electron flow. Results similar to those shown in Table I were obtained with *R. rubrum* cells.

CCCP and gramicidin, by rendering the bacterial membranes permeable to protons, will eliminate both the ΔpH and $\Delta\psi$ components of $\Delta\bar{\mu}_{H^+}$ [34]. The roles of these two components in Ca^{2+} efflux can be examined separately by using specific ionophores that eliminate either $\Delta\psi$ or ΔpH, but not both. Table II shows the effect of valinomycin, a reagent that eliminates $\Delta\psi$ by rendering the membrane permeable to K^+ [34], on light-dependent Ca^{2+} efflux by *C. vinosum* cells at three external pH values. Valinomycin becomes increasingly effective as an inhibitor of Ca^{2+} efflux at more alkaline pH. Table III shows the effect of nigericin on light-dependent Ca^{2+} efflux in *C. vinosum*. Nigericin catalyzes an electroneutral exchange

TABLE II

THE EFFECT OF pH AND VALINOMYCIN ON LIGHT-DEPENDENT Ca^{2+} EFFLUX BY *C. VINOSUM* CELLS

Reaction conditions were as in Table I except that 50 μM potassium phosphate buffers of pH 5.5 or 7.5 were substituted for pH 6.5 buffer where indicated. Valinomycin was present at a concentration of 20 μM where indicated.

pH	Sample	Ca^{2+} content (μmol Ca^{2+} per μmol BChl)	Percent Inhibition by Valinomycin
5.5	(1) Zero Time	2.02	–
	(2) 15 Min, Light	0.94	–
	(3) (2) + Valinomycin	1.22	25.4
6.5	(1) Zero Time	2.10	–
	(2) 15 Min, Light	1.48	–
	(3) (2) + Valinomycin	1.85	60.1
7.5	(1) Zero Time	1.60	–
	(2) 15 Min, Light	0.71	–
	(3) (2) + Valinomycin	1.45	84.1

TABLE III

THE EFFECTS OF NIGERICIN ON LIGHT-DEPENDENT Ca^{2+} EFFLUX BY *C. VINOSUM* CELLS

Reaction conditions were as in Table II. Nigericin was present at a concentration of 0.5 μM where indicated.

pH	Sample	Ca^{2+} content (μmol Ca^{2+} per μmol BChl)	Percent Inhibition by Nigericin
5.5	(1) Zero Time	0.145	–
	(2) 15 Min, Light	0.099	–
	(3) (2) + Nigericin	0.124	54.3
7.5	(1) Zero Time	2.04	–
	(2) 15 Min, Light	0.42	–
	(3) (2) + Nigericin	0.55	7.6

of H^+ for K^+ [34] and thus collapses ΔpH. In contrast to the effects obtained with valinomycin, nigericin is considerably more effective as an inhibitor of light-dependent Ca^{2+} efflux at acid pH than at alkaline pH. The results of Tables II and III suggest that ΔpH contributes significantly to the driving force for Ca^{2+} efflux at acid pH values, but makes essentially no contribution at pH 7.5. Similar results had been obtained previously in our laboratory for light-dependent alanine uptake by *C. vinosum* [22].

The demonstration that cells of purple photosynthetic bacteria can exchange internal Ca^{2+} for external H^+ leads to the prediction that chromatophores prepared from these bacteria, because of their opposite sidedness, should be able to exchange external Ca^{2+} for internal H^+. The protons would be initially taken up during cyclic electron flow. We have tested this prediction by following the effect of Ca^{2+} on transmembrane proton movements in *C. vinosum* chromatophores, utilizing the technique of 9-aminoacridine fluorescence quenching [27] to monitor ΔpH. Fig. 2 shows that illumination of *C. vinosum* chromatophores produces a ΔpH (inside acid), as indicated by the quenching of 9-aminoacridine fluorescence. Control experiments (data not shown) showed that the rate of decay of the quenching on cessation of actinic illumination was very slow and that the quenching was immediately reversed by addition of low concentrations of CCCP or nigericin. Also, as would be expected, prior addition of either CCCP or nigericin abolished the light-dependent 9-aminoacridine fluorescence quenching. *C. vinosum* chromatophores lose the soluble electron carrier, cytochrome *c*-551, during preparation [33,35], thus it was necessary to add the nonphysiological electron carrier phenazine methosulfate in order to observe ΔpH. The 9-aminoacridine fluorescence quenching by actinic light showed the same dependence on the concentration of added phenazine methosulfate as did the light-dependent phosphorylation of ADP by *C. vinosum* chromatophores.

