BBA 46290

# LIGHT REQUIREMENTS FOR PROTON MOVEMENT BY ISOLATED CHLOROPLASTS AS MEASURED BY THE BROMOCRESOL PURPLE INDICATOR

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(Received October 29th, 1971)

SUMMARY

ATP formation by isolated chloroplasts is due to the proton gradient phenomena, according to Mitchell. The number of protons moved per electron pair transported and per photon absorbed is related to the number of protons required to produce each ATP. Thus, a critical test of the Mitchell hypothesis is the quantum yield of $H^+$ transport. Bromocresol purple, a pH indicator, can be used to measure the pH external to isolated chloroplasts accurately and rapidly. The action spectrum (with pycocyanine as the electron acceptor) appears to be that of a System I-linked reaction (high above 700 nm). The quantum yield has been calculated to be $3.5 \pm 0.1$ $H^+/h\nu$ from 640 nm to 690 nm and $6.7 \pm 0.4$ $H^+/h\nu$ above 700 nm. The action spectrum of the efflux of $H^+$ occurring in the dark, which is usually identified as being equivalent to the steady-state influx, has the same shape as that of the influx. The quantum yield, however, is reduced by 0.5. Therefore, Photosystem II seems to affect both the initial influx and dark efflux. The $H^+$/photon and $H^+/e_2$ for the initial influx are too high for the Mitchell hypothesis. Only the $H^+$ efflux in the dark from 640–690 nm has an $H^+/h\nu$ of 1.6 which agrees with the theory of Mitchell.

## INTRODUCTION

The Mitchell hypothesis for ATP formation[1] rests most firmly upon the proton gradient phenomena observed in the isolated chloroplasts[2,3]. The theoretical energetics of the process suggest that a ratio exists for the number of protons moved into the grana per ATP molecule formed. This ratio is related to the number of $H^+$ transferred per electron transported and, hence per photon absorbed. Therefore, a critical test of the Mitchell hypothesis is the quantum yield for proton transport. DILLEY AND VERNON[4] have previously measured this yield. However, their spectrum for the yield has a peculiar shape in the region of 700–710 nm[5]. In addition, IZAWA AND HIND[6] have found that the initial $H^+$ movement often cannot be accurately observed with the standard pH electrode due to large time constants associated with the electronics.

Recently organic dyes have been used to measure $H^+$ movement. CHANCE and coworkers[7,8] have pioneered such use for the measurement of $H^+$ gradients in mitochondria. One such dye, Bromocresol purple (5,5′-dibromo-*o*-cresol sulfonphthalein),

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; MES, 2-(*N*-morpholino)-ethanesulfonic acid.

has been used to monitor $H^+$ movement in bacterial chromatophores by JACKSON AND CROFTS[9]. Bromocresol purple can also easily measure the $H^+$ transport in chloroplasts[10]. Bromocresol purple is not bound appreciably to chloroplasts, measures only the external pH, and does not interfere with normal cyclic electron transport.

In this paper the action spectrum and quantum yield of $H^+$ movement, as measured by the bromocresol purple method, have been investigated for the initial influx of $H^+$ and the dark efflux of $H^+$ observed immediately after the light is extinguished. This dark efflux has been previously equated with the steady-state influx occurring in continuous illumination[6,11]. In addition, the variation of $H^+$ movement with light intensity is examined in order to determine the kinetics of the influx and efflux of $H^+$.

## METHODS

The chloroplasts from commercially grown spinach were isolated as previously described[10]. Chloroplasts (at a chlorophyll concentration of 10 $\mu$g/ml) were suspended in 0.8 M sucrose, 0.03 M NaCl, 50 $\mu$M pyocyanine and 2.0 $\mu$M bromocresol purple (initial pH, 6.00) at 16° for the experiments. The absorbance changes of bromocresol purple were monitored using an Amino-Chance Double Beam Spectrophotometer with the reference wavelength at 490 nm and the measuring wavelength at 575 nm. The spectrophotometer was equipped with ports for actinic illumination of the sample compartment. The actinic light was provided by 350-W projectors using either a 645 or 715-nm Baird-Atomic filters (half power band width, 7 nm), or, for the action spectra, Baird-Atomic filters (half power band width, 15 nm) with a Corning red cut-off filter (No. 2-54). The light intensity was varied with Kodak neutral density filters and was measured with a radiometer (Yellow Springs Instrument Co.) previously calibrated with an Eppley Thermopile.

