LIGHT-INDUCED CHANGE IN THE BUFFER CAPACITY OF SPINACH CHLOROPLAST SUSPENSIONS

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Summary: Illumination of spinach chloroplast suspensions in the presence of a redox dye causes large changes in the buffer capacity of the suspensions. The light-induced changes of buffer capacity above pH 6.4 can be completely reversed in the dark, while buffer capacity changes at pH values less than 6.4 are partly or completely irreversible. While the light-induced buffer capacity change at pH 7.2 and light-induced net proton uptake show similar responses to uncouplers and similar light requirements, the buffer capacity change at pH 6.2 has a much lower light intensity requirement than the other phenomena. The demonstration of light-induced buffer capacity changes in chloroplast suspensions calls for quantitative reappraisal of the literature dealing with light-induced net proton uptake by chloroplasts.

Isolated chloroplasts take up protons when illuminated under conditions of electron transport, especially in the region from pH 5 to 7 (Jagendorf and Hind, 1963; Neumann and Jagendorf , 1964). This phenomenon has come to be considered as evidence consistent with the chemiosmotic theory for photophosphorylation as elaborated by Mitchell (Mitchell, 1966, 1967; see also Jagendorf and Uribe, 1966a; Jagendorf, 1967). Other phenomena supporting this concept are those of post-illumination ATP formation (Shen and Shen, 1962; Hind and Jagendorf, 1963) and ATP synthesis driven by an acid-base gradient in the dark (Jagendorf and Uribe, 1966b). However, alternative explanations for these phenomena are available, and critical evidence testing predictions from the theory have therefore been sought.

One prediction of the chemiosmotic model is that of a stoichiometry of one proton taken up for every electron passing through a coupling site. Accordingly, attempts have been made to measure rates of net proton uptake and to correlate these with concomitant electron transport flux. The primary measurements in these experiments have usually been of the rate of change of pH as measured by a glass electrode or by the absorbance change of a dye. These pH

changes are translated into units of net proton uptake by reference to the titration curve of the chloroplast suspension. However our results presented below demonstrate appreciable changes in the buffer capacity of chloroplast suspensions due to illumination, so that a titration curve determined in the dark is not neccessarily applicable to the relation between a pH change and the net proton uptake in the light. These results shed uncertainty on the quantitative significance of past determinations of H^+/e^- ratios.

Swollen chloroplasts suspended in 10 mM NaCl were prepared from market spinach as described previously (Hind and Jagendorf, 1963). Chloroplast suspensions were titrated with freshly prepared NaOH solutions (prepared with boiled H_2O) in a water-cooled vessel. pH changes were monitored using a Corning glass electrode and a Corning Model 10 pH meter, and recorded using a Moseley Autograf Model 7100B strip chart recorder. Light was provided by a Sylvania Sun Gun and filtered through water traps and a Corning 2418 red filter. Light intensities were measured using a YSI-Kettering Model 65 Radiometer. Titrations of illuminated chloroplasts were commenced when the pH of the suspension had reached a steady value after turning on the light. Buffer capacity of the suspension was routinely estimated from the pH shift on addition of a known aliquot of NaOH solution. Light-induced proton uptake was quantitated from the steady-state light-induced pH shift by means of titration curves determined in the same conditions of illumination.

Fig. 1a shows the suspension buffer capacity as a function of the pH at which it is measured. Curves are shown for the buffer capacities in the dark initially, in the light, and in the dark after the light has been turned off. It is apparent that illumination causes large changes in the buffer capacity. The light buffer capacity curve has a maximum at pH 6 and a shoulder in the vicinity of pH 7; a large number of experiments have confirmed the qualitative reproducibility of these phenomena. It is clear that the buffer capacity change in the vicinity of pH 6 is not as readily reversible in the dark as is the buffer capacity change in the vicinity of pH 7. Fig. 1b shows the result

