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The Effect of Redox Potential on the Coupling Between Rapid Hydrogen-Ion Binding and Electron Transport in Chromatophores from *Rhodopseudomonas Spheroides*

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Introduction

The light-driven hydrogen-ion pump of chromatophores from photosynthetic bacteria is related to the processes of electron transport and the phosphorylation of ADP.[1,2,3] Using pH indicator techniques[4,5,6] a rapid component of H^+-binding, whose kinetic parameters lie within the time range of the chromatophore electron transport reactions[7] has been detected. A study of the effects of inhibitors, uncoupling agents and ion-transporting antibiotics on this phenomenon has led to the suggestion that hydrogen-ions bind at two distinct sites in the electron transport chain.[6] Two models have been proposed to explain rapid H^+-binding.

(i) The H^+ disappearing is directly involved in the reduction of a H-accepting redox component; the H^+ carrier is envisaged as one of a series of alternating electron-carriers and hydrogen carriers, which are arranged anisotropically within the membrane such that the transport of reducing equivalents is coupled to H^+-binding on the outside and H^+-release on the inside of the chromatophore vesicle.[1,6,8]

(ii) Initiation of coupled electron transport converts the chromatophore membrane to an activated state that is necessary but not sufficient for energy coupling and which is characterized by enhanced proton binding; the "membrane Bohr effect".[5]

Recently, the thermodynamic properties of the cytochromes of *Rhodopseudomonas spheroides* chromatophores have been characterized by redox titration,[7] and the dependence of flash-induced electron flow and carotenoid spectral shift on the ambient redox potential of the chromatophore suspension have been investigated.[9] It was therefore

Non-Standard Abbreviations: FCCP—carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; BChl—bacteriochlorophyll; BCP—bromocresol purple.

of some importance to examine the redox potential dependence of the rapid H^+-binding reactions. The results of this investigation, which are presented in this communication, give further support to the chemiosmotic mechanism of proton translocation but are not easily reconciled with a "membrane Bohr effect."

Methods

Batch cultures of *Rps. spheroides*, strain Ga (the "green" mutant) were grown anaerobically in the light as previously described.[1] Chromatophores were prepared with a French Press. The chromatophores were washed and resuspended in a medium containing 100 mM choline chloride and 1 mM N-morpholino ethanesulphonic acid at pH 6·5. This low buffer medium was used throughout the preparation which was otherwise similar to the standard procedures employed in this laboratory.[1]

The colour changes of the pH indicator were measured with a sensitive and rapidly responding single beam spectrophotometer which has been described elsewhere.[10] Actinic illumination was provided by either a 20 ns Q-switched ruby laser pulse or a 200 μs xenon flash. A redox titration vessel, similar to that designed by Dutton[11] was positioned in the spectrophotometer in a cell housing modified to permit stirring by a magnetic flea. The following dyes were employed to facilitate redox equilibration between the electron transport carriers of the chromatophores and the platinum electrode; potassium ferri/ferrocyanide ($E_{m\,7\cdot0} = +430$ mV), diaminodurol ($E_{m\,7\cdot0} = +240$ mV), phenazine methosulphate ($E_{m\,7\cdot0} = +80$ mV), phenazine ethosulphate ($E_{m\,7\cdot0} = +55$ mV), pyocyanine ($E_{m\,7\cdot0} = -34$ mV). The oxidation/reduction potential of the suspension was controlled by injecting small volumes of freshly prepared potassium ferricyanide and sodium dithionite solutions through the rubber septum of the side arm of the vessel.

Small changes in pH of the suspension resulting from additions, were corrected with dilute HCl or KOH, thus keeping the total transmittance of the sample, at 583 nm, constant. In this way it was possible to maintain the pH of the chromatophore suspension within $\pm 0{\cdot}02$ units of the desired value. Control experiments showed that transmittance changes at 583 nm resulting from the direct effects of pH or redox potential on the chromatophores and redox dyes were negligible. Repeated additions of sodium dithionite and KOH solutions produced a gradual increase in the buffering capacity of the chromatophore suspension. For this reason we divided each titration, as shown in Figs. 3A and 3B, into three parts and, by making use of the chromatophore "endogenous reductant",[7] avoided as far as possible the use of dithionite. Calibrations of the phenol red change with dilute HCl and

KOH before and after each experiment ensured that the buffering capacity did not change significantly during the course of the titration.

Choice of pH Indicator

In contrast to bromothymol blue[12] bromocresol purple remains largely unbound by the chromatophore membrane and accurately reflects the pH of the extra-vesicular medium, at least during the first second of illumination.[4] In preliminary experiments (with P. L. Dutton and B. Chance) it was found that bromocresol purple was irreversibly destroyed by exposure to low redox potentials. We have therefore screened a number of other indicators for their accuracy and reliability under a variety of experimental conditions. The screening procedures have been described in detail elsewhere[4] for bromothymol blue and bromocresol purple. On the basis of the experiments summarized below, phenol red (phenol-sulphonphthalein) was selected as the most suitable pH indicator for the present series of experiments.

