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ON THE EXTENT OF THE ELECTRICAL POTENTIAL ACROSS THE THYLAKOID MEMBRANE INDUCED BY CONTINUOUS LIGHT IN *CHLORELLA* CELLS

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Summary

The extent of the electrical potential $\Delta\varphi_{ss}$ across the thylakoid membrane of *Chlorella* cells was estimated under steady state conditions. This has been achieved by comparing the absorption change which occurs after continuous light is switched off with a calibrated field indicating absorption change induced by flash light. Under saturating light conditions $\Delta\varphi_{ss}$ is in the order of 100 mV.

Electrical potentials $\Delta\varphi$ across the thylakoid membrane can be measured by field indicating absorption changes [1—3]. These optical changes are caused by a shift of the absorption bands of the membrane pigments in the transmembrane field (electrochromism). These changes can most easily be measured at 515 nm when they are induced by flash light. The component which indicates $\Delta\varphi$ rises in < 20 ns and decays in approx. 100 ms. The potential indicating optical change is proportional to $\Delta\varphi$ [4, 5]. The extent of the absorption change at 515 nm in a saturating single turnover flash corresponds to a transmembrane voltage of approx. 50 mV. In saturating long flashes the value is 200 mV [4]. According to earlier results [6] the extent of the change at 515 nm in saturating continuous light corresponds to a steady state voltage of $\Delta\varphi_{ss} \approx 100$ mV. The values are of importance in respect to the mechanism of phosphorylation [7, 8]. In continuous light, slower changes due to other events (light scattering etc.) can, however, interfere with the changes caused by $\Delta\varphi_{ss}$. It has also been discussed that in chloroplasts the steady state

voltage may be small (approx. 10 mV) [9]. Because of these uncertainties about $\Delta\varphi_{ss}$ in steady-state light we extended our measurements.

In isolated chloroplasts the optical changes are disturbed in continuous light by a strong drift of the zero line. This is not the case with whole *Chlorella* cells. Therefore, *Chlorella* cells were used as the subject. Furthermore, in *Chlorella* cells the measurements are based on completely intact thylakoid membranes. We measured the absorption change at 515 nm when the continuous light (2 min) was switched off (see Fig. 1). Whether this change (B in Fig. 1) is due to the field indicating absorption change or not has been analysed as follows. During the permanent light and 4 s after the light, a saturating long flash was fired (A and C in Fig. 1). (The long flashes have been realized by a group of 5 saturating single turn-over flashes with a distance of 2 ms.) Such measurements have been carried out at different intensities of continuous light. Fig. 1 illustrates that the absorption changes A decrease with

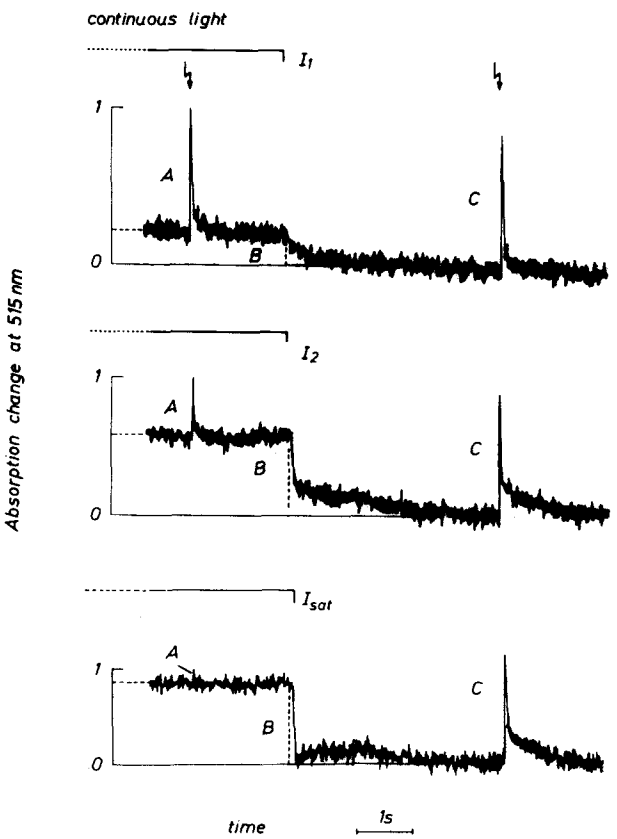

Fig. 1. Relative absorption changes at 515 nm in continuous light plus flash light (A and B) and in flash light 4 s after the continuous light is switched off (C). Subject, *Chlorella* cells in carbonate buffer, pH 9. 20 °C. Intensities $I_1 = 4.5 \cdot 10^2$, $I_2 = 4 \cdot 10^3$, $I_{sat} = 2.3 \cdot 10^4$ $\mu W/cm^2$. I_{sat} is saturating. Details see text.

increasing intensities I and the changes B increase. The change (A) by the flash during illumination is smaller than that (C) after illumination. Probably this is because the electron acceptors on the membrane outside and electron donors on the membrane inside are partly kept reduced and oxidized, respectively, by the continuous light. If we assume that the number of these reduced and oxidized states is proportional to a steady base potential indicated by B — an assumption which is not self-evident — it is expected that the voltages are additive. In other words the maximal voltage which can be set up in continuous light + flash (A + B) is the same as the voltage which can be produced in a saturating long flash alone (C). The results in Fig. 1 and Table I

TABLE I

RELATIVE EXTENT OF THE ABSORPTION CHANGE AT 515 nm IN SATURATING FLASH LIGHT AND CONTINUOUS LIGHT OF DIFFERENT INTENSITIES (I)

Extent of absorption change: A, induced by a flash during continuous light; B, when continuous light is switched off; C, by a flash 4 s after continuous light. Subject, *Chlorella* cells. Details see Fig. 1 and text.

I	$4.5 \cdot 10^2$	$1.5 \cdot 10^3$	$4 \cdot 10^3$	$2.3 \cdot 10^4$ $\mu W/cm^2$
A	5.2	4.0	2.3	0.2
B	1.3	1.5	3.5	3.6
A + B	6.5	5.5	5.8	3.8
C	5.7	5.7	5.0	4.6

illustrate that A + B is within ± 20% in fair agreement with C. From this agreement we assume that the essential part of the change B reflects an electrical potential. This is supported by the following observation. The potential indicating absorption change induced by flash light has an opposite sign at 475 nm (negative absorption change, [2, 3]). In experiments analogous to Fig. 1 but carried out at 475 nm, besides A and C, indeed B also changes the sign. This has already been shown in ref. 6.

When we compare the amplitude of B in saturating continuous light with the amplitude in saturating flash light of C in the dark, which has been calibrated in potential differences [4], it results in a steady state potential of $\Delta\varphi_{ss} \geqslant 100$ mV.

The traces of the absorption changes in Fig. 1 give further information. Obviously no significant diffusion potential (opposite sign) is set up. If this were the case, a temporary undershoot of the traces below the zero line should have been noticed after switching off the light. This is, however, not the case. (Only after short illumination of approx. 10 s duration this can sometimes be observed [6].)

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