DECREASE OF PROTON PERMEABILITY OF CF_1-DEFICIENT CHLOROPLAST PARTICLES BY TRIPHENYLTIN

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Received 30 June 1975

1. Introduction

Removal of the mitochondrial ATPase (F_1) has been reported to increase the proton permeability of sub-mitochondrial particles [1,2]. Mitchell [3,4] suggested that removal of F_1 unblocked a natural proton conducting channel through the F_0 component of the coupling membrane. Oligomycin, an inhibitor of the membrane-bound mitochondrial ATPase has been found to decrease the high proton conductivity of F_1-deficient sub-mitochondrial particles and bacterial chromatophores [1,4,5,6,7,8,9]. Similar studies on isolated chloroplasts and bacteria [10,11] have shown that removal or absence of the coupling factors rendered these particles permeable to protons and that titration with *N,N'*-dicyclohexylcarbodiimide restored them to a 'pseudo-coupled' state. Unlike oligomycin, triorganotin compounds have been found to inhibit photosynthetic as well as oxidative phosphorylation reactions [12,13,14,15,16,17,18]. Kahn [15] showed that chloroplasts which had been uncoupled by treatment with EDTA had a low residual level of light-induced proton uptake which was increased by the addition of tributyltin and he also found that the slow residual rate of photophosphorylation in EDTA-uncoupled chloroplasts was enhanced by tributyltin. However, Kahn did not show whether blockage of proton leakage or restoration of proton pumping was responsible for the increased level of proton uptake observed when tributyltin was added to EDTA-uncoupled chloroplasts.

The results presented in this paper confirm and extend the work of Kahn and show that triphenyltin is able to restore the electron transport reactions of EDTA-uncoupled chloroplasts to a more coupled state by reducing the high proton permeability of the CF_1-deficient chloroplast particles. The observation that both electron transport and proton permeability of EDTA-uncoupled chloroplasts were decreased by triphenyltin while the level of light-induced proton uptake was increased eliminates the possibility that the increase in the level of proton uptake observed by Kahn [15] was due to an increase in the rate of electron transport. The concentration of triphenyltin which produced maximum restoration of the light-induced proton uptake in EDTA-uncoupled chloroplasts is the same as that concentration which produced maximum inhibition of photophosphorylation in untreated chloroplasts [18]. It is suggested that triphenyltin acts at the same site in both coupled and uncoupled chloroplast preparations. In the former the blockage of proton movement by triphenyltin prevents ATP formation by the proton translocating ATPase. In the latter preparation the blockage of proton permeability prevents short-circuiting of the electron transport driven proton pump via the opened F_0 channel. In addition, observations of the light scattered from chloroplast suspensions show that triphenyltin restored actinic light induced conformational and osmotic responses of EDTA-uncoupled chloroplasts. In contrast to triphenyltin, the smaller and less hydrophobic trimethyltin was not effective in decreasing the proton permeability of EDTA-uncoupled chloroplasts.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FCCP, *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone, Hepes, *N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulphonic acid.

2. Methods and materials

Pea chloroplasts from which the outer membranes had been removed were prepared as described previously [18]. EDTA-uncoupled chloroplast particles were prepared according to the method of Shoshan and Shavit [19], removal of the coupling factor was achieved by incubating chloroplasts in 1 mM EDTA pH 7.8 at a concentration of 100 μg chlorophyll/ml. A Clark-type oxygen electrode was used for measurements of oxygen evolution [18]. Observations of the light scattered at 90° angles from chloroplast suspensions were carried out as described [20] except that an improved amplifier system was used [18]. Proton flux measurements were made on chloroplast suspensions in a cylindrical glass vessel with an integral water jacket. The vessel was enclosed in an aluminium box and actinic light was provided by a Rank Aldis 2000 projector with a Wratten 70 filter. A pH electrode (micro-combination electrode type 33.1140.200 Electronic Instruments Ltd., Chertsey, Surrey, U.K.) was mounted in the lid of the box with an opaque sleeve designed to minimise light entry and facilitate making additions to the reaction medium.

Reagents were obtained as described earlier [18] and in addition, FCCP was obtained from The Boehringer Corporation (London), London, U.K., and EDTA Analar grade from BDH Chemicals Ltd., Poole, U.K.

