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THE ROLE OF THE MEMBRANE POTENTIAL IN ACTIVE TRANSPORT BY THE PHOTOSYNTHETIC BACTERIUM *CHROMATIUM VINOSUM*

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1. Introduction

The photosynthetic purple sulfur bacterium *Chromatium vinosum* has been shown to accumulate a variety of metabolites in energy-dependent reactions [1]. The observation that this metabolite accumulation was inhibited by uncouplers known to abolish transmembrane electrochemical proton gradients ($\Delta\bar{\mu}_{H^+}$), suggested the possibility that the driving force for active transport in *C. vinosum* may be $\Delta\bar{\mu}_{H^+}$ [1]. Our initial results suggested that both the pH gradient (ΔpH) and membrane potential ($\Delta\psi$) components of $\Delta\bar{\mu}_{H^+}$ may be involved in transport [1]. In this investigation, we have concentrated on the role of $\Delta\psi$. Using the lipophilic cation TPP^+, we have confirmed the finding [2] that *C. vinosum* cells generate $\Delta\psi$ (exterior positive) in the light. We have also shown that *C. vinosum* maintains a $\Delta\psi$ of similar polarity in the dark, apparently using energy from ATP hydrolysis. Evidence has been obtained that a $\Delta\psi$, in the absence of other energy sources, is capable of supporting alanine uptake by *C. vinosum* cells.

2. Materials and methods

C. vinosum was grown and alanine uptake assayed as in [1]. Flow dialysis assays were performed under anaerobic conditions as in [3,4]. *C. vinosum* cells at a concentration equivalent to 175 μM bacteriochlorophyll (estimated according to [5]) in 0.4 ml 50 mM potassium phosphate buffer (pH 6.6) were used with a 6.5 ml/min flow rate. Fractions of ~1.7 ml were collected. Illumination during flow dialysis was provided by a Photographic Research Organization high intensity movie lamp 30 cm from the sample compartment with 2% $CuSO_4$ solution used as a heat filter and a 1 M $NaNO_2$ solution used as an ultraviolet filter. [^{3}H]TPPBr (6.3 mCi/mmol) was provided by the Isotope Synthesis Group of the Hofman-LaRoche Co. [^{14}C]Alanine (168 mCi/mmol) was purchased from New England Nuclear. CCCP, HOQNO, and DCCD were purchased from Calbiochem. Nigericin was a gift of Dr D. Berger. The last 4 reagents were added as concentrated stock solutions in dimethylsulfoxide.

Abbreviations: TPP^+, tetraphenylphosphonium; DCCD, *N,N'*-dicyclohexylcarbodiimide; HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone

3. Results and discussion

Uptake of several metabolites by *C. vinosum* cells has been shown to occur in the dark although at lower rates than in the light [1]. This uptake in the dark was inhibited by DCCD [1], a specific inhibitor of the membrane-bound ATPase in *C. vinosum* [2,6], suggesting that ATP hydrolysis provided the energy for uptake in the dark. If, as suggested by the uncoupler sensitivity of transport, ATP hydrolysis produced an electrochemical proton gradient which in turn drove transport, it should be possible to demonstrate a DCCD-sensitive $\Delta\psi$ in *C. vinosum* cells in the dark. Figure 1 shows that adding CCCP, an uncoupler known

