Further Evidence for an Optical Response of Chloroplast Bulk Pigments to a Light Induced Electrical Field in Photosynthesis

H. M. EMRICH, W. JUNGE, and H. T. WITT

Max-Volmer-Institut, I. Institut für Physikalische Chemie, Technische Universität Berlin

(Z. Naturforschg. **24 b**, 1144—1146 [1969]; eingegangen am 5. April 1969)

1. In the primary processes of photosynthesis a rather strong electric field ($\sim 10^5$ V/cm) is set on across the thylakoid membrane. This field has been detected by absorption changes attributed to chlorophyll-b (1).
2. In this paper it is demonstrated that the optical response to the field is not restricted to chlorophyll-b. Responses of the other bulk pigments which are embedded in the thylakoid membrane, as several types of chlorophyll-a and carotenoids, are detected.

It has been shown that the light induced absorption changes in photosynthesis at 475, 515 and 648 nm which have been tentatively attributed to chlorophyll-b [2] indicate a light induced electric field across the thylakoid membrane [1]. In a short flash of light ($\ll 6 \cdot 10^{-4}$ s) in which one electron is transferred through one electron chain the produced voltage has been estimated to about 50 mV [3]. Since the nonaqueous isolating lipid layer of the thylakoid membrane has a thickness of about 30 Å, the field strength in this area amounts to about several 10^5 V /cm. In dyes which are exposed to an electric field of this magnitude electrochromic effects can be observed (for a summary see l. c. [4]) which induce a shift of absorption bands in the order of 0.1 Å. Since chlorophyll-b is not the only bulk pigment in the thylakoid membrane, field indicating absorption changes have to be expected in the spectral regions of the other bulk pigments, too.

The superposition of the field indicating absorption changes with all the other types of absorption changes which indicate electron transfers etc. requires a special method of separation (see below). With this method the separation of the field indicating absorption changes from the other ones has been performed in the spectral region from 430 to 720 nm. It is shown that besides chlorophyll-b also the other bulk pigments of chloroplasts, as the different types of chlorophyll-a and carotenoids, contribute to this spectrum.

Results

According to l. c. [1] the decay rate of the absorption changes at 478, 515, and 648 nm which have been attributed to chlorophyll-b [2] reflect the discharge of the thylakoid capacity by ion fluxes across the thylakoid membrane. An increase in the membrane's ionic conductivity as indicated by an acceleration of the decay of the three absorption changes has been induced for instance by the antibiotic gramicidin D. It has been tested that with concentrations of gramicidin below 10^{-9} M/l this acceleration does not occur to the well-known absorption changes that indicate the electron transfer [1, 3]. In this way the field indicating absorption changes are kinetically labelled. In order to separate them from the other absorption changes the difference between the absorption changes with and without gramicidin has been taken into account.

In Fig. 1 the dependence of the absorption changes on the concentration of gramicidin D is depicted for two typical wavelengths (515 and 700 nm).

Neglecting the small fast peak in Fig. 1 (top) it can be concluded from the time course that nearly the whole signal at 515 nm can be accelerated by addition of gramicidin D, that means that nearly the whole signal is field indicating.

The fast peak has been discussed in l. c. [1]. It represents those thylakoids in the chloroplasts which are already highly permeable to ions due to damaging during the preparation process.

[1] W. JUNGE and H. T. WITT, Z. Naturforschg. **23 b**, 244 [1968].
[2] B. RUMBERG, Nature [London] **204**, 860 [1964].
[3] W. SCHLIEPHAKE, W. JUNGE, and H. T. WITT, Z. Naturforschg. **23 b**, 1571 [1968].
[4] H. LABHARD, in: Advances in Chem. Phys. **XIII**, 179, Interscience Publishers, London 1967; W. LIPTAY, Angew. Chem. **6**, 195 [1969].

At 700 nm in Fig. 1 (bottom) a much smaller part of the whole signal can be accelerated, i. e. a much smaller part of the signal is field indicating. This smaller part is superimposed on a well-known negative absorption change at 700 nm representing a redox reaction of chlorophyll-a_I.

