Biochimica et Biophysica Acta, 347 (1974) 264–272

BBA 46743

H^+ UPTAKE BY CHROMATOPHORES FROM *RHODOPSEUDOMONAS SPHEROIDES*

THE RELATION BETWEEN RAPID H^+ UPTAKE AND THE H^+ PUMP

RICHARD J. COGDELL* and ANTONY R. CROFTS

Department of Biochemistry, Medical School, University of Bristol, Bristol BS8 1TD (U.K.)

(Received November 21st, 1973)

SUMMARY

1. H^+ uptake induced by repeated flash excitation approached the full extent of H^+ uptake induced by continuous light. At low repetition rates, the H^+ uptake was seen to consist of repeated occurrences of rapid H^+ uptake.

2. The effects of ionophores and uncoupling agents on H^+ uptake induced by continuous light could be adequately accounted for in terms of their effects on the flash induced changes. It is concluded that the reaction disclosed by rapid H^+ uptake is an integral part of the process observed on continuous illumination, and therefore, in view of the association between rapid H^+ uptake and the reduction of a hydrogen-carrying secondary acceptor, that the electron transport system is an integral part of the mechanism of the H^+ pump.

3. When the frequency of repetition of the flashes was increased, the full extent of H^+ uptake or of the carotenoid change was seen only after the first few flashes. Thereafter, the extent decreased, and depended on the dark time between flashes. The full extent of the change could be restored even at high frequencies if uncoupling agents or valinomycin were present.

4. It is concluded that the recovery of the extent of H^+ uptake or the carotenoid change between flashes reflected the turnover of the electron transport chain, and that the increased recovery in the presence of uncoupling agents or valinomycin reflected the stimulation of electron flow under uncoupled conditions, or on dissipation of the membrane potential.

INTRODUCTION

Upon illumination, chromatophores prepared from photosynthetic bacteria take up H^+ from the suspending medium into the intravesicular space [1, 2]. Though it is now generally accepted that this light induced H^+ uptake represents the activity

Abbreviation: FCCP, carbonyl cyanide-*p*-trifluoromethoxy-phenylhydrazone.

* Present address: Section of Genetics, Development and Physiology, and Department of Applied Physics, Cornell University, Ithaca, New York. 14850.

of an electrogenic H^+ pump [3], there is still considerable argument about the exact mechanism of the pump.

While studying the kinetics of light induced H^+ uptake, an initial fast phase was discovered [4]; recent investigations into the mechanism have been concerned with characterising this initial phase [5–8]. Before information gained from these studies on rapid H^+ uptake can be extended to help with an understanding of the total light induced change, it is necessary to prove that both the initial rapid phases and the subsequent slower ones reflect the same overall process.

We have investigated this problem by comparing H^+ uptake induced by continuous illumination to that induced by a repetitive series of single turnover flashes. It has also been possible, by exciting the chromatophores with flash groups of sufficiently high frequency, to demonstrate how the rate of electron flow is controlled by the degree of coupling.

A preliminary report of this work has appeared elsewhere [9].

METHODS

Cells of *Rhodopseudomonas spheroides* Ga mutant were grown in batch culture and chromatophores prepared from them as previously described [4]. The experiments were performed in an anaerobic redox cuvette similar to that described by Dutton [10], which was gassed with oxygen free nitrogen. This procedure minimised the slow pH drifts normally encountered with unbuffered suspensions open to the air. The cuvette was housed in a rapidly responding single beam spectrophotometer, and the pH changes were monitored from absorbancy changes of the pH indicator bromocresol purple [8]. In some experiments over an extended time scale the pH change was measured with an electrode. H^+ uptake was routinely calibrated by standard addition of 10 mM HCl.

Excitation was provided by either a repetitive series of 10 μs xenon flashes, a group of 20 μs xenon flashes (flash group) or a 55 W quartz iodine lamp (continuous illumination).

Nigericin was a kind gift from Dr R. L. Harned of Commercial Solvents Ltd, Ind. FCCP was a kind gift from Dr P. G. Heytler and valinomycin and antimycin A were purchased from Sigma. All other chemicals used were of AnalaR grade or of the highest purity commercially available.

RESULTS

The time course of H^+ uptake by chromatophores from *Rps. spheroides* induced by a series of 10 μs xenon flashes (at a frequency of 2 Hz) was "staircase" like (Fig. 1). The slope of the "staircase" depended on the frequency of the flashes (Fig. 2). It increased with increasing frequency until saturation, where the change when observed over a slow time-scale was essentially indistinguishable from that induced by continuous illumination.

