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TEMPERATURE DEPENDENCE OF THE LIGHT-INDUCED SPECTRAL SHIFT OF CAROTENOIDS IN *CYANIDIUM CALDARIUM* AND HIGHER PLANT LEAVES

EVIDENCE FOR AN EFFECT OF THE PHYSICAL PHASE OF CHLOROPLAST MEMBRANE LIPIDS ON THE PERMEABILITY OF THE MEMBRANE TO IONS*

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SUMMARY

1. Kinetics of the light-induced spectral shift of carotenoids were studied by changing temperature of cells of the thermophilic alga *Cyanidium caldarium*. Below the temperature of phase transition of thylakoid membrane lipids at 9 °C, the dark recovery of the carotenoid shift after several seconds of illumination was composed of a fast and a slow decay component. Above the phase transition temperature only the slow decay component was seen.

2. In chloroplasts of a desert plant, *Tidestromia oblongifolia*, the fluorescence versus temperature curve indicated that the phase transition of the thylakoid membrane lipids occurred at about 5 °C. Below the phase transition temperature the kinetics of dark decay of the spectral shift of carotenoids at 520 nm in leaves was composed of a fast and a slow component. Above the phase transition temperature only the slow decay component was present.

3. The light-induced spectral shift of carotenoids was measured at 520 nm in leaves of the chilling-sensitive plants, tomato and bean. The Arrhenius plots of dark recovery of the light-induced changes showed characteristic breaks at 10 and 12 °C in tomato leaves grown at 15 and 25 °C, respectively, and at 15 °C in a bean leaf. In leaves of the chilling-resistant plants lettuce and spinach, on the other hand, the dark recovery of the carotenoid shift showed no breaks in the Arrhenius plots.

4. If the light-induced spectral shift of carotenoids in the photosynthetic membrane is caused by a production of a transmembrane electric field, then the dark recovery of this shift is an indicator of the disappearance of this accumulated electric charge. It is concluded that the diffusion of ions through thylakoid membranes is

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greatly affected by the physical phase of membrane lipids and that the membrane becomes leaky to the ions when the lipids are in the phase separation state.

INTRODUCTION

Major components of thylakoid membranes are lipids and proteins. Physiological activities of thylakoid membranes are thus significantly affected by the fluidity and the physical state of membrane lipids. At the temperature of transition of the physical phase of the membrane lipids from the liquid crystalline to the phase separation states [1], there appear characteristic changes in the rates of photosynthetic electron transport [2–4], the State 1-State 2 shift [3], in the intensity of delayed fluorescence [5], and in membrane permeability to non-electrolytes [6]. In the present study, we investigated the temperature dependence of the light-induced spectral shift of carotenoids in order to elucidate the effect of the physical phase of membrane lipids on the production of a membrane potential and on the permeability of the membrane to ions.

Absorbance changes are produced at 520 nm (positive) and at 475 nm (negative) upon illumination of intact cells of green algae [7–9] and leaves of higher plants [10]. The changes appear to be produced by a spectral shift of photosynthetic pigments (mainly β-carotene) in the thylakoid membrane [11–13]. Light-induced absorbance changes with three maxima and minima due principally to a spectral shift of carotenoids have been observed in a number of different classes of algae such as the red [14], brown [14, 15], yellow-green algae, as well as the Eustigmatophyceae, a new class of algae having no accessory chlorophyll [15, 16]. These changes have been observed in the photosynthetic purple bacteria [17–19] but not in the blue-green algae [13]. Similar shapes of absorbance changes are observed also in isolated higher plant chloroplasts [20, 21] and in chromatophores of photosynthetic bacteria [22–24].

The light-induced spectral shift of carotenoids (and other pigments) has been explained as being due to electrochromism [11, 25], that occurs when an electric field (or membrane potential) is produced across the thylakoid membrane. This electric field is generated upon absorption of light by the photosynthetic reaction center that produces positive and negative charges at the inside and the outside of thylakoid membranes, respectively [25–27]. This has been demonstrated in experiments using short flashes as actinic illumination.

