Evidence for two calcium transport systems in the photosynthetic bacterium *Chromatium vinosum*

Cindy R. Peach, Andrea D. Cobb*, James A. Smith and David B. Knaff+

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409, USA

Received 17 March 1986

Light-dependent Ca^{2+} efflux via the Ca^{2+}/H^+ antiport in the photosynthetic purple sulfur bacterium *Chromatium vinosum* was inhibited by three phenothiazines: chlorpromazine; trifluoperazine and phenothiazine. The inhibitors had no effect on Ca^{2+} uptake by *C. vinosum* in the dark nor any effect on the light-dependent efflux of either Na^+ or Tl^+ catalyzed, respectively, by the *C. vinosum* Na^+/H^+ or K^+/H^+ antiports. Ruthenium red and $LaCl_3$, neither of which inhibited light-dependent Ca^{2+} efflux in *C. vinosum*, markedly inhibited Ca^{2+} uptake in the dark by *C. vinosum* cells. Ruthenium red had no effect on the uptake of either Na^+or the K^+ analog Tl^+ by *C. vinosum* cells in the dark. These results have been interpreted in terms of two separate Ca^{2+} transport systems in *C. vinosum*: (i) a phenothiazine-sensitive and ruthenium red, La^{3+}-insensitive Ca^{2+}/H^+ antiport responsible for Ca^{2+} efflux in the light; and (ii) a ruthenium red and La^{3+}-sensitive but phenothiazine-insensitive Ca^{2+} uptake system.

Ca^{2+} transport *La^{3+}* *Ruthenium red* (C. vinosum)

1. INTRODUCTION

Relatively little information is available concerning the mechanism(s) of cation transport in photosynthetic bacteria [1,2]. However, the photosynthetic purple sulfur bacterium *Chromatium vinosum* has been shown to carry out energy-dependent efflux of Ca^{2+}, Na^+ and K^+ catalyzed by 3 separate cation/H^+ antiports [3–5]. Evidence was also obtained for the presence of Na^+/H^+ and Ca^{2+}/H^+ antiports in the photosynthetic purple non-sulfur bacterium *Rhodospirillum rubrum* [3,4]. In these earlier experiments on Ca^{2+} efflux from *C. vinosum* conducted in our laboratory [4], cells were loaded with Ca^{2+} by prolonged incubation with $^{45}Ca^{2+}$ in the dark but the characteristics of Ca^{2+} uptake (e.g. energy requirements and inhibitor sensitivity) were not investigated. We have since re-examined Ca^{2+} uptake by *C. vinosum* and obtained evidence that Ca^{2+} uptake appears to be catalyzed by a second *C. vinosum* Ca^{2+} transport system, distinct from the Ca^{2+}/H^+ antiport. Furthermore, we have demonstrated that Ca^{2+} efflux via the *C. vinosum* Ca^{2+}/H^+ antiport, but not Ca^{2+} uptake, is highly sensitive to inhibition by phenothiazine drugs known to inhibit other Ca^{2+}-transport or -binding proteins, such as the Ca^{2+}-ATPase of the sarcoplasmic reticulum [6,7] and calmodulin [8,9].

* Present address: Department of Biochemistry, Texas Tech University School of Medicine, Lubbock, TX 79409, USA

+ To whom reprint requests should be addressed

Abbreviations: BChl, bacteriochlorophyll; CCCP, carbonyl cyanide-*m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide

2. MATERIALS AND METHODS

C. vinosum cells were grown on a malate-containing medium and harvested essentially as in [10]. Ca^{2+}, Na^+ and Tl^+ efflux and uptake were assayed under an N_2 atmosphere by the previously described membrane filtration technique [3–5,11].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

In uptake experiments, all inhibitors were allowed to incubate with the cells for 5 min prior to the initiation of transport by substrate addition except for arsenate, which was allowed to incubate for 30 min. In efflux experiments, inhibitors were added after the 75 min dark-loading period, immediately before efflux was initiated by the start of the 20 min illumination period. BChl *a* was measured after extraction into 7:2 (v/v) acetone/methanol as described by Clayton [12].