When a chromatophore suspension was treated with between 1–100 μM phenol red, 95–97% of the indicator was recovered in the supernatant after complete centrifugation of the particles. Lack of appreciable binding of the indicator was also suggested by the failure of the chromatophores to shift the pK_a from its usual value of 7·8. Illumination of an unbuffered suspension of *Rps. spheroides* chromatophores containing 30–100 μM phenol red produced a colour change at 587–625 nm (isobestic in the absence of phenol red) in the direction of increasing alkalinity. The kinetics of the change on a slow time-scale were indistinguishable from those measured simultaneously with a glass electrode, and the extents of the changes estimated after calibration with dilute HCl were similar in each case. Addition of buffer to the suspension suppressed the phenol red and glass electrode changes in parallel. Treatment of the chromatophores with valinomycin, nigericin or FCCP had quantitatively the same effect on the light-induced phenol red change and the glass electrode response.[1,4]

In the rapidly responding single beam spectrophotometer, flash excitation elicited a rapid absorption change of phenol red at 583 nm. At this wavelength there was no light induced absorbancy change in the absence of phenol red, or at high buffer concentrations when phenol red was present. The spectrum of the flash-induced change, computed from either the difference between the flash-induced change in the presence and absence of indicator or the difference between the flash-induced change at high and low buffer concentrations, closely resembled the absorption spectrum of the alkaline form of phenol red.

Phenol red was not chemically changed by exposure to redox potentials down to at least −200 mV, and is therefore an ideal indicator for studying the dependence of rapid hydrogen-ion binding upon the oxidation-reduction potential of a chromatophore suspension.

Results

Rapid H^+-binding Indicated by Phenol Red

The kinetics and effects of inhibitors and ionophores on the rapid H^+-binding of aerobic chromatophore suspensions at pH 7·6 indicated by phenol red were essentially the same as those previously reported for changes indicated by bromocresol purple (Fig. 1). Some of these parameters are summarized in Table I.

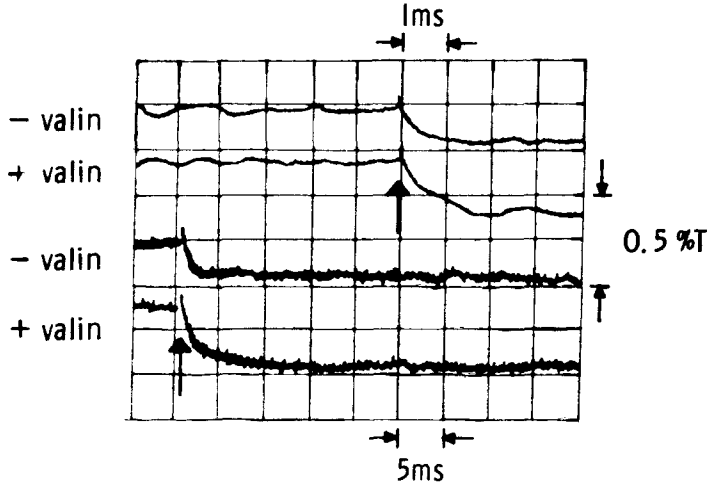

Figure 1. Laser-induced phenol red changes in an aerobic suspension of chromatophores from *Rps. spheroides*. Chromatophores, containing 0·125 mg BChl were suspended in 2·5 ml of 100 mM KCl and 50 μM phenol red at pH 7·6 and 25°C in an aerobic cuvette. The upper two oscilloscope traces were recorded at 1 ms/cm, the lower two at 5 ms/cm. The amplifier RC = 100 μs. The suspension was exposed to a 20 ns Q-switched ruby laser pulse at the time indicated by the arrows. An upward change of 0·5% transmission was equivalent to the addition of 1·34 ng ion H^+. Valinomycin, at a concentration of 4 μM was present where indicated.

As with the rapid H^+-binding indicated by bromocresol purple, a relatively high concentration of valinomycin (0·4 μM for a half maximal effect) was required for stimulation of the phenol red change. The stimulation of the laser-induced response was seen as a slower phase of H^+-binding following the 300 μs rapid phase, giving a ratio of $1H^+$/125 BChl as compared with $1H^+$/180 BChl in the untreated chromatophores (Fig. 1). Antimycin A was without effect on the laser-induced phenol red change in the absence of valinomycin but completely inhibited the slow phase resulting from treatment with the ionophore. These data are completely in accordance with the results previously reported for bromocresol purple (see Table I).

The pH Dependence of Rapid H^+-binding

The finding of two indicators of differing pK_a values, which accurately reflect the pH of a chromatophore suspension has enabled us to

TABLE I. Characteristics of rapid H^+-binding by chromatophores of *Rps. spheroides* in aerobic suspension

Preparation	Flash	Indicator	Additions	Phase I $t\frac{1}{2}$	Phase I extent	Phase II $t\frac{1}{2}$	Phase II extent	Total H^+/BChl
A	Laser	BCP	None	300 μs	1·0			1/170
B	Laser	Phenol red	None	300 μs	1·0			1/180
A	Xenon	BCP	None	360 μs	1·4			1/120
B	Xenon	Phenol red	None	470 μs	1·85			1/100
A	Laser	BCP	Valinomycin	250 μs	1·6			1/100
B	Laser	Phenol red	Valinomycin	600 μs	1·55			1/125
A	Xenon	BCP	Valinomycin	500 μs	2·3	2·5 ms	1·1	1/50
B	Xenon	Phenol red	Valinomycin	560 μs	2·3	2·5 ms	1·45	1/50
A	Xenon	BCP	Val. + antimycin	250 μs	1·4			1/120
B	Xenon	Phenol red	Val. + antimycin	250 μs	1·9			1/100

(A) *Rps. spheroides* chromatophores containing 0·163 mg BChl were suspended in 2·5 ml of 100 mM Kcl, 40 μM BCP at pH 6·3 and 25°C.
(B) As in figure 1. When present valinomycin and antimycin A were at a concentration of 4 μM. All the extents have been normalized so that the laser induced extent with no additions was 1·0.