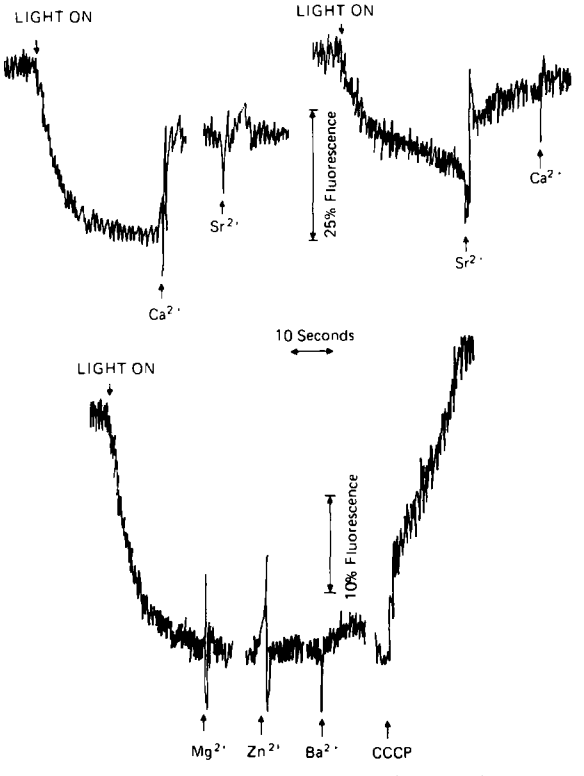

Fig. 2. The effect of divalent cations and uncoupler on ΔpH in *C. vinosum* chromatophores. The reaction mixtures (2.0 ml volume) contained *C. vinosum* chromatophores, equivalent to 23 μM BChl, 25 mM Tricine-KOH buffer (pH 7.5), 50 mM choline chloride, 30 μM phenazine methosulfate and 10 μM 9-aminoacridine. The divalent cations were added to a final concentration of 3 mM where indicated. CCCP was added to a final concentration of 2 μM where indicated. 2 μM valinomycin was present in all samples.

Fig. 2 shows that addition of Ca^{2+} to *C. vinosum* chromatophores which have produced a ΔpH leads to a rapid efflux of H^+, as indicated by the rapid reversal of 9-aminoacridine fluorescence quenching. This result would be expected if a Ca^{2+}/H^+ antiport were present to catalyze the exchange of external Ca^{2+} for internal H^+. Fig. 2 shows that Sr^{2+} was as effective as Ca^{2+} in reversing the 9-aminoacridine fluorescence quenching. The fact that prior addition of Sr^{2+} eliminated the Ca^{2+} effect and vice versa (see Fig. 2) suggests that these two divalent cations affect the same Ca^{2+}/H^+ antiport. Similar effects were observed with Ca^{2+} and Mn^{2+} (data not shown), suggesting that the Ca^{2+}/H^+ antiport has an affinity for Sr^{2+} and Mn^{2+} similar to that for Ca^{2+}. Ba^{2+} caused only a barely detectable, but reproducible, reversal of 9-aminoacridine fluorescence quenching. The effect of Ba^{2+} was abolished by prior addition of Ca^{2+}. As can be seen from Fig. 2, Mg^{2+} and Zn^{2+} caused no increase in 9-aminoacridine fluorescence. Three monovalent ions (Na^+, K^+ and Rb^+) were tested and they neither