The p$K$ for bromocresol purple was measured by fitting the logarithm of $(A_0-A)/A$, where $A_0$ is the absorbance in alkaline solution and $A$ is the absorbance at the specific pH, and the pH to a line of unity slope by the least square method. Bromocresol purple (21 $\mu$M) was added to 0.8 M sucrose 0.03 M NaCl, and 0.02 M 2-($N$-morpholino)ethanesulfonic acid (MES) buffer at the required pH. Twenty-five experiments were run from pH 7.80 to 5.05. Results for 3 separate days of experiments were 6.24 $\pm$ 0.04, 6.26 $\pm$ 0.02 and 6.25 $\pm$ 0.03 at 22°, closely agreeing with that previously determined by CLARK AND LUBS[13]. The heat of ionization was measured by using a high concentration of bromocresol purple (470 $\mu$M) and monitoring the change in pH as a function of temperature. The heat was found to be 7.8 $\pm$ 0.4 kcal/mole from 12 to 28°. Thus, the p$K$ of bromocresol purple at 16° is 6.09.

The absorption spectrum of the chloroplasts was measured using the method of SAUER AND BIGGINS[12], which employs opal glass plates behind the cuvettes in a Cary spectrophotometer (Model 15). The $H^+$ flux in $\mu$equiv was calculated using the mass-action equation for the pH equilibrium of bromocresol purple and a differential extinction coefficient (575 nm–490 mm) for bromocresol purple of 49.5 $mM^{-1} \cdot cm^{-1}$ (see ref. 10). The buffer capacity of the chloroplast containing suspension was determined by titration in the dark using a Corning expanded scale pH meter. The buffer capacity used was that at pH 6 for the initial rate and that at a higher pH (calculated from the $\Delta A$ induced by the light) for the efflux rate in the dark.

## RESULTS

Previous work[10,14] indicated that there are four kinetic regions of $H^+$ movement; (1) an initial rate of influx in the light, (2) a secondary rate of influx in the light (after about 3–5 sec), (3) the extent or steady-state level, and (4) the efflux rate in the dark (about 0.5 sec after the light has been extinguished). Experiments reported here have been carried out with nonsaturating light and, typically, only the initial rate, extent and efflux rate can be observed.

The dependence of the extent and dark efflux upon the light intensity is shown in Figs. 1A and 1B. The light intensity for the dark efflux is, of course, the intensity prior to the dark period. Fig. 1 is a log–log plot of the extent of pH change or of the efflux of protons and the intensity of light. The solid line in Fig. 1A has a slope of 0.5 indicating that the extent varies as the square root of light intensity. The solid line in Fig. 1B, however, has a slope of 1.0; this indicates that the efflux varies linearly with light intensity up to about 0.7–0.8 neinstein·$cm^{-2}$·$sec^{-1}$. The curves indicate that the yield for the efflux must be measured at light intensities below 0.7 neinstein·$cm^{-2}$·$sec^{-1}$ for 645 nm light and that any quantum yield calculations for extent measurements must utilize the dependency upon the square root of light intensity.

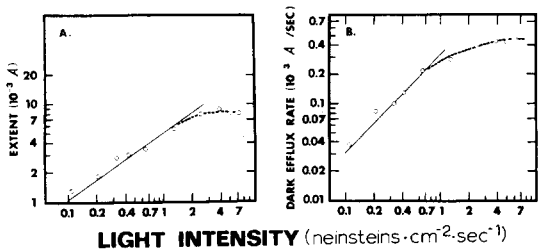



Fig. 1. Dependence of the steady-state extent and rate of efflux in the dark upon light intensity. (A) Steady-state extent. (B) Rate of efflux. Chloroplasts were suspended as described in METHODS and illuminated with 645 nm light.

The initial rate also depends upon the light intensity; however, the initial rate does not reach a maximum until high light intensities are reached. In fact, the maximum rate for the initial light response (not shown here) can be extrapolated to $4.0 \cdot 10^{-3}$ $A$ unit/sec whereas the efflux rate extrapolates to about $6 \cdot 10^{-4}$ $A$ unit/sec. Fig. 2 also shows a log–log plot of the initial rate of $H^+$ movement and the intensity. The slope of the line is unity below about 1 neinstein·$cm^{-2}$·$sec^{-1}$. Also it was found that the rate of the initial response is linearly dependent upon the chloroplast concentration (up to 12 $\mu$g/ml chlorophyll) at the intensities of light shown here.

