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Light- and temperature-induced changes in the distribution of excitation energy between Photosystem I and Photosystem II in spinach leaves

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The influence of light quality and temperature on the distribution of the absorbed quanta between Photosystem I (PS I) and Photosystem II (PS II) in spinach leaves has been studied from the characteristics of chlorophyll fluorescence at 77 K. Leaves were preilluminated at different temperatures with either PS I light (to establish State 1) or with PS II light (to establish State 2), then cooled to 77 K and measured for fluorescence. In State 1, energy distribution appeared to be unaffected by temperature. A transition to State 2 resulted in an increase in PS I fluorescence and a decrease in the PS II fluorescence, indicating that a larger fraction of energy becomes redistributed to PS I. However, the extent of this redistribution varied: it was only small at 5°C to 20°C, but it largely increased at temperatures exceeding 20°C. This variation in the extent was related to a change in the mechanism of the state transition: at 15°C only the 'initial' distribution of energy was affected, while at 35°C an additional increase in the spill-over constant, $k_{T\,(II \rightarrow I)}$, was included. It is assumed that under physiological conditions $k_{T\,(II \rightarrow I)}$ is under the control of temperature rather than of light quality, whereby in leaves adapted to high physiological temperatures, the probability of energy spill-over from closed PS II centres to PS I is enhanced. In darkened leaves, the spill-over constant has been manipulated by preincubation at different temperatures. Then, the light-induced 'energization' of thylakoid membranes has been tested by measuring the light-induced electrochromic absorbance change at 515 nm (and light-induced light-scattering changes) in these leaves. The flash-induced 515 nm signal as well as the initial peak during a 1 s illumination were not affected by energy distribution. However, the amplitude of the pseudo-steady-state signal (as established during 1 s illumination) was considerably enhanced in leaves in which a larger fraction of the absorbed energy is distributed to PS I at the expense of PS II excitation. The results have been interpreted in such a way that an increase in energy spill-over from PS II to PS I favours a cyclic electron transport around PS I. It is discussed that changes in energy distribution (via spill-over) may serve to maintain a suitable balance between non-cyclic and cyclic electron transport in vivo.

Introduction

Rapidly responding changes in the distribution of the adsorbed-light energy between PS I and PS II upon variation in the light quality were first observed in green and blue-green algae (e.g., Refs. 1–3), but the same mechanism, called State 1–State 2 transition, seems to occur in higher plants as well [4,5]: upon illumination with light I (preferentially absorbed by PS I) state I is established, where the energy absorbed by LHC and PS II is mainly channeled to PS II centers, while illumination with light II (absorbed by LHC and PS II) induces a

Abbreviations: PS I (II), Photosystem I (II), LHC, chlorophyll *a/b* light-harvesting complex; F_I (F_{II}), fluorescence emitted at 735 (695) nm; F_0, fluorescence with open Photosystem II centers; F_m, fluorescence with closed Photosystem II centres; Q, first stable electron acceptor of PS II, PQ, plastoquinone

State 2, where a larger share of the absorbed energy is delivered to PS I. In a recent paper [6] it has been shown that in spinach leaves variation also in temperature can induce changes in energy distribution.

It has often been discussed that the serial connection of the two photosystems requires a regulation of energy distribution in leaves to maintain a balance in the rate of excitation of PS I and PS II and also to maintain an optimal linear electron transport between these photosystems during variations in the spectral quality of the absorbed light (e.g., Ref. 4). Allen and Bennett [7,8] introduced a further aspect as they discussed state changes to be involved in the regulation of the coupling between the photosynthetic electron transport and ATP synthesis. They assumed that state changes can produce a specific imbalance in the rate of excitation of the photosystems, such as to change the ratio between non-cyclic and cyclic electron transport: to promote a cyclic transport at the expense of a noncyclic pathway it may be nessesary to overexcite PS I. The ATP synthesis coupled to the cyclic transport would serve to maintain an appropriate ATP/NADP ratio in the stroma. However, this concept is based on theoretical assumptions rather than on experimental evidence.

