LIGHT INDUCED H^+ UPTAKE CATALYSED BY PHOTOCHEMICAL REACTION CENTRES FROM *RHODOPSEUDOMONAS SPHEROIDES* R26

R.J. COGDELL, R.C. PRINCE and A.R. CROFTS
Department of Biochemistry, Medical School, University of Bristol, Bristol, BS8 1TD, England

Received 20 July 1973

1. Introduction

Although it is now generally accepted that light induced H^+ uptake in bacterial chromatophores reflects the activity of an electrogenic pump, the mechanism of this pump remains a subject of considerable controversy [1,2].

Studies on the initial kinetics of H^+ uptake revealed a small initial burst of H^+ uptake (rapid H^+ binding) before the slower 'steady state' uptake [3]. The properties of this rapid H^+ binding have been studied in detail following activation with short flashes of light [4–6].

It has been suggested that H^+ uptake reflects the reduction of a hydrogen carrying redox component during electron flow [4,5].

Recently two important lines of evidence have favoured this hypothesis: a) Callis et al. [7] have shown that in *Chromatium* chromatophores the laser induced rapid H^+ binding has very similar kinetics to the reduction of the secondary electron acceptor; b) Flash induced rapid H^+ binding is attenuated in chromatophores from *Rps. spheroides, Rps. capsulata* and *Rps. viridis* as a redox component, which is distinct from the primary electron acceptor, is chemically reduced before the flash [5,8,9]. The variation with pH of the midpoint of attenuation shows that the reduction of this component involves a H^+.

The isolation of photochemical reaction centres [10] allows more detailed investigation of this phenomenon. If the redox component associated with rapid H^+ binding is present in the reaction centres then a light induced H^+ uptake should still be observed, and the relation between the two should be more easily studied.

We have chosen to use photochemical reaction centres prepared by the method of Clayton and Wang [10] since they contain a minimal complement of accessory components apart from the photochemical reactants.

2. Materials and methods

Cells of *Rps. spheroides* R26 were grown in batch culture and chromatophores prepared from them as previously described [1]. Photochemical reaction centres were isolated from the chromatophores with the zwitterionic detergent LDAO, according to the method of Clayton and Wang [10]. The concentration of the reaction centres was determined using a value for E_{mM} 865 of 113 [10].

The concentration of ubiquinone in the reaction centres was determined from a total lipid extract of the reaction centres according to the method of Griffiths et al. [11].

Light induced H^+ uptake was monitored with the pH indicator Phenol red, and recorded in a rapidly responding single beam spectrophotometer [5]. The experiments were performed in an anaerobic redox cuvette, (Dutton and Jackson, [12]) which was flushed with O_2-free nitrogen. This minimised the pH drifts normally encountered with unbuffered solutions open to the air. The extent of the pH change was

North-Holland Publishing Company – Amsterdam

routinely calibrated with standard additions of 10 mM HCl. Excitation was provided by a xenon flash (Mecablitz 182, Metz, Germany, pulse width at half height of 200 μ sec) screened with two layers of Wratten 87A gelatin filter.

Mammalian cytochrome *c* was purchased from Sigma Chemical Co., Ltd. LDAO was a generous gift from A.B.M. Industrial Products Ltd, Stockport, Cheshire, and all other chemicals used were of analar grade or of the highest purity commercially available.

3. Results and discussion

Isolated photochemical reaction centres will oxidise added mammalian cytochrome *c* [13–16]. Following excitation with a saturating single turnover flash the rate of cytochrome *c* oxidation exactly equals the rate of decay of the oxidised reaction centre change [15]. The rate of this interaction is very dependent on ionic strength. The ionic strength of the suspending medium in the experiments was therefore kept low at 10 mM.

At 556 nm the xenon flash induced no net absorbance change in reaction centres supplemented with cytochrome *c* (fig. 1A). When Phenol red was added to the reaction mixture the xenon flash induced an increase in absorbance at 556 nm (fig. 1B). This absorbance change could be buffered out by changing the suspending medium to 10 mM Tris–HCl pH 7.5, indicating that the change represented a true Phenol red absorbance change in response to a small pH increase in the medium.

Although in the absence of Phenol red there was no net absorbance change at 556 nm, there were some initial interfering transients. This made it impossible to follow the initial kinetics of the H^+ uptake; nevertheless, half rise time of the change was less than 2 msec.

The extent of the H^+ uptake was slightly stimulated by addition of 1,4-napthoquinone (fig. 1C), and strongly inhibited by 3 mM *o*-phenanthroline (fig. 1D). *o*-Phenanthroline has been shown to inhibit electron flow within these photochemical reaction centres between the primary and secondary electron acceptors. In the presence of *o*-phenanthroline cytochrome *c* oxidation induced by a single turnover flash was unaffected, but subsequent turnovers were strongly inhibited [15, 16].