For a specific light intensity, there is a direct relation between the extent reached (not necessarily steady-state) and the dark efflux (Fig. 3). Once the indicated extent was reached, the actinic light was turned off and the dark efflux (as a rate) was measured. As seen the rate of the efflux in the dark is linearly dependent upon the extent reached[11].

The action spectrum for the initial rate, measured for several preparations of chloroplasts with light intensities below 1 neinstein·$cm^{-2}$·$sec^{-1}$, is shown in Fig. 4. In order to average the results from several preparations of chloroplasts the rate at

670 nm has been normalized to unity. While the maximum activity of the preparations vary slightly, the variation of the data points at a given wavelength is only 23 % (with an error of the mean of 9 %). The spectrum is typical of a chlorophyll-driven light reaction with a broad peak between 670 and 685 nm. The initial rate is still about 45 % at 700 nm, indicating that System I is active in the process of $H^+$ movement[4,5,12].

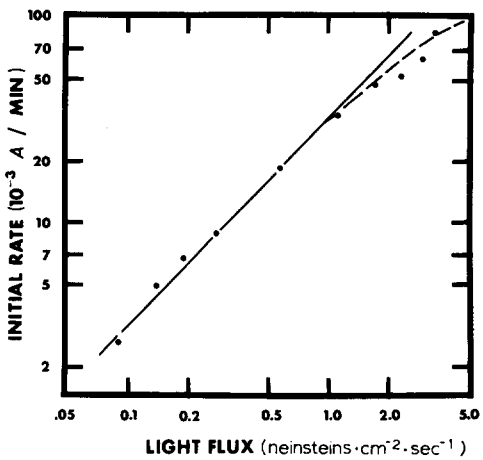



Fig. 2. Dependence of the efflux in the dark upon the extent of pH rise. Chloroplasts were suspended as described in METHODS. Intensity of the 645 nm light was 6.5 kergs·cm$^{-2}$·sec$^{-1}$.

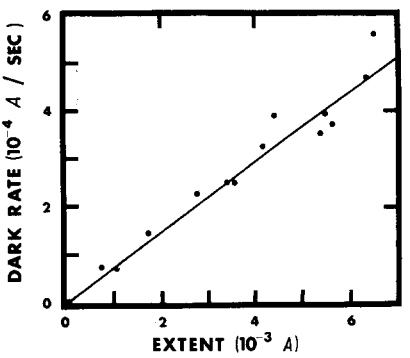



Fig. 3. Dependence of the rate of the initial influx of $H^+$ upon light intensity. Chloroplasts were suspended as described in METHODS. Illumination was by 645 nm light.

The action spectrum for the rate of the $H^+$ efflux in the dark is the same as that for the initial rate, as seen in Fig. 5. Each point on the graph indicates a separate experiment at given wavelength (with the initial rate of influx and rate of efflux given on the axes). If the action spectra were different for both light and dark processes, a curved line would result. The least squares fit of Fig. 5 indicates that the slope is 0.47 $\pm$ 0.05 with an intercept of the ordinate of 0.03 $\pm$ 0.02. Thus, both action spectra are equivalent and the same spectrum would result if the rate of efflux in the dark were used in Fig. 4 instead of the rate of $H^+$ influx in the light. The ratio of dark efflux to light influx is very near 0.5, for the light intensities used here.

The action spectrum for the extent could also be calculated by dividing the steady-state extent reached by the square root of the light intensity (see Fig. 1). It is difficult to understand what this would measure, however. A more telling calculation is shown in Table I. The yield ratio of normalized extent to normalized dark rate is tabulated for all wavelengths and for the red and far-red regions. There is no significant difference between the yield ratio in the red and far-red region. This indicates that the dependency of the rate of proton efflux in the dark is linearly related to the extent reached (see Fig. 3). Thus, the same action spectrum would result if the extent was used in Fig. 4 rather than initial influx rate.

The quantum yield (*i.e.* number of protons moved per photon absorbed) can be determined from Figs. 4 and 5 and the absorption spectrum of the chloroplasts.

