Biochimica et Biophysica Acta, 459 (1977) 412–417

BBA 47257

FORMATION OF ELECTRICAL FIELD ACCOMPANYING TEMPERATURE JUMP IN ISOLATED SPINACH CHLOROPLASTS

MICHIKO SHIMIZU and MITSUO NISHIMURA

Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812 (Japan)

(Received August 23rd, 1976)

SUMMARY

Temperature-jump-induced absorbance changes of spinach chloroplasts in the dark were studied. After the temperature rise, a fast absorbance decrease and a succeeding slow absorbance increase were observed at the wavelength of 515 nm. The spectrum of the fast phase had positive maxima (increase in absorbance) at 430, 470 and 673 nm and a negative maxima (decrease in absorbance) at 525 nm. Permeant ions, tetraphenylboron$^-$, tetraphenylarsonium$^+$, and tetraphenylphosphonium$^+$, decreased the extent of the fast absorbance change and increased the rate of slow recovery. Additions of inorganic potassium salts had a similar effect. Valinomycin, added in the presence of potassium ion, also increased the rate of slow recovery. These ions and ionophore had a parallel effect also on the recovery of flash-induced 515-nm absorbance change in chloroplasts. Electroneutral nigericin did not affect the temperature-jump-induced absorbance change. These results suggest the formation of electrical field across the thylakoid membrane in the dark accompanying the temperature rise. A possible involvement of the movement of water molecules (thermo-osmosis) in the observed absorbance changes is also discussed.

INTRODUCTION

Light-induced 515-nm absorbance change is considered to be an indicator of electrical field across thylakoid membrane [1]. There are many data which show relationships between the translocation of ions, the formation and decay of electrical field and the 515-nm absorbance change. Acceleration of the recovery of membrane-potential sensing absorbance changes (the 515-nm absorbance change in chloroplasts and the "red shift" of carotenoid in photosynthetic bacteria) by ionophoretic antibiotics has been observed [1–4]. Effects of various inorganic anions on the light-induced 515-nm absorbance change and proton uptake were explained in terms of the difference in permeability of anions across the thylakoid membrane [5]. Lipophilic permeant ions, tetraphenylboron$^-$, tetraphenylarsonium$^+$ and tetraphenylphosphonium$^+$ were also shown to diminish the light-induced 515-nm absorbance change in chloroplasts [6].

The introduction of diffusion potential across the photosynthetic membrane systems in the dark has been known to induce an absorption band shift of carotenoids (in bacterial chromatophores) and a spectral change near 520 nm (in chloroplasts) [7, 8]. In the present paper, we report temperature-jump induced absorbance changes of chloroplasts. Some evidences that suggest the formation of membrane potential accompanying temperature jump in the dark were obtained.

MATERIALS AND METHODS

Chloroplasts were isolated from spinach leaves obtained from a local market. Depetiolated leaves were homogenized in 5 mM Tris · HCl (pH 7.4) containing 0.4 M sucrose in a Waring blendor at 4 °C. The homogenate was filtered through a layer of cheesecloth. The filtrate was centrifuged at $200 \times g$ for 30 s. The supernatant was filtered through four layers of gauze and centrifuged at $2000 \times g$ for 15 min. The sediment was suspended in the isolation medium and centrifuged again at $2000 \times g$ for 15 min. The sediment was suspended in 10 mM glycylglycine (pH 7.4) and used for experiments. Chlorophyll concentration was determined using absorption coefficients reported by Mackinney [9].

Temperature-jump-induced absorbance changes and flash-induced absorbance changes were measured using a rapid single-beam spectrophotometer as described previously [10]. Temperature rise (3.2 °C) by Joule heating took place in a discharge of electricity stored in a 200-meter coaxial cable (0.02 μF) [11]. The discharge voltage applied for the temperature jump was 20 kV. The 90 % risetime for the data in Figs. 1 to 3 was 49 μs, and that for the data in Fig. 4 was 30 μs. Flash light (7 μs) from a xenon discharge tube, passed through a Toshiba glass filter VR-68 (> 660 nm), was used for pulse excitation of chloroplasts. A blue filter, Corning 9782, was placed in front of the photomultiplier to protect it from the excitation light.

All experiments were carried out at temperature 10–15 °C.