Carrier-free $^{45}CaCl_2$ was obtained from ICN Radioisotope Division. Carrier-free $^{22}NaCl$ and $[^{204}Tl]Tl_2SO_4$ were obtained from Amersham. Valinomycin, CCCP, DCCD, chlorpromazine, phenothiazine and trifluoperazine were obtained from Sigma. Ruthenium red and $LaCl_3$ were generous gifts from Professor P.S. Song. Inhibitors were added as small aliquots of stock solutions using either water or DMSO as the solvent. The volume of DMSO was always <2% (v/v), at which concentration DMSO itself was shown to have no effect on cation transport.

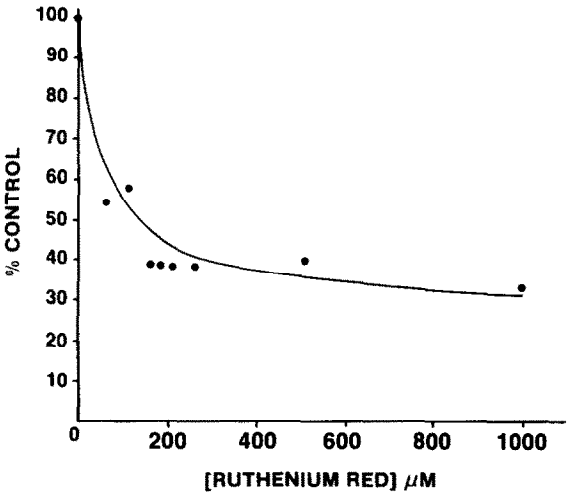

Fig.1. Effect of ruthenium red on Ca^{2+} uptake by *C. vinosum* cells in the dark. The reaction mixtures (100 μl volume) contained *C. vinosum* cells, at a concentration equivalent to 150 μM BChl, in 50 mM potassium phosphate buffer (pH 6.5), 10 mM $^{45}CaCl_2$ (spec. act. 1×10^3 dpm/nmol Ca^{2+}) and ruthenium red, as indicated, at 0°C. Uptake was measured after 75 min.

3. RESULTS

As indicated above, in previous experiments in our laboratory, *C. vinosum* cells were loaded with $^{45}Ca^{2+}$ during 75 min incubations in the dark under an N_2 atmosphere. It was not known whether loading occurred through non-specific, limited Ca^{2+} permeability across the membrane or was catalyzed by a specific Ca^{2+}-transport system. To investigate this question, the effect of $[Ca^{2+}]$ on the initial rate of Ca^{2+} uptake in the dark was studied. Uptake obeyed Michaelis-Menten kinetics with $K_m = 7.0 \pm 0.7$ mM and $V_{max} = 150$ nmol $Ca^{2+} \cdot min^{-1} \cdot \mu mol\ BChl^{-1}$ (not shown). This observation, indicative of carrier-mediated Ca^{2+} uptake in the dark, suggested an examination of the effect of ruthenium red (an inhibitor of Ca^{2+} fluxes through Ca^{2+}-specific channels in eukaryotic membranes [13]) on Ca^{2+} uptake by *C. vinosum* cells. Fig.1 shows that Ca^{2+} uptake in the dark was markedly inhibited by ruthenium red, with 50% inhibition of uptake observed in the range 60–65 μM ruthenium red. Maximal inhibition of uptake, even at ruthenium red concentrations as high as 2 mM, was typically in the 65–70% range. This observation suggests that while 65–70% of the observed Ca^{2+} uptake by *C. vinosum* occurs via a specific, ruthenium red-sensitive channel, a portion of the uptake (i.e. 30–35%) may occur via non-specific leakage across the cell membrane. If this conclusion is correct, 50% inhibition of Ca^{2+} uptake via the ruthenium red-sensitive channel occurs at a ruthenium red concentration of 20 μM. This concentration is higher than that required to give 50% inhibition of Ca^{2+} transport in ruthenium red-sensitive eukaryotic systems [13]. However, it is possible that the outer cell wall of *C. vinosum* may impose some permeability barrier against this inhibitor and that the ruthenium red-sensitive *C. vinosum* system is in fact of comparable sensitivity to eukaryotic systems. This possibility is supported by the observation that several ionophores (e.g. valinomycin, nigericin, monensin and CCCP) require 10-fold higher concentrations for effectiveness in whole cells of *C. vinosum* than in cell wall-free chromatophores [4,5,11]. Ruthenium red had no effect (<5% inhibition), even at a concentration of 1 mM, on the uptake of either $^{22}Na^+$ [3] or $^{204}Tl^+$ by *C. vinosum* cells in the dark (Tl^+ serves as a K^+ analog in the *C. vinosum* K^+/H^+ antiport [5] and in an ATP-energized K^+-uptake system [5,14]). These results suggest that