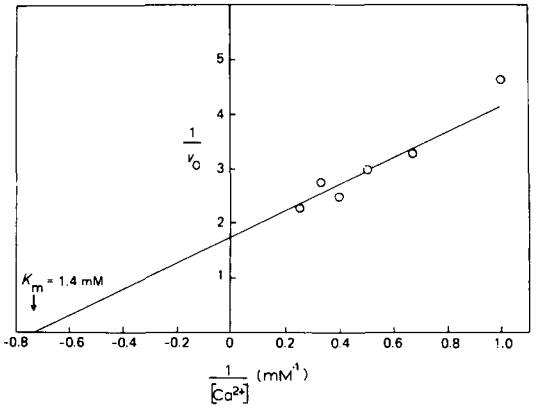

Fig. 3. The effect of Ca^{2+} concentration on the rate of H^+ efflux from *C. vinosum* chromatophores. Reaction conditions were as in Fig. 2. v_0 was measured as the initial rate of percent change in 9-aminoacridine fluorescence. Illumination times for each sample were adjusted to give the same extent of 9-aminoacridine fluorescence quenching, and thus the same ΔpH, prior to Ca^{2+} addition.

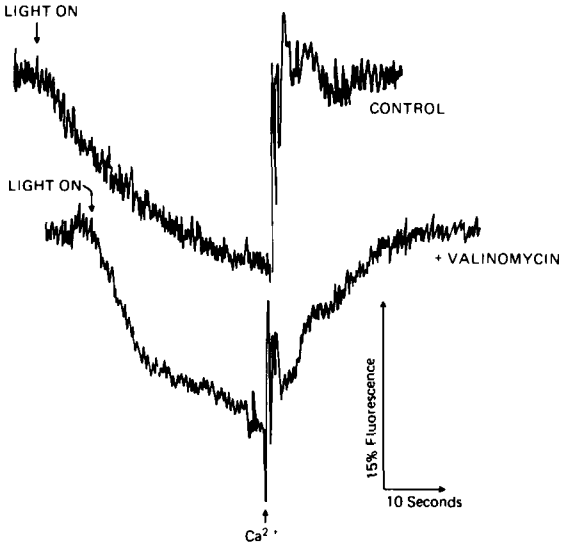

Fig. 4. The effect of valinomycin on light-dependent 9-aminoacridine fluorescence quenching and its reversal by Ca^{2+} in *C. vinosum* chromatophores. Reaction conditions were as in Fig. 2. 2 μM valinomycin was present where indicated.

affected the 9-aminoacridine fluorescence intensity nor blocked the reversal of 9-aminoacridine fluorescence quenching caused by Ca^{2+}, Sr^{2+} or Mn^{2+}. Fig. 2 also shows that the addition of the uncoupler CCCP caused an immediate increase in 9-aminoacridine fluorescence, usually to a level above that prior to illumination, suggesting the possibility that a small ΔpH exists in the dark. Similar results were obtained with chromatophores prepared from *R. rubrum*, suggesting that both bacteria possess a Ca^{2+}/H^+ antiport with specificity: $Ca^{2+} \approx Sr^{2+} \approx Mn^{2+} \gg Ba^{2+}$.

Further investigation is needed to establish the exact order of relative affinities. However, the widespread existence of Ca^{2+}/H^+ antiports [13,36–38] suggests that Ca^{2+} is the physiologically significant cation. Fig. 3 shows that the K_m value for Ca^{2+} is 1.4 mM. There appears to be some divergence from Michaelis-Menten behavior at the lowest calcium concentration. Such deviation was not unanticipated in the light of the observations of Brey and Rosen [38], who reported sigmoidal dependence on $[Ca^{2+}]$ for the *Escherichia coli* Ca^{2+}/H^+ antiport. We are currently attempting to refine our measurements so that we can be sure that any deviations from Michaelis-Menten kinetics are significantly larger than the experimental uncertainty.

Fig. 4 shows the effects of eliminating Δψ (by addition of valinomycin plus K^+) on light-induced 9-aminoacridine fluorescence quenching and its reversal by Ca^{2+} in *C. vinosum* chromatophores. Two effects of valinomycin are apparent. The rate of light-induced formation of ΔpH is increased by valinomycin (in the presence of K^+). This is to be expected, since elimination of Δψ (inside positive) makes it easier to pump additional positively-charged protons inside the chromatophores. Such increases in the rate of ΔpH formation on abolition of Δψ have been previously observed in other systems [1]. Of greater concern in our investigation was the effect of eliminating Δψ on the rate of Ca^{2+}-H^+ exchange via the antiport. Fig. 4 shows that eliminating Δψ causes a large decrease in the rate of antiport reaction (as monitored by the efflux of H^+ caused by Ca^{2+} addition). The experiments described in Fig. 4 were performed at pH 7.5. Similar inhibition of the Ca^{2+}/H^+ antiport reaction on eliminating Δψ was also observed at pH 6.5 and pH 7.0. Similar results were also obtained with *R. rubrum* chromatophores.