The carotenoid shift is also produced by artificially inducing an ion gradient across the thylakoid membrane. Addition of salts to bacterial chromatophores or to chloroplasts isolated from higher plants produces a spectral shift of carotenoids [28–30]. This has been interpreted as being caused by the formation of a transient diffusion potential across the membrane due to different membrane mobilities of certain cations and anions [28]. On illumination with continuous light, protons are incorporated into the inner space of the thylakoid [31–33]. In isolated chloroplasts without the addition of phosphorylating and uncoupling reagents, the carotenoid shift induced by prolonged illumination has a decay time of seconds [34], which is close to the half time of spontaneous H^+ release from the thylakoid to the outer medium [31–33], while the flash-induced carotenoid shift had a decay time of about 0.1 s [26]. These findings suggest that the carotenoid shift appearing during continuous illumination results from the production of an ion gradient in the light.

The unicellular thermophilic alga, *Cyanidium caldarium*, possesses a chloroplast membrane structure like that of a red alga [35]. This alga shows characteristic light-induced absorption changes produced by carotenoids (personal communication of B. Diner) with three positive and three negative peaks appearing at the same wavelength region as in other red algae [4, 14]. In leaves of higher plants, the light-induced absorbance increase at 520 nm can easily be measured, although the associated absorbance decrease at 475 nm is somewhat distorted [10].

Measurements of fluorescence versus temperature curves in our previous study [4] showed that the phase transition of membrane lipids occurred at 8–9 °C in *C. caldarium* grown at 38 °C. The decay kinetics of the light-induced carotenoid shift and the dark reduction of cytochrome *f* showed breaks in their Arrhenius plots at this temperature [4]. Raison [36] studied the transition of the physical phase of membrane lipids of chloroplasts of chilling-sensitive and chilling-resistant plants by means of a spin-labeling technique, and found that the phase transition occurred around 10 °C in the chilling-sensitive plants, but not at room temperature in chilling-resistant plants.

METHODS

C. caldarium, obtained from Carolina Biological Supply Co., Burlington, N. C., was grown at 38 °C in Allen's medium [37] bubbled with air enriched with 3 % CO_2. Cells in the logarithmic phase were diluted with the same culture medium to give a chlorophyll concentration of approx. 5 μg/ml.

Romaine lettuce (*Lactuca* sp. var. Romaine, purchased at Germain's Seed Co., Los Angeles) and tomato (hybrid tomato N-65, University of Hawaii) were cultivated in a growth cabinet with a supply of nutrient solution. The temperature in the cabinet was controlled to give the same temperature day and night. Light and dark periods were 14 and 10 h, respectively. The leaves were harvested in the light period. Bean was grown in the field of the Carnegie Institution at Stanford, California. *Tidestromia oblongifolia* was collected in Death Valley, Calif., in the morning and used for the measurements in the afternoon. Spinach leaves were purchased at a local market.

To prepare chloroplasts of *T. oblongifolia*, the leaves were ruptured with a Waring Blender in a medium containing 0.4 M sucrose, 0.01 M NaCl and 0.05 M phosphate buffer, pH 7.4. After centrifugation at $200 \times g$ for 1 min to remove cell walls, nuclei and unbroken leaf pieces, the chloroplasts were collected by centrifugation at $1000 \times g$ for 5 min. The precipitate was suspended in the same medium and the suspension was again centrifuged at $1000 \times g$ for 5 min. The precipitated chloroplasts were resuspended in the same medium and used for fluorescence measurements after centrifugation at $200 \times g$ for 1 min. The concentration of chloroplasts was adjusted by diluting with the same medium to give a chlorophyll concentration of approx. 5 μg/ml.

For measurements of absorbance changes, the algal cell suspension was placed in a lucite cuvette of 1 mm optical path length. This cuvette was held in a horizontal position and submerged in 8 ml of water contained in a vessel that was jacked with a stainless steel coil through which coolant fluid was circulated from a temperature-controlled bath. Leaves were cut to an appropriate size and submerged in the same vessel and held in a horizontal position. Uniform temperature on both sides of the leaf or the cuvette was achieved by stirring the water in the vessel with a small propeller. Temperature was measured by inserting a small copper-constantan thermocouple into

the cuvette or by attaching the thermocouple to the leaf. The measuring and the actinic beams came from above the vessel and the photomultiplier was put underneath the vessel. During the measurement of the temperature dependence of light-induced absorbance changes, the temperature was first decreased from about the growth temperature to about the freezing temperature of water and then increased. The rate of temperature change was about 1 °C/min. A mechanical shutter controlled by an automatic timer gave repeated cycles of light and dark of 4–5 s and 4–8 s, respectively. Absorbance changes were measured at appropriate intervals as the temperature changed.