ruthenium red is inhibiting a Ca^{2+}-specific process in *C. vinosum*. Furthermore, ruthenium red had no inhibitory effect on $^{45}Ca^{2+}$ efflux in the light from pre-loaded *C. vinosum* cells (not shown).

La^{3+}, another well-investigated inhibitor of carrier-mediated Ca^{2+} fluxes [13], affected Ca^{2+} transport by *C. vinosum* in a manner similar to that observed with ruthenium red. $LaCl_3$ had no effect on light-dependent Ca^{2+} efflux but was a potent inhibitor of Ca^{2+} uptake in the dark. As was the case with ruthenium red, 100% inhibition of Ca^{2+} uptake in the dark could not be obtained, even at La^{3+} concentrations as high as 500 μM. However, in typical experiments 80–85% inhibition was observed with half-maximal inhibition found at 35 μM La^{3+} (not shown). These results are consistent with the majority of the observed uptake occurring via a Ca^{2+}-specific carrier but with some uptake (~20%) occurring through a non-specific leak. The observation that La^{3+} and ruthenium red inhibit Ca^{2+} uptake without affecting Ca^{2+} efflux strongly suggests that two separate Ca^{2+} systems exist in *C. vinosum*: one sensitive to La^{3+} and ruthenium red that results in Ca^{2+} uptake in the dark and a second, unaffected by ruthenium red or La^{3+}, that catalyzes Ca^{2+} efflux during illumination. As light-driven Ca^{2+} efflux from *C. vinosum* cells had previously been shown to occur via an electrogenic Ca^{2+}/H^+ antiport [4], it can be concluded that the *C. vinosum* Ca^{2+}/H^+ antiport is inhibited neither by ruthenium red nor by La^{3+}.

Ca^{2+} uptake via ruthenium red-sensitive channels in eukaryotes is electrogenic [13] and thus can be driven by a membrane potential ($\Delta\psi$), outside positive. As *C. vinosum* cells can utilize ATP hydrolysis via a DCCD-sensitive F_0F_1 ATPase [15] to maintain a $\Delta\psi$ of ~80 mV (outside positive) in the dark [15,16], it was of interest to determine the effect of reagents known to eliminate $\Delta\psi$ in *C. vinosum* cells on Ca^{2+} uptake in the dark. Table 1 shows that eliminating $\Delta\psi$ by inhibiting the ATPase with DCCD [15–17], by depleting the internal pool of ATP with arsenate [14] or by using ionophores that collapse $\Delta\psi$ in *C. vinosum* [16,17] by rendering the membranes permeable to either protons (CCCP [18]) or K^+ (valinomycin [19]), had only a small inhibitory effect on Ca^{2+} uptake by *C. vinosum* cells in the dark. These results suggest little if any involvement of $\Delta\psi$ in providing energy for Ca^{2+} uptake in the dark (ΔpH cannot serve as a possible energy source since *C. vinosum* cells maintain no ΔpH in the dark [17]).

