Biochimica et Biophysica Acta, 440 (1976) 261—265

BBA Report

BBA 41282

TEMPERATURE-JUMP-INDUCED RELEASE OF HYDROGEN IONS FROM CHLOROPLASTS AND ITS RELAXATION CHARACTERISTICS IN THE PRESENCE OF IONOPHORES

UMEO TAKAHAMA, MICHIKO SHIMIZU and MITSUO NISHIMURA

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

(Received April 5th, 1976)

Summary

Temperature-jump-induced absorption changes of bromocresol purple in chloroplast suspensions in the dark were studied. After a rapid rise in temperature (<10 μs), a slow absorbance decrease of bromocresol purple ($t_{1/2} \approx 0.2$ s) following a fast absorbance decrease of chloroplasts and bromocresol purple ($t_{1/2} < 1$ ms) was observed. The slow absorbance decrease corresponds to acidification of the suspending medium, indicating H^+ efflux from chloroplasts after the temperature jump. Nigericin and gramicidin D suppressed the slow absorbance change completely in the presence of 10 mM KCl, while valinomycin did not affect it. The fast absorbance change was not affected by the above ionophores. 3-(3,4-dichlorophenyl)-1,1-dimethylurea also diminished the slow absorbance change.

Since Neumann and Jagendorf [1] reported light-induced H^+ uptake by chloroplasts, many investigators have observed light-induced H^+ uptake or release in various types of photosynthetic membranes [2—6]. The pH gradient and membrane potential formed can be regarded as being the driving forces of ATP synthesis [7]. In chloroplasts, a H^+ gradient across the thylakoid membrane may be formed by electron transfer involving plastoquinone and by water oxidation by Photosystem II [8]. Recently, a hypothesis was proposed on the mechanism of proton translocation across mitochondria or thylakoid membrane [9]. We report here a temperature-jump-induced pH decrease in chloroplast suspension using bromocresol purple, which is known to respond to external pH changes [10,11].

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 500 × *g* for 1 min. The supernatant was centrifuged at 2000 × *g* for 10 min. The sediment was suspended in the isolation medium and centrifuged again at 2000 × *g*

for 10 min. The sediment obtained was suspended in 50 mM NaCl at a concentration of 1—2 mg of chlorophyll/ml. The chloroplast suspension was kept at 0°C under weak illumination (room light) until use. Chloroplasts which had been kept in total darkness had low activity of the temperature-jump-induced H^+ efflux.

Temperature-jump-induced absorption change of bromocresol purple was measured at the wavelength of 580 nm at 12—15°C in the dark using a rapid single-beam spectrophotometer combined with temperature-jump and pulse-flash-excitation devices (Union Giken RA-1201). Signals from the photomultiplier were processed by a high-speed A-D converter and stored in a digital memory (Union Giken RA-108T). The output from the digital memory was displayed on an X-Y oscilloscope or X-Y recorder (Riken Denshi F-42C). A temperature jump (3.2°C) was achieved within 10 μs (90% risetime in 10 mM KCl) by Joule heating accompanying the discharge of electricity stored in a 200-meter coaxial cable (0.02 μF) [12,13].

The basic reaction mixture contained chloroplasts equivalent to 37.5 μg of chlorophyll, 10 mM KCl and 16 μM bromocresol purple in 1.5 ml. The final pH of the reaction mixture was about 6. The pH was adjusted by adding small amounts of 0.01 M NaOH.

Figs. 1 and 2 show typical traces of temperature-jump-induced absorbance changes of bromocresol purple at 580 nm in two different time ranges. Accompanying the temperature jump, a fast absorbance decrease was observed. The fast decrease was also observed in chloroplast suspensions without bromocresol purple or in the presence of bromocresol purple only (Fig. 1, traces E and F). The temperature-jump-induced absorption spectrum changes of chloroplast suspensions will be the subject of a subsequent paper (Shimizu and Nishimura, in preparation). The very rapid (<20 μs) absorption decrease of bromocresol purple in the absence of chloroplasts is explained by a change in pK of bromocresol purple accompanying the temperature rise (apparent lowering of pH). When chloroplasts were subjected to a temperature jump in the presence of bromocresol purple, a slight absorbance increase (half-risetime several ms) and a much larger slow absorbance decrease (half-risetime 200 ms) were observed.