3. Results and discussion

Removal of the coupling factor by treatment with EDTA yielded resolved chloroplasts which exhibited a much greater rate of electron flow than the rate given by non-treated chloroplasts. In contrast to the stimulatory effect of FCCP on basal electron flow in coupled chloroplasts (fig.1a) FCCP did not increase electron flow in EDTA-uncoupled chloroplasts (fig.1b). However, a 'pseudo-coupled' state of the EDTA-uncoupled chloroplasts was achieved by the addition of 1.1 μM triphenyltin (fig.1c). This concentration of triphenyltin decreased the rate of electron flow almost to the

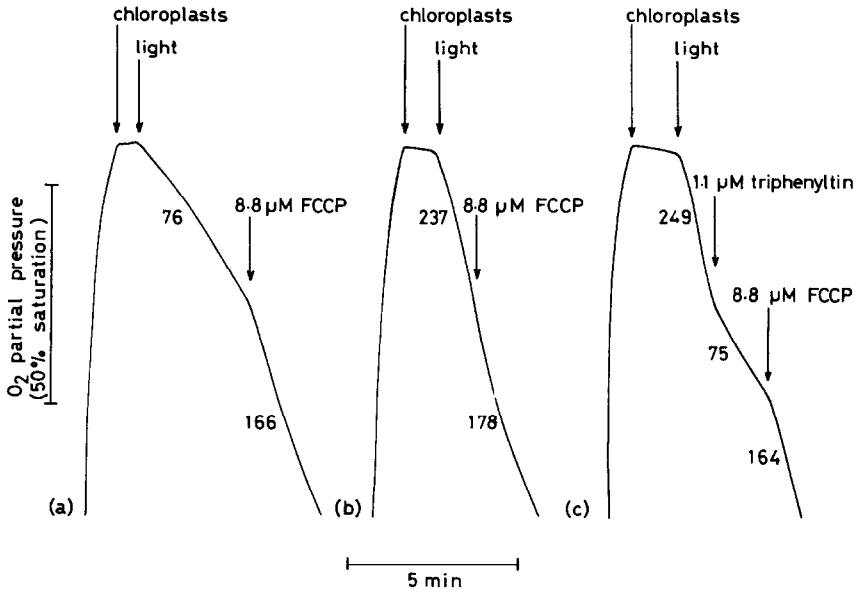

Fig.1. Oxygen evolution from EDTA-uncoupled and non-treated chloroplasts. Chloroplasts at a concentration of 60 μg chlorophyll/ml were added to a medium, deoxygenated by bubbling with N_2, containing 300 mM sorbitol, 12.5 mM Hepes adjusted to pH 7.6 with NaOH, 4.4 mM K_3 Fe $(CN)_6$, 3.3 mM $MgCl_2$, at a temperature of 18°C in a total volume of 4.5 ml. (a) non-treated chloroplasts. (b) and (c) EDTA-uncoupled chloroplasts. Numbers along the traces refer to rates of 0_2 evolution expressed as μg-atoms of oxygen/hr per mg of chlorophyll.

rate of basal electron flow in non-treated chloroplasts but on addition of FCCP a high rate of electron flow was produced (fig.1c). Trimethyltin was unable to substitute for triphenyltin in this 'pseudo-recoupling' process. These results suggest that triphenyltin is able to interact with the thylakoid membrane and to restrain electron flow by reducing the proton permeability, which was presumably increased on removal of the coupling factor. The observation that FCCP stimulated electron flow in triphenyltin-recoupled chloroplasts shows that low concentrations of triphenyltin do not inhibit electron transport and it therefore seems unlikely that the 'pseudo-recoupling' activity of triphenyltin involves its direct interaction with components of the electron transport chain. The inability of trimethyltin to act like triphenyltin in restoring coupling to EDTA-uncoupled chloroplasts suggests that the site(s), which may be occupied by an organotin compound effectively reducing proton leakage, are located in a somewhat hydrophobic region of the thylakoid membrane.

