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SLOW TRANSIENTS OF LIGHT-INDUCED pH CHANGES AT THE OUTER SURFACE OF PHOTOSYNTHETIC MEMBRANES

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SUMMARY

Non-artificial transients, with a halftime of about 10 sec., of light-induced pH changes occur in fresh suspensions of spinach chloroplast fragments. The transients were observed with rapid direct pH measurements as well as the fluorescent probe technique.

Ageing of the preparations for 2–3 hrs. at room temperature annihilates the transients, whereas photoinduced pH changes still occur.

1. INTRODUCTION

Illumination of unbuffered chloroplast suspensions results in a marked rise in the pH of the suspending medium, cf. JAGENDORF & HIND (1963) and NEUMANN & JAGENDORF (1964). In accordance with the chemiosmotic hypothesis of MITCHELL (1966, 1974) this phenomenon is ascribed to proton uptake, cf. TREBST (1974). A number of techniques, cf. ROTTENBERG (1975), were used for measuring the resulting proton gradients. However, due to e.g. differences in time resolution of these methods, cf. AUSLÄNDER & JUNGE (1975), the results varied. According to SCHLIEPHAKE et al. (1968) a proton uptake occurs within 8 msec. A low time resolution may result in the registration of artifical, slow, transients occurring in between 5 and 30 sec. after dark-light and light-dark transitions, cf. IZAWA & HIND (1967), KARLISH & AVRON (1968), and DE KOUCHKOVSKY (1975c). Still, rapid measuring techniques, cf. DE KOUCHKOVSKY (1975c), indicate that non-artificial transients may occur as well. Such indications can be obtained from some control experiments by DE KOUCHKOVSKY, cf. (1974) fig. 1, (1975a) fig. 4, and (1975b) fig. 6. This author used the fluorescent probes fluorescein or fluorescein isothiocyanate (FITC) allowing rapid recordings with a half-response time of 0.2 sec., cf. DE KOUCHKOVSKY (1975a, 1975c). Both fluorescent probes do not penetrate the membranes and, thus, only the external pH is measured.

In the mentioned experiments red light of saturating intensity was used. However, it cannot be ruled out that non-artificial transients show up in a

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more pronounced way at non-saturating intensities. In order to find out whether non-artificial transients actually occur, measurements of photo-induced proton translocations are studied under non-saturating light conditions. To this purpose use is made of rapid direct pH measurements as well as the mentioned fluorescent probe technique. The results are presented below.

2. MATERIALS AND METHODS

Upon washing with 10 mM Tris buffer containing 10 mM NaCl, pH 8.0, freshly collected leaves of spinach, about 3 weeks old and grown at the institute, were minced in an ice-cooled Sorvall Omnimixer in the same buffer for 8 sec. The suspension was then centrifuged at $4.2 \cdot 10^3 \times g$ for 10 min. The sediment was taken up in a 50 mM NaCl solution and adjusted to about pH 6.3 with HCl. Fluorescein or FITC, and in a number of experiments also pyocyanine, were added at concentrations mentioned in the legends of the figures. The final chlorophyll concentration of the suspension, determined according to the method of ARNON (1949), was adjusted to about $3 \cdot 10^{-5}$ M. The total procedure, performed under weak light, required about 45 min. For direct pH measurement a Radiometer PHM22p in combination with a BD10 Kipp recorder was used. The measurements with fluorescent probes were done as described by DE KOUCHKOVSKY (1975a). The signals were recorded with a half-response time of 0.2. sec.

Fluorescence was excited by weak 482 nm light, 25 $\mu W \cdot cm^{-2}$, using an interference filter. It was measured at 527 nm. Actinic, incandescent light, 25 $mW \cdot cm^{-2}$, was obtained by the use of a filter cutting off wavelengths smaller than 640 nm. It was checked that this light intensity was not saturating.

3. RESULTS

An example of distinct slow transients occurring upon illumination and darkening of a fresh spinach chloroplast fragment suspension is shown in *fig. 1*. In all, 17, experiments the transient due to an initial excess proton uptake, termed below hyperalcalification, upon illumination was observed, whereas the transient upon darkening indicating an increased proton release, called below hyperacidification, showed up in only 60% of the experiments. The reason why the latter transient did not always show up is not clear. The occurrence of both types of transients was not markedly affected by variations of the mentioned experimental conditions.