Following the tripartite model [9], there are two ways of energy transfer from LHC · PS II to PS I. (1) Energy absorbed by LHC can be initially delivered to PS I before reaching PS II. The fraction of energy initially distributed to PS I (by direct absorption as well as by initial transfer of energy from LHC to PS I) is given by the distribution coefficient α. (2) Energy can be transferred from closed PS II centers to PS I ('spill-over'). The probability of spill-over is expressed by the transfer constant $k_{T\,(II \rightarrow I)}$.

The lateral displacement concept [10,11] is based on the assumption that most of LHC-PS II complexes are concentrated in the appressed regions of thylakoid membranes, whereby the close connectivity between these structures allows energy transfer between PS II units while the transfer from PS II to PS I (localized in the non-appressed regions) is less efficient. A gradual loss of stacking would result in a more intimate mixing of LHC · PS II and PS I, thus allowing energy transfer to PS I. In isolated membranes, an increase in energy transfer to PS I can be experimentally induced either by suspending the membranes in a medium having a low concentration of cations (see Ref. 12) or by activating the enzymatic phosphorylation of the LHC protein [13]. In vitro, LHC phosphorylation may be operative depending on the background level of charge-screening cations: at high cation level, when the membranes are tightly stacked, LHC phosphorylation solely affects the initial distribution of energy from the LHC to the two photosystems, while at a lower cation level it probably increases the spill-over of energy from PS II to PS I as well [14,15,31].

In the intact system, State 1–State 2 transitions are assumed to be linked to the protein-phosphorylation system [30] which requires ATP and might be controlled by the stromal-reducing potential [8]. However, little is known about the characteristics of the in vivo state changes. While in intact leaves evidence for the exsistence of spill-over was provided by measuring photooxidation of P-700 [16], it is not clear at present whether changes in spill-over are involved in the state regulation in leaves.

In the present study, attention is focussed on two aspects of the regulation of energy distribution in intact spinach leaves. (1) The relative contribution of changes in α and in $k_{T\,(II \rightarrow I)}$ on light- and temperature-induced state-changes has been evaluated by measuring the characteristics of the 77 K fluorescence. A special technique has been used to minimize artifacts arising from self-absorption properties of the leaves (see Ref. 17). (2) The influence of changes in energy distribution on the light-induced 'energization' of thylakoids as mediated by cyclic or non-cyclic electron transport has been studied by measuring the field-indicating electrochromic absorbance change at 515 nm in leaves that are in different states of energy distribution. The relation between spill-over changes and the in vivo regulation of the cyclic electron transport has been discussed.

Materials and Methods

Plant material

Plants of *Spinacia oleracea* L. were grown in a glasshouse (October–April) equipped with supplemental light (10 h photoperiod) at a temperature adjusted to 17–20°C (day) and 14–17°C (night).

Pretreatments of leaves

Freshly cut leaves were placed at room temperature into vapour-saturated chambers which then were slowly (about 1°C per min) adjusted to the indicated temperatures. To establish 'State 1' or 'State 2' the transparent temperated chambers were illuminated with far-red (Schott RG 715 filter; 4 $W \cdot m^{-2}$ and blue-green (Corning 4-96; 2.5 $W \cdot m^{-2}$) light, respectively. Far-red light (absorbed by PS I pigments) was called 'light I', blue-green light (preferentially absorbed by LHC/PS II) was called 'light II'. After the indicated time, a leaf was removed from the chamber and stored for 1.5 min at room temperature in the dark before cooling in liquid N_2 or measuring for absorbance changes.

77 K fluorescence

To avoid spectral distortion connected to self-absorption properties of the leaf tissue (see Ref. 17), fluorescence emission spectra at 77 K were obtained from samples of 'diluted leaf particles' (diluted leaf particle samples) which were prepared from the frozen leaf tissue as follows. Small discs of pretreated leaves were rapidly frozen in liquid N_2. Then the frozen tissue was ground to small subcellular particles which were – without rethawing – mixed with ice and quartz particles ('diluted'), as described elsewhere (Ref. 18; see also Ref. 6). The resulting diluted leaf particle samples contained no more than about 5 μg chlorophyll per cm^3. A small aliquot of a diluted leaf particle sample was put into a cylindric Dewar cuvette filled with liquid N_2. The emission spectra were measured with a spectrofluorimeter (Farrand Mark 1) which was corrected for fluorimeter sensitivity. The fluorescence was sensitized at 480 nm (5 nm bandwidth) and measured in the F_m state between 650 and 800 nm (1nm bandwidth).

