ELECTRICAL EVIDENCE FOR THE FIELD INDICATING ABSORPTION CHANGE IN BIOENERGETIC MEMBRANES*

H.T. WITT and A. ZICKLER

Max-Volmer-Institut für Physikalische Chemie und Molekularbiologie, Technische Universität Berlin, 1 Berlin 12, Strasse des 17. Juni 135, GFR

Received 15 August 1973

1. Introduction

The generation of an electrical field across the membrane of bioenergetic systems as in green plants and bacteria has been demonstrated since 1967 by the shift of absorption bands of membrane pigments in the field (electrochromism) [1–8]. Such shifts cause absorption changes of all the bulk pigments incorporated in the membrane (chlorophyll-*a*, -*b* and carotenoids) [4, 7, 8]. The rise of these so-called 'field indicating absorption changes' takes place in < 20 nsec [5]. It is caused by a light-induced transfer of electrons perpendicular to the bioenergetic membrane. The decay in the dark occurs in about 10–100 msec and is due to the breakdown of the field by ion fluxes across the membrane. The extent of the changes indicates a transmembrane voltage of about 100 mV [3].

There are three lines of evidence for the correctness of these interpretations: (a) kinetically, (b) spectroscopically and (c) by salt jump experiments.

(a) The decay of the field indicating absorption changes can be accelerated up 100-fold by increasing the membrane permeability for ions through addition of ionophores [1]. Absorption changes during photosynthesis attributed to other events are, however, not accelerated by ionophores.

(b) The spectra of the field indicating absorption changes of chloroplasts and bacteria correspond to the spectrum of electrochromic changes of multilayers of chlorophyll-*a*, -*b* and carotenoids which have been exposed between two electrodes to field up to 10^6V/cm [7, 8].

(c) The spectrum of the field indicating absorption changes in bacteria is in accordance with that induced with fields generated by salt jumps across the inner membrane [6].

From the relationship between the field indicating absorption changes and ATP formation important conclusions have been made for mechanism of phosphorylation [9–13]. It is therefore of interest to find alternative evidence for the electrochromic nature of these optical changes. So that in this communication the kinetics of the field across the bioenergetic membranes has been measured electrically by electrodes and compared with the kinetics of the field indicating absorption changes.

2. Methods

The scheme of the equipment for the electrical detection of the electrical events in bioenergetic membranes is depicted in fig. 1. In a suspension of spinach chloroplasts two electrodes (e.g. Ag–AgCl) are located in a distance *d*. The electrodes are connected to an oscilloscope via a voltage amplifier. When a non-saturating red light flash ($\lambda > 610$ nm) is fired from one side of the electrodes, e.g. from the top, the pigments in the upper part of each thylakoid membrane absorb more light ($\sim 1\%$ at 670 nm) than those in the lower part. Therefore the upper part is more charged ($\sim 1\%$) than the lower (asymmetrical charging). The difference of the transmembrane voltage $\Delta\varphi$ between the upper and lower part (~ 1 mV) gives rise to a net

* Presented at the International Biochemical Congress in Stockholm, 6 July 1973.

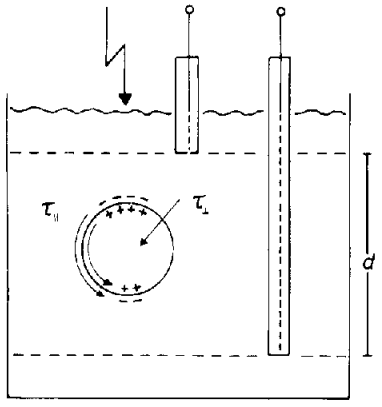

Fig. 1. Scheme for the electrical measurement of potential changes on the thylakoid membrane of chloroplasts. For details see text.

voltage $\Delta\Delta\varphi$ which can be detected by the electrodes. In this way Fowler and Kok [14] have measured electrical signals in chloroplast suspensions. They observed that the extent of these signals is diminished by one half when one of the two light reactions in photosynthesis is chemically blocked. This confirms our results derived from field indicating absorption change measurements that each light reaction contributes one half to the potential generation [3]. However, there exist two considerable discrepancies:
(i) the electrical signal decays in a physiological reaction mixture in about 10 μsec (depending on the ion concentration). The signal measured by the field indicating absorption changes, however, decays in ~100 msec, i.e. 10^4 times slower; (ii) the decay time of the electrical signal is independent of the presence of ionophores, whereas the decay time of the field indicating absorption changes is modified by the addition of ionophores.

3. Results and discussion

The discrepancies outlined above have been explained as follows [15]. The electrically measured change of voltage indicates *not* the breakdown of the transmembrane voltage $\Delta\varphi$ but only the quenching of the net voltage $\Delta\Delta\varphi$ by equilibration of the overshoot charges at the upper part of the membrane with the undershoot at the lower part. This occurs by ion fluxes *parallel* to the plane of the membrane in the water phase in $\tau_{\parallel} \approx 10\,\mu$sec (fig. 1). This ion flux is independent of ionophores. (Ionophores act only in the lipoprotein phase of the membrane). The result is a symmetrical charged membrane. In addition to this event the transmembrane voltage $\Delta\varphi$ decays by ion fluxes perpendicular to the plane of the membrane through the lipoprotein phase. This process is much slower and occurs in $\tau_{\perp} \approx 100$ msec (fig. 1); ionophores accelerate this flux. Because the $\Delta\varphi$ decay occurs under conditions where the membrane has already reached the symmetrical charged state (see above), this decay is not detected by the electrodes.