investigate the pH dependence of the rapid H^+-binding reactions. Figure 2 shows good agreement between the extent of the change measured with either bromocresol purple or phenol red in the overlap region between pH 6·5 to 7·0. Between pH 6·0 and 7·8 both the extent and half risetime of the reaction were constant. Above pH 7·8 the extent of the change was attenuated but measurement of the rate of change at these pH values was unreliable owing to the high background

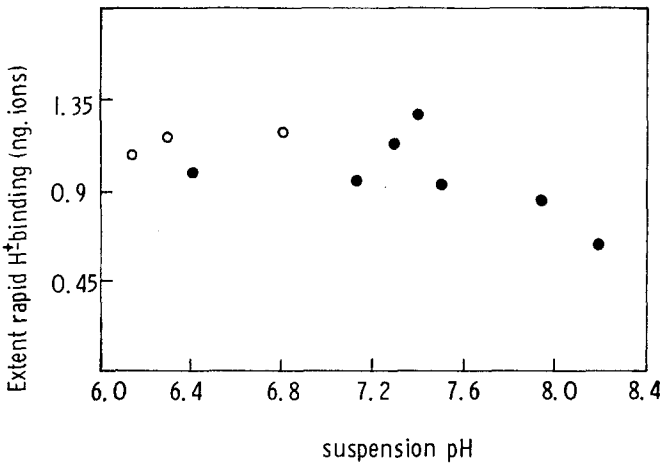

Figure 2. The pH dependence of chromatophore laser-induced rapid H^+-binding. The experimental conditions were similar to those for Fig. 1, except that phenol red was present at 465 μM (●) or BCP at 160 μM (○) and the chromatophores contained 0·13 mg BChl. The pH of the suspension was adjusted with HCl or KOH by comparison with solutions of indicator dye in buffer and was measured after each experiment.

absorption of the alkaline form of phenol red. Chance *et al.*[5] found a twofold difference in the risetime of the bromocresol purple change between pH 5·1 and 7·1 in chromatophores prepared from *Chromatium*.

Rapid H^+-binding as a Function of the Redox Potential of the Chromatophore Suspension

The extent of the rapid H^+-binding reactions of *Rps. spheroides* chromatophores elicted by a 20 ns ruby laser pulse or a 200 μs xenon flash depended on the ambient redox potential of the suspension as shown in Fig. 3A. The laser-induced change, in the absence of valinomycin was attenuated below a midpoint value of $E_{m\,7\cdot5} = +5$ mV and above approximately $E_{m\,7\cdot5} = +430$ mV. These values compare with midpoint potentials of −40 mV for the primary acceptor and +450 mV for the reaction centre bacteriochlorophyll at the same pH.[7, 13] The low potential midpoint of attenuation of the phenol red change was consistently 40 mV more positive than the midpoint potential of the primary acceptor,[7, 14, 15, 16, 17] but within the limits of experimental

error, the high potential midpoint was similar to that of the reaction centre bacteriochlorophyll.[7, 13] A small but reproducible (25–40%) stimulation of the extent of H^+-binding was observed as the potential of the suspension was lowered through the 350–300 mV range (see