The temperature dependence of chlorophyll *a* fluorescence in chloroplasts of *T. oblongifolia*, was measured as described previously [38].

RESULTS

Cyanidium caldarium

The previous study on chlorophyll *a* fluorescence and the light-induced absorbance changes in *C. caldarium* [4] has indicated that the transition of the lipid phase of the thylakoid membrane occurs at 8–9 °C when this alga is grown at 38 °C. It has also suggested that the spectral shift of carotenoids is most clearly observed by the absorbance change at 483 nm.

Fig. 1 shows time courses of the light-induced absorbance change due to the carotenoid shift measured at 483 nm. At 33 °C, above the phase transition, a small spike appeared at the onset of actinic illumination and was followed by a second gradual increase in the light to a higher level. At 4 °C, below the phase transition, the spike was not clear because of the fast second increase. During the illumination period at 4 °C the absorbance change declined to a lower level, while it stayed at almost the same level at 33 °C. After turning off the actinic illumination a clear difference between the decay rates was noted at these two temperatures.

Fig. 2 shows the decay kinetics in the dark after 4.0 s of illumination. At 10, 14 and 20 °C, the decay seemed to be composed of a single component. Over this temperature range the higher the temperature, the faster the decay rate. At 4 °C a fast

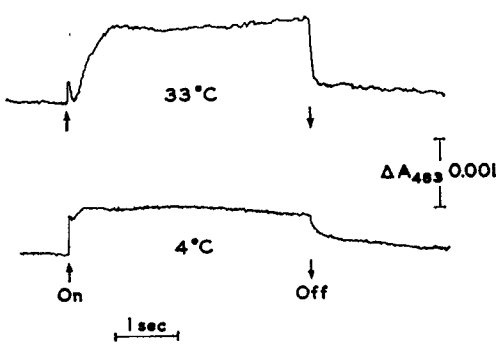

Fig. 1. Time courses of the light-induced absorbance changes at 483 nm due to the carotenoid shift at 33 and 4 °C in *C. caldarium*. Actinic light, 630–750 nm, $1.8 \cdot 10^5$ ergs/cm^2 per s. Repeated light and dark periods were 4.0 and 4.2 s, respectively.

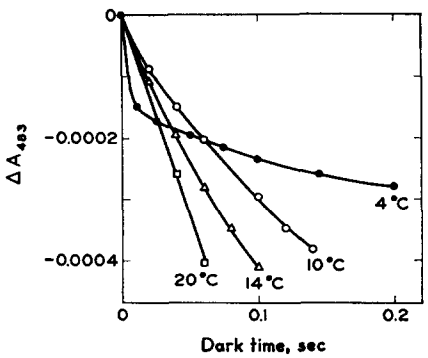

Fig. 2. Dark decay kinetics of the light-induced absorbance change at 483 nm at different temperatures in *C. caldarium*. Experimental conditions were the same as in Fig. 1.

decay component appeared in addition, although the decay rate of the slow component was lower than those of the higher temperatures. The fast decay component began to appear when the temperature was decreased below 8 °C. Fig. 3 shows the temperature dependence of the amounts of the fast and slow decay components after 4 s of actinic illumination. The fast decay component existed only below 8 °C. Since the phase transition of thylakoid membrane lipids occurs at 8–9 °C in these algal cells [4], it can be inferred that the fast decay component appears below the phase transition temperature, in other words, when the membrane lipids are in the phase-separation state [1].

Fig. 4 shows the Arrhenius plot of the slow phase of the dark decay of the absorbance change produced by the carotenoid shift. At lower temperatures where the two decay components existed, the decay rates of the slow component were taken. In this experiment reciprocals of the half decay time were taken as representing the decay rates, even though the dark decay did not follow perfect first-order kinetics. It can be