Components which can be accelerated are observed at several wavelengths in the visible spectral region. These have been evaluated from the difference between signals with and without gramicidin D, for instance in Fig. 1 between signal 1 – 4. The results

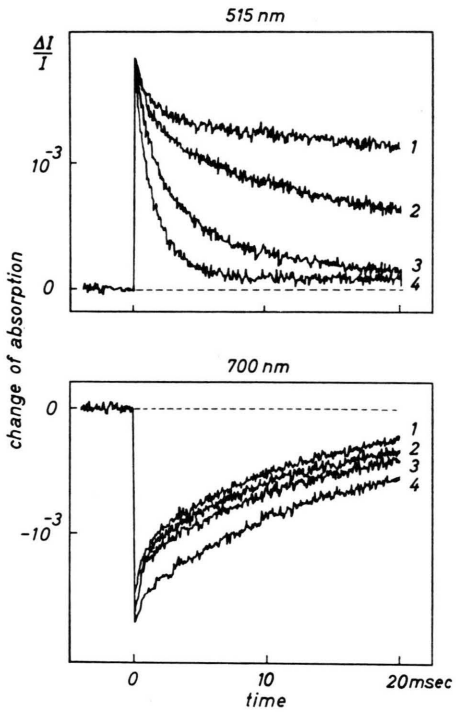

Fig. 1. Time course of absorption changes in chloroplasts of spinach at 515 nm and 700 nm at different concentrations of gramidicin D; 1: 0 M, 2: 10^{-10} M, 3: 5×10^{-10} M, 4: 10^{-9} M. *Chlorophyll content* 10^{-5} M, *buffer:* tricine-NaOH, 10^{-3} M, pH 7,4, *electron acceptor:* benzyl viologen 5×10^{-5} M, *uncoupler:* NH_4Cl 10^{-2} M; *further addition:* sucrose 4×10^{-1} M, KCl 10^{-2} M. *Temperature* 22 °C. Optical path: 1 mm. *Excitation:* 630 – 680 and 380 – 500 nm respectively, duration $1{,}5 \times 10^{-5}$ sec, saturating intensity, *repetitive* pulse technique: 4 cps, 1450 flashes, *measuring light:* grating monochromator, optical bandwidth 5 nm, 500 erg/cm² sec, electric bandwidth 12×10^3 sec^{-1}.

of such an analysis at different wavelengths between 430 – 720 nm is depicted in Fig. 2. This spectrum represents the field indicating absorption changes. Besides the already known field indicating changes at 478, 515 and 648 nm additional changes can be characterized by the wavelengths at 430, 445, 460, 660, 668, 680 and 700 nm. These new field indicating bands have not been observed before since they were masked by the signals of the electron transfers.

In order to test, if the kinetic behaviour of all the absorption changes in Fig. 2 is identical, the half life times of the field indicating components in

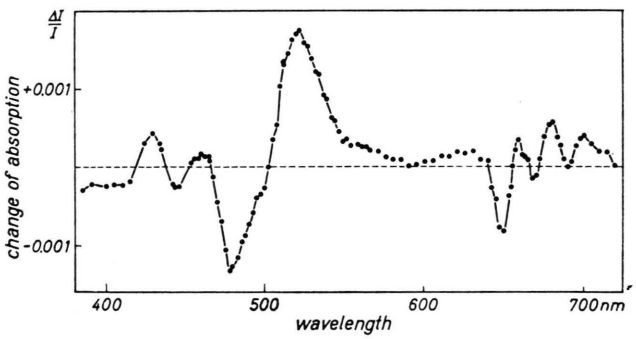

Fig. 2. Spectrum of field indicating absorption changes in chloroplasts of spinach. Details see text.

dependence of the gramicidin concentration have been compared with each other (Table I). The agreement is fairly good in the limits given by the resolution and the reproduciblity, especially of the smaller components. This demonstrates clearly that these absorption changes indicate one and the same physical process.