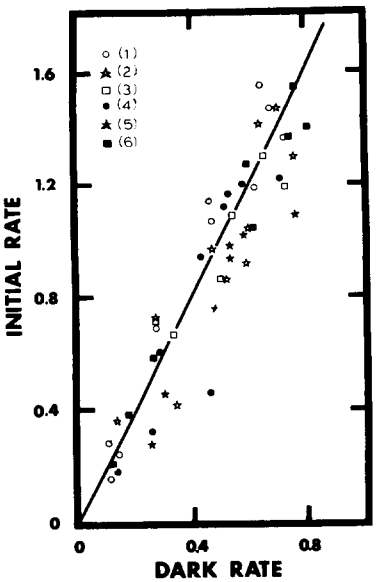



Fig. 4. Action Spectra of $H^+$ movement as measured by bromocresol purple. Chloroplasts were suspended as described in METHODS. The symbols represent the following experimental conditions:

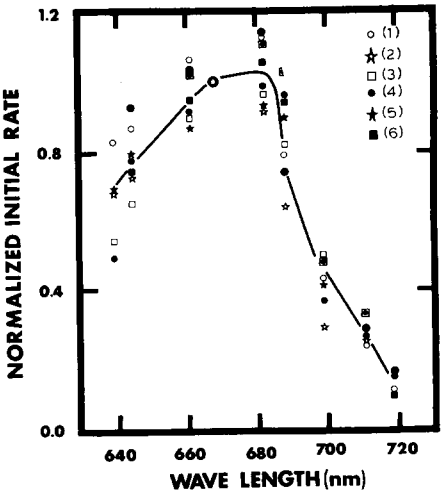



Fig. 5. Comparison of the action spectra of the efflux in the dark and initial influx. Chloroplasts were suspended as described in METHODS. Each point represents a different wavelength or preparation from Fig. 4. Line is fitted by method of least squares.

| *Symbol No.* | *Light intensity (neinstein·cm$^{-2}$·sec$^{-1}$)* | *Preparation* | *Rate at 670 nm* |
|---|---|---|---|
| (1) | 0.70 | I | 0.70 |
| (2) | 0.35 | | 0.72 |
| (3) | 0.53 | | 0.68 |
| (4) | 0.70 | | 0.70 |
| (5) | 0.75 | II | 0.59 |
| (6) | 0.75 | | 0.53 |

TABLE I

CORRELATION BETWEEN EXTENT AND RATE OF PROTON EFFLUX IN THE DARK WITH RESPECT TO ACTION SPECTRUM

| *Expt. No.* | *Yield ratio* (neinsteins$^{1/2}$·sec$^{1/2}$·cm$^{-1}$) for wavelength region (nm)* | | |
|---|---|---|---|
| | *640–720* | *640–690* | *700–720* |
| 1 | 19 ± 5 (9)** | 22 ± 5 (6) | 14 ± 2 (3) |
| 2 | 16 ± 3 (8) | 16 ± 2 (6) | 15 ± 6 (2) |
| 3 | 19 ± 5 (10) | 15 ± 2 (6) | 24 ± 5 (4) |
| 4 | 15 ± 4 (8) | 13 ± 2 (6) | 21 ± 1 (2) |
| 5 | 20 ± 7 (8) | 15 ± 1 (5) | 28 ± 4 (3) |
| Average | | 16 ± 3 | 20 ± 6 |

* Yield ratios were calculated according to the formula:

Ratio = [Extent/(Intensity)$^{\frac{1}{2}}$]/[Dark rate/Intensity]

for wavelengths of light shown in Fig. 4.

** Number in parentheses indicates number of experiments at different wavelengths.

Table II compares the absorption of chloroplasts obtained by this laboratory and others[12,15] over the wavelengths of interest. The method described in METHODS is similar to that used by SAUER AND BIGGINS[12], while the absorption spectrum obtained by SCHWARZ[15] used an integrating-sphere.

TABLE II

COMPARISON OF THE ABSORPTION SPECTRUM OF ISOLATED CHLOROPLASTS

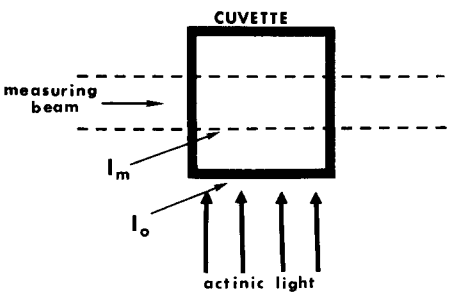
| *Wavelength (nm)* | *Absorbance* | | |
|---|---|---|---|
| | *Measured** | *cf.* SCHWARZ** | *cf.* SAUER AND BIGGINS*** |
| 640 | 0.144 | 0.135 | 0.115 |
| 650 | 0.188 | 0.182 | 0.153 |
| 660 | 0.252 | 0.218 | 0.212 |
| 670 | 0.376 | 0.329 | 0.378 |
| 680 | 0.362 | 0.368 | 0.403 |
| 690 | 0.130 | 0.176 | 0.146 |
| 700 | 0.062 | 0.061 | 0.042 |
| 710 | 0.022 | 0.026 | 0.014 |
| 720 | 0.010 | 0.010 | 0.007 |
| Maximum | 678 | n.r.+ | 678 |

