CO_2 FIXATION BY *HALOBACTERIUM HALOBIUM*

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Received 5 January 1977

1. Introduction

Photosynthesis has been described in a general way as 'a series of processes in which electromagnetic energy is converted to chemical free energy which can be used for biosynthesis' [1]. In the usual photosynthetic organsims the photoactivation of the chlorophyll molecule causes electrons to flow from an electron donor to an electron-acceptor; anaerobic oxido–reduction reactions take place to which the reductive assimilation of CO_2 is linked. In *Halobacterium halobium* the photoactivation of the bacteriorhodopsin molecule results in the transfer of protons from the inside of the cell to the outside [2]. The electrochemical potential gradient thus established across the cell membrane drives ATP synthesis [3]. The fact that bacteriorhodopsin-mediated photophosphorylation is insensitive to the usual inhibitors of the electron-transport chain [3], while oxidative phosphorylation is inhibited by them, suggested that the new type of photophosphorylation could not be linked to oxido–reduction reactions and no reducing power should be formed during the process. However, it will be shown here that the anaerobic *H. halobium* cells are able to assimilate CO_2 into acid-stable products. The requirements for the reaction suggest that it is linked to a reversed electron-flow.

Abbreviations: DCCD *N,N'*-dicyclohexyl carbodiimide, NQNO 2-*n*-nonyl-4-hydroxyquinoline-*N*-oxide, CCCP carbonyl cyanide *m*-chlorophenyl hydrazone, DSPD disalicylidene propanediamine, PIPES piperazine-*N,N'*-bis [2-ethanesulfonic acid], MES 2-[*N*-morpholino]-ethanesulfonic acid

2. Materials and methods

Halobacterium halobium R1 was used [4]. The growth medium and conditions were as described previously [3]. After 2 days of semi-anaerobic growth in the light, the cells were harvested by low speed centrifugation, washed once with basal salt (i.e., all the salts of the medium but without nutrients) and buffered, at pH 6.5, with 50 mM PIPES–MES buffer. They were then suspended in basal salt solution at a concentration of $4–5 \times 10^8$ cells/ml. The suspension was kept in the dark under an argon stream for one hour before starting an experiment. Any additions required were added at that time. The experiment started by the addition of [^{14}C]bicarbonate at a final concentration of 5 mM. Aliquots were taken at intervals and the reaction stopped by adding trichloroacetic acid to a final concentration of 3% [5]. The suspension was centrifuged and a sample of the supernatant used to determine the amount of CO_2 incorporated into acid-stable products. For this each sample of supernatant (1.5 ml) was placed in a vial and the labile CO_2 allowed to evaporate under vacuum overnight. The samples were counted in a Packard scintillation counter using a dioxane-based scintillating fluid. The amount of purple membrane was determined as described previously [3].

PIPES, MES, DCCD, NQNO and CCCP were from Sigma, DSPD was a generous gift from Dr G. Ben-Haim of Tel-Aviv University.

3. Results

3.1. *Fixation of CO_2 into acid-stable products*

Figure 1 shows that anaerobic cells in the dark do

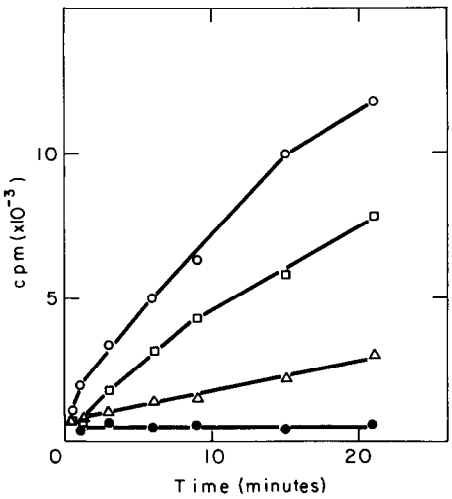

Fig.1. CO_2 fixation by *H. halobium* kept under anaerobic conditions in the dark and in the light. Cells were treated as described under Materials and methods. The suspension contained 26.5 μg bacteriorhodopsin/ml. The reaction was started by adding the labelled bicarbonate (5 mM final concentration, 120 000 cpm/μmol). (•—•) $NaH^{14}CO_3$ added at time 0 in the dark, (△—△) $NaH^{14}CO_3$ added in the dark after 3 min pre-illumination, (□—□) $NaH^{14}CO_3$ added in the light without pre-illumination, (○—○) $NaH^{14}CO_3$ added in the light after 3 min pre-illumination.

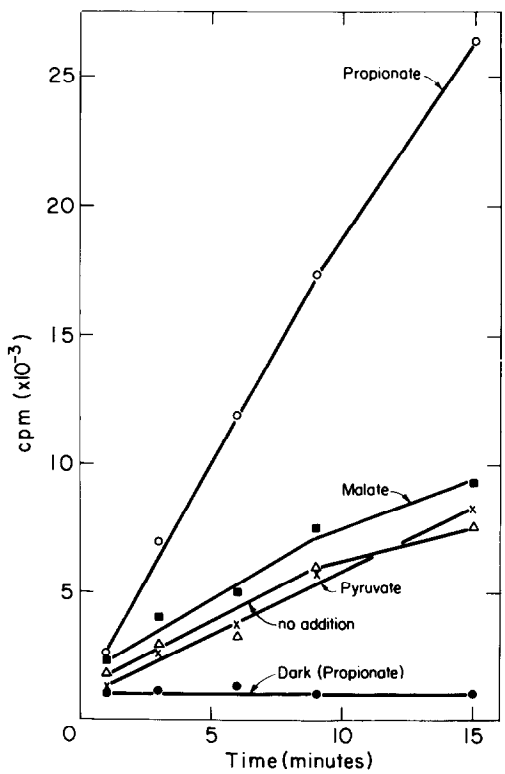

Fig.2. Effect of different substrates added to the cell-suspension in buffered basal salt (see Materials and methods). The substrates were added at a concentration of 10 mM one hour before the start of the CO_2 fixation. (•—•) Propionate dark control, (○—○) propionate, (■—■) malate, (X—X) pyruvate and (△—△) no addition. In all cases $NaH^{14}CO_3$ added in the light after 3 min pre-illumination.