If the signal from the photomultiplier was recorded using the monitoring oscilloscope in its AC-coupled mode, then the "steps" in the "staircase" could be superimposed. Using a fast sweep speed (2 ms division) it could be seen that at relatively low frequencies of repetition each "step" corresponded kinetically to a single

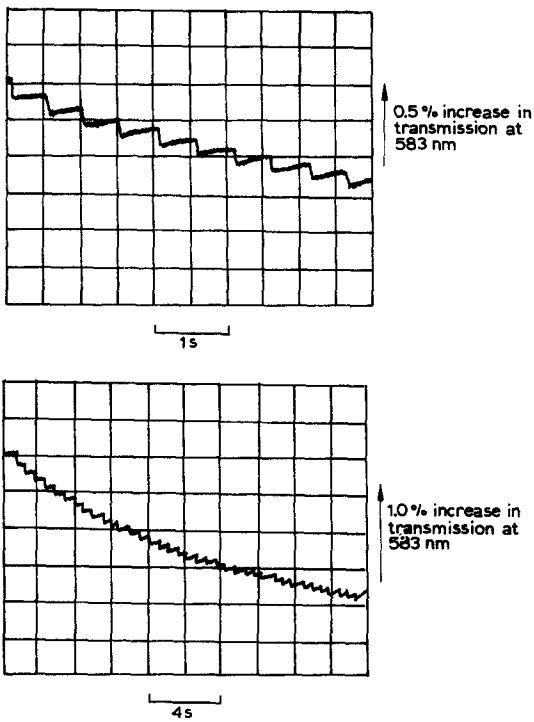

Fig. 1. H^+ uptake induced by repeated 10 μs xenon flashes. Chromatophores from *Rps. spheroides* (12 μg/ml bacteriochlorophyll) were suspended in 100 mM KCl at pH 6.2 containing 25 μM bromocresolpurple. The flash frequency was 2 Hz.

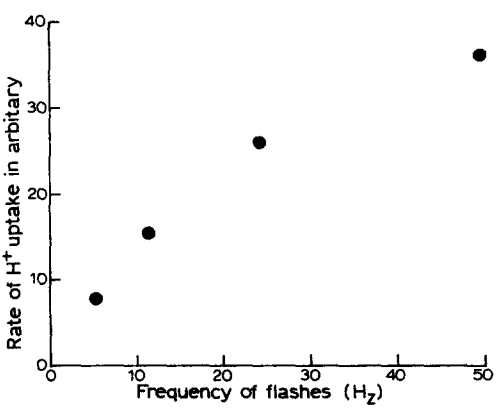

Fig. 2. The effect of flash frequency on the initial slope of the H^+ uptake induced by repeated flashes. Chromatophores from *Rps. spheroides* (52 μg/ml bacteriochlorophyll) were suspended in 100 mM KCl at pH 6.3, containing 80 μM bromocresolpurple. 20 μs xenon flashes were provided at the frequencies indicated.

occurrence of rapid H^+ uptake. The extent of the "step" declined after the first few flashes, but the rate of the change remained the same. A series of superimposed "steps" recorded when the change was approaching saturation was similar in time course but of smaller extent. At higher frequencies the decline of the extent of each "step" became apparent after fewer flashes, possibly because the turnover of the electron transport system which was possible following each flash was reduced, so that the extent of each "step" declined (see below). It is interesting to note (Fig. 1) that the rate of decay of each "step" increased as the cumulative extent of the change increased.

When the extent of H^+ uptake induced by continuous illumination and that induced by a repetitive series of 10 μs xenon flashes at 50 Hz were recorded with a glass electrode, the extent of H^+ uptake induced by the repetitive flash reached 50–60% of the change induced by continuous light in the absence of valinomycin, and 80–90% of the change in the presence of valinomycin. At lower frequencies, the extent of the change achieved was rather less; the failure to reach the full extent observed with continuous illumination may be ascribed mainly to non-saturation with the intensities and the geometry of the apparatus available.