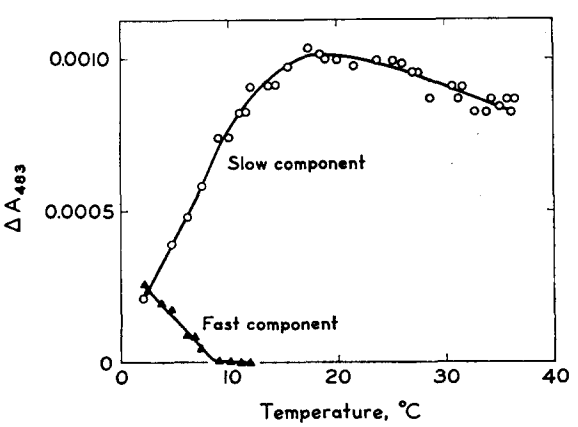

Fig. 3. Temperature dependence of the extents of the fast decay and the slow decay components of the absorbance change at 483 nm after illumination of *C. caldarium* for 4.0 s. Experimental conditions were the same as in Fig. 1. The temperature was changed from low to high.

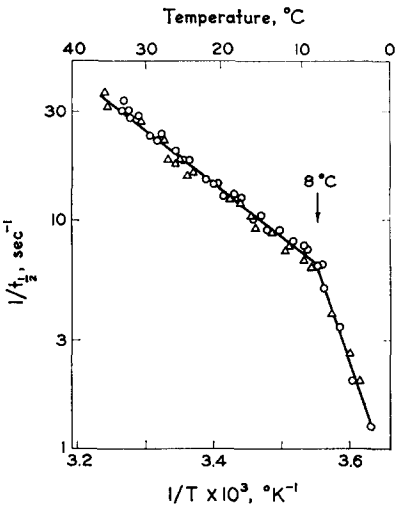

Fig. 4. Arrhenius plot of the decay kinetics of the slow component of the light-induced absorbance change at 483 nm in *C. caldarium*. Reciprocals of half decay time were taken as representing decay rates. Open circles, decreasing temperature. Open triangles, increasing temperature. Experimental conditions were the same as in Fig. 1.

noted that the Arrhenius plots followed essentially the same line on decreasing and increasing the temperature. A break in the line appeared at 8 °C, the phase transition temperature. The apparent activation energy was 11 and 40 kcal/mol above and below 8 °C, respectively.

Tidestromia oblongifolia

Since *T. oblongifolia* grows at very high temperatures ($\geqq$ 50 °C) in Death Valley, California [39], the phase transition of membrane lipids in the chloroplasts would be expected to occur at a much higher temperature than in the chloroplasts of mesophilic plants.

It was suggested in our previous study [38] that the yield of chlorophyll *a* fluorescence is a useful measure of the occurrence of a phase transition. In the present study measurements of fluorescence versus temperature produced curves for chloroplasts of *T. oblongifolia* that had a shoulder or in some cases a maximum around 5 °C (not shown in the figures). This can be taken as evidence for the occurrence of a phase transition of membrane lipids at this temperature. In our previous study [38], however, we failed to find such an indication of a phase transition in the fluorescence versus temperature curve in the chloroplasts of this plant. In that case the plant was grown in a growth cabinet at much lower temperatures than those occurring during the growth season in Death Valley. The plant used in the present study was harvested in the summer season in Death Valley in the morning and used in the afternoon. The difference between these two fluorescence versus temperature curves appears to be due to this difference of growth temperature.

In the chloroplasts of higher plants the light-induced absorbance increase at 520 nm is caused by a spectral shift mainly of carotenoid and also of chlorophyll [11, 25]. In a study to relate the transition of the physical phase of membrane lipids in

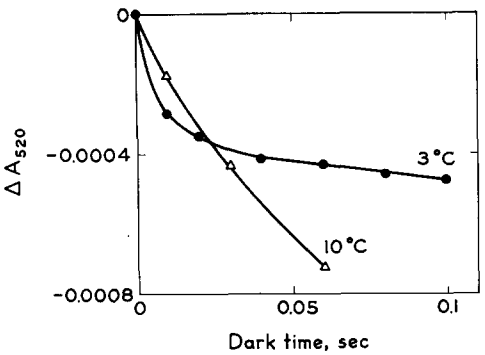

Fig. 5. Dark decay kinetics at 3 and 10 °C of the light-induced absorbance change at 520 nm in a leaf of *T. oblongifolia*. Repeated light and dark periods were 5.1 and 7.4 s, respectively. Actinic light, 630–750 nm, $1.8 \cdot 10^5$ ergs/cm^2 per s.

chloroplasts to the temperature dependence of the 520 nm change, there is a great advantage in using leaves of higher plants because the absorbance change can be measured easily in leaves and thus damage produced during isolation of chloroplasts can be avoided.