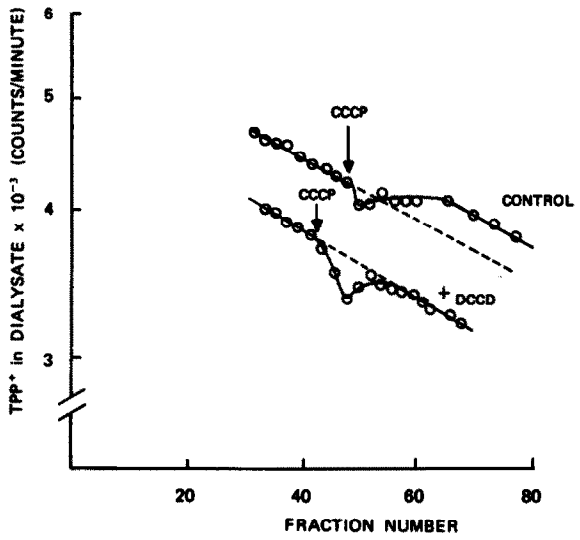

Fig.1. The effect of DCCD on [^{3}H]TPP$^+$ uptake by *C. vinosum* cells in the dark. Flow dialysis was conducted as in section 2 with 0.8 mM TPP$^+$ and 0.5 μM nigericin present. DCCD was present at 200 μM where indicated. CCCP was added, as indicated by the arrow, to give final conc. 20 μM.

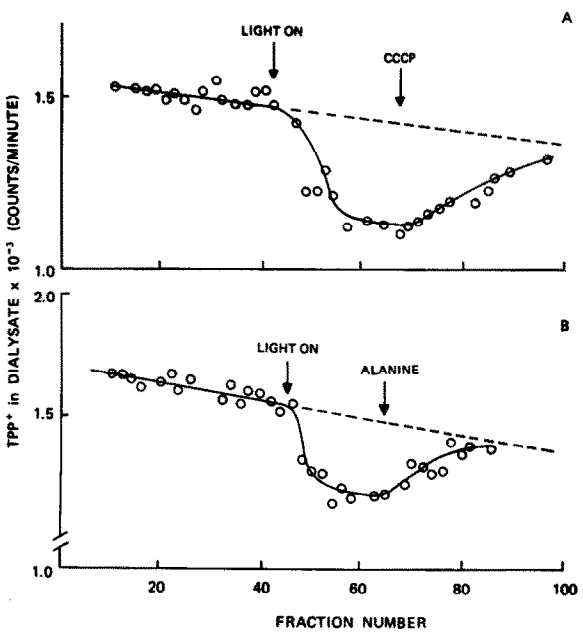

Fig.2. The effect of CCCP and alanine on [^{3}H]TPP$^+$ uptake by *C. vinosum* cells in the light. Conditions as in fig.1 with 200 μM DCCD present in both samples. CCCP (A) or alanine (B) was added, where indicated, to give final conc. 20 μM and 200 μM, respectively.

to eliminate $\Delta\psi$, causes the release of [^{3}H]TPP$^+$ from *C. vinosum* cells kept in the dark. Release of this lipophilic cation on addition of CCCP implies that the cation had been pre-accumulated by *C. vinosum* in response to a $\Delta\psi$ (interior negative). If *C. vinosum* was pre-incubated with DCCD, no release of TPP$^+$ was observed on addition of CCCP. It, thus, appears that ATP hydrolysis, catalyzed by a DCCD-sensitive enzyme, results in a $\Delta\psi$ (interior negative), detectable by the uptake of a lipophilic cation.

Figure 2A shows that even in the presence of DCCD, an additional light-induced uptake of TPP$^+$ can be detected. Adding CCCP causes release of the accumulated TPP$^+$. The migration of a lipophilic cation, such as TPP$^+$, into the *C. vinosum* cells is consistent with the formation of a light-dependent $\Delta\psi$ (interior negative) and confirms our earlier results using the light-induced carotenoid band-shift to monitor $\Delta\psi$. Addition of HOQNO at concentrations known to inhibit cyclic electron flow [7] and active transport [1] in *C. vinosum* cells eliminated light-induced TPP$^+$ uptake (data not shown), suggesting that the light-dependent $\Delta\psi$ originates from cyclic electron flow.

If $\Delta\psi$ can indeed serve as a source of energy for transport in *C. vinosum*, use of this energy during metabolite uptake should lead to a decrease in the magnitude of $\Delta\psi$. Figure 2B shows that $\Delta\psi$ decreases when uptake is initiated by addition of the transportable metabolite, alanine. It should be pointed out that the experiments in both fig.1,2 were performed in the presence of nigericin, an ionophore that will eliminate ΔpH but not $\Delta\psi$ [3,4,8]. This was done chiefly so that the relationship of $\Delta\psi$ to active transport could be studied independently of any contribution from ΔpH. However, it should be mentioned that the extent of TPP$^+$ uptake was always much greater in the presence than in the absence of nigericin. In some preparations, no TPP$^+$ uptake was detected unless nigericin was present. The reasons for this variability are under further investigation. It is worth noting that substantial increases in the size of $\Delta\psi$ on nigericin addition have been observed with *E. coli* vesicles [3,4].