The observation of Ca^{2+}-dependent H^+ efflux from chromatophores is consistent with the operation of a Ca^{2+}/H^+ antiport. If such antiports do exist, one

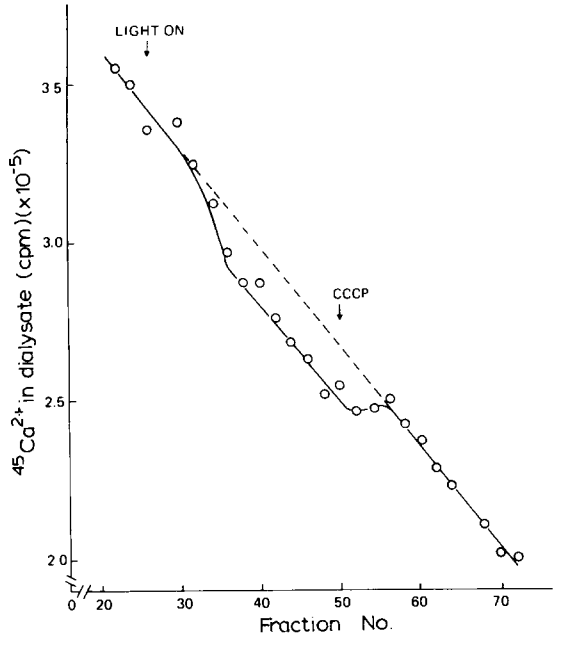

Fig. 5. Light-dependent $^{45}Ca^{2+}$ uptake by *R. rubrum* chromatophores. The upper chamber of the flow dialysis cell contained *R. rubrum* chromatophores equivalent to 340 μM BChl, 25 mM Tricine-KOH buffer (pH 7.5), 50 mM choline chloride and 30 μM phenazine methosulfate. $^{45}CaCl_2$ was added at time zero to give a final $CaCl_2$ concentration of 10 mM (specific activity as in Fig. 1). The dialysis buffer which contained 25 mM Tricine-KOH (pH 7.5), 50 mM choline chloride and 30 μM phenazine methosulfate, was kept anaerobic by continuous bubbling with N_2 gas. The flow rate was 6 ml per min and 1.5-ml fractions were collected. 10 μM CCCP was added where indicated.

should also be able to observe light-dependent Ca^{2+} uptake by chromatophores. Fig. 5 shows a flow-dialysis experiment in which $^{45}Ca^{2+}$ uptake by *R. rubrum* chromatophores can be observed in the light. As expected, addition of CCCP to eliminate $\Delta\bar{\mu}_{H^+}$ causes the efflux of previously accumulated $^{45}Ca^{2+}$. The reason for the lag between the onset of illumination and any measurable decrease in the $^{45}Ca^{2+}$ content of the dialysate is under further investigation.

Discussion

Intact cells of both *C. vinosum* and *R. rubrum* exhibit Ca^{2+} efflux that is dependent on light-driven electron flow and is inhibited by uncouplers that eliminate $\Delta\bar{\mu}_{H^+}$. Chromatophores prepared from these bacteria show H^+ efflux on addition of Ca^{2+} and take up Ca^{2+} in an uncoupler-sensitive reaction. These results provide excellent evidence for the presence of Ca^{2+}/H^+ antiports in representative species of both photosynthetic purple sulfur and nonsulfur bacteria. The studies reported above represent the first direct evidence for Ca^{2+}/H^+ antiports in any photosynthetic bacteria. The advantage of such antiports to these bacteria may lie in the ability they confer on cells to maintain sufficient $\Delta\bar{\mu}_{H^+}$ to drive endergonic reactions (e.g., ATP formation), without relying on a ΔpH so large that it would raise the internal pH beyond the limits consistent with activity for internal enzymes.