Effects of ionophores and inhibitors

Valinomycin has previously been shown to stimulate the rate and extent of H^+ uptake induced by continuous illumination [1, 2]. The effect of valinomycin on

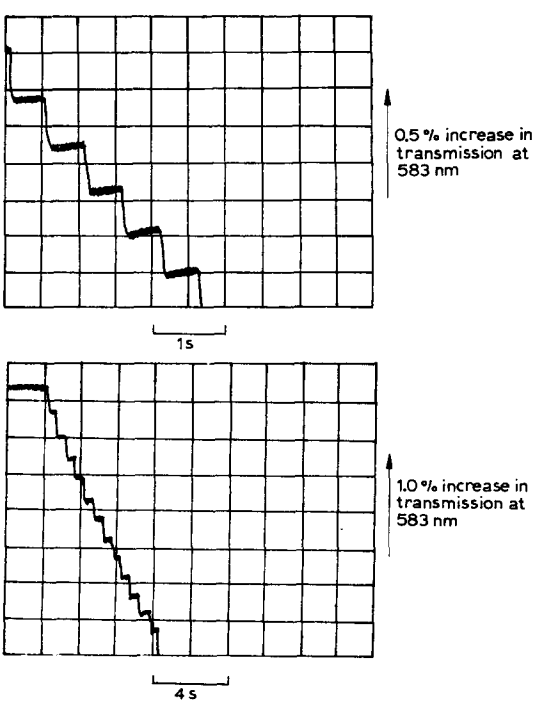

Fig. 3. The effect of valinomycin on H^+ uptake induced by repeated 10 μs xenon flashes. The conditions were exactly the same as those of Fig. 1. except that the medium was supplemented with 2 μM valinomycin.

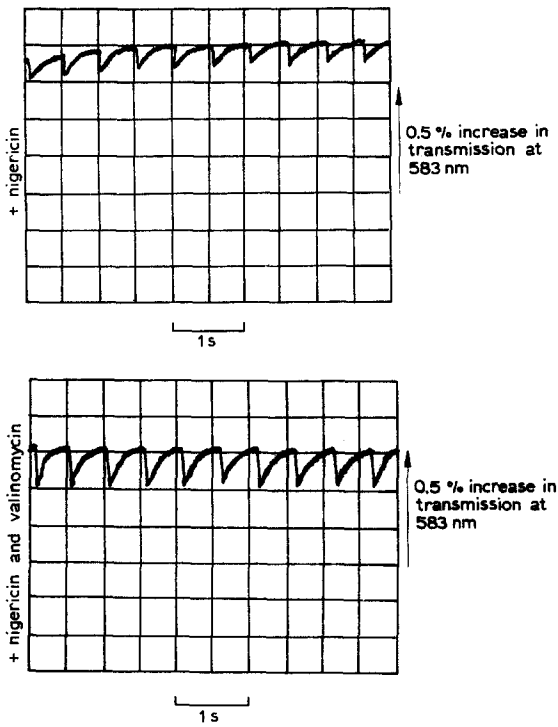

Fig. 4. The effect of nigericin, in the presence and absence of valinomycin, on H^+ uptake induced by repeated 10 μs xenon flashes. The conditions were exactly the same as those in Fig. 1, except that 2 μM nigericin and 2 μM valinomycin were added where indicated.

the repetitive flash induced H^+ uptake is depicted in Fig. 3. In the presence of valinomycin (2 μM) the extent of each "step" was larger (double the extent in the absence of valinomycin) and the slope of the "staircase" was consequently steeper.

Nigericin inhibits the light induced H^+ uptake by chromatophores [1, 11]. On addition of nigericin [0.2 μM] the "staircase" like H^+ uptake induced by repetitive excitation was replaced by a "sawtooth" trace, in which the rapid H^+ uptake was followed by a slower decay, the rate of which could be varied by varying the concentration of nigericin (Fig. 4). At the concentration used here the rate of decay of each "step" was such that the change decayed completely between flashes. A similar effect on the decay of the rapid H^+ uptake induced by repetitive flashes was also observed when FCCP replaced nigericin. With either ionophore, at higher frequencies of excitation when the time between flashes was shorter than the decay time, a net H^+ uptake was seen, but the total extent was less than that in the absence of the ionophore. In the presence of high concentrations of nigericin a small absorbancy change in the direction of acidification of bromocresolpurple was observed; this was not seen when valinomycin was also present (Fig. 4), or when FCCP replaced nigericin, or when the change was monitored with phenol red. The change was not reduced by high buffering and could be ascribed to changes associated with the binding of the indicator to the membrane (for example see refs 4, 12). Bromocresolpurple does bind