As demonstrated in *fig. 2*, ageing of the preparations for 2–3 hrs. at room temperature in the dark annihilates the transients. The pH and ΔpH values, indicated in both figures, were measured with the glass electrode. The hyperalcalification varies in between ΔpH 0.1, as shown in *fig. 1*, to 0.03 in other experiments. The hyperacidification transient was always smaller than the other one. In both figures the ΔpH of the steady state level is about the same. In some experiments it was smaller for the aged preparation than for the fresh

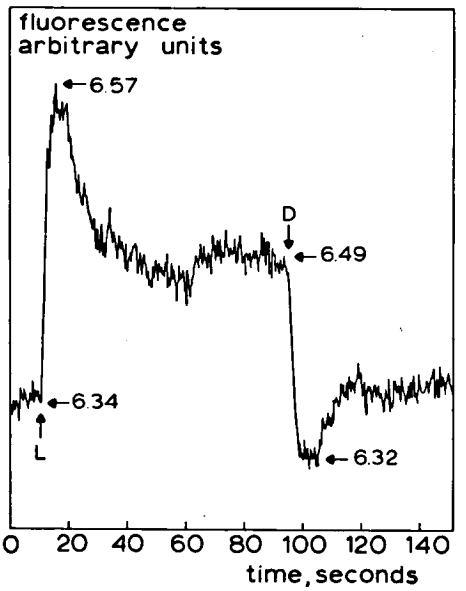

Fig. 1. Slow transients of photoinduced pH changes in a suspension of spinach chloroplast fragments, measured with 25 μM fluorescein. Initial pH 6.3, maximum ΔpH 0.2. No pyocyanine. Room temperature. For details see Materials and Methods. L; light, D; dark. Fresh suspension.

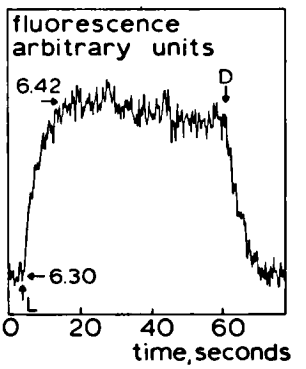

Fig. 2. Absence of transients as shown in *fig. 1* upon ageing of the suspension for 2–3 hrs. at room temperature in the dark. Fluorescein 13 μM. Initial pH 6.3, ΔpH 0.1. For remaining data see legends of *fig. 1*.

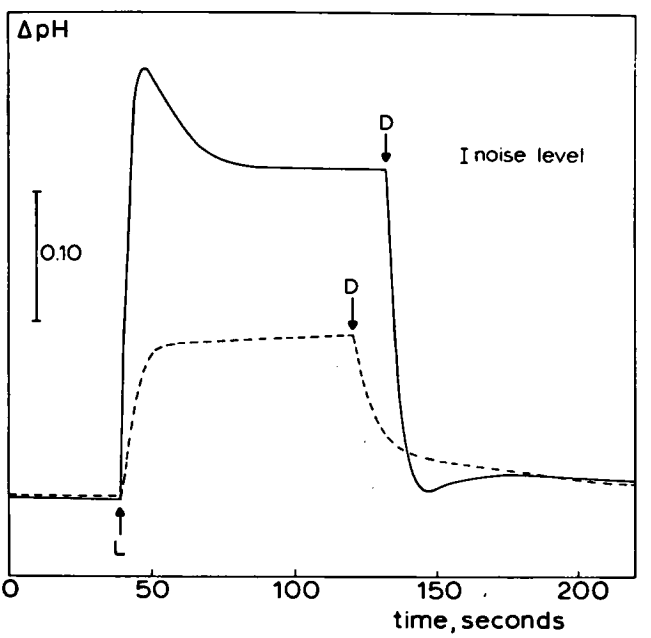

Fig. 3. Photoinduced pH changes in a suspension of spinach chloroplast fragments, measured with the glass electrode. Room temperature. L; light, D; dark. ———: Fresh suspension. Fluorescein 1.7 μM, pyocyanine 17 mM. Initial pH 6.2, maximum ΔpH 0.3. - - - - - -: 2–3 hrs. aged suspension. Fluorescein 18 μM, no pyocyanine. Initial pH 6.4, ΔpH 0.1.

one. An extreme case is given in the next figure.

Fig. 3 shows measurements with the glass electrode method. Also here both transients show up provided the suspensions are fresh. The hyperacidification transient was observed only when it was also found with the fluorescent probe technique.

4. DISCUSSION

In all, 17, experiments a slow transient upon irradiation was observed only with fresh chloroplast fragment preparations. According to ROTTENBERG et al. (1972) the proton uptake depends on the internal buffer capacity of the photosynthetic membrane. It is suggested by DE KOUCHKOVSKY (1974) to be due to the reduction of the plastoquinone pool A by photosystem 2, at least under aerobic conditions and in the absence of pyocyanine, cf. *figs. 1, 2*, and the aged preparation of *fig. 3*. Since it was shown by RUMBERG et al. (1965) that the reaction cycle of photosystem 2 is much more sensitive towards ageing than that of photosystem 1, the transients possibly reflect photosystem 2 activity. However, other possibilities exist as well. For instance, the transients may be due to the presence of a diffusion barrier for protons at the outer surface of the thylakoids, which is sensitive to various treatments, cf. AUSLÄNDER & JUNGE (1974). Ageing may affect this barrier as well.

The absence of the considered transients in aged preparations demonstrates that these effects are real, instead of artificial, phenomena. Future research may provide more insight into the processes involved in the production of these transients.

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