The Ca^{2+}/H^+ antiport of *C. vinosum* exhibits 50% maximal rate at 1.4 mM Ca^{2+}, higher than the 0.28 mM value reported for the *E. coli* antiport [34]. The divalent cation specificity of the *C. vinosum* and *R. rubrum* antiports is similar to that of the *E. coli* system with Ca^{2+}, Sr^{2+} and Mn^{2+} being considerably more effective than Ba^{2+}, and Mg^{2+} being ineffective [38]. Zn^{2+}, a divalent cation that was not tested in the *E. coli* system, was ineffective in the photosynthetic bacteria employed in this study.

A question of considerable interest is the $H^+ : Ca^{2+}$ stoichiometry of the photosynthetic bacteria Ca^{2+}/H^+ antiports. The fact (see Fig. 4) that $\Delta\psi$ (inside positive) greatly stimulates the rate of H^+ efflux coupled to Ca^{2+} uptake in both *C. vinosum* and *R. rubrum* chromatophores suggests the possibility that the antiport is electrogenic with $H^+ : Ca^{2+} > 2$. While there are other possible explanations for the effect of $\Delta\psi$ on the antiport, the data obtained in this study on Ca^{2+} efflux from whole cells of *C. vinosum* and *R. rubrum* are consistent with an electrogenic antiport with $H^+ : Ca^{2+} > 2$. The driving force for Ca^{2+} efflux from the cells via the Ca^{2+}/H^+ antiport can arise from three conditions: (1) $[Ca^{2+}]_{in} > [Ca^{2+}]_{out}$; (2) $[H^+]_{in} < [H^+]_{out}$; or (3) the antiport is electrogenic with $H^+ : Ca^{2+} > 2$ and the cells maintain a $\Delta\psi$ (outside positive). Possibility 1 is unlikely. If incomplete equilibration occurs in the 1.5 h period of calcium loading, $[Ca^{2+}]_{in}$ would be expected to be less than, not greater than $[Ca^{2+}]_{out}$. The possibility did remain that *C. vinosum* cells possessed high endogenous $[Ca^{2+}]$, making $[Ca^{2+}]_{in}$ actually greater than

$[Ca^{2+}]_{out}$. We have been able to determine the total Ca^{2+} content in *C. vinosum* cells using atomic absorbance spectroscopy and estimate the free Ca^{2+} by determining the Ca^{2+} lost after incubation in Ca^{2+}-free medium in the presence of the Ca^{2+} ionophore A23187. Combining this information with the internal cell volume, we can estimate the internal $[Ca^{2+}]$ to be 0.51 mM. Thus, in the efflux experiments where the external $[Ca^{2+}]$ is 10 mM, Ca^{2+} movement is not from high to low $[Ca^{2+}]$. As far as possibility 2 is concerned, Table III suggests that although ΔpH contributes to the driving force for Ca^{2+} efflux at pH 5.5, it does not contribute significantly at pH 7.5. The inhibition of Ca^{2+} efflux by valinomycin at all pH values tested (see Table II) suggests that possibility 3 may be correct. However, a direct measurement of the $H^+ : Ca^{2+}$ stoichiometry must be made before a definite conclusion can be drawn.

The greater inhibitory effectiveness of valinomycin on Ca^{2+} efflux from *C. vinosum* cells at alkaline pH, where nigericin has little effect, than at acid pH, where nigericin shows substantial inhibition, is similar to the results previously observed in our laboratory for light-dependent alanine uptake by *C. vinosum* cells [22]. The explanation proposed at that time [22] suggested that the $\Delta\bar{\mu}_{H^+}$ produced by *C. vinosum* cells in the light consisted of both ΔpH and $\Delta\psi$ components at $pH_{out} < 6.5$, but only of $\Delta\psi$ at pH_{out} substantially above 6.5. Qualitatively similar dependencies of ΔpH, $\Delta\psi$ and $\Delta\bar{\mu}_{H^+}$ on external pH had previously been reported in whole cells [39] and vesicles [40] from *E. coli* and other nonphotosynthetic bacteria. The results obtained in this study of Ca^{2+} efflux in *C. vinosum* are also consistent with the suggestion that $\Delta\psi$ is the major component of $\Delta\bar{\mu}_{H^+}$ over most of the bacterium's viable pH range.

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