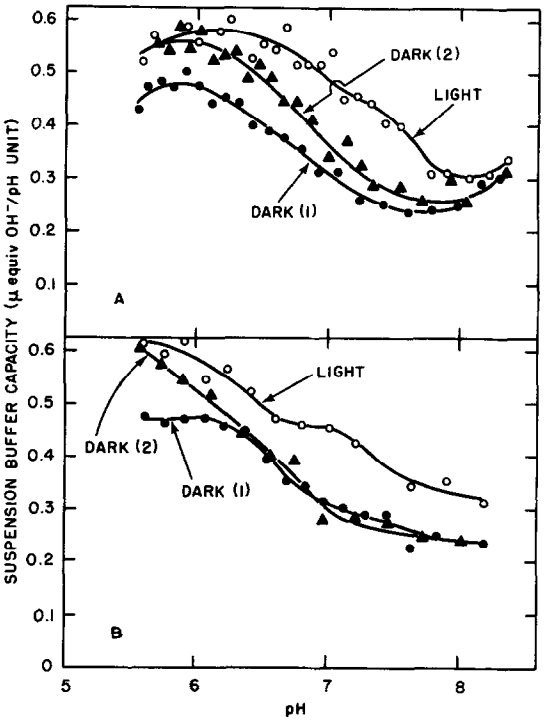

Figure 1. (a)Buffer capacity of a suspension of chloroplasts (125 μg chl/ml) in 10 mM NaCl-10 μM pyocyanine (total vol. 4 ml) at 5.5°, determined by titration in the dark before illumination (DARK 1, ●), when illuminated with red light (21.5 x 10^5 ergs cm^{-2} sec^{-1}) (LIGHT, ○), and from 7 mins after the cessation of illumination (DARK 2, ▲). The chloroplast suspension used to determine all 3 curves was illuminated for a total of 31 mins. (b)Buffer capacity of chloroplast suspensions (125 μg chl/ml) in 10 mM NaCl-25 μM pyocyanine at 5.5°, titrated in the dark (DARK 1, ●), in the dark from 5 mins after a prior 5 min illumination with red light (10.3 x 10^5 ergs cm^{-2} sec^{-1}) (DARK 2, ▲), and while illuminated with red light (10.3 x 10^5 ergs cm^{-2} sec^{-1}) (LIGHT, ○).

of a similar experiment in which a lower light intensity and a shorter time of exposure of the chloroplast suspension to light resulted in a complete reversibility of the buffer capacity change over the range of pH 6.4 to pH 8.2 while the buffer capacity change in the vicinity of pH 6 was again largely irreversible.

Other experiments have shown that the buffer capacity at pH 7.2 is a linear function of chloroplast concentration in the light and in the dark and that the lines extrapolate to the same buffer capacity in the absence of chloroplasts. This rules out a contribution of added redox dye to the extent

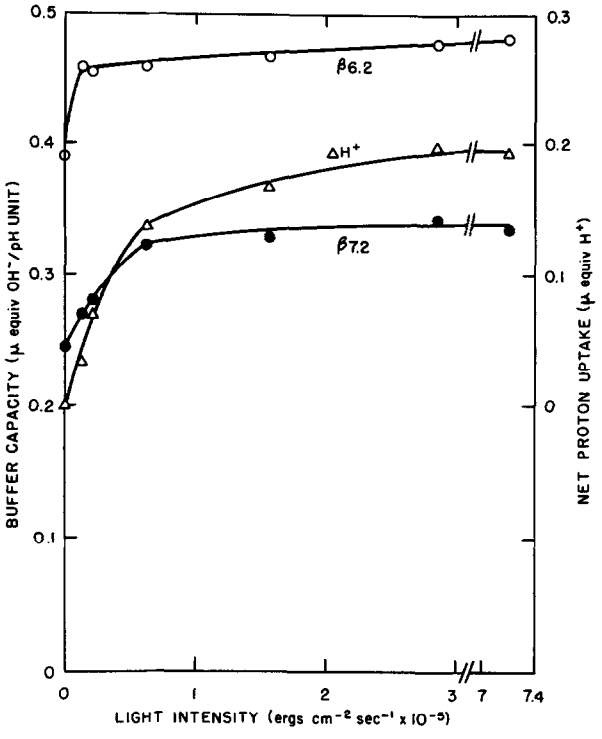

Figure 2. Light intensity dependence of the light-induced buffer capacity changes at pH 6.2 (-○-) and pH 7.2 (-●-), and the light-induced net proton uptake (initial pH 6.20 ± 0.04) (-△-), determined for chloroplast suspensions (125 μg chl/ml) in 10 mM NaCl-25 μM phenazine methosulfate (total vol. 4 ml) at 5.5°. Curves describing buffer capacity in the light and in the dark as functions of pH were determined for all experimental situations and the buffer capacity changes at pH 6.2 and pH 7.2 determined by interpolation.

of the buffer capacity change at pH 7.2 in the light.