RESULTS AND DISCUSSION

Fig. 1 shows typical traces of temperature-jump-induced absorbance changes at 515 nm on two different time scales. Accompanying the temperature rise, a rapid absorbance decrease followed by a slow absorbance increase was observed. The fast absorbance change consisted of two kinetically distinguishable first-order phases. Actual measurement of temperature-jump-induced absorbance change in a longer time range (> 1 s) was difficult with the instrument used because of the convection of solution after the temperature rise.

It is known that the pH of a buffer solution depends on the temperature. In an experiment to examine the extent of pH change after a temperature jump (3.2 °C), a very small apparent alkalinization (Δ pH = 0.015) was observed in 10 mM glycylglycine (pH 7.4). It is likely that this value is too small to induce a pH-jump-induced absorbance change [7]. The effect of pH of the suspending medium on the temperature-jump-induced absorbance changes in chloroplasts was examined using 10 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 4.3–5.5) and 10 mM glycylglycine (pH 5.5–7.5). The extent of the absorbance change was larger and the rate of the slow absorbance increase was slower at pH 5.0–5.5 than in any other pH range (data not shown).

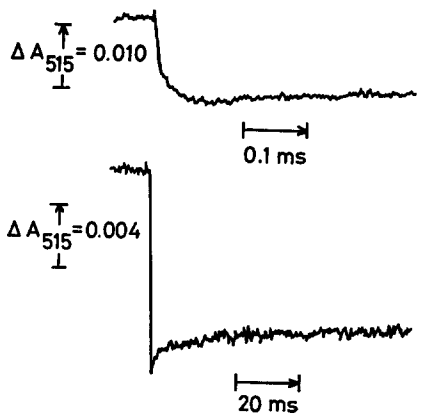

Fig. 1. Typical time courses of temperature-jump-induced absorbance change at 515 nm. The reaction mixture (1.5 ml) contained chloroplasts equivalent to 45 μg of chlorophyll in 10 mM glycylglycine (pH 7.4). Rise in temperature was 3.2 °C.

The spectrum of the fast phase of temperature-jump-induced absorbance change is shown in Fig. 2. Maximal absorbance changes were observed at wavelengths of 430, 470 and 673 nm (positive maxima) and 525 nm (negative maximum). A similar absorption spectrum change, with inverted signs, was obtained in the slow phase in a time range of 50–100 ms after the temperature rise. An addition of KCl, KNO_3 or K_2SO_4 did not affect the shape of the difference spectra in both fast and slow phases.

Permeant lipophilic ions, tetraphenylboron$^-$, tetraphenylarsonium$^+$ and tetraphenylphosphonium$^+$ inhibited the light-induced 515-nm absorbance change in chloroplasts [6]. This effect was interpreted as the result of partial dissipation of electric field formed during illumination. The acceleration of recovery of the flash-induced 515-nm absorbance change by tetraphenylboron$^-$ (0.1–10 μM) was ascertained as shown in Fig. 3A. A similar acceleration by tetraphenylboron$^-$ of the decay of the electric field across the thylakoid membrane has been reported [12]. Tetraphenylarsonium$^+$ and tetraphenylphosphonium$^+$ (0.1–10 μM) also shortened half-recovery time of the flash-induced 515-nm absorbance change (data not shown).

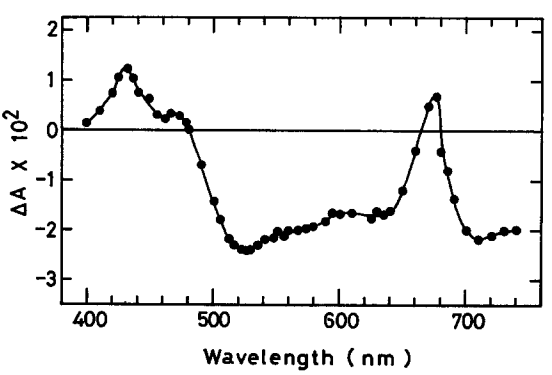

Fig. 2. Spectrum of temperature-jump-induced absorbance change. The basic mixture was the same as in Fig. 1. Extent of the fast phase of absorbance change is plotted.