Fig. 3 shows spectra of the temperature-jump-induced absorption change. In the wavelength range examined (550—620 nm), no distinct spectral feature was observed in the temperature-jump-induced absorption change of chloroplasts in the absence of bromocresol purple. When chloroplasts were subjected to the temperature rise in the presence of bromocresol purple, a decrease in absorption with a maximum at 585—590 nm was observed, which corresponds to the formation of the protonated form of bromocresol purple. The same spectral shape was observed both in the very rapid (<1 ms) and slow (half-risetime $\approx$ 200 ms) phases. This indicates that H^+ is released from chloroplasts accompanying a rapid temperature rise in the time range of the slow phase.

Effects of different ionophores on the temperature-jump-induced absorption change were examined in the presence of 10 mM KCl (Figs. 1 and 2). Valinomycin, which increases K^+ permeability and decreases membrane potential, did not affect the temperature-jump-induced absorbance change.

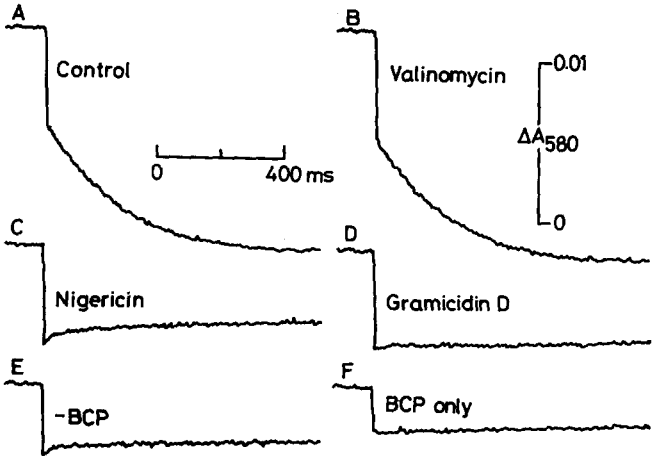

Fig. 1. Effects of ionophores on temperature-jump-induced absorbance change of bromocresol purple (BCP) in chloroplast suspension. A, control; B, 0.45 μM valinomycin; C, 34 nM nigericin; D, 1 μM gramicidin D; E, in the absence of bromocresol purple (chloroplasts only); F, in the absence of chloroplasts (bromocresol purple only). ΔA = 0.01 corresponds to 14 nmol of OH^-.

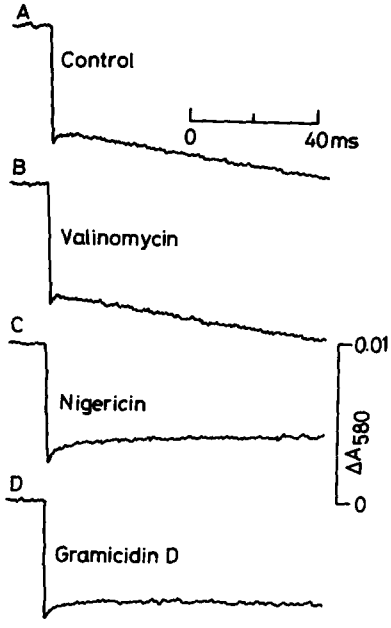

Fig. 2. Effects of ionophores on temperature-jump-induced absorbance change in a shorter time range. A, control; B, 0.45 μM valinomycin; C, 34 nM nigericin; D, 1 μM gramicidin D. ΔA = 0.01 corresponds to 14 nmol of OH^-.

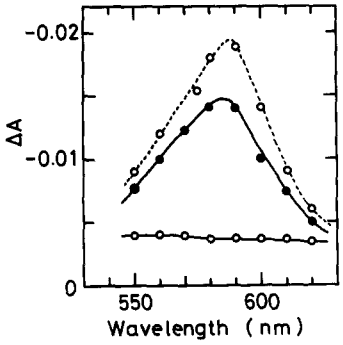

Fig. 3. Spectra of temperature-jump-induced absorption changes. Extent of absorbance change at 800 ms after temperature jump was plotted. ○——○, absorbance change in the absence of bromocresol purple; ●——●, in the presence of 16 μM bromocresol purple; ○- - - -○, in the presence of 32 μM bromocresol purple.

Nigericin and gramicidin D, which accelerate decay of ΔpH across the membrane, decreased the temperature-jump-induced slow absorbance change without affecting the rapid absorbance change. Gramicidin D also decreased the slow absorbance change in the presence of 50 mM NaCl. Concentration dependence of the effects of nigericin and gramicidin D on the slow absorbance decrease are shown in Fig. 4. As the concentration of these ionophores was increased, the slow absorbance decrease was gradually diminished. When the concentrations of nigericin and gramicidin D were higher than 6.8 nM and 1 μM, respectively, the temperature-jump-induced slow absorbance change was completely abolished.