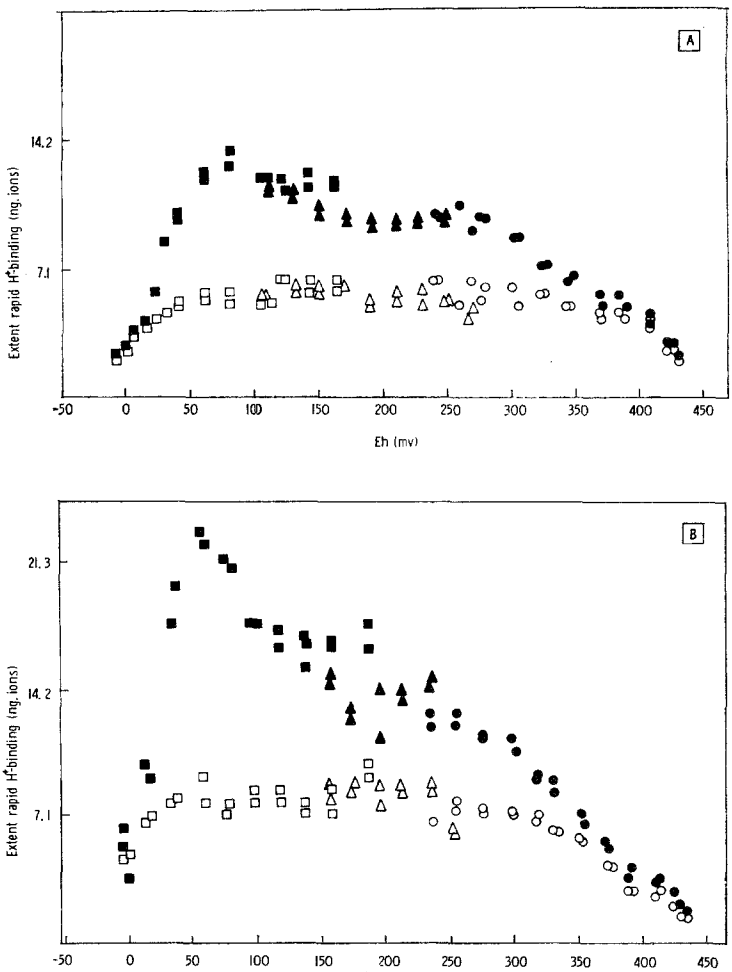

Figure 3. The dependence of rapid H^+-binding upon the redox potential of the chromatophore suspension. *Rps. spheroides* chromatophores (0·72 mg BChl) were suspended in 7·5 ml of a medium containing 100 mM KCl and 465 μM phenol red, final pH 7·5, in the anaerobic redox titration vessel. Each titration was performed in three parts with the following mediating oxidation/reduction dyes: □, ■ 10 μM phenazine methosulphate, 10 μM phenazine methosulphate, 10 μM diaminodurol, 7 μM pyocyanine; ▲, △ 10 μM phenazine methosulphate, 10 μM phenazine ethosulphate, 10 μM diaminodurol; ○, ● 250 μM potassium ferricyanide and, below +300 mV, 10 μM diaminodurol. The open symbols show the extent of H^+-binding after a laser flash, the closed symbols H^+-binding after a 200 μs xenon flash. Following addition of dithionite or ferricyanide, and compensating KOH addition the system was left to equilibrate for at least 3 min. The titrations were mainly carried out in the direction of reduction, making use of the slow endogenous reductant. Occasional calibrations with standard HCl and KOH were performed to ensure that the suspension buffering capacity remained constant. A, no valinomycin; B, in the presence of 2 μM valinomycin.

Fig. 3A and Table II), when the chromatophore cytochrome *c* ($E_{m\,7\cdot0}$ = +293 mV) was becoming chemically reduced before the flash.

After a 200 μs flash, H^+-binding was most extensive when the redox potential of the suspension was poised between +70 and +100 mV. With further lowering of the oxidation/reduction poise, the H^+-binding reactions were suppressed in two steps; first around a midpoint value of +40 mV and finally across the span that resulted in attenuation of the laser-induced change (at $E_{m\,7\cdot5} = +5$ mV). To the high potential side of the +90 mV maximum, the binding reactions were attenuated in three steps, $E_{m\,7\cdot5} = +130$ mV, +320 mV and +430 mV. These features may be correlated with certain chromatophore electron transport carriers the "+160 mV component" or "*Z*" ($E_{m\,7\cdot0} = +160$ mV), cytochrome *c* ($E_{m\,7\cdot0} = +293$ mV) and reaction centre bacteriochlorophyll ($E_{m\,7\cdot0} = +450$ mV).[7] There was an interesting similarity between this profile of rapid H^+-binding (Fig. 3A) and that of the flash-induced carotenoid shift as a function of redox potential.[9]

Preliminary observations (J. B. Jackson and R. J. Cogdell, unpublished work) have shown that in chromatophores prepared from *Chromatium* the rapid H^+-binding reaction, indicated by phenol red is attenuated below $E_{m\,6\cdot9} \simeq -70$ mV. This compares with the data of Case and Parson for the midpoint potentials of the primary acceptor X, $E_{m\,7\cdot7} \simeq -130$ mV and the secondary acceptor *Y*, $E_{m\,7\cdot7} \simeq -90$ mV.

The pH Dependence of the Low Potential Attenuation Midpoint of the Laser-induced Rapid H^+-binding

The indicator-dye technique for measuring rapid hydrogen-ion changes is only satisfactory within a narrow pH range on either side of the indicator p*K*. With phenol red we have been able to perform suitably accurate redox titrations between pH 6·7 and 7·7 (see Fig. 4A). Figure 4B shows that the E_m of the component associated with attenuation of the rapid H^+-binding reaction at low potentials increases with decreasing pH with a slope of approximately 60 mV/pH unit. We have attempted to fit theoretical curves to the data as shown in Fig. 4A. The points shown for pH = 6·75, 7·0 and 7·2 approximate to a theoretical one electron transfer ($n = 1$) but at pH = 7·4 and 7·6, an $n = 2$ curve is a better fit. This discrepancy reflects the degree of scatter in the data, especially at pH values approaching the indicator pK_a.

When the chromatophore suspension was poised in the redox potential range +60 to +100 mV and below approximately pH 7·0, the decay of the laser or flash induced phenol red change was slightly increased. The increased decay may be a reflection of a higher intrinsic permeability of the chromatophore to ions at low pH,[1] or possibly of interaction with the oxidation reduction dyes. The experimental approach pre-supposes that the oxidation/reduction dyes do not react so rapidly with the chromatophore electron transport systems as to