For measuring fluorescence induction at 695 and 735 nm, leaf discs were put in the dark on the transparent bottom of a Dewar vessel which was filled with liquid N_2. Fluorescence was measured in a front-to-front arrangement trough a triple-arm fiber-optic arrangement, as previously described [6]. As demonstrated previously [18], the F_m/F_0 ratio obtained from such induction curves was not affected from self-absorption properties of the frozen leaf tissue.

Absorbance changes at 515 nm

Discs (2 cm in diameter) from pretreated leaves were put in the dark into a water-filled temperated cuvette. The temperature was adjusted at 0–2°C. The absorbance changes were measured in a vertical optical arrangement. The distance between the lower surface of the leaf and the front of the photomultiplier (EMI 8995 B, equipped with Cs 4-96 and BG 18 filters) was small so that most of the measuring light passing through the leaf was collected from the photomultiplier, including scattered light. The weak measuring light (515 nm) was provided by a monochromator (Bausch and Lomb). The excitation light was filtered through heat-absorbing filters and either Schott RG 630, Balzers K65, Calflex c (red light, 630–680 nm) or Schott RG 9 (far-red light, less than 715 nm).

Slow fluorescence and light-scattering changes

Leaves were put into a cuvette gassed with CO_2-free air (20 l/h). Light-induced light-scattering changes were measured in this cuvette at 20°–22°C as apparent absorbance changes at 535 nm in an arrangement similar to that described above, except that the distance between the lower surface of the leaf and the front of the photomultiplier was increased. Simultaneously, light emission was collected with a light pipe from the upper surface of the leaf, and measured at 740 nm (RG 9 and Balzers B-20, 740 nm filter). Red light (RG 630 plus K65) was used for excitation. At room temperature the emission at 740 nm largely consists of PS II fluorescence (see Ref. 17).

Results

Fluorescence at 77 K

Changes in the overall distribution of excitation energy between the photosystems can be demonstrated by fluorescence emission spectra at 77 K, as the relative exciton density within LHC, PS II antennae and PS I antennae is indicated by the amplitude of the related emission bands at 685 nm (LHC), 695 nm (PS II) and 735 nm (PS I); for a review, see Ref. 17. Since relative changes in the self-absorption properties of an optically dense leaf-tissue can simulate alterations in the F_I/F_{II}-ratio, a technique has been introduced [6] and characterized in detail [18] to obtain emission

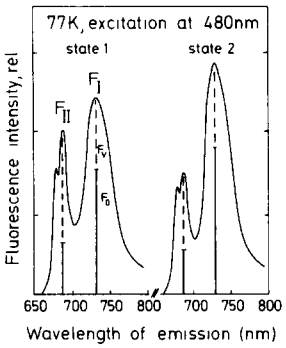
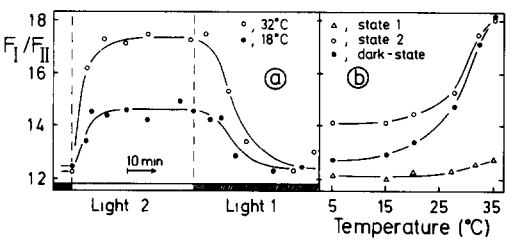


Fig 1. Fluorescence emission spectra at 77 K of diluted leaf particle samples, prepared from rapidly frozen spinach leaves. The leaves were preilluminated at 32°C for 30 min with light I (State 1) or with light II (State 2), then 1.5 min darkened (to allow relaxation of high-energy state that quenches fluorescence) and rapidly frozen liquid N_2. The fluorescence was excited at 480 nm. The emission spectra were normalized setting the 'pure' PS I fluorescence (sensitized in each individual sample at 700 nm and measured at 735 nm) as 1. In parallel measurements F_m/F_0 ratios were determined from fluorescence induction curves measured directly from frozen leaf discs at 695 and 735 nm. These ratios were used to indicate the different level of fluorescence in the respective emission bands of spectra obtained from diluted leaf particle samples. $F_v = F_m - F_0$.

spectra at 77 K from leaf material, free from self-absorption artifacts (diluted leaf particle samples). The emission spectra (sensitized with 480 nm light, preferentially absorbed by LHC) of such diluted leaf particle samples were normalized on a 'pure' PS I emission, as described elsewhere [18].