If $\Delta\psi$ (exterior positive) does indeed provide the

driving force for alanine uptake, the creation of a $\Delta\psi$ of proper polarity should result in alanine uptake independently of any other energy input. Figure 3 shows the results of an experiment in which a $\Delta\psi$ across the *C. vinosum* membrane is created by a K^+ diffusion gradient in the dark and the presence of DCCD. *C. vinosum* cells washed in buffer containing KCl were placed in a K^+-free medium and the experiment was initiated by adding valinomycin to render the cells permeable to K^+. As can be seen in fig.3, the $\Delta\psi$ (exterior positive) produced by K^+ diffusion out of the cell in the presence of valinomycin results in substantial alanine uptake. No uptake of alanine was observed in the absence of K^+ gradient. This can be seen from the experiment shown in fig.3, in which the cells were placed in a medium with a KCl concentration equal to that in the medium used to wash the cells. Similar metabolite uptake driven by an artificially created $\Delta\psi$ has been observed in non-photosynthetic bacteria [9–14] but has not been reported in an obligate phototroph.

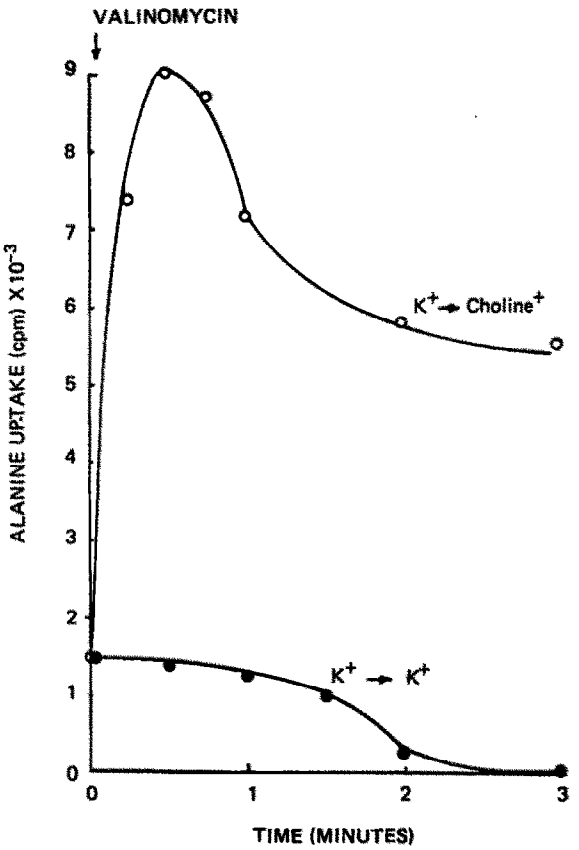

Fig.3. K^+-diffusion potential driven alanine uptake by *C. vinosum* cells. *C. vinosum* cells were washed twice with 100 mM KCl/50 mM Tris·HCl (pH 7.3) and resuspended to final bacteriochlorophyll 150 μM in a reaction mixture containing 250 μM DCCD, 20 μM [^{14}C]alanine, 50 mM Tris·HCl (pH 7.3) and either 100 mM choline chloride (○) or 100 mM KCl (●). The experiment was initiated (t = 0) by adding valinomycin to final conc. 15 μM.

The above results suggest that *C. vinosum* cells use energy (either from light-driven cyclic electron flow or ATP hydrolysis catalyzed by a DCCD-sensitive ATPase) to maintain a membrane potential (exterior positive). The observations that the size of $\Delta\psi$ decreases as energy is used to accumulate alanine and that $\Delta\psi$ created by a K^+-diffusion potential supports alanine uptake both strongly suggest that $\Delta\psi$ (exterior positive) can provide the energy for physiological alanine transport in *C. vinosum*.

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