[nm]	Gramicidin D Concentration 1×10^{-10} M [msec]	2×10^{-10} M [msec]	5×10^{-10} M [msec]	1×10^{-9} M [msec]
385	3.3	1.7	1.0	0.4
440	—	1.6	0.8	0.4
457	5.0	1.7	0.7	0.3
515	5.1	1.5	0.8	0.3
685	3.3	—	0.6	0.3
700	3.2	1.7	0.6	0.2

Table I. Half life times of the field indicating signals at different wavelengths and different concentrations of gramicidin D.

Discussion

Since the electric field has been shown to be a collective property of one whole thylakoid [1] electrochromic effects of all bulk pigments have been expected. Indeed, the spectrum in Fig. 2 reveals maxima in the blue where the carotenoids and chlorophylls a and b absorb in vivo, and in the red where chlorophyll-a and b have further absorption bands (Chl-b (653), Chl-a (673), Chl-a (683), Chl-a (695)).

However, superepositions make it rather difficult to attribute the maxima to the different types of pigments. This is additionally complicated since in vivo the absorption bands of carotenoids and of chlorophylls in the blue are as yet not precisely known in contrast to the red bands of chlorophylls.

1. The two maxima in the green at 478 and 515 nm have been attributed to a change in the *blue* absorption band of *chlorophyll-b* [2]. Probably these changes are not caused by chlorophyll-b exclusively. A contribution of carotenoids has to be considered (see below).

The two maxima in the red at 648 and 660 nm are located antisymmetrically to a center wavelength at 653 nm. This shape is similar to the derivative of the *red chlorophyll-b* band at 653 nm. It corresponds to the expectation for an absorption change due to a band shift to the red. From the assumption of a homogeneous shift of the whole chlorophyll-b band the magnitude of the shift can be calculated approximately using the spectral profile and the extinction coefficient of the in vitro band [5]. The shift amounts to about 0.1 Å. This is, however, the order of magnitude that has been measured for the electrochromic shift of the Soret band of porphyrin in vitro due to an electric field of about 10^6 V/cm [6].

The differences of the magnitude of the optical response in the blue of chlorophyll-b at 478 and 515 nm and in the red region of chloroplyll-b at 648 and 660 nm may be caused by different angles between the transition moments of the blue and red bands with respect to the direction of the electric field.

2. The two maxima at 668 and 680 nm are antisymmetrically to a center wavelength at 673 nm. This shape is similar to the derivative of the *red chlorophyll-a* band at 673 nm. The minor height of the absorption change at 660 nm as compared with the 648 nm change may be due to a superposition with the negative change at 668 nm.

3. The absorption changes at 430, 445 and 460 nm are probably caused by shifts of the *blue* absorption band of *chlorophyll-a* and by the absorption bands of *carotenoids*. (The blue absorption band of chlorophyll-a is around 435 nm. The carotenoids have three absorption bands between 400 – 500 nm.)

It is difficult as yet to know definitely which properties of the molecules are influenced by the electric field. At such high field strength numerous properties of the molecules can be strongly affected. Molecules may respond by a shift of electronic bands (electrochroism) or by a change of chemical equilibrium or by conformational changes. Furthermore, a rigorous interpretation of the response has to account for a possible bias due to local fields as well as for geometric factors (see above).

Despite of this complexity the absorption changes in Fig. 2 can be used as a valuable means to study the biological function of the light induced field in chloroplasts. Since the absorption changes at 475 and 515 nm are induced practically by the electric field only and not by the electron transfers (see above), it is reasonable to use these changes for the analysis of the electric phenomena in photosynthesis. The results obtained by analysis of these changes have been published recently [1, 3, 7].

[5] W. D. Bellamy and M. E. Lynch, G. E. Res. Lab. Rep. 63-RL-3469 G (1963).

[6] M. Malley, G. Feher, and D. Mauzerall, J. molecular Spectroscopy **25**, 544 [1968].

[7] H. T. Witt, B. Rumberg, and W. Junge, in: 19. Mosbach Colloquium p. 262, Springer-Verlag, Berlin-Heidelberg-New York 1968.