Fig. 1 shows normalized emission spectra (recorded in the F_m-state) of two diluted leaf particle samples prepared from leaves that are either in State 1 or in State 2. The State 1–State 2 transition was reflected in an increase in F_I and a concomitant decrease in F_{II}, thus showing the typical pattern of redistribution of absorbed energy in favour of PS I, at the expense of PS II. Using the F_I/F_{II}-ratio as a relative measure for the overall distribution of energy between PS I and PS II, Fig. 2b shows the kinetics of a State 1–State 2–State 1 transition at 15°C and at 32°C. At both temperatures, the steady state was reached within 5–10 min upon illumination with light II; however, the extent of this change was increased at 32°C relative to that at 15°C. Fig. 2b shows a complete temperature profile of the steady-state ratios, including the ratios established in the dark ('dark state'). While no change or only a small one in the State 1 ratio (1.2 to 1.4) was observed over the temperature range studied (5°C–35°C), the State 2 ratio increased from about 1.4 at 5°C to 2.0 at 35°C, the increase being most pronounced at temperatures exceeding 20°C. The experiment shows that at a high temperature rather than at a low one a larger fraction of the absorbed energy is redistributed in State 2 in favour of PS I.

The dark state shifted from a low ratio (near that of state I) at low temperature to a high ratio (identical with that of state II) at 35°C. Reversible temperature-induced changes in the dark state were



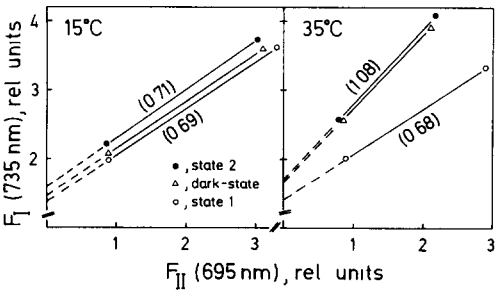


Fig. 2 (a) Time-course of the change in the F_I/F_{II} ratio during a State 1–State 2–State 1 transition Spinach leaves were incubated at the indicated temperatures, 30 min illuminated with light I to establish at State 1, and then illuminated as indicated. F_I/F_{II} ratios were obtained from emission spectra as shown in Fig. 1 (b) Temperature dependency of the F_I/F_{II} ratio of leaves being in different states At the indicated temperatures leaves were illuminated for 30–35 min with light I (State 1) or light II (State 2) To establish a dark state, leaves were preincubated for about 5 h at the indicated temperatures

Fig 3 F_I versus F_{II} plots obtained from spinach leaves being in different states F_0- and F_m-values of F_I and F_{II}, obtained as shown in Fig 1, were plotted and connected with a straight line The plotted values are mean values from 7–9 leaves The standard deviation never exceeded with approx 5%

characterized in a previous paper [6]. The half-time of such changes was in the range of 0.5 h.

All changes in fluorescence characteristics shown in Fig. 2 were reversible. The absolute value of F_I/F_{II} often varied between different batches of plants.