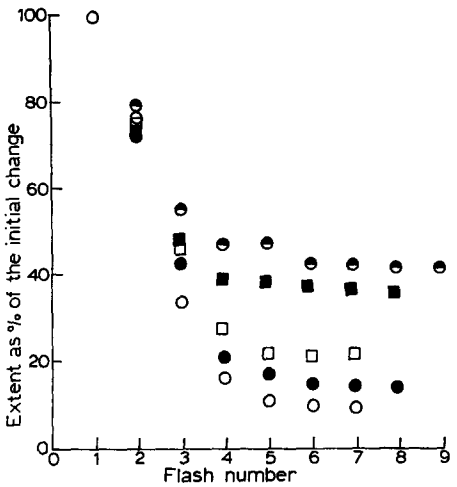

Fig. 5. The effect of flash frequency upon the amount of turnover per flash, monitoring the xenon flash induced carotenoid shift. The carotenoid shift was monitored single ended at 490 nm. The cuvette contained 3 cm^3, 100 mM KCl, 20 mM 2-(*N*-morpholino)ethanesulphonic acid, pH 6.3 and chromatophores of *Rps. spheroides* (15 μg/ml bacteriochlorophyll). The extent of the change as a percentage of the extent of the change induced by the first flash in the group is plotted against the flash number. The following flash frequencies were used. (○) 100 Hz, (●) 50 Hz, (□) 25 Hz, (■) 12 Hz, (◓) 6 Hz. Excitation was provided by a 20 μs xenon flash.

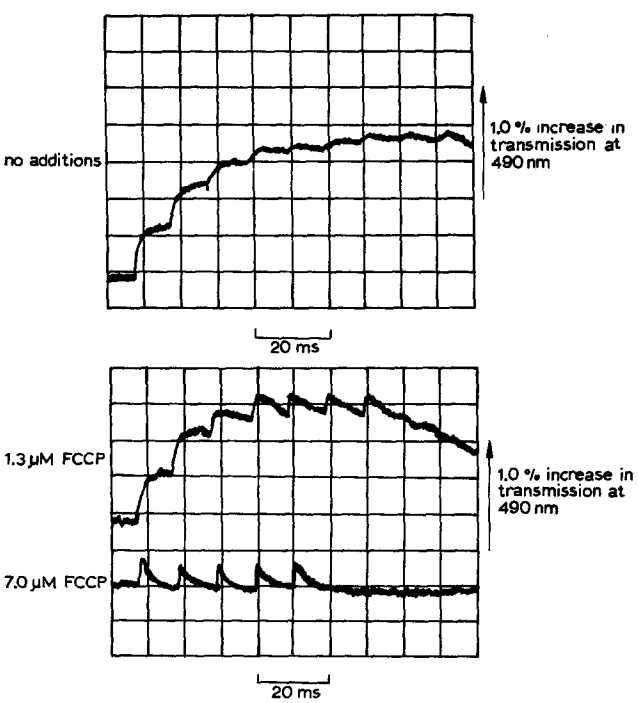

Fig. 6. The effect of FCCP upon the amount of turnover per flash monitoring the carotenoid band shift. The conditions were as described in Fig. 5 except that FCCP was added where indicated. The flash group frequency was 100 Hz. (A) A group of nine flashes. (B) Top trace, a group of seven flashes, Bottom trace, a group of five flashes.

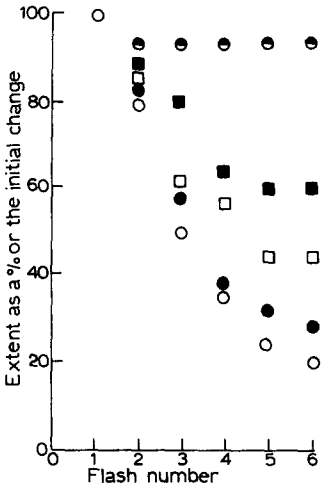

Fig. 7. The effect of FCCP upon the amount of turnover per flash, monitoring the carotenoid band shift at constant flash frequency. The experimental conditions were the same as described in Fig. 5 with FCCP added as indicated below; (●) 0.66 μM FCCP, (□) 1.3 μM FCCP, (■) 2.6 μM FCCP, (◓) 7.0 μM FCCP, (○) no FCCP. The flash frequency was 100 Hz. The extent of the change as a percentage of the extent induced by the 1st flash is plotted against flash number.

weakly to the chromatophore membrane and it is of interest to note that the "acid" change was observed only under conditions where a high membrane potential might be expected to exist across the chromatophore membrane.

Antimycin A and *o*-phenanthroline both inhibited H^+ uptake induced either by a repetitive series of xenon flashes, or by continuous illumination.

Flash group excitation

When rapid H^+ uptake or the carotenoid band shift was induced by a group of single turn-over flashes, the amount of turnover induced by each flash, as indicated by the extent of the change relative to that observed after the first flash, depended on the time between flashes. As the frequency of the flashes within the groups was increased, the amount of turnover per flash decreased. These effects are shown in Figs 5, 6 and 7 for the carotenoid change; exactly analogous results were obtained when measuring the rapid H^+ change. Under normal coupled conditions the recovery time for dark electron flow was relatively long ($>$ 125 ms, Fig. 5). However if the degree of coupling was decreased by addition of the uncoupler FCCP the extent of the change per flash increased, indicating a more rapid turnover of the dark electron flow (Figs 6 and 7). With increasing amounts of FCCP the amount of turnover occurring per flash increased to a maximum, where further additions of FCCP had no additional effect.