Fig. 2 shows the light intensity dependence of net proton uptake at pH 6.2, of the change in buffer capacity at pH 7.2, and of the change in buffer capacity at pH 6.2 in the presence of 25 μM phenazine methosulfate. Similar data were obtained using pyocyanine as cofactor. Table I lists the light intensities required for half-maximal saturation of these 3 phenomena, measured with 2 different cofactors. It is clear that the light intensities for half-maximal buffer capacity change at pH 7.2 and net proton uptake at pH 6.2 are approximately the same, but considerably greater than that required for half-maximal change in buffer capacity at pH 6.2.

TABLE I

LIGHT INTENSITY DEPENDENCE OF NET PROTON UPTAKE AND BUFFER CAPACITY CHANGE

The data were obtained as described in the legend to Fig. 2 and derive from 2 separate experiments.

Phenomenon	Light intensity for half-maximal effect (ergs cm^{-2} sec^{-1} x 10^{-5})
(1) Cofactor: 25 μM phenazine methosulfate	
Buffer capacity change (pH 7.2)	0.29
Buffer capacity change (pH 6.2)	0.04
Net proton uptake (initial pH 6.20 ± 0.04)	0.33
(2) Cofactor: 25 μM pyocyanine	
Buffer capacity change (pH 7.2)	0.69
Buffer capacity change (pH 6.2)	0.10
Net proton uptake (initial pH 6.16 ± 0.04)	0.63

Effects of uncouplers on net proton uptake and on buffer capacity change at pH 7.2 are shown in Table II. The inhibition of light-induced net proton uptake (at an initial pH of 6.25) is qualitatively parallelled by inhibition of the buffer capacity change at pH 7.2. Effects of uncouplers on the buffer capacity change in the vicinity of pH 6 are quite complex and will be described elsewhere.

These pH-dependent changes in buffer capacity were predictable as an inevitable consequence of a light-induced proton uptake whose extent varies with pH; the two types of phenomena are simply different aspects of the same thing. This is best illustrated by reference to the titration curves for chloroplasts in the light and in the dark shown in Fig. 3. Starting with any pH in the dark (pH_D), the light-induced pH rise is given by the vertical line (A), its termination being a point (pH_L) on the light titration curve. Altern-

TABLE II

EFFECTS ON UNCOUPLERS ON LIGHT-INDUCED NET PROTON UPTAKE AND BUFFER CAPACITY CHANGE IN SPINACH CHLOROPLAST SUSPENSIONS

Buffer capacity changes as functions of pH were determined for suspensions of chloroplasts (125 μg chlorophyll/ml) in 10 mM NaCl-25 μM pyocyanine at 5.5° in each of the experimental situations and the buffer capacity change at pH 7.2 determined by interpolation. Net proton uptake (initial pH 6.25 ± 0.03) was quantitated from the extent of the steady-state pH rise and the titration curves of the suspensions in each experimental situation, determined in the same conditions of illumination (10.3 x 10^5 ergs cm^{-2} sec^{-1} in all cases).

	Treatment	Net Proton Uptake (μequiv/mg chl)	Buffer Capacity Change (pH 7.2) (μequiv/pH/mg chl)
1.	Control	0.718	0.412
	0.25 mM chlorpromazine	0.136	-0.058
	1 mM NH_4Cl	0.552	0.116
2.	1% EtOH	0.644	0.448
	1% EtOH-5μM CCCP	0.472	0.232
	1% EtOH-10μM CCCP	0.348	0.164
	1% EtOH-25μM CCCP	0.134	0.062
	1% EtOH-50μM CCCP	0.036	0.072

atively, the intercept on the horizontal line (B) between the two titration curves indicates the amount of acid required to keep the pH of the suspension constant once the light has been turned on, i.e. the net proton uptake in the light at that pH. Thus the pH-dependence of the light-induced pH rise, superimposed on the dark titration curve defines the light titration curve. The pH-dependent buffer capacity curves in turn simply obtain from the first derivatives of these titration curves.