Fig. 5 shows decay kinetics of the 520 nm absorbance change after a light exposure of 5.1 s in a leaf of *T. oblongifolia*. The decay was monophasic at 10 °C, but a fast decay component appeared at 3 °C in addition. Fig. 6 shows the temperature dependence of the extents of the fast decay and the slow decay components of the 520 nm change after 5.1 s of actinic illumination. The fast decay component disappeared above 8 °C. Since the fluorescence versus temperature curve indicated that the phase transition of membrane lipids occurred around 5 °C, the experimental results suggest that the fast decay component of the 520 nm change only appears when the membrane lipids are in the phase separation state.

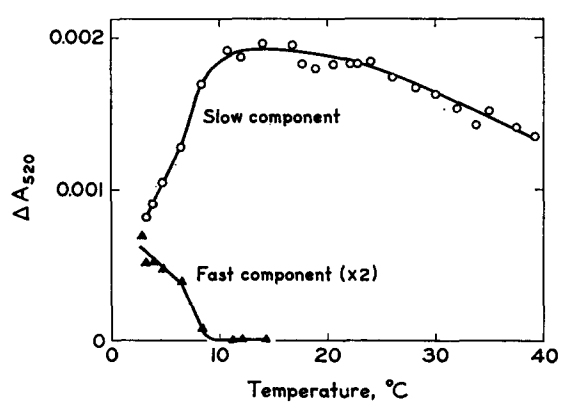

Fig. 6. Temperature dependence of the extents of the fast decay and the slow decay components of the absorbance change at 520 nm in *T. oblongifolia*. Experimental conditions were the same as in Fig. 5.

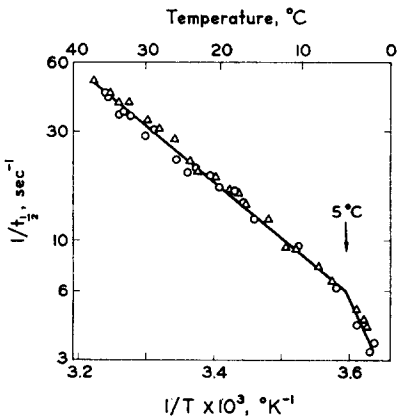

Fig. 7. Arrhenius plot of the decay kinetics of the slow component of the absorbance change at 520 nm in *T. oblongifolia*. Reciprocals of the half decay time were taken as representing decay rates. Open circles, decreasing temperature. Open triangles, increasing temperature. Experimental conditions were the same as in Fig. 5.

Fig. 7 shows the Arrhenius plot of the dark decay of the slow component of the 520 nm change in a leaf of *T. oblongifolia*. It can be noted that the plots on decreasing and increasing the temperature followed the same line, and that a break in the straight lines appeared at 5 °C, the phase transition temperature. The apparent activation energy was 11 and 30 kcal/mol above and below 5 °C, respectively.

Tomato and bean: chilling-sensitive plants

Raison [36], in his study using spin labels with isolated chloroplasts, suggested that the phase transition of membrane lipids occurs around 10 °C in the chloroplasts of chilling-sensitive plants such as tomato and bean, but not in those of chilling-resistant plants. Therefore it was of interest to study the temperature dependence of the kinetics of the 520 nm change in these plants.

Light-induced difference spectra measured at 24 and 5 °C in a tomato leaf showed both a positive peak at 520 nm and a negative peak at 475 nm typical of that produced by the electrochromic shift of photosynthetic pigments in higher plant chloroplasts [7]. The negative peak at 475 nm was somewhat distorted, probably because of an artifact caused by strong light absorption in this wavelength region [16]. A negative peak at 555 nm was also seen in these difference spectra and is due to a light-induced oxidation of cytochrome *f*. Light-induced difference spectra were also measured in leaves of other plants used in the present study. These spectra were essentially similar to those of tomato and had a large positive peak at 520 nm and a relatively small negative peak at 475 nm.