* Measured, as in METHODS with a correction applied [$A$ (measured) = $A$ (observed) − $A$ (observed at 750 nm)]. This, in fact, sets the absorbance at 750 nm equal to zero.

** Data from SCHWARZ[15], for 10 μg/ml chlorophyll concentration.

* Data from SAUER AND BIGGINS[12]; also from SAUER AND PARK[19] with 678 nm peak set to be equal to 0.410 (from author's measured value).

+ Not reported.



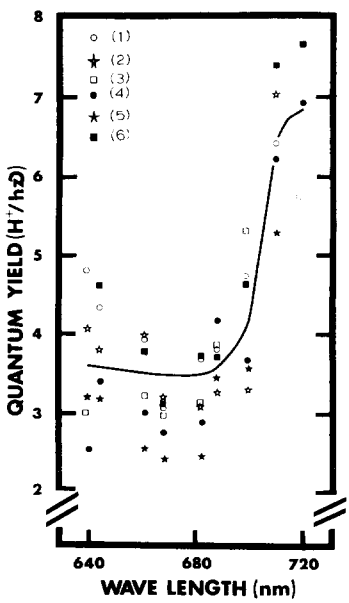




Fig. 6. Geometry of the spectrophotometer used in calculating the quantum yield. See text for complete discussion.

Fig. 7. Wavelength dependence of the quantum yield of the initial influx of $H^+$. Chloroplasts were suspended as described in METHODS. The symbols correspond to those used in Fig. 4.

The actual amount of light absorbed by the chloroplasts is calculated as follows. The measuring beam of the spectrophotometer only travels through the middle third of the cuvette with respect to actinic illumination and, hence, the rates were determined in this region (see Fig. 6). The light absorbed by material (in units of neinstein·$cm^{-2}$·$sec^{-1}$) in the middle third of the cuvette ($\Delta I$) using the Beer–Lambert Law is equal to:

$$\Delta I/I_m = (1 - 10^{-E/3})$$

where $I_m = I_0 10^{-E'/3}$ is the amount of light absorbed by the material in the first third of the cuvette or incident light on middle third of solution; $E$, absorption of the chloroplasts for path length of 1 cm; $E'$, absorption of (chloroplasts + pyocyanine + bromocresol purple) for path length of 1 cm; $I_0$ = incident actinic light on the solution at front of cuvette.

The above equation with the data from Fig. 4 and Table I is used to calculate the quantum yield as a function of wavelengths as shown in Fig. 7. The percentage of light absorbed by the middle third of the chloroplasts suspension is never greater than 16% of the incident light. Fig. 7 shows that the average quantum yield ($H^+$/photon) is 3.5 ± 0.1 from 640 to 690 nm. However, for 710–720 nm region the yield rises to 6.7 ± 0.3. The ± values are standard error of the data points. From Fig. 5 the quantum yield for $H^+$ efflux in the dark would be exactly the same except the yields are reduced by 0.47 to 1.6 $H^+$/photon between 640 and 690 nm and 3.2 $H^+$/photon between 710 and 720 nm. This experimental number is the minimum average value which has been observed. The argument could be made that the yield would be still higher if a more active preparation could be made or that the maximum values observed here should be used.