The light-induced change in buffer capacity can be explained in terms of 2 extreme models or a combination of both. One major possibility is that it arises from light-induced conformational changes of membrane polyelectrolytes and does not neccessarily involve proton translocation across the thylakoid

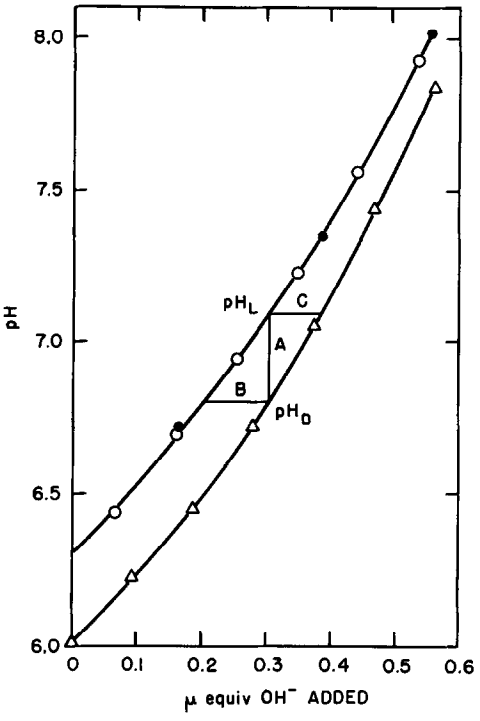

Figure 3. Portions of titration curves of chloroplast suspensions (125 μg chl/ml) in 10 mM NaCl-10 μM pyocyanine (total vol. 4 ml), determined at 5.5° in the dark (-△-), or when the suspension was illuminated with red light (21.5 x 10^5 ergs cm^{-2} sec^{-1}) (-○-). Also shown is the superimposition on the dark titration curve of the light-induced pH rise (-●-), determined in the same experimental conditions. The quantitation of the light-induced pH rise (pH_L-pH_D) in terms of proton equivalents clearly derives from line (B) rather than line (C).

membrane. The alternative view is that there is a net translocation of protons across the thylakoid membrane in the light and equilibrium is attained through equivalence of an active proton influx and a passive proton efflux. Either model can produce the results demonstrated here.

The fact that the buffer capacity increases in the light has important consequences with respect to attempts to quantitate light-induced proton uptake. If the net proton uptake is titrated at constant pH in the light (Neumann and Jagendorf, 1964; Galmiche, Girault, Tyszkiewicz and Fiat, 1967) the steady-state extent and kinetics of proton uptake can be measured with validity. However, if the steady-state extent of the proton uptake is calculated from

primary recordings of pH change, then clearly the titration to quantitate the pH change in terms of proton equivalents must be performed in identical experimental conditions (i.e. of illumination etc.). The details of such requisite quantitating titrations have not been specified in any of the published reports of such experiments. Our data indicate that such results are in error by minimizing the actual net proton movement if in fact they derive from calculations based on dark titration curves. The extent of the error is more difficult to estimate in the case of attempts to measure the initial kinetics of proton flux from pH vs. time recordings, because it is not clear how rapidly the buffer capacity is changing after the light is turned on or off.

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REFERENCES

Galmiche, J. M., Girault, G., Tyszkiewicz, E., and Fiat, R. (1967), C. R. Acad. Sci., Paris 265, 374.
Hind, G., and Jagendorf, A. T. (1963), Proc. Nat. Acad. Sci. 49, 715.
Jagendorf, A. T. (1967), Fed. Proc. 26, 1361.
Jagendorf, A. T., and Hind, G. (1963), Nat. Acad. Sci., Nat. Res. Council, Publ. No. 1145, 599.
Jagendorf, A. T., and Uribe, E. (1966a), Brookhaven Symp. in Biol. 19, 215.
Jagendorf, A. T., and Uribe, E. (1966b), Proc. Nat. Acad. of Sci. 55, 170.
Mitchell, P. (1966), Biol. Rev. 41, 445.
Mitchell, P. (1967), Fed. Proc. 26, 1370.
Neumann, J., and Jagendorf, A. T. (1964), Arch. Biochem. Biophys. 107, 109.
Shen, Y. K., and Shen, G. M. (1962), Scientia Sinica 11, 1097.