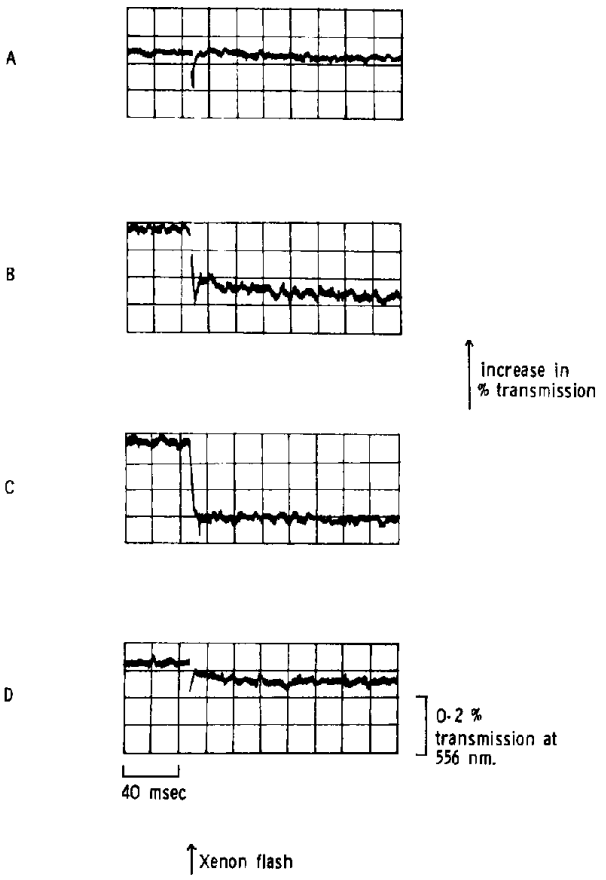

Fig. 1. The xenon flash induced H^+ uptake with photochemical reaction centres from *Rps. spheroides* R26. The H^+ uptake was monitored from the flash induced Phenol red change at 556 nm. The anaerobic cuvette contained 8 cm^3 of 10 mM KCl pH 7.5, 1.3 μM reaction centres and 25 μM reduced mammalian cytochrome *c*: A) with no further additions; B) with 52 μM Phenol red; C) as for B but with 22 μM 1,4-napthoquinone; D) as for C but with 3 mM *o*-phenanthroline. Addition of 20 μl of 10 mM HCl gave a deflection of 5.2% transmisson.

Interestingly, there was no H^+ uptake in the absence of added cytochrome *c*. It seems that the presence of the oxidised reaction centre prevents the reduced primary acceptor from releasing its reducing equivalent to other secondary acceptors capable of binding a proton. Kononenko et al. [17] came to similar conclusions with whole cells from *Ectothiorhodospiria shaposhnikouii*.

When a suspension of reaction centres with a large excess of added cytochrome *c* was activated with