Table 1

The effect of inhibitors on Ca^{2+} uptake by *C. vinosum* cells in the dark

Sample	Uptake (% control)
(1) Control	100
(2) + 20 μM valinomycin	75 (±6)
(3) + 30 μM CCCP	79 (±8)
(4) + 300 μM DCCD	84 (±5)
(5) + 5 mM arsenate	73 (±2)

Reaction conditions as in fig.1 except that the indicated inhibitors replaced ruthenium red and the specific activity of $^{45}CaCl_2$ was 2.97×10^7 dpm/nmol Ca^{2+}. The results represent the average of 5 experiments with the average deviations given in parentheses

In an attempt to provide further evidence for a Ca^{2+}-transport system in *C. vinosum* distinct from the Ca^{2+}/H^+ antiport, the effect of other inhibitors on Ca^{2+} uptake in the dark and on Ca^{2+} efflux via the antiport in the light was investigated. Three phenothiazine drugs (chlorpromazine, phenothiazine and trifluoperazine) were tested and shown, even at concentrations as high as 100 μM, to have no effect (<5% inhibition) on Ca^{2+} uptake by *C. vinosum* cells in the dark. However, as shown in fig.2, all 3 phenothiazines were potent inhibitors of Ca^{2+} efflux from pre-loaded *C. vinosum* cells in the light, with 50% inhibition of efflux being observed at 12, 14 and 25 μM phenothiazine, trifluoperazine, and chlorpromazine, respectively. The 3 phenothiazine drugs, even at 100 μM, had no inhibitory effect (<5% inhibition) on either light-driven Na^+ efflux via the *C. vinosum* Na^+/H^+ antiport [3] or Tl^+ efflux via the *C. vinosum* K^+/H^+ antiport [5] (not shown), as would be expected if a Ca^{2+}-specific system were being inhibited. The observation that light-driven Ca^{2+} efflux via the *C. vinosum* Ca^{2+}/H^+ antiport is unaffected by ruthenium red and La^{3+} but is inhibited by phenothiazines, while Ca^{2+} uptake in the dark shows the opposite response to the two classes of inhibitors, further supports the presence of two separate Ca^{2+}-transport systems in *C. vinosum*.

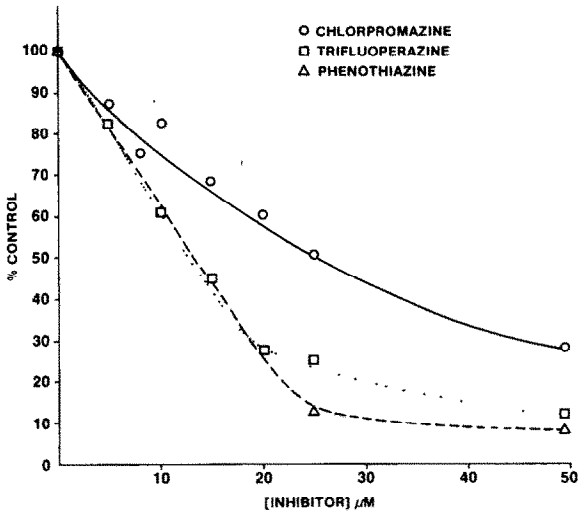

Fig.2. Effect of phenothiazines on Ca^{2+} uptake by illuminated *C. vinosum* cells. Reaction mixtures were as in table 1 except that chloropromazine (○), phenothiazine (△) or trifluoperazine (□) replaced ruthenium red. Cells were pre-loaded with $^{45}CaCl_2$ in the dark for 75 min at 0°C and Ca^{2+} efflux assayed after a subsequent 20 min exposure to saturating light at ambient temperature.

4. DISCUSSION

The data presented above provide the first evidence for multiple Ca^{2+}-transport systems in a photosynthetic bacterium. However, there is precedent for a similar situation in non-photosynthetic bacteria. For example, *Azotobacter vinelandii* contains a ruthenium red-insensitive Ca^{2+}/H^+ antiport that catalyzes energy-dependent Ca^{2+} efflux and a ruthenium red- and La^{3+}-sensitive transport system that catalyzes Ca^{2+} uptake [20]. Unlike the case with *C. vinosum*, the ruthenium red-sensitive, *A. vinelandii* Ca^{2+}-uptake system appeared to be driven by $\Delta\psi$ (outside positive). The data of table 1 suggest that while $\Delta\psi$ may make a small contribution to driving Ca^{2+} uptake by *C. vinosum* in the dark, the ruthenium red, La^{3+}-sensitive system in *C. vinosum* may catalyze Ca^{2+} equilibration across the membrane without coupling $\Delta\psi$ to Ca^{2+} accumulation.