In order to investigate a possible interaction between Systems I and II an enhancement-type experiment was tried, and is summarized in Table III. The intensity for System I light (715 ± 5 nm) is constant while intensity of System II light (645 ± 5 nm) is varied. Within experimental error, the response with light given

TABLE III

LACK OF ENHANCEMENT OF THE INITIAL RATE OF $H^+$ MOVEMENT BY SYSTEM II LIGHT

| *Intensity* (*neinstein·cm⁻²·sec⁻¹*) | *Initial rate** (*10⁻³ A unit·min⁻¹*) *for light of:* | | | |
|---|---|---|---|---|
| | *645 nm* | *715 nm** + 645 nm* | *Calculated (715 + 645)* | *Enhancement**** |
| 1.36 | 35.1 | 39.3 | 41.9 | 0.94 |
| 0.68 | 21.7 | 29.3 | 27.5 | 1.04 |
| 0.34 | 11.4 | 17.7 | 17.2 | 1.01 |
| 0.27 | 9.3 | 14.4 | 15.1 | 0.95 |
| 0.14 | 3.2 | 11.7 | 9.0 | 1.30 |
| 0 | 0 | 5.8 ± 0.3 | | |

* Measured as in METHODS with initial pH 5.96, and 2.1 μM bromocresol purple and the intensity of 645 nm light as given.

** The intensity of 715 nm light = 1.2 neinsteins·$cm^{-2}$·$sec^{-1}$. However, only 10% of the 715 nm light is absorbed compared with 38% of the 645 nm light. The same number of photons are absorbed at 715 nm and 645 nm when the 645 nm light has an intensity of 0.34 neinstein·$cm^{-2}$·$sec^{-1}$.

*** Enhancement = (715 nm + 645 nm)/((715 + 645) calculated).

simultaneously for both System II and System I is the same as the sum of the response with System II light and the response with System I light. One must conclude no enhancement can be observed; therefore, no interaction between Systems I and II exist for $H^+$ movement.

IZAWA AND HIND[5] found using a flow pH electrode system that the initial proton movement can be inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU). HEATH AND HIND[10], using bromocresol purple as a pH indicator, also found that the initial rate, as well as the extent, was inhibited by DCMU. The inibition of the extent by DCMU is examined in Fig. 8. DCMU progressively inhibits the extent upon repeated cycles of illumination (also see ref. 10). The rate of this progressive inhibition is slower for lower concentration of DCMU.

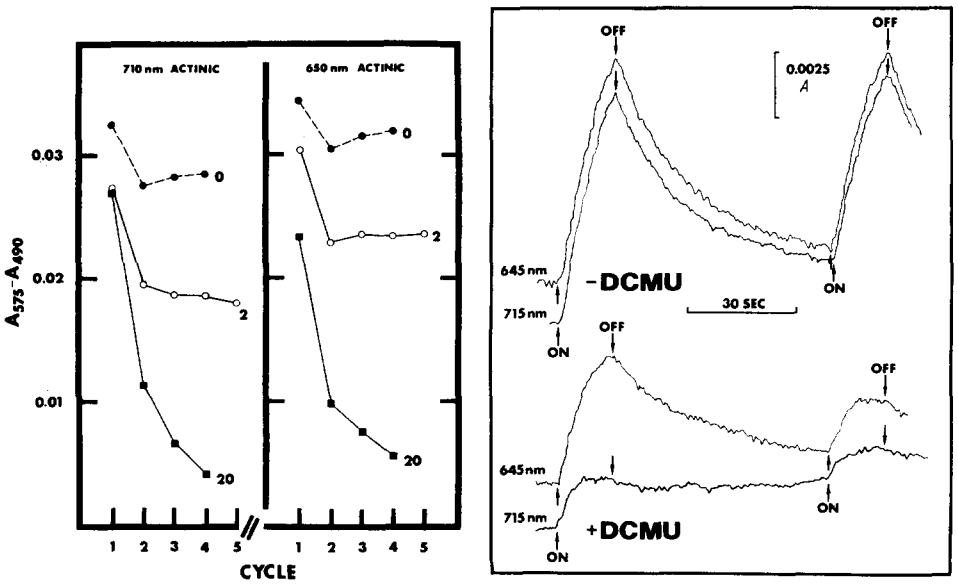



Fig. 8. Progressive inhibition of steady-state extent by repeated periods of illumination for DCMU-poisoned chloroplasts. Chloroplasts (50 $\mu$g/ml) were suspended as described in METHODS and illuminated by repeated periods of saturating actinic light (at indicated wavelength). The cycle period was 15 sec light and 80 sec dark. The number beside curves represents the concentration of added DCMU in $\mu$M.