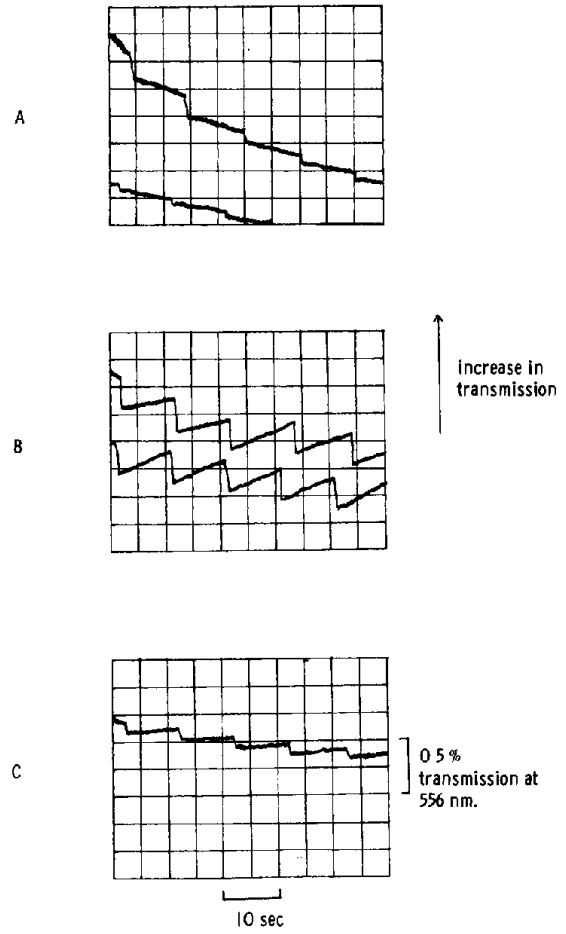

Fig. 2. H^+ uptake with photochemical reaction centres induced by xenon flashes every 10 sec. The H^+ uptake was monitored from the flash induced Phenol red change at 556 nm. The anaerobic cuvette contained 8 cc of 10 mM KCl pH 7.5, 1.3 μM reaction centres, 25 μM reduced mammalian cytochrome *c* and 52 μM Phenol red: A) with no further additions; B) with 22 μM 1,4-napthoquinone; C) as for B but with 3 mM *o*-phenanthroline. Addition of 20 μl of 10 mM HCl gave a deflection of 6.3% transmisson. Note that each trace was triggered twice and that the lower trace is a direct continuation of the upper one;

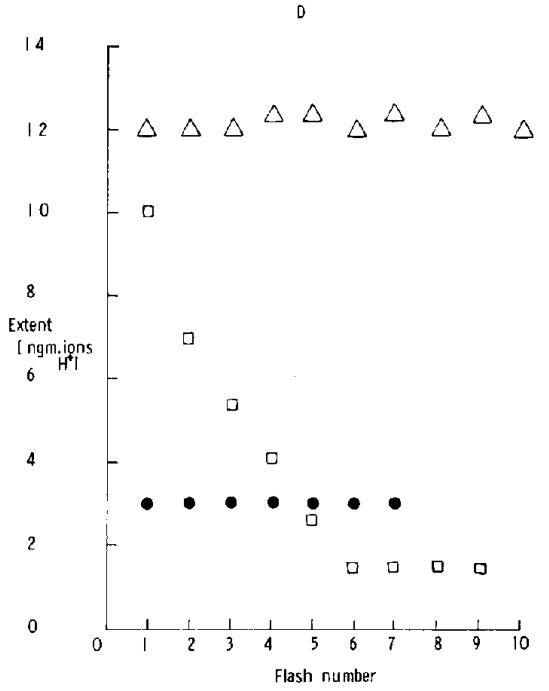

Fig. 2. D) The extent of each of the flash induced changes is plotted against the flash number: (△) with napthoquinone; (□) no additions; (●) with *o*-phenanthroline.

xenon flashes every 10 sec, the extent of the H^+ uptake declined after the first flash (fig. 2A). There was a small amount of decay after each change (seen as a decrease in the slope of the pH drift) which may be attributed to cyclic electron flow from the acceptors to cytochrome *c*; because of this the extent of H^+ uptake only declined to a minimum, and not to zero. If napthoquinone was added to increase the size of the secondary acceptor pools, then the extent of the H^+ uptake did not decrease, at least for the first ten flashes (fig. 2B). However there was now a marked decay of the H^+ uptake, showing that the 1,4-napthoquinone also catalysed the cyclic electron flow. In the presence of *o*-phenanthroline the repetitive flashes each induced the small inhibited H^+ uptake (fig. 2C).

The absolute amount of flash induced H^+ uptake was 8 nmoles H^+ in the absence of added 1,4-napthoquinone, and 11 nmoles H^+ with 1,4-napthoquinone. The concentration of the reaction centres was equivalent to 10.4 nmoles P870 so that the extent of the H^+ uptake was $\sim 1H^+$/reaction centre.

The ubiquinone analysis showed that there were 1.5 ubiquinones/reaction centre. From fig. 2A, adding the successive extents of the flash induced H^+ uptake (in the absence of added quinone) up to the point where the slow cycling occurs, gives a total extent ~ 3 times the initial extent. As the initial extent of the H^+ uptake was $\sim 1H^+$/reaction centre this means that the pool size of the component responsible for

Table 1
The extent of the H^+ uptake seen under the various conditions in fig. 1 and fig. 2.

Change	Extent (nmoles H^+)	mole/mole reaction centres
Flash induced H^+ uptake		
With cytochrome *c*	8.0	0.75–0.85
With cytochrome *c* + napthoquinone	11.0	1.0 –1.1
With cytochrome *c* + napthoquinone, + orthophenanthroline	2.1	0.25–0.30
Total H^+ uptake induced by repetitive xenon flashes	24.0	2.25–2.55
Total content of ubiquinone		1.5

The experimental conditions were exactly the same as given for fig. 1 and fig. 2.

H^+ uptake is ~ three equivalents/reaction centre. This is exactly the equivalent pool size of ubiquinone. The pool sizes revealed by the extents of H^+ uptake under a variety of conditions are compared with the ubiquinone content in table 1.