To obtain more information about the characteristics of energy distribution $F_{o\,(I)}$ and $F_{m\,(I)}$ (obtained from induction curves and normalized as indicated in Fig. 1) were plotted against $F_{o\,(II)}$ and $F_{m\,(II)}$ (Fig. 3). As the analysis of such plots is based on a method developed by Kitajiama and Butler [19], the slope is proportional to the spill-over constant, $k_{T\,(II\to I)}$, while the extrapolated value, $F_{I\,(\alpha)}$, represents that part of F_I which is related to energy initially distributed to PS I, i.e., changes in $F_{I\,(\alpha)}$ are proportional to changes in the distribution coefficient, α. The following conclusions can be drawn from the experiment shown in Fig. 3. In State 1 $k_{T\,(II\to I)}$ and α remained almost unaffected by the leaf temperature. After transition to state II, $F_{I\,(\alpha)}$ increased by about 15% (15°C) or 20% (35°C). The relative contribution of spill-over changes strongly varies with temperature; while no change was observed at 15°C, $k_{T\,(II\to I)}$ increased at 35°C by about 60%. Normally, it was necessary to preadapt the leaves for a short time (at least 15 min) at the respective temperature to find the typical pattern of energy distribution shown in the figure for State 1 and State 2. Somewhat longer exposure times were necessary to establish the dark states.

The data show that the pigment apparatus of leaves adapted to low temperature is characterized by a relative low spill-over constant, while in leaves adapted to high temperature, spill-over of energy from PS II to PS I appears to be enhanced, except that a State 1 is established. However, the State 1 at 35°C was quite unstable and rapidly returned to a state with high $k_{T\,(II\to I)}$, even in the dark. Sometimes, especially after extended preincubation at 35°C (more than 1 h), illumination with light I did not result in a complete transition to a 'state 1 pattern' of fluorescence, but it became possible again after lowering the temperature (not shown).

Absorbance changes at 515 nm

The above results demonstrate that different $k_{T\,(II\to I)}$ can be established by preincubating leaves in the dark at different temperatures. Using this phenomenon to manipulate $k_{T\,(II\to I)}$ experimentally it became possible to study the light-induced 'energization' of thylakoid membranes in dependence of the rate of spill-over. In the light, an electrical-field gradient, connected to electrogenic reactions of the non-cyclic and cyclic electron transport, is built up across the thylakoid membrane. Any increase in the rate of electron transport will enhance this gradient, provided its dissipation by ATP-synthesis and ion-leakage remains constant. The light-induced electrochromic pigment absorbance change at 515 nm (for a review, see Ref. 20) has been used as a relative measure for light-induced field gradients in two different types of leaf, which will be called 15°C leaves (3–5 h preincubated at 15°C to establish a low $k_{T\,(II\to I)}$) and 35°C leaves (3–5 h at 35°C to establish high $k_{T\,(II\to I)}$). The preincubated leaves were rapidly cooled and measured for absorbance changes at 0°C due to the fact that at this temperature photosynthetic reactions other than primary photoreactions and electron transport will be only slight, i.e., the Calvin cycle cannot efficiently act as a sink for electrons, and the light-induced elec-

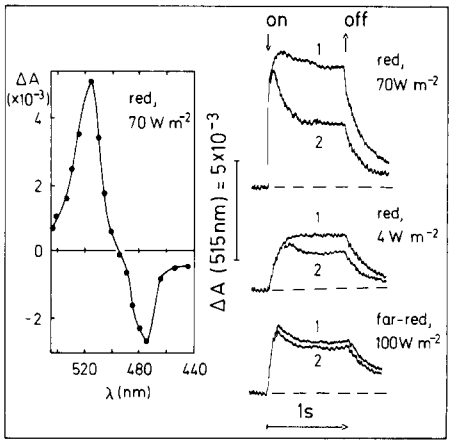


Fig. 4 Light-induced absorbance changes at 515 nm in leaves, measured at 0°C The leaves were 3–5 h preincubated in the dark at 35°C (curves 1) or at 15°C (curves 2), then rapidly cooled to 0°C and measured within 1 min Illumination with red (630–680 nm) or with far-red (less than 715 nm) actinic light as indicated The inset shows the spectral characteristic of the amplitude of the light-off signal (red actinic light, 70 W m^{-2}) in leaves preincubated at 35°C Downward arrows, light on, upward arrows, light off

trochemical gradient across the thylakoid membrane will be only slightly dissipated via ATP-synthesis. In addition, it was found that light-scattering changes, peaking around 535 nm [21] and at 520 nm [22] and interfering with absorbance changes at 515 nm, tend to decline with decreasing temperature, while the extent of the electrochromic reaction rather increases (not shown).