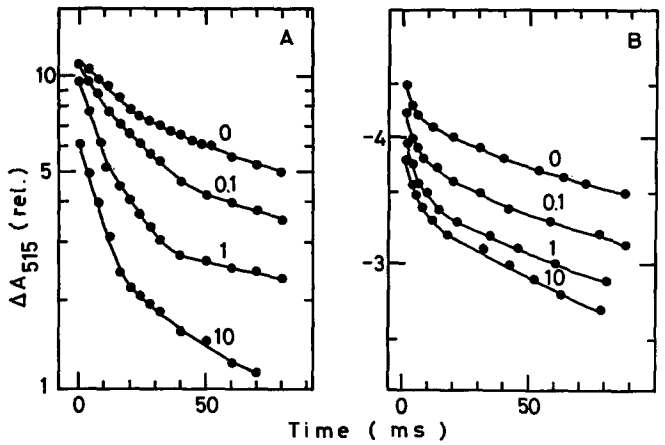

Fig. 3. Effect of tetraphenylboron$^-$ on flash-induced and temperature-jump-induced absorbance changes at 515 nm. The basic reaction mixture was the same as in Fig. 1. A, flash-induced absorbance change; B, temperature-jump-induced absorbance change. The inserted figures indicate concentrations of tetraphenylboron$^-$ in μM.

The extent of the fast absorbance decrease at 515 nm was decreased by the lipophilic ions, tetraphenylboron$^-$, tetraphenylarsonium$^+$ or tetraphenylphosphonium$^+$. The rate of slow absorbance increase after the temperature jump was accelerated by these ions (Fig. 3B). Accompanying a sudden rise in temperature, a new steady state of electron carriers will be established producing a shift of electrical field across the thylakoid membrane [10, 18]. As it is considered that tetraphenylboron$^-$, tetraphenylarsonium$^+$ or tetraphenylphosphonium$^+$ migrates electrophoretically to diminish membrane potential, the slow phase of absorbance change may be reflecting the dissipation of membrane potential.

Various potassium salts (KCl, KNO_3 and K_2SO_4) decreased the temperature-jump-induced fast absorbance decrease and increased the rate of slow absorbance increase. The fast absorbance decrease was reduced to about one-half of the control (glycylglycine only) in the presence of 10 mM KCl, 10 mM KNO_3 or 5 mM K_2SO_4. The order of effectiveness was $SO_4^{2-} > NO_3^- > Cl^-$ on the basis of anionic charges (with SO_4^{2-} at the concentration half of other anions). Schuldiner and Avron [13] showed that the order of permeability of various anions in chloroplasts was $NO_3^- > Cl^- > SO_4^{2-}$. Ikehara and Nishimura [5] reported that light-induced 515-nm absorbance change was diminished by anions in the same order. At present, we cannot explain the effect of SO_4^{2-} on the temperature-jump-induced absorbance change in terms of permeability across the thylakoid membrane. It may be noted that more or less specific effects of SO_4^{2-} on the electron flow and photophosphorylation have been reported [14–17].

Fig. 4 shows the effect of valinomycin on the slow absorbance increase after temperature was raised in the presence of potassium ion. A low concentration of KNO_3 (1 mM) was used here to minimize the effect of salt itself, as an acceleration of the slow phase became noticeable at higher salt concentrations in the absence of valinomycin. Valinomycin, which accelerates decay of the flash-induced 515-nm absorbance change, increased the rate of the slow absorbance increase. When effects of valino-

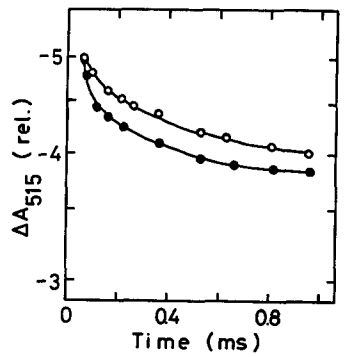

Fig. 4. Effect of valinomycin on temperature-jump-induced absorbance change. The basic reaction mixture (1.5 ml) contained chloroplasts equivalent to 45 μg of chlorophyll, 1 mM KNO_3 and 10 mM glycylglycine (pH 7.4). ○—○, no addition; ●—●, 0.9 μM valinomycin.

mycin on flash-induced and temperature-jump-induced absorbance changes were compared, maximal stimulation of the decay of flash-induced electrical field was observed at valinomycin concentrations of less than 100 nM, while about 1 μM valinomycin was needed to obtain maximal stimulation of temperature-jump-induced electrical field, both at the same KNO_3 concentration (1 mM).