Fig. 9. Effect of pre-illumination on the inhibition of the bromocresol purple response by DCMU. Chloroplasts were suspended as described in METHODS and pre-illuminated by either 645 nm light (2.0 neinstein·cm$^{-2}$·sec$^{-1}$) or 715 nm (1.8 neinstein·cm$^{-2}$·sec$^{-1}$) for 30 sec. During the 90 sec dark interval, DCMU (20 $\mu$M) in ethanol (0.1 %) or ethanol alone was added to the chloroplasts. Subsequently the suspension was illuminated with 645 nm light (3.5 neinstein·cm$^{-2}$·sec$^{-1}$).

If a slow Mehler-type electron flow[16] continuously drains electrons from System I (to $O_2$), any observed phenomena utilizing these electrons must have them continuously supplied by a source. This situation is similar to that described by HAUSKA *et al.*[17]. When the supply of electrons from System II is blocked by DCMU, System I is gradually depleted of electrons by the Mehler-type, non-cycle electron flow[10] and cyclic flow ceases. Pre-illumination of the chloroplast suspension (Fig. 9) shows that this is the effect for the DCMU-induced progressive inhibition of $H^+$ movement. The top two traces of $H^+$ movement are controls with no DCMU added to them. The

samples have been previously pre-illuminated (not shown) by either 645 and 715 nm light for 20 sec; after 60 sec of darkness, they are illuminated by 645 nm light. The traces are nearly identical (initial rates of $7.5 \cdot 10^{-4}$ $A$ unit$\cdot$sec$^{-1}$ for 645 nm pre-illumination and of $7.1 \cdot 10^{-4}$ $A$ unit$\cdot$sec$^{-1}$ for 715 nm pre-illumination). On the other hand, the bottom two traces have had DCMU added to the chloroplasts during the dark period between the pre-illumination and the 645 nm light. The chloroplasts pre-illuminated with 645 nm light (which partially fills System I with electrons by allowing electron flow from System II) shows an inhibition over the controls; however, it is much less than that for the chloroplasts pre-illuminated with 715 nm light (which drains System I of electrons). The initial rate for chloroplasts pre-illumination with 645 nm light (+ DCMU) is $6.1 \cdot 10^{-4}$ $A$ unit$\cdot$sec$^{-1}$ (or 16 % inhibition) while that for chloroplasts pre-illumination with 715 nm light is $3.3 \cdot 10^{-4}$ $A$ unit$\cdot$sec$^{-1}$ (or 55 % inhibition).

## DISCUSSION

It is clear from the action spectrum for the initial rate of $H^+$ movement (Fig. 4) that a long wavelength form of chlorophyll (System I) is active in the proton transport. The apparent System II involvement in cyclic $H^+$ movement[6,10] is most probably related to the role of System II in the maintenance of the proper reduction/oxidation poise of the components of System I, as described by HAUSKA *et al.*[17] (see Figs. 8 and 9). Thus, we will focus our attention on Fig. 7 and the quantum yield, and its relation to the MITCHELL hypothesis[1].

There are six areas of concern in the calculations of the quantum yield of $H^+$ movement. The determination of the intensity of incident light by the radiometer is accurate to within $\pm$ 5 % as determined by the Eppley Thermopile. The p$K$ of bromocresol purple was measured as 6.25 $\pm$ 0.03 (error of mean) at room temperature, agreeing quite closely with the value previously determined of 6.3[13]. Although, if the p$K$ were off by only 0.1 unit, the yield would be altered by about 20–25 %. Any systematic error in the extinction coefficient would be canceled by use of the ratio of the initial and light-induced change in concentration of bromocresol purple from the mass action law[10]. The initial pH of the chloroplast suspension for the quantum yield measurements was always adjusted to 6.00 $\pm$ 0.02 and calculations were done from the actual amount of added bromocresol purple, measured to within 0.2 %. However, an initial pH misadjustment could have altered the yield by about 3 %. The buffer capacity of the chloroplasts can be titrated to within 5 %; however, if that capacity changes in the light, as suggested by POLYA AND JAGENDORF[18], this would tend to raise the yield. The buffer capacity also changes with pH (ref. 6); thus, only the initial rate is accurate with respect to the buffer capacity. The quantum yield depends upon how the correction for the residual light scattering is made. The correction used[12], (see METHODS) rest upon the light scattering properties of a broken chloroplasts preparation (called a quantasome preparation by the authors[19]). However, absorption measured by SCHWARZ[15] using an integrating sphere, absorption measured by SAUER AND BIGGINS[12] and the absorption measured by the author (Table II) all agree well in the red region (to within 15–20 % up to 690 nm). Beyond 690 nm a sizable variation makes quantum yields in this region highly uncertain.