Fig. 4 shows ΔA_{515} transients during 1 s illumination. At high light intensity (70 $W \cdot m^{-2}$ short-wavelength red light, mainly absorbed by LHC/PS II), the absorbance rapidly increased to an initial peak, followed by a decay to a quasi-steady-state level, at which it remained until illumination was determined. The basic result was that the peak level showed only a small difference between the two types of leaf, while the steady-state level was considerably higher in 35°C leaves.

The spectral characteristics of ΔA_{515} (Fig. 4, inset) was quite similar to a pure electrochromic response observed in isolated thylakoids [23]. Whilst there is still some unresolved controversy about the precise meaning of 515 nm transients, it is widely accepted that such absorbance changes are connected with transmembrane-field formation that is closely related to energy-transducing processes (for a review, see Ref. 20). During 'continuous' illumination, 515 nm changes may largely coincide with the 'slow' field change which has been attributed by a number of authors (e.g., Ref. 24–26) to a localized field connected to a domain of an intramembrane buffer, which, however, equilibrates with the overall transmembrane field. Following Velthuys' explanation [32], the slow field change is associated with the electron flow out of the PQ pool, and its driving force is provided by PS I. Under appropriate conditions it may be related to a cyclic reaction via ferredoxin around PS I (e.g., Refs. 27 and 28).

From the chlorophyll fluorescence measured simultaneously with the absorbance change (not shown), it has been concluded that only during the first phase of illumination (low fluorescence) when ΔA_{515} reaches its peak level, is the rate of electron transport throught PS II high. During the quasi steady state (high fluorescence level) the PQ pool is highly reduced and the non-cyclic electron transport will be slowed down. Under such conditions the steady-state level of ΔA_{515} may be largely attributed to a cyclic electron transport around PS I, which then would be enhanced in 35°C leaves.

The most pronounced difference between the two types of leaf was observed after exciting with relative high light intensity. When low light was used for excitation (4 $W \cdot m^{-2}$; red light) the overall amplitude of the absorbance signals was reduced and the relative difference was smaller (Fig. 4).

No difference in the absorbance transients was observed after exciting with far-red light (100 $W \cdot m^{-2}$; Fig. 4, lower part), which is mainly absorbed directly by PS I pigments. This indicates that the electrogenic reaction itself, driven by PS I, was not affected by the temperature pretreatment.

When the leaves were preilluminated with light II to establish State 2, the dark decay of ΔA_{515} was accelerated and in both types of leaf the signal amplitude was somewhat lower than in the dark state. However, the relative difference in the extent of the steady-state signal persisted (Fig. 5a). No difference appeared when a State 1 (where $k_{T\,(II \rightarrow I)}$ is low in both types of leaf; see Fig. 3) was established, and in this case the extent of the steady-state signal in 35°C leaves was as low as in

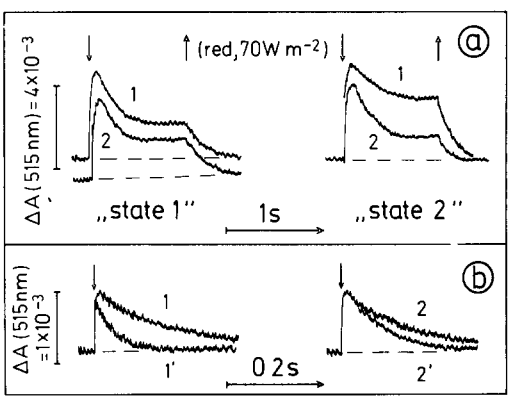


Fig 5 (a) Absorbance changes at 515 nm in leaves, preincubated at 35°C (curves 1) or at 15°C (curves 2) in light I (State 1) or in light II (State 2) The absorbance changes were induced with red actinic light (630–680 nm; 70 W m^{-2}) Downward arrows, light on; upward arrows, light off (b) Absorbance changes at 515 nm following a 1.5 ms light pulse (red light, 70 W m^{-2}) The leaves were preincubated for 4 h in the dark at 35°C