Nigericin (3.4 nM), in the presence of 10 mM KCl, did not show any effect on the temperature-jump-induced absorbance change, indicating that the facilitated electroneutral translocation of H^+ and K^+ did not affect the characteristics of the temperature-jump-induced physical changes of membrane. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea, an inhibitor of electron transfer from the primary electron acceptor of Photosystem II (Q) to plastoquinone, did not show any significant effect at 10 μM. This indicates that the shift of the redox steady state of electron carriers in the vicinity of Photosystem II by the temperature jump is not necessary for the observed absorbance change. However, the shift in redox levels of electron carriers near Photosystem II was found to be linked to the H^+ translocation induced by the temperature jump [10, 18]. From the effect of permeant ions and ionophores to reduce the extent of fast phase and accelerate the slow recovery, it is evident that the membrane potential formation is involved in the observed absorption change, but at the same time it is highly probable that the change also reflects the movement of water molecules across the thylakoid membranes. Movement of water molecules across erythrocyte membranes accompanying the temperature jump by Joule heating has been observed [19, 20]. It is noted that the temperature-jump-induced spectral change of chloroplast suspension differs considerably from the difference spectrum of light-induced electrochromic absorption changes [21]. The movement of water molecules may introduce the rather unspecific light scattering and apparent absorbance change. Some changes in the permeability of membranes have been observed, when chloroplasts were subjected to the temperature jump (unpublished observations). The nature of possible involvement of charged molecular species (permeant ions, ionophore plus K^+, etc.) in the permeability change is under study. The participation of electron transfer (establishment of a new steady-state level of electron carriers after the temperature jump) in these processes is also open to further investigation.

REFERENCES

1 Witt, H. T. (1971) Quart. Rev. Biophys. 4, 356–477
2 Junge, W. and Witt, H. T. (1968) Z. Naturforsch. 23b, 244–254
3 Nishimura, M. (1970) Biochim. Biophys. Acta 197, 69–77
4 Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130
5 Ikehara, N. and Nishimura, M. (1973) Plant Cell Physiol. 14, 77–90
6 Wakamatsu, K. and Nishimura, M. (1974) Plant Cell Physiol. 15, 587–600
7 Jackson, J. B. and Crofts, A. R. (1969) FEBS Lett. 4, 185–189
8 Strichartz, G. R. and Chance, B. (1972) Biochim. Biophys. Acta 256, 71–84
9 Mackinney, G. (1941) J. Biol. Chem. 140, 315–322
10 Takahama, U., Shimizu, M. and Nishimura, M. (1976) Biochim. Biophys. Acta 440, 261–265
11 Eigen, M. and DeMaeyer, L. (1963) in Technique of Organic Chemistry (Friess, S. L., Lewis, E. S. and Weissberger, A., eds.), Vol. VIII, Part II, pp. 895–1054, Wiley (Interscience), New York
12 Erixon, K. and Renger, G. (1974) Biochim. Biophys. Acta 333, 95–106
13 Schuldiner, S. and Avron, M. (1971) Eur. J. Biochem. 19, 227–231
14 Asada, K., Deura, R. and Kasai, Z. (1968) Plant Cell Physiol. 9, 143–149
15 Ikehara, N. and Nishimura, M. (1973) Plant Cell Physiol. 14, 61–76
16 Sakurai, H. (1972) Plant Cell Physiol. 13, 857–866
17 Ryrie, I. J. and Jangendorf, A. T. (1971) J. Biol. Chem. 246, 582–588
18 Takahama, U., Shimizu, M. and Nishimura, M. (1977) Plant Cell Physiol., Takamiya Memorial Issue, in the press
19 Owen, J. D., Bennion, B. C., Holmes, L. P., Erying, E. M., Berg, M. W. and Lords, J. L. (1970) Biochim. Biophys. Acta 203, 77–82
20 Tsong, T. Y. and Kingsley, E. (1975) J. Biol. Chem. 250, 786–789
21 Emrich, H. M., Junge, W. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1144–1146