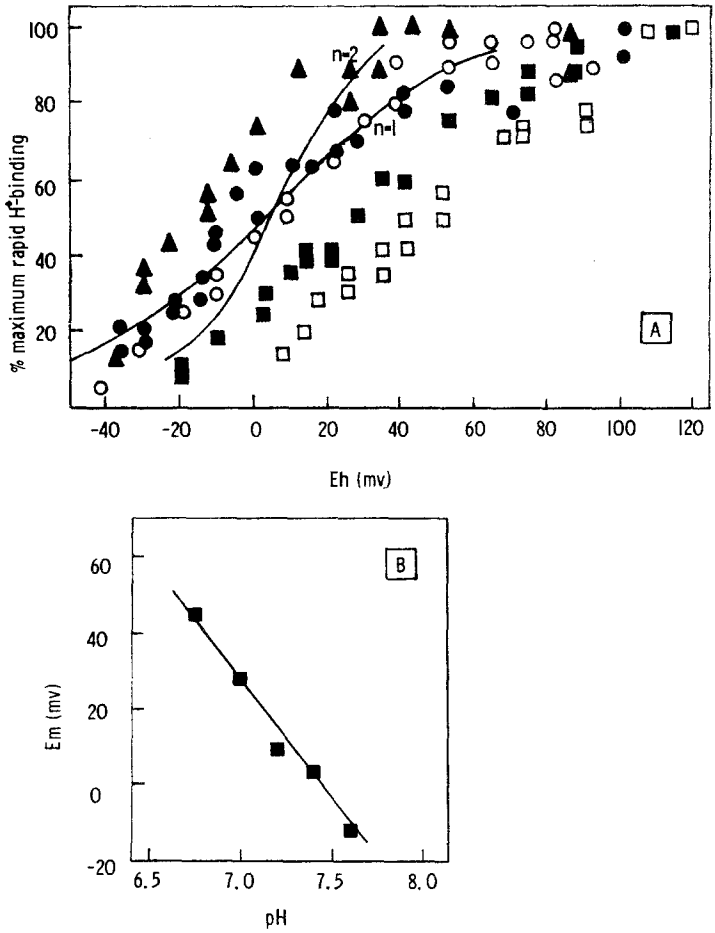

Figure 4. The pH dependence of the potential of the oxidation/reduction component responsible for rapid H^+-binding. The conditions were as described for Fig. 3 except that the chromatophores contained 0·70 mg BChl and 10 μM phenazine methosulphate, 10 μM phenazine ethosulphate and 7 μM pyocanine were used as mediators. A; □ pH 6·75, maximum extent of H^+-binding, 4·3 ng ion H^+; ■ pH 7·0, maximum extent of H^+-binding, 4·2 ng ion H^+; ○ pH 7·2, maximum extent of H^+-binding, 3·3 ng ion H^+; ● pH 7·4, maximum extent of H^+-binding, 3·2 ng ion H^+; ▲ pH 7·6, maximum extent of H^+-binding, 3·6 ng ion H^+. The solid lines are theoretical $n = 1$ and $n = 2$ plots for the data at pH 7·2 ($E_{m7·2} = +6$ mV). B; the midpoint potentials from A, plotted as a function of pH.

modify the response immediately after the flash. To avoid ambiguity we have confined our data to measurements within 20 ms of flash excitation, during which time dye interference is minimal.[7, 9]

The Redox Potential Dependence of the Valinomycin Stimulation of Rapid H^+-binding

A comparison of Figs. 3A and 3B shows that valinomycin stimulation of the rapid H^+-binding occurs only when the redox poise of the suspension is such that cytochrome *c* is partially reduced before the

TABLE II. The effects of valinomycin, antimycin A and *ortho*-phenanthroline on the kinetics of laser-induced rapid H^+-binding

Preparation	Additions	o/r potential (mv)	H^+-binding (ng. ion/mg BChl)	o/r potential (mv)	H^+-binding (ng. ion/mg BChl)	o/r potential (mv)	H^+-binding (ng. ion/mg BChl)	o/r potential (mv)	H^+-binding (ng. ion/mg BChl)
A	None	385	8·9	241	12·4	166	13·0	84	13·9
A	Valinomycin	384	10·0	241	14·3	166	19·5	85	26·0
A	Valinomycin plus Antimycin A	383	10·0	242	12·8	166	14·5	85	17·4
B	None	380	8·1	248	11·7	160	12·4	99	10·0
B	Antimycin A	378	6·5	248	11·6	160	10·5	99	8·4
B	None	378	7·9	235	13·4	159	12·9	95	12·7
B	*o*-phenanthroline	378	3·9	238	8·9	159	8·6	93	8·3

The experimental conditions for this table are described in Fig. 5. Preparation A was that used in frames A → D and preparation B was used in frames E → J.

flash. Between +50 and +300 mV the ratio of hydrogen ions bound following a laser pulse to the number of chromatophore bacteriochlorophyll molecules was increased from 1/130 to 1/95 by treatment with valinomycin. Table II shows that in some preparations the extent