Support for the view that triphenyltin decreased proton leakage in EDTA-uncoupled chloroplasts comes from observations of the development and decay of the light induced proton gradients. Fig.2 shows the light induced uptake of protons by EDTA-uncoupled chloroplasts suspended at pH 7.5 in a medium containing NO_3^- ions. In the first period of illumination the extent of proton uptake was small and there was a rapid decay of the gradient in the dark. Addition of 1.5 μM triphenyltin increased the level of proton uptake during a second period of illumination and decreased the rate constant of decay of the gradient in the dark. 2 μM FCCP greatly reduced these light induced proton movements. Titration of EDTA-uncoupled chloroplasts with increasing concentrations of triphenyltin (fig.3a) revealed that maximum blockage of proton leakage was produced by 0.5 μM triphenyltin but that maximum increase in the level of proton uptake was produced by 1.5 μM triphenyltin. This discrepancy may be due to a low level of endogenous chloride which becomes effective in organotin mediated chloride-hydroxide antiport [21] at the higher levels of triphenyltin. This descrepancy became more pronounced when the concentration of Cl^- ions was increased and at 12 mM Cl^- triphenyltin produced no measurable decrease of proton permeability but the increase in the level of proton uptake remained. Triphenyltin was also able to restore the light induced proton uptake activity of EDTA-uncoupled chloroplasts which had completely lost the ability to take up measurable amounts of protons during actinic illumination. When the pH of the suspending medium was lowered to pH 6.0 there was no measurable blockage of proton leakage in the dark from EDTA-uncoupled chloroplasts (fig.3b). But there remained the

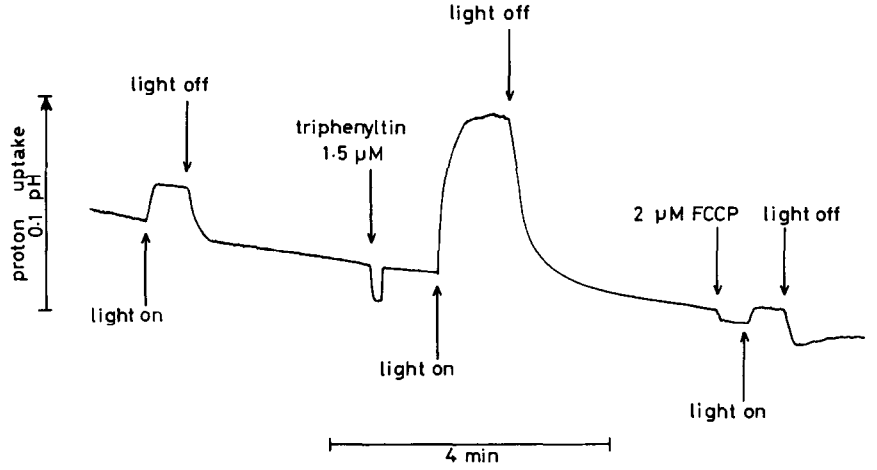

Fig.2. Proton uptake in EDTA-uncoupled chloroplasts during periods of actinic illumination. Chloroplasts were suspended at a concentration of 60 μg chlorophyll/ml in 150 mM sorbitol, 5 mM KNO_3, 2 mM $Mg(NO_3)_2$, 20 μM phenazine methosulphate at 17°C in a total volume of 5.0 ml. The pH of the suspending medium was adjusted to pH 7.5 with dilute HNO_3.