not fix CO_2. When the light was switched on, fixation started with a lag of about one minute. If the cells were pre-illuminated for 3 min before addition of the label, no lag was observed. However, pre-illumination of the cells, with addition of labelled bicarbonate, in the dark, failed to stimulate the incorporation. If the light were switched off at different times after the start of the experiment, the level of the CO_2 incorporated remained constant for at least 30 min (not shown).

3.2. *Effect of substrates*

Succinate, malate, citrate, acetate, pyruvate and propionate were tested as substrates. Only propionate had a noticeable stimulatory effect above the level of the CO_2 incorporated by cells suspended in basal salt alone. Representative results are shown in fig.2.

3.3. *Effect of different inhibitors*

The energy-transfer inhibitor DCCD and the proton-transporting uncoupler CCCP, which were both shown to strongly inhibit photophosphorylation in *Halobacterium halobium* [3], also inhibit CO_2 fixation (fig.3). NQNO, KCN and sodium azide, which have no effect on the photophosphorylation, decrease the assimilation of CO_2 by at least 50%. DSPD is known to prevent the photoreduction of NADP by inhibiting the catalytic function of ferredoxin [6,7]. In our system DSPD did not prevent photophosphorylation but did impair CO_2 fixation.

4. Discussion

The reductive assimilation of CO_2 in photosynthetic bacteria is linked to energy-yielding reactions, viz., photoreduction of ferredoxin and photophos-

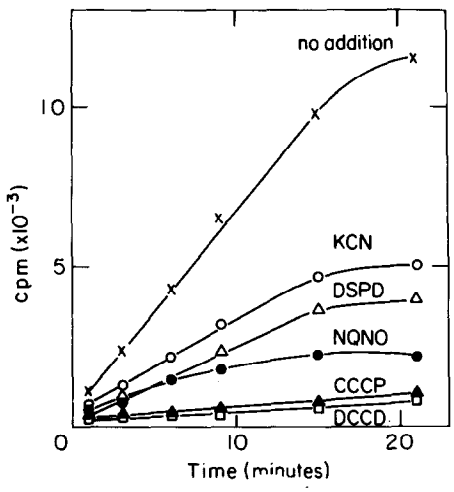

Fig.3. Effect of different inhibitors on the CO_2 fixation. The inhibitors were added as an alcoholic solution (except for KCN). The dilution of the stock solution into the cell-suspension was in each case 500-fold. An equivalent amount of alcohol was added to the control. The final concentrations were, KCN 1 mM, DSPD 1 mM, CCCP 5 × 5 10^{-6} M, DCCD 10^{-4} M, NQNO 10^{-5} M. (×—×) No addition, (○—○) KCN, (^—^) DSPD, (●—●) NQNO, (▲—▲) CCCP and (□—□) DCCD.

phorylation. We see here that *Halobacterium halobium* is able to incorporate CO_2 into acid-stable products. The reaction requires light, ATP and apparently the integrity of the electron-transport chain. Indeed NQNO, which prevents electron-transport between cytochromes *b* and *c* and KCN or sodium azide, which act at the terminal oxygen oxidase, all inhibited the fixation of CO_2 although they had no affect on the photophosphorylation. The inhibition by DSPD suggests that ferredoxin might be involved in the process. Large amounts of ferredoxin have been found in halophilic bacteria but no function is known as yet [8]. However, since the photo-excitation of the bacteriorhodopsin results in a proton-transport, rather than an electron-transport as is the case in chlorophyll systems, it is not immediately obvious how the ferredoxin could be photoreduced. This difficulty may be overcome by remembering that the proton electrochemical-potential gradient generated by the bacteriorhodopsin could in principle drive a reversed electron-flow, as has been shown to occur in mitochondria [9,10] and in chloroplasts [11,12]. In turn this backward electron transport would generate the reducing power necessary to activate the reductive carboxylic acid cycle known to be the major pathway for the assimilation of CO_2 in photosynthetic bacteria [13].

The light inhibition of respiration observed in aerobic *Halobacterium halobium* [14] suggests that a reversed electron-flow, impeding the normal oxido-reduction reactions of the respiratory chain, may very well be brought about by the proton-pump. In the above experiments this mechanism agrees with the requirement of continuous illumination for the incorporation of CO_2 and the strong inhibition produced by CCCP. These effects indicate that a continuous flow of protons is necessary in addition to the presence of ATP. Since of all the substrates we tested only propionate had a substantial stimulatory effect, the reaction which takes place is probably of the type [15]

$$Z_{red} + CO_2 + \text{propionyl-CoA} \rightarrow Z_{ox} +$$

$$\alpha\text{-ketobutyrate} + \text{CoA}$$

where *Z* may be ferredoxin. Identification of the products of the CO_2 fixation will shed some light on the mechanism of the reaction and is currently under investigation.

Acknowledgements

This study was supported by grants from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel and from the German Ministry of Research and Technology.

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