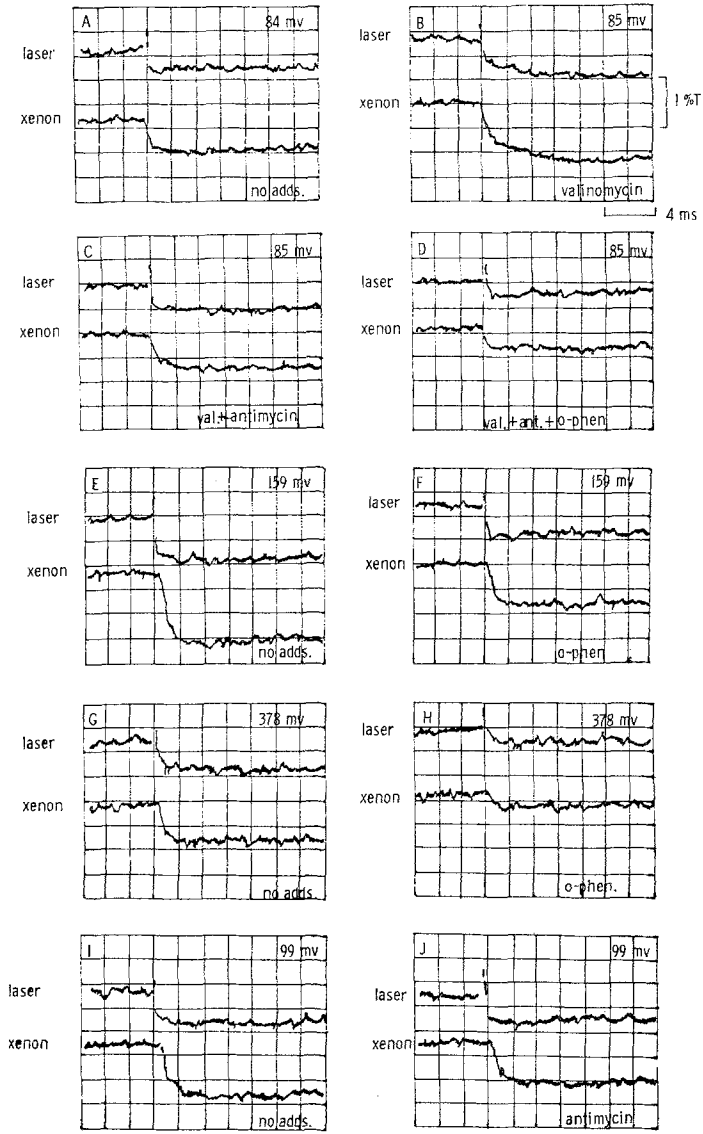

Figure 5. The kinetics of rapid H^+-binding; the effect of valinomycin, antimycin A and *o*-phenanthroline. Conditions as for Fig. 3 except that chromatophores contained 0·46 mg BChl in experiments A–D and 0·42 mg BChl in experiments E–J. Calibration with dilute HCl showed that 1% ΔT was equivalent to 8·3 ng ion H^+ in experiments A–D and 6·1 ng ion H^+ in experiments E–J. Where shown valinomycin was present at 2 μM, antimycin at 2 μM and *o*-phenanthroline at 4 mM. The redox potential of the suspension is shown in the top right-hand corner of each frame.

of valinomycin stimulation of the laser-induced phenol red change was greater between +90 and +160 mV than at higher potentials. This was always the case for H^+-binding following a xenon flash (Figs. 3A and 3B). At +70 mV the H^+-BChl ratio reached 1/30 following a xenon flash in the presence of valinomycin.

The laser induced H^+-binding reaction of chromatophores untreated with valinomycin, was complete is less than 1 ms (Fig. 5). The half-risetime of the change was about 300 μs but was not a simple first-order change. Further kinetic resolution will not be possible without the application of repetitive pulse techniques. The extra H^+-binding following valinomycin treatment was slower, $t_{\frac{1}{2}} \simeq 2$ ms (Fig. 5) in agreement with earlier work on aerobic chromatophore suspensions.

The Inhibitory Effects of Antimycin A and ortho-Phenanthroline on the rapid H^+-binding reactions of Rps. spheroides chromatophores

In the absence of valinomycin, antimycin A was without significant effect on the H^+-binding reactions elicited by a laser pulse, regardless of the redox poise of the chromatophore suspension (Table II). However, low concentrations of antimycin A inhibited the valinomycin-stimulated phenol red change, and this was especially apparent at low redox potentials. Approximately 75% of the extra H^+-binding resulting from valinomycin treatment was inhibited by the antimycin A. Figure 5 shows that the inhibitor removed the slow phase of the H^+-binding, leaving the faster phase unaffected.

Inhibition of the rapid H^+-binding by ortho-phenanthroline was also dependent on the ambient redox potential of the chromatophore suspension. The change was 50% inhibited by 4 mM *ortho*-phenanthroline when the potential was poised such that cytochrome *c* was chemically oxidized before the flash (above +350 mV) but only 30–35% inhibited by the same concentration at redox potentials between 40 and 300 mV (Fig. 5 and Table II). Other effects of *o*-phenanthroline at lower potentials are discussed separately elsewhere.[17]

Discussion

Rapid H^+-binding at the Level of a Secondary Electron Acceptor

Rapid H^+-binding was elicited by laser pulse or xenon flash activation of a *Rps. spheroides* chromatophore suspension whenever the reaction centre bacteriochlorophyll ($E_{m\,7.5} = +450$ mV) was reduced and a component of $E_{m\,7.5} = +5$ mV was oxidized, before the flash. The dependence of the midpoint potential on pH indicates that this component accepts $1H^+$/electron on reduction.

The potential of the component ($E_{m\,7.5} = +5$ mV) was 40 mV more positive than the midpoint potential of the primary acceptor and 20 mV more negative than cytochrome *b*, so that it cannot be identified with

any of the previously characterized electron transport carriers of *Rps. spheroides* chromatophores.

There is kinetic as well as thermodynamic justification for placing the component responsible for rapid H^+-binding (henceforth called Y^1) at the level of the secondary acceptor pools. The reduction of Y^1 after a flash must have a half risetime of less than 300 μs. This is intermediate between the very rapid reaction between the primary donor and acceptor (10^{-11} s)[18] and the oxidation/reduction reactions of the *b*-type cytochromes (1–2 ms).[7]

The double pulse technique of Parson and Case [19] has shown that the half rise-time of the reaction between the primary acceptor (X) and secondary acceptor (Y) in *Chromatium* chromatophores is between 60 and 80 μs. Unfortunately species differences between *Chromatium* and *Rps. spheroides* forbid comparison of the double pulse data with the phenol red experiments reported here. However, the similarity between the rates of the two processes is encouraging and may point to the chemical identity of Y and Y^1. Our preliminary data for the midpoint potential of the hydrogen carrier of *Chromatium* chromatophores, $E_{m\,6.9} \simeq -70$ mV are in good agreement with the value for Y. $E_{m\,7.7} \simeq -90$ mV reported by Case and Parson,[20] since the value changes to the extent of 30 mV per pH unit.

H^+-binding and H^+-translocation

No rapid appearance of hydrogen ion was ever observed under the circumstances of these experiments (Figs. 1–5). This suggests that if any H^+-releasing reactions accompany electron transport, as might occur for example upon reduction of a cytochrome by a flavoprotein or quinone, then either the resulting protons must be released on the inside of the chromatophore vesicle, or the release of H^+ is fortuitously balanced by uptake of H^+ from the medium. This latter possibility seems unlikely. Further evidence for a proton translocation mechanism is shown in Fig. 6, where the dependence on uncoupler concentration of the decay the phenol red change was the same as that for the decay of the field-indicating carotenoid shift. Uncoupling agents of the FCCP type have been shown to act as proton conductors through natural and artificial membranes.[21,22,23] It appears that dissipation of the membrane potential generated by the light pulse is achieved by outward, electrophoretic H^+ translocation mediated by FCCP. Since the decay of the phenol red change is associated with a *trans-membrane* H^+-efflux, we may assume that the light-driven rapid H^+-binding is associated with an inward translocation of H^+.