DISCUSSION

The results presented above show that the extent of H^+ uptake induced by continuous illumination could be approached by a series of discreet reactions of rapid H^+ uptake following each of a series of repetitive flashes. Moreover the effects

of ionophores, uncouplers, and electron flow inhibitors on rapid H^+ uptake, and on H^+ uptake induced by repetitive or continuous illumination show clearly that the latter effects can be adequately accounted for in terms of the former. These results indicate that rapid H^+ uptake is the elementary event contributing to light induced H^+ uptake. We can therefore conclude that information gained from studies of rapid H^+ uptake are of direct relevance to our understanding of the mechanism of the H^+ pump.

We have previously shown that attenuation of flash induced rapid H^+ uptake in these chromatophores is associated with chemical reduction of a H-carrier, and concluded that the flash induced change may represent H^+ binding by this component on its reduction by the primary acceptor [8]. If this is the case, our present results indicate that the reactions of electron flow are involved directly in the mechanism of the H^+ pump, as proposed by Mitchell [13].

Following excitation of coupled chromatophores with a group of closely spaced flashes the amount of turnover per flash (monitored from the extent of either the carotenoid shift or rapid H^+ uptake) rapidly declined after the initial flash. Even at flash frequencies of 6 Hz, dark electron flow did not completely recover between the flashes. However, if the degree of coupling was decreased by small additions of the uncoupler FCCP, dark electron flow was accelerated, until at a concentration of 7 μM FCCP, dark electron flow could completely recover within 10 ms. This illustrates how the rate of dark electron flow can be controlled by the back pressure of the high energy state. Jackson and Dutton [14] have shown that, following activation of chromatophores of *Rps. spheroides* with flash groups, electron flow between cytochromes *b* and *c* was accelerated by the addition of FCCP. It seems therefore that a rate limiting step of dark electron flow occurs between these two cytochromes, which is sensitive to the extent of the high energy state. By implication this locates a site of energy conservation between the two cytochromes. Further elucidation of this problem must await a more detailed analysis of the relative rates of electron flow through the cytochrome region, the turnover of electron transport, and the decay of the carotenoid shift and H^+ uptake, under a variety of conditions of coupling.

ACKNOWLEDGEMENTS

We would like to thank the Science Research Council for support and equipment grants, and Mrs J. Fielding and Mr G. Tierney for their expert technical assistance. We are also grateful to Drs P. L. Dutton and J. B. Jackson for allowing us access to their unpublished work.

REFERENCES

1 Jackson, J. B., Crofts, A. R. and von Stedingk, L-V. (1968) Eur. J. Biochem. 6, 41–54
2 Von Stedingk, L-V. and Baltscheffsky, H. (1966) Arch. Biochem. Biophys. 117, 400–404
3 Walker, D. A. and Crofts, A. R. (1970) Annu. Rev. Biochem. 39, 389–428
4 Jackson, J. B. and Crofts, A. R. (1969) Eur. J. Biochem. 10, 226–237
5 Chance, B., Crofts, A. R., Nishimura, M. and Price, B. (1970) Eur. J. Biochem. 13, 364–374
6 Chance, B., McCray, J. and Thornber, J. P. (1970) Abstr. Biophys. Soc. 124(e)
7 Crofts, A. R., Cogdell, R. J. and Jackson, J. B. (1973) in Mechanisms in Bioenergetics (Quagliariello, E., Papa, S. and Rossi, C. S., eds), pp. 337–346, Academic Press, New York and London

8 Cogdell, R. J., Jackson, J. B. and Crofts, A. R. (1973) J. Bioenerg. 4, 412–429
9 Cogdell, R. J. and Crofts, A. R. (1973) in Abstr. 9th Int. Congr. Biochem., Stockholm, 4j10
10 Dutton, P. L. (1971) Biochim. Biophys. Acta 226, 63–80
11 Shavit, N., Thore, A., Keister, D. L. and San Pietro, A., (1968) Proc. Natl. Acad. Sci. U.S. 59, 917–922
12 Montal, M. and Gitler, C. (1973) J. Bioenerg. 4, 363–382
13 Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation, Glynn Res. Ltd, Bodmin, Cornwall
14 Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102–113