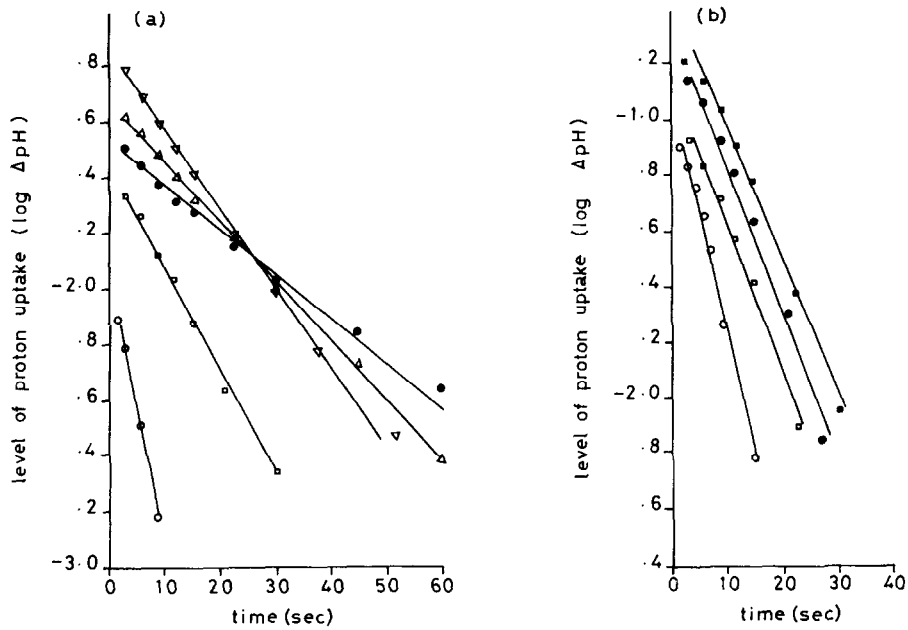

Fig.3. Logarithmic plot of dark decay of the levels of protons taken up during illumination. (a) chloroplasts were suspended at pH 7.5 under the conditions described in Fig.2. (○) EDTA-uncoupled chloroplasts; (□) EDTA-uncoupled chloroplasts plus 0.25 μM triphenyltin; (△) EDTA-uncoupled chloroplasts plus 0.5 μM triphenyltin; (▽) EDTA-uncoupled chloroplasts plus 1.5 μM triphenyltin; (●) untreated chloroplasts. (b) chloroplasts were suspended at pH 6.0 in media containing either NO_3^- or Cl^- ions. (○) EDTA-uncoupled chloroplasts suspended at pH 6.0 under the conditions described in fig.2., (●) as open circles but with the addition of 1 μM triphenyltin, (□) EDTA-uncoupled chloroplasts suspended at pH 6.0 under the conditions described in fig.2 except that Cl^- ions were present instead of NO_3^- ions, (■) as open squares but with the addition of 1 μM triphenyltin.

triphenyltin-induced increase in the level of proton uptake on illumination and this occurred in the presence of either NO_3^- or Cl^- ions. The inability of triphenyltin to block proton leakage from EDTA-uncoupled chloroplasts at pH 6.0 was also apparent from the distinct lack of triphenyltin-induced 'pseudo-recoupling' of electron flow at pH 6.0 and this is in contrast to the activity of triphenyltin at pH 7.5 as seen in fig.1. The different effects of triphenyltin at pH 6.0 and pH 7.5 may result from differences in the light induced movements of ions at the two pH values.

It is generally accepted that changes in light

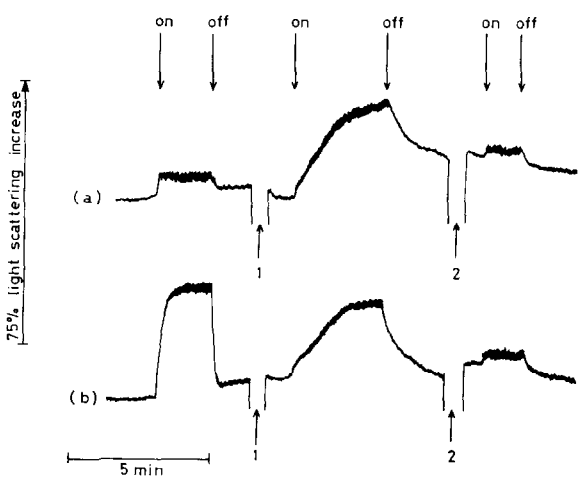

Fig.4. 90° angle light scattering from chloroplasts during periods of actinic illumination. (a) EDTA-Uncoupled chloroplasts at a concentration of 10 μg chlorophyll/ml were suspended in 150 mM sorbitol, 2 mM $MgCl_2$, 5 mM KCl, 20 μM phenazine methosulphate, 4 mM 2-*N*-morpholinoethanesulphonic acid (Mes) adjusted to pH 6.0 with NaOH in a total volume of 2.5 ml at room temperature. Arrows indicate the addition of 1, 1.5 μM triphenyltin, 2, 8 μM FCCP. (b) Non-treated chloroplasts under the same conditions as described in (a).

scattered from chloroplast suspensions are indicative of changes in osmotic and conformational properties of these organelles. Chloroplasts lacking the coupling factor did not show a light scattering response to actinic illumination (fig.4a), however, addition of 1.5 μM triphenyltin, which restored proton uptake, also restored the ability of EDTA-uncoupled chloroplasts to show a light scattering response to actinic illumination. The light scattering response of triphenyltin-recoupled chloroplasts was similar in nature to the response shown by non-treated chloroplasts in the presence of triphenyltin (fig.4b) and in both cases the addition of FCCP completely abolished these responses to actinic light. In view of the sensitivity to FCCP and the slower rise and decay times of light scattering when compared with proton uptake these light scattering changes appear to be secondary to but dependent on proton uptake and may be the result of movements of other ions and molecules in response to pH and electrical gradients across the membranes.

Acknowledgements

We thank Dr. A. P. Dawson for discussion and the Science Research Council for financial support.

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