A scheme which accounts for many of our observations on the H^+-binding reaction is shown in Fig. 7. Since the photochemical reaction involves the transfer of electrons across the chromatophore membrane,

giving rise to a membrane potential,[10,24] it is possible that the H^+-binding reaction at the level of a secondary electron acceptor Y^1 serves to stabilize the electric field. These reactions may constitute the first energy conserving site or loop[8] in the chromatophore electron transport chain.

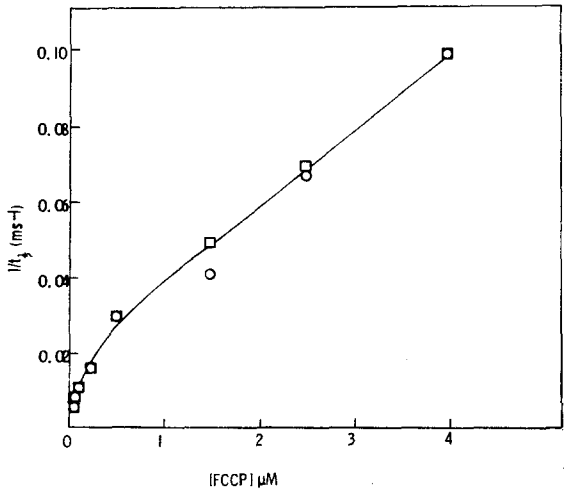

Figure 6. The effect of FCCP on the decay of the rapid H^+-binding and the carotenoid shift. ○, The reciprocal of the half decay time of phenol red change following a xenon flash measured under conditions similar to those described in Fig. 1 but on a longer time scale. The chromatophores contained 0·062 mg BChl and the phenol red concentration was 305 μM. □, The reciprocal of the half decay time of the xenon flash induced carotenoid shift, measured on a single beam spectrophotometer at 490 nm.[10] The same amount of chromatophore preparation was used as in the phenol red experiment. The suspending medium contained 100 mM KCl, 20 mM tricine, pH 7·6.

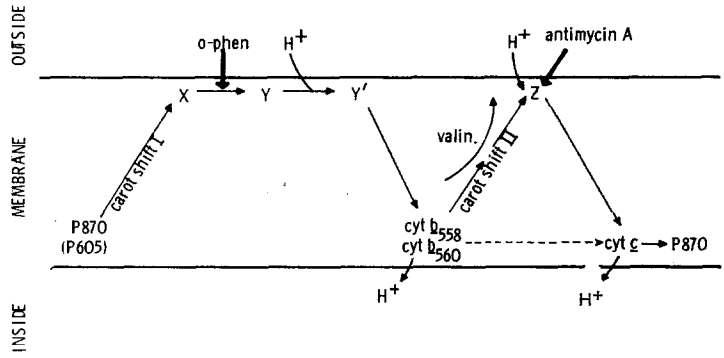

Figure 7. Model for rapid H^+-binding to *Rps. spheroides* chromatophores. Based on the model proposed in ref. 6 with additional data from this report and from refs. 7, 9 and 17. (See text.)

The Second "Site" of Photosynthetic Energy Conservation in Rps. spheroides Chromatophores

There is evidence that electrical work is done at two sites in the electron transport chain of chromatophores from *Rps. spheroides*,[9] and that coupling occurs at site II only if cytochrome *c*, $E_{m\,7\cdot0} = +293$ mV[7] is chemically reduced before the flash.[9] It is likely that the extra H^+-binding following a laser pulse at potentials below +300 to +350 mV (see Fig. 3 and Table II) is associated with the second site of energy conservation. Complete expression of site II H^+-binding however, was only observed in the presence of valinomycin. If we assume that valinomycin acts simply as a trans-membrane K^+ transporter,[25] then it could only directly stimulate those oxidation/reduction reactions which involve the transfer of electrical charge across the membrane. Previous studies[9] have shown that the re-reduction of cytochrome *c* after a laser pulse was accelerated by valinomycin. Cytochrome *c* oxidation and oxidation/reduction reactions of the *b* cyotchromes and reaction centre bacteriochlorophyll were unaffected. It is probable therefore that an electrogenic reaction and an H^+-binding reaction are located at the second site of energy conservation to the low potential side of cytochrome *c*.

The failure to detect significant site II H^+-binding after a laser pulse in the absence of valinomycin is puzzling since redox titration of the carotenoid shift[9] suggests that, at a redox poise between +40 and +120 mV, at pH 7·0, one electron per reaction centre bacteriochlorophyll traverses a second electrogenic site. It is conceivable however, that two electron equivalents per hydrogen carrier participate in the H^+-binding reaction. For example, if the hypothetical site II hydrogen carrier (see Fig. 7) is a quinone or a flavoprotein

$$FP + 2e^- + 2H^+ \rightleftharpoons FPH_2$$

valinomycin would facilitate transfer of the second electron to Z by lowering the electrical potential against which the reaction must do work.