Fig. 8 (upper part) shows time courses of light-induced absorbance changes at 520 nm in a tomato leaf grown at 25 °C. The extent of the absorbance change during the light exposure varied remarkably depending upon the temperature of measurement. By contrast, the height of the initial rise produced upon onset of actinic illumination was rather unaffected by a change of temperature. At 2 °C, and also at 4 °C, the increase of the second slow phase of the 520 nm change disappeared, resulting in a

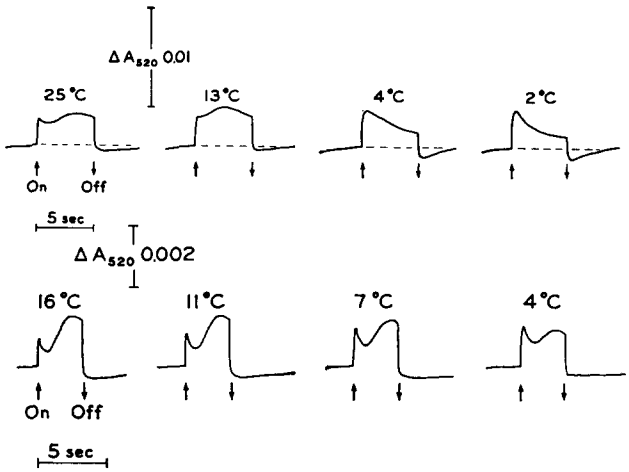

Fig. 8. Time courses of light-induced absorbance changes at 520 nm at different temperatures in a tomato leaf grown at 25 °C (upper part), and in a lettuce leaf grown at 15 °C (lower part). Repeated light and dark periods were 5.0 and 7.5 s, respectively, for tomato and 3.5 and 9.0 s, respectively, for lettuce. Actinic light, 630–675 nm, $1.8 \cdot 10^5$ ergs/cm^2 per s.

steady-state level after 5 s of actinic illumination that was very close to the dark steady-state level. After turning off the actinic illumination, the absorption change became transiently negative and dipped below the dark steady-state level. The difference spectra for the initial rise, the decline during the light period and the decay after turning off the actinic light were all the same. This suggests that such characteristic changes in the time courses are all due to a variation of the 520 nm change with temperature. A similar temperature dependence of the time courses of the 520 nm change were also observed in a bean leaf.

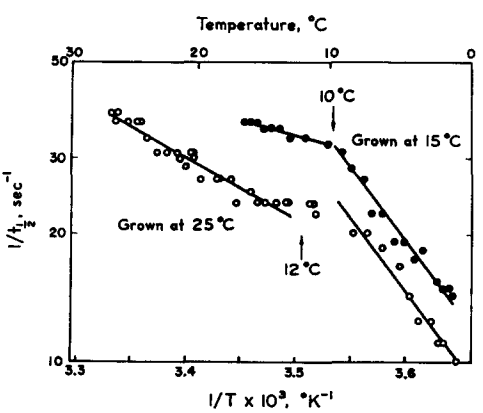

Fig. 9. Arrhenius plots of the dark decay of the absorbance change at 520 nm in leaves of tomato grown at 15 and 25 °C. Reciprocals of the half decay time were taken as representing decay rates. Measurement was done by decreasing the temperature. Experimental conditions were the same as in Fig. 8.

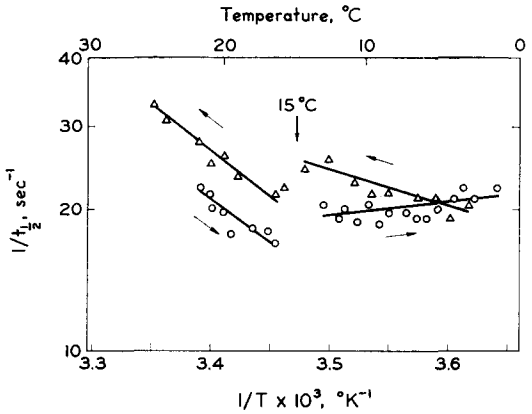

Fig. 10. Arrhenius plots of the dark decay of the absorbance change at 520 nm in a bean leaf. Reciprocals of the half decay time were taken as representing decay rates. Arrows indicate the direction of temperature change. Experimental conditions were the same as in Fig. 8. Light and dark cycles of 5.0 and 7.5 s were used, respectively.

The dark decay kinetics were studied at different temperatures in leaves of tomato and bean. Even on decreasing the temperature close to 0 °C, the fast decay component such as seen in *C. caldarium* and *T. oblongifolia* was not observed.