The sensitivity of the *C. vinosum* Ca^{2+}/H^+ antiport to phenothiazine drugs suggests that the antiport may have some structural similarities to other proteins involved in Ca^{2+} transport or binding such as the Ca^{2+}-ATPase of the sarcoplasmic reticulum [6,7] and calmodulin [8,9], since these proteins are also affected by phenothiazines. In the case of calmodulin, it appears that the hydrophobic phenothiazines act by competing with calmodulin's normal target enzymes for hydrophobic portions of calmodulin [21–23]. It may be possible that these inhibitors act in a similar fashion in so far as the *C. vinosum* Ca^{2+}/H^+ antiport is concerned. Experiments to test this possibility are currently underway in our laboratory.

ACKNOWLEDGEMENT

This work was supported by a grant (to D.B.K.) from the National Science Foundation (PCM-8408564).

REFERENCES

[1] Knaff, D.B. and Davidson, V.L. (1983) Photochem. Photobiol. 36, 721–724.
[2] Knaff, D.B. (1986) Photosynth. Res., in press.
[3] Knaff, D.B., Davidson, V.L. and Pettitt, C.A. (1981) Arch. Biochem. Biophys. 211, 234–239.
[4] Davidson, V.L. and Knaff, D.B. (1981) Biochim. Biophys. Acta 637, 53–60.
[5] Davidson, V.L. and Knaff, D.B. (1982) Photobiochem. Photobiophys. 3, 167–174.
[6] Volpe, P., Costello, B., Chu, A. and Fleischer, S. (1984) Arch. Biochem. Biophys. 233, 174–179.
[7] Chiesi, M. and Carafoli, E. (1982) J. Biol. Chem. 257, 984–991.
[8] Levin, R.M. and Weiss, B. (1977) Mol. Pharmacol. 13, 690–697.
[9] Cheung, W.Y. (1984) Fed. Proc. 43, 2995–2999.
[10] Knaff, D.B. and Buchanan, B.B. (1975) Biochim. Biophys. Acta 376, 549–560.
[11] Knaff, D.B. (1978) Arch. Biochem. Biophys. 189, 225–230.
[12] Clayton, R.K. (1963) in: Bacterial Photosynthesis (Gest, H. et al. eds) pp.495–500, Antioch, Yellow Springs, OH.
[13] Carafoli, E. and Sottocasa, G. (1984) in: Bioenergetics (Ernster, L. ed.) pp.269–289, Elsevier, Amsterdam, New York.
[14] Davidson, V.L. and Knaff, D.B. (1982) Arch. Biochem. Biophys. 213, 358–362.
[15] Gephstein, A., Carmeli, C. and Nelson, N. (1978) FEBS Lett. 85, 219–223.
[16] Knaff, D.B., Whetstone, R. and Carr, J.W. (1979) FEBS Lett. 98, 283–285.

[17] Davidson, V.L. and Knaff, D.B. (1982) Photochem. Photobiol. 36, 551–558.
[18] Heytler, P.C. (1979) Methods Enzymol. 55, 463–472.
[19] Reed, R.W. (1979) Methods Enzymol. 55, 435–444.
[20] Zimniak, P. and Barnes, E.M. jr (1980) J. Biol. Chem. 255, 10140–10143.
[21] La Porte, D.C., Wierman, B.M. and Storm, D.R. (1980) Biochemistry 19, 3814–3819.
[22] Vogel, H.J., Lindahl, L. and Thulin, E. (1983) FEBS Lett. 157, 241–246.
[23] Krebs, J., Buerkler, J., Guerini, D., Brunner, J. and Carafoli, E. (1984) Biochemistry 23, 400–403.