The Action of Antimycin A and o-Phenanthroline on Rapid H^+-binding

The model of Fig. 7, discussed above, is consistent with the effects of antimycin shown in Table II and Fig. 5. This inhibitor has been shown to act on the electron transport reactions at the level of the second site of energy conservation.[7,9] In the absence of valinomycin or in the presence of valinomycin when the suspension was poised above about +300 mV (when site II H^+-binding is inoperative) antimycin A had little effect on the phenol red change following a laser pulse. In the presence of valinomycin, when the redox potential was below 300 mV, antimycin A inhibited by about 75% that portion of the H^+-binding associated with site II.

It is significant that *o*-phenanthroline, an inhibitor of the reaction between the primary and secondary electron acceptor pools[17,19,26] has a more pronounced inhibitory effect at redox potentials above +300 to +350 mV where site I H^+-binding alone was observed. At lower redox potentials, where site II H^+-binding was operating, the inhibitory action was less marked.

The Membrane Bohr Model for Rapid H^+-binding

In the terms of the membrane Bohr effect two changes in membrane conformation must be postulated, one for each energy conserving site, directly or indirectly driven by electron transport at either site.

H^+ uptake by chromatophores measured by a glass electrode under conditions of continuous illumination is generally considered to be a trans-membrane, electrogenic process[1,27] and is too extensive to be stoichiometrically related to a membrane conformation change of the type discussed by Chance *et al.*[5] Rapid H^+-binding and H^+-uptake would therefore be unrelated phenomena despite their similar response to treatment with uncoupling agent unless the H^+-binding is assumed to be a cyclic process reflecting an electrogenic H^+-pump.

The light-induced red shift of carotenoid absorption of the photosynthetic bacteria has been shown to be a response to the formation of a membrane potential[10,24,28] and Fig. 6 suggests that it is closely related to the rapid H^+-binding reactions. The nature of this relationship must account for the following observations: (i) the kinetics of the two phenomena are very different (compare Figs. 1 and 5 with ref. 10); (ii) valinomycin increases the extent of the rapid H^+-binding but stimulates the decay of the carotenoid shift; (iii) nigericin stimulates the decay of the rapid H^+-binding and increases the extent of the carotenoid shift.[10]

While these observations follow naturally from the chemiosmotic model proposed here, the postulated membrane Bohr effect can only account for them by additional hypotheses.

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References

1. J. B. Jackson, A. R. Crofts and L.-V. von Stedingk, *European J. Biochem.*, **6** (1968) 41.
2. P. Scholes, P. Mitchell and J. Moyle, *European J. Biochem.*, **8** (1969) 450.
3. A. Thore, D. L. Keister, A. Shavit and A. San Pietro, *Biochemistry*, **7** (1968) 3499.
4. J. B. Jackson and A.R. Crofts, *European J. Biochem.*, **10** (1969) 226.
5. B. Chance, A. R. Crofts, M. Nishimura and B. Price, *European J. Biochem.*, **13** (1970) 364.
6. A. R. Crofts, R. J. Cogdell and J. B. Jackson, in: *Energy Transduction in Respiration and Photosynthesis*, E. Quagliariello, S. Papa, and C. S. Rossi (eds.), pp. 883–901, Adriatica Editrice, Bari, 1971.

7. P. L. Dutton and J. B. Jackson, *European J. Biochem.*, submitted.
8. P. Mitchell, in: *Chemiosmotic Coupling in Oxidative and Photophosphorylation*, Glynn Research Laboratories, Bodmin, Cornwall, 1966.
9. J. B. Jackson and P. L. Dutton, *Biochim. Biophys. Acta*, submitted.
10. J. B. Jackson and A. R. Crofts, *European J. Biochem.*, **18** (1971) 120.
11. P. L. Dutton, *Biochim. Biophys. Acta*, **226** (1971) 63.
12. B. Chance, M. Nishimura, M. Avron and M. Baltscheffsky, *Arch. Biochem. Biophys.*, **117** (1966) 158.
13. I. D. Kunz, P. A. Loach and M. Calvin, *Biophys. J.*, **4** (1964) 227.
14. P. A. Loach, *Biochemistry*, **5**, (1962) 592.
15. D. W. Reed, K. L. Zankel and R. K. Clayton, *Proc. Natn. Acad. Sci. U.S.*, **63** (1969) 42.
16. W. A. Cramer, *Biochim. Biophys. Acta*, **189** (1969) 54.
17. J. B. Jackson, R. J. Cogdell and A. R. Crofts, in preparation.
18. K. L. Zankel, D. W. Reed and R. K. Clayton, *Proc. Natn. Acad. Sci. U.S.*, **61** (1968) 1243.
19. W. W. Parson and G. D. Case, *Biochim. Biophys. Acta*, **205** (1970) 232.
20. G. D. Case and W. W. Parson, *Biochim. Biophys. Acta*, **253** (1971) 187.
21. J. Bielawsky, T. E. Thompson and A. L. Lehninger, *Biochem. Biophys. Res. Commun.*, **24** (1966) 948.
22. V. P. Skulachev, A. A. Sharof and E. A. Liberman, *Nature*, **216** (1967) 718.
23. P. J. F. Henderson, J. D. McGivan and J. B. Chappell, *Biochem. J.*, **111** (1969) 521.
24. J. B. Jackson and A. R. Crofts, *FEBS Letters*, **4** (1969) 185.
25. P. Mueller and D. O. Rudin, *Biochem. Biophys. Res. Commun.*, **26** (1967) 398.
26. B. Chance, J. McCray and P. Thornber, *Biophys. Soc. Abstr.*, 124(a) (1970).
27. M. Nishimura and B. C. Pressman, *Biochemistry*, **8** (1969) 1360.
28. S. Schmidt, R. Reich and H. T. Witt, in: *Abstracts for the Second International Congress on Photosynthesis Research*, Stresa, Italy, 1971.