Fig. 9 shows Arrhenius plots of the dark decay of the 520 nm change in tomato leaves grown at 15 and 25 °C. A discontinuity point was found at 10 °C in the leaf grown at 15 °C and at 12 °C in a leaf grown at 25 °C. It appeared at 13 °C in a leaf grown at 35 °C (not shown in the figure). Fig. 10 shows Arrhenius plots of the dark decay of the 520 nm change in a bean leaf. Although the line on decreasing and increasing the temperature did not completely follow each other, the discontinuity point appeared at the same temperature, 15 °C for both directions of temperature change.

Lettuce and spinach: chilling-resistant plants

Fig. 8 (lower part) shows time courses of light-induced absorbance changes at 520 nm in a leaf of lettuce grown at 15 °C. In contrast to the tomato leaf, the pattern of time courses was not so markedly altered by changing the temperature of measurement; and the second increase during the light exposure still remained at 4 °C. In a lettuce leaf grown at 25 °C and a spinach leaf purchased in a local market, the same tendency was found in the temperature dependence of time courses of the 520 nm change.

Fig. 11 shows Arrhenius plots of the dark decay of the 520 nm change in leaves of lettuce grown at 15 and 25 °C and in a spinach leaf. There were no break points in these lines. The lack of breaks in the Arrhenius plots of data obtained from lettuce and spinach leaves is consistent with the finding that no phase transition of membrane lipids occurs over this temperature range in chloroplasts of these chilling-resistant plants [36].

Fig. 12 shows the temperature dependence of the extent of the light-induced

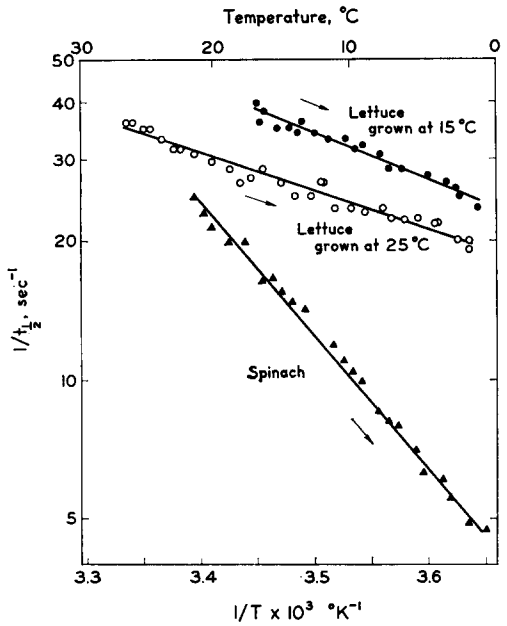

Fig. 11. Arrhenius plots of the dark decay of the absorbance change at 520 nm in leaves of lettuce grown at 15 and 25 °C and in a spinach leaf. Reciprocals of the half decay time were taken as representing the decay rates. Arrows indicate the direction of temperature change. Experimental conditions were the same as in Fig. 8.

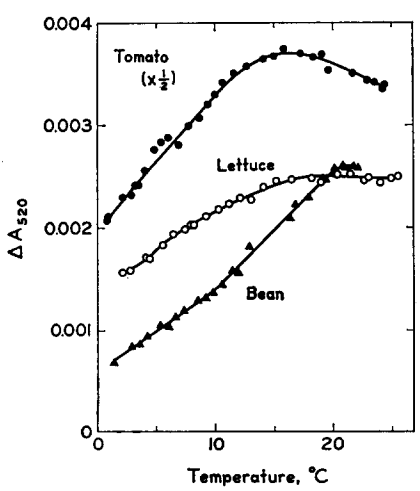

Fig. 12. Temperature dependence of the extents of the light-induced absorbance change at 520 nm after a light exposure of 5.1 s in leaves of tomato, lettuce both grown at 25 °C and in a bean leaf. Components which decayed within 1 s after the light exposure were taken. Measurements were performed by decreasing the temperature.

absorbance change measured at 520 nm in leaves of tomato, bean and lettuce. No great difference was found in these curves between the chilling-sensitive and chilling-resistant plants. This suggests that the extent of the light-induced change at 520 nm cannot be used as a measure of the phase transition of thylakoid membrane lipids.