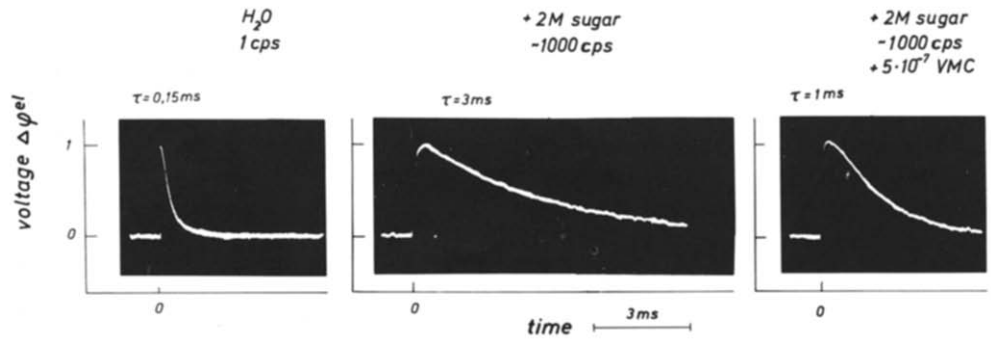

Fig. 2. Electrical measured potential change as a function of time in a chloroplast suspension of spinach: left, in water solution, viscosity ~ 1 cps; center, in 2 M sucrose solution, viscosity ~ 10^3 cps; right, in 2 M sucrose solution with 5×10^{-7} M valinomycin, viscosity ~ 10^3 cps. The reaction mixture contains: 10^{-4} M chlorophyll; 10^{-4} M KCl; 10^{-2} Tris, pH 7; 10^{-4} M benzylviologen as electron acceptor. At $\tau = 0$ a single turnover flash (2.10^{-5} S) is fired.

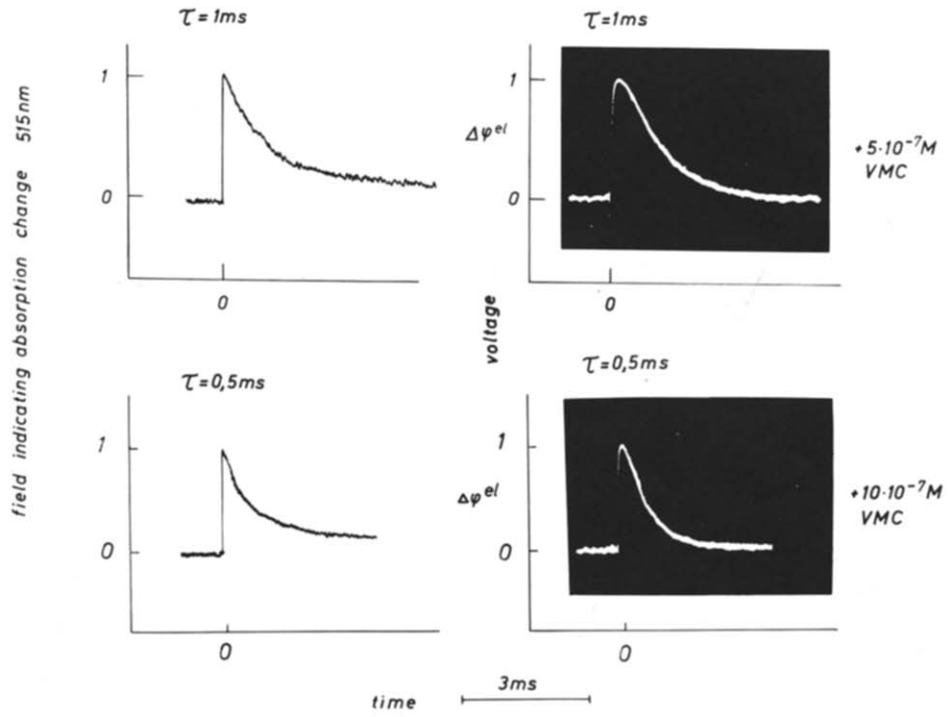

Fig. 3. Right, electrical measured potential change (fig. 1); left, optical measured potential change (by field indicating absorption changes at 515 nm, see text); top, with 5×10^{-7} M valinomycin + 2 M sucrose; bottom, with 10×10^{-7} M valinomycin + 2 M sucrose; reaction mixture, see fig. 2.