H^+ uptake could also be measured with a glass electrode on continuous illumination in the presence of excess 1,4-napthoquinone and cytochrome *c*. Simultaneous recording of the coupled cytochrome *c* oxidation showed that for every electron leaving cytochrome *c*, one H^+ was removed from the medium (R.J. Cogdell and R.C. Prince, unpublished observation).

The action of FCCP, valinomycin, nigericin and antimycin, either alone or in combination were tested on the reaction centre changes described above. In no case did the reagents have any significant effect.

4. Conclusions

The experiments reported here show that rapid H^+ uptake can be readily demonstrated using photochemical reaction centres. From the results we arrive at the following conclusions:

i) Rapid H^+ uptake is associated with electron flow to secondary electron acceptors. In these reaction centres the endogenous secondary acceptor is ubiquinone, and the maximal extent of H^+ uptake is equivalent to the capacity of the ubiquinone pool. Clayton et al. [16] have shown that these reaction centre preparations will photoreduce ubiquinone. The results thus support the hypothesis that light induced H^+ uptake in chromatophores reflects the reduction of a hydrogen carrying redox component (Y, cf [5]) during electron flow, and strongly suggests that this component is ubiquinone.

ii) The failure of ionophores and uncoupling agents to affect the rapid H^+ uptake by reaction centres suggests that their effects on the chromatophore reaction cannot be accounted for in terms of effects on the initial electron transport reaction, but must reflect the organisation of the electron transport system within the membrane. Similarly the effect of antimycin on the chromatophore H^+ uptake must reflect electron flow through a site distinct from that between primary and secondary acceptor, since this was unaffected by antimycin.

iii) If the rapid H^+ uptake indicates reduction of ubiquinone then oxidised ubiquinone is unlikely to be acting as a primary acceptor. This follows from:
a) the inhibitory effect of orthophenanthroline and
b) the stoichiometric inequality between H^+ uptake and the P605 change on the second and subsequent flashes.

This conclusion leaves open the question as to how a full reduction of quinone occurs, when initially the reaction must involve a single electron. Possibly collision between reaction centre molecular assemblies allows two semiquinones to undergo a dismutation reaction. This possibility has not yet been tested.

References

[1] Jackson, J.B., Crofts, A.R. and Stedingk, L.-V., von (1968) European J. Biochem. 6, 41–54.
[2] Walker, D.E. and Crofts, A.R. (1970) Ann. Rev. Biochem. 39, 389–428.
[3] Jackson, J.B. and Crofts, A.R. (1969) European J. Biochem. 10, 226–237.
[4] Chance, B., Crofts, A.R., Nishimura, M. and Price, B. (1970) European J. Biochem. 13, 364–374.

[5] Cogdell, R.J., Jackson, J.B. and Crofts, A.R. (1973) J. Bioenergetics 4, 412–429.
[6] Cogdell, R.J., Ph.D. Thesis (1973) University of Bristol, England.
[7] Callis, J.B., Parson, W.W. and Gouterman, M. (1972) Biochim. Biophys. Acta 267, 348–362.
[8] Cogdell, R.J., Jackson, J.B. and Crofts, A.R., Abs. of the VIth International Congress on Photobiology, Bochum, Germany No. 264 (1972).
[9] Crofts, A.R., Evans, E.H. and Cogdell, R.J. (1973) in: The Proceedings of the Conference on the Mechanism of Energy Transduction in Biological Systems, New York, in press.
[10] Clayton, R.K. and Wang, R.T. (1971) in: Methods in Enzymology (San Pietro, A., ed), Vol. 23A, pp. 696–704, Academic Press, New York and London.
[11] Griffiths, W.T., Threfall, D.R. and Goodwin, T.W. (1967) Biochem. J. 103, 589–600.
[12] Dutton, P.L. and Jackson, J.B. (1972) European J. Biochem. 50, 495–510.
[13] Ke, B., Chaney, T.H. and Reed, D.W. (1970) Biochim Biophys. Acta 216, 373–383.
[14] Clayton, R.K., Fleming, H. and Szuts, E.Z. (1972) Biophys. J. 12, 46–63.
[15] Prince, R.C., Cogdell, R.J. and Crofts, A.R., in preparation.
[16] Clayton, R.K., Szuts, E.Z. and Fleming, H. (1972) Biophys. J. 12, 64–79.
[17] Kononenko, A.A., Lukashev, E.P., Rubin, A.B. and Venediktov, P.S. (1972) Biochim. Biophys. Acta 275, 130–133.