DISCUSSION

The chlorophyll *a* fluorescence versus temperature curve revealed that the transition of the physical phase of thylakoid membrane lipids occurs at 8–9 °C in *C. caldarium* grown at 38 °C [4] and at 5 °C in *T. oblongifolia* that was growing in Death Valley, California. Based on studies with electron paramagnetic resonance signals from spin probes, Raison [36] has suggested that the phase transition occurs around 10 °C in the chilling-sensitive plants, tomato and bean; while it does not occur above 0 °C in the chilling-resistant plants such as lettuce and spinach. These phase transitions of the thylakoid membrane lipids are supposed to be between the liquid crystalline and the phase-separation states [3, 38].

In *C. caldarium* and *T. oblongifolia*, the dark decay of the light-induced absorbance change of carotenoids was monophasic when the membrane lipids were in the liquid-crystalline state, while a fast decay component appeared in addition when they were in the phase separation state. In tomato and bean, on the other hand, the fast decay component did not appear. The decay rate of the slow component showed breaks at the phase transition temperatures in *C. caldarium*, *T. oblongifolia* and tomato, the apparent activation energy being higher below than above the phase transition temperature. By contrast, in spinach and lettuce neither the fast component nor the break in the Arrhenius plot occurred in the temperature region between 0 and 25 °C.

The light-induced absorbance change at 483 nm in *C. caldarium* is due to an electrochromic shift of the absorption spectrum of carotenoids just as in the chromatophores of photosynthetic purple bacteria [27, 28]. The light-induced absorbance change at 520 nm in leaves of higher plants is also produced by an electrochromic shift (mainly of carotenoids) just as in isolated chloroplasts [11, 25, 26]. As described in the Introduction, the spectral shift of carotenoid absorption after a prolonged illumination is formed mainly by the ion gradients produced initially by the H^+ influx from the outer medium to the inner space of the thylakoid [28, 30–33]. The dark decay of the absorbance change seems to provide an indication of the dissipation of the ion gradient established across the thylakoid membrane in the light. It should be noted here that in intact cells or leaves, the relaxation of this ion gradient is probably coupled to phosphorylation. This would explain the rather fast decay rate of approx. 100 ms after continuous illumination (Figs. 4, 7, 9, 10, and 11) compared to the value of approx. 10 s as seen in isolated chloroplasts [34].

Based on studies made with model membranes, it has been suggested that the rate of self-diffusion of small molecules and ions across the membranes is maximum when the lipids composing the membranes are in the phase separation state [40–44]. This observation can be used to explain the experimental results obtained in the present study with *C. caldarium* and *T. oblongifolia*, in that the fast component of the dark decay appeared only when the lipids of the thylakoid membrane were in the phase separation state. Since the electrochromic shift of carotenoid absorption after a long period of actinic illumination can be considered to be due mainly to the produc-

tion of an ion gradient, the fast decay of the absorbance change is a reflection of the rapid degradation of this ion gradient. This suggests that the thylakoid membrane becomes leaky to the ions, when the membrane is in the phase separation state.

In leaves of tomato and bean, on the other hand, the fast decay component did not appear. One possible explanation for the lack of the fast decay component below the phase transition point is that ion diffusion due to leakage in the phase separation state is so fast that it competes with the formation of an ion gradient.

Breaks in the Arrhenius plots or changes in the apparent activation energies of the slow decay component appeared at the temperature of phase transition in *C. caldarium*, *T. oblongifolia*, and tomato. Since the slow component of dark decay of the carotenoid change is supposed to result from a coupling to phosphorylation in photosynthesizing intact chloroplasts, these results suggest that the coupling process itself is also affected by the occurrence of a phase transition in the membrane lipids. This is supported by a finding in a recent study by Ono and Murata (unpublished) that a clear break appears at the phase transition temperature in the Arrhenius plot of photophosphorylation in thylakoid membrane preparations of *Anacystis nidulans*.

Nobel [6] studied the temperature dependence of membrane permeability of chloroplasts to glycerol and erythritol and found discontinuity points at about 10 °C when chloroplasts from the chilling-sensitive plants tomato and bean were used, but no breaks when chilling-resistant plants were used. He assumed that this phenomenon was related to the phase transition of membrane lipids. These findings suggest that membrane permeability to non-electrolytes is also affected by the physical phase of lipids in the chloroplasts.

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