Nevertheless, in order to be able to measure the $\Delta\varphi$ change by the electrodes, it is necessary to prevent the flash-induced asymmetrical charging from equilibrating parallel to the surface in $\tau_{\parallel}$. This holds when $\tau_{\parallel} \gg \tau_{\perp}$. In this case the net voltage $\Delta\Delta\varphi$ decays to zero not by equilibration along the surface but only due to the decay of the transmembrane voltage $\Delta\varphi$ via fluxes perpendicular to the membrane, i.e. within $\tau_{\perp}$. Under these conditions $\Delta\Delta\varphi(t)$ is proportional to $\Delta\varphi(t)$. Because the electrodes response to $\Delta\Delta\varphi(t)$, the electrical signal reflects the time course of $\Delta\varphi(t)$. $\tau_{\parallel} \gg \tau_{\perp}$ can be realized as follows. It is necessary on the one hand to increase the viscosity of the water phase and on the other hand to decrease the resistance of the lipoprotein layer, both to such an extent that $\tau_{\parallel} \gg \tau_{\perp}$. A viscosity increase from 1 cps (H_2O) to ~1000 cps by addition of sucrose to give 2 M markedly increase the decay time $\tau_{\parallel}$ of the electrical signal up to 3 msec (fig. 2, left and center). At the same time sucrose decreases the resistance of the lipoprotein layer of the membrane by holes due to osmotic forces. $\tau_{\perp}$ has additionally been decreased by addition of ionophores. If under this condition the decay of the electrical signal is caused by transmembrane ion fluxes, i.e. $\tau_{\parallel} \gg \tau_{\perp}$, the decay time should then depend on the presence of ionophores. Indeed upon addition of 5×10^{-7} M valinomycin the decay is accelerated from 3 msec to 1 msec (fig. 2, right). A consequence of the above interpretations is: (a) that in the presence of 2 M sucrose and 5×10^{-7} M valinomycin the electrically measured time course should be identical with that measured by the field indicating absorption changes and (b) that the decay time of both signals should depend in the same way on the concentration of ionophores. Fig. 3 shows that the time course of both signals are in fair agreement. This is also valid at higher concentrations of valinomycin. The small slow phase of the decay of the optical signal in fig. 3 belongs to other events and is not accelerated by valinomycin. The results can be obtained also by addition of gramicidin D. The results indicate:

(i) the kinetics of the potential changes perpendicular to bioenergetic membranes can be measured under the outlined conditions electrically by electrodes; (ii) the agreement with the kinetics of the 'field indicating absorption changes' provides further support that these changes are indicating electrical potentials; (iii) the proportionality between the electrical signal and the electrochromic changes confirms our earlier conclusion that $\Delta\varphi$ is indicated linearly by the optical changes [7]. (iv) Because the electrodes respond only to potentials perpendicular to the membrane the agreement excludes the possibility that the field indicating absorption changes respond to fields adjusted only in the plane of the membrane.

Acknowledgements

We thank Dr. P. Gräber and Dipl-Chem. S. Saphon for the optical measurements; also Dr. J.B. Jackson and Dr. W. Junge for critizing the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 244.

[2] Witt, H.T. (1967) in: Fast Reactions and Primary Processes in Chemical Kinetics, Nobel Symp. V (Claesson, S., ed.), p. 81, Almqvist & Wiksell, Stockholm; Interscience, New York, London, Sydney.

[3] Schliephake, W., Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23b, 1571.

[4] Emrich, H.M., Junge, W. and Witt, H.T. (1969) Z. Naturforsch. 24b, 1144.

[5] Wolff, Ch., Buchwald, H.-E., Rüppel, H., Witt, K. and Witt, H.T. (1969) Z. Naturforsch. 24b, 1038.

[6] Jackson, J.B. and Crofts, A.R. (1969) FEBS Letters 4, 185.

[7] Schmidt, S., Reich, R. and Witt, H.T. (1971) Naturwiss. 58, 414.

[8] Schmidt, S., Reich, R. and Witt, H.T. (1972) in: Proc. 2nd Internatl. Congr. Photosynthesis Res., Stresa 1971 (Forti, G., Avron, M. and Melandri, A., eds.), p. 1087, Dr. W. Junk, The Hague.

[9] Witt, H.T., Döring, G., Rumberg, B., Schmidt-Mende, P., Siggel, U. and Stiehl, H.H. (1966) in: Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. in Biology, 19, 161.

[10] Rumberg, B. and Siggel, U. (1968) Z. Naturforsch. 23b, 239.

[11] Junge, W., Rumberg, B. and Schröder, H. (1970) Eur. J. Biochem. 14, 575.

[12] Witt, H.T. (1971) Quart. Rev. Biophys. 4, 365.

[13] Boeck, M. and Witt, H.T. (1972) in: Proc. 2nd Internatl. Congr. Photosynthesis Res., Stresa 1971 (Forti, G., Avron, M. and Melandri, A., eds.), p. 903, Dr. W. Junk, The Hague.

[14] Kok, B., in: VI. Internatl. Congr. Photobiol., Bochum (1972) (Schenck, ed.), in preparation.

[15] Witt, H.T. Discussions remark in: VI Internatl. Congr. Photobiol. Bochum 1972 (Schenck, ed.), in preparation.