3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of electron transfer from the primary electron acceptor of Photosystem II (Q) to plastoquinone, also inhibited the temperature-jump-induced slow H^+ efflux (data not shown). The effects of ionophores and 3-(3,4-dichlorophenyl)-1,1-dimethylurea sug-

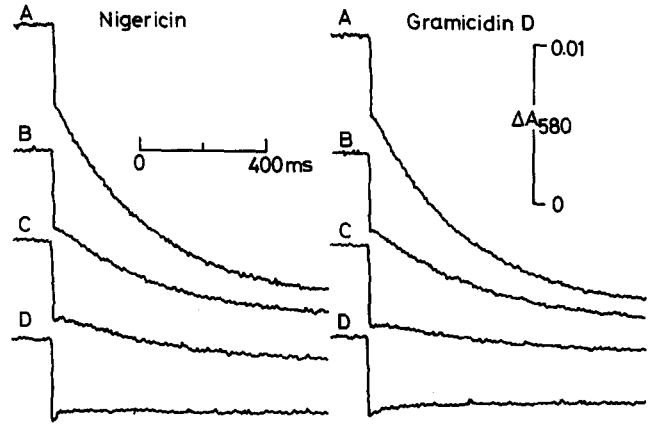

Fig. 4. Concentration dependences of effects of nigericin and gramicidin D on temperature-jump-induced absorbance change. Nigericin: A, none; B, 0.9 nM; C, 2.25 nM; D, 6.8 nM. Gramicidin D: A, none; B, 0.13 μM; C, 0.67 μM; D, 2 μM. ΔA = 0.01 corresponds to 14 nmol of OH^-.

gest that electron transfer, especially between Photosystems I and II, is necessary to establish a new level of ΔpH across thylakoid membrane accompanying the temperature jump. Actually, a lowering of the level of the light-induced ΔpH by the temperature jump was shown by illumination/temperature-jump combination experiments (data not shown).

The results reported here suggest that the temperature jump causes the establishment of a new dark steady state of electron carriers producing a shift in the distribution of H^+ across the membrane. ATP- or ΔpH-driven reverse electron flow between Photosystems I and II (reduction of Q and oxidation of cytochrome *f*) may be regarded as a somewhat analogous phenomenon [14,15]. Dark relaxation of the H^+ gradient seems to be controlled by ΔpH but much less by membrane potential. Thus, the temperature-jump method may provide us with useful information concerning the mechanism of the coupling of electron transfer to proton translocation in chloroplasts, as well as the relaxation characteristics of the physical and chemical processes accompanying the temperature jump. Further experiments are now in progress.

References

1 Neumann, J. and Jagendorf, A.T. (1964) Arch. Biochem. Biophys. 107, 109—119.
2 von Stedingk, L.-V. and Baltschefsky, H. (1966) Arch. Biochem. Biophys. 117, 400—404
3 Edwards, G.E. and Bovell, C.R. (1966) Biochim. Biophys. Acta 172, 126—133.
4 Chance, B., Crofts, A.R., Nishimura, M. and Price, A. (1970) Eur. J. Biochem. 13, 364—374.
5 Kobayashi, Y. and Nishimura, M. (1973) J. Biochem. Tokyo 74, 1217—1226.
6 Oesterhelt, D. and Stoeckenius, W. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2853—2857.
7 Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation, Glynn Research Ltd., Bodmin, Cornwall.
8 Witt, H.T. (1971) Q. Rev. Biophys. 4, 356—477.
9 Robertson, N.R. and Boardmann, N.K. (1975) FEBS Lett. 60, 1—6.
10 Jackson, J.B. and Crofts, A.R. (1969) Eur. J. Biochem. 10, 226—237.
11 Heath, R.H. and Hind, G. (1972) J. Biol. Chem. 247, 2917—2925.
12 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.
13 French, T.C. and Hammes, G.G. (1969) in Methods in Enzymology (Kustin, K., ed.), Vol. XVI, pp. 3—30, Academic Press, New York.
14 Rienits, K.G., Hardt, H. and Avron, M. (1974) Eur. J. Biochem. 43, 291—298.
15 Shahak, Y., Hardt, H. and Avron, M. (1975) FEBS Lett. 54, 151—154.