A slightly lower quantum yield (15 %) is observed in the region of the peak of

chlorophyll absorption (Fig. 7). The light intensity used for the action spectra at the wavelengths of 670–680 nm (Fig. 4) must have been in some cases, too high; such that the rate–intensity relationship (Fig. 2) was slightly non-linear. The percent of the light absorbed at 670–680 nm is nearly twice as much as that at 645 nm. However, lower intensities of light were difficult to use due to the low absorption at wavelengths greater than 700 nm. Thus, one must judge the peak in Fig. 7 to be nearly 15 % higher. Due to the variation in the preparation and band-width of the actinic light used here, the position of the peak of the action spectrum, often used to determine System I and System II activity[12], is only approximate.

Pyocyanine catalyzes a cyclic flow of electrons around Photosystem I (ref. 2); therefore, the quantum yield should be one electron transported per photon absorbed. From Fig. 7 a $H^+/e_2$ ratio of 7.0 can be calculated for pyocyanine-catalyzed $H^+$ movement at low light (from 640–690 nm). This is above that measured with saturating light intensity ($H^+/e_2 = 4.0$) by a factor of nearly 2 (ref. 6), but close to that measured by DILLEY AND VERNON[4] at low light for System I using reduced trimethyl benzoquinone → cytochrome *c* ($H^+/e_2 = 10$). It is unclear whether Dilley and Vernon corrected for the $H^+$ involved in the oxidation of reduced trimethyl benzoquinone; if not, the yield then becomes $H^+/e_2 = 8$. Thus, the quantum yield measured here correlates well with that observed by DILLEY AND VERNON[4].

The $H^+$ efflux in the dark is assumed to be the $H^+$ influx in the light at steady-state[10,11]; that is, the steady-state level or extent is a balance between light-driven influx and an efflux. The difference in the quantum yield between these two rates (see Fig. 4) could represent an increased pH gradient through which the $H^+$ must be moved or a change in the buffer capacity of the chloroplasts[18]. The quantum yield (640–690 nm) at steady state conditions in continuous illumination is about 1.6 $H^+$/photon or 3.2 $H^+/e_2$, similar to those reported elsewhere[6] at saturating light intensity. DILLEY AND VERNON[4,5] measured a yield of 2.5 $H^+$/photon.

For wavelengths above 700 nm, the picture of the yield changes. The yield is nearly 7 $H^+$/photon which calculates to approximately 14 $H^+/e_2$. There seems to be no peculiarities in either the action spectrum (Fig. 4) or the yield spectrum (Fig. 7) in the 700 or 710 nm as previously noted by DILLEY AND VERNON[5]; hence, their action spectrum must be in error. However, the results here agree well with DILLEY AND VERNON[4] [5 $H^+$/photons] as to the increased yield.

It is obvious from the yield for $H^+$ transport (Fig. 7) and the yield for ATP production[15,20] that for wavelengths shorter than 700 nm, about half of the quanta absorbed are "wasted" presumably upon System II-linked reactions. In the far-red region the yields for both cases nearly double. Another interpretation is that the redox poise generated by System II affects, by some unknown manner, the number of $H^+$ transported per photon.

The quantum yield for ATP production (at pH 8) is low; SCHWARZ[15] has measured ATP/photon = 0.15 at 700–710 nm. However, recently under more rigorously controlled redox conditions AVRON[20] and AVRON AND NEUMANN[21] have measured an ATP per photon ratio of 0.33. If cyclic electron flow has one ATP producing site per chain, one would expect a yield of unity (or 0.5 if 2 electrons are transported per site). This low measured yield may be due to imperfect conditions. Thus, if one can compare $H^+$ movement at pH 6 (yield of dark efflux) to ATP production at pH 8, the $H^+$/ATP is 10 experimentally[20] (or 3.5 for 1 electron/ATP site theoretically). All of these values

are too high for the MITCHELL[1] hypothesis which predicts $H^+$/ATP of not more than 2. It may be, however, that some transported $H^+$ are not involved in ATP production. The reason for the transport of these $H^+$ is yet to be understood.

ACKNOWLEDGEMENTS

The author would like to thank Dr. W. M. Dugger, Jr. for his encouragement and helpful discussions and Dr. J. Olson for many helpful comments. This work was supported in part by grants from U.S. Public Health Service (AP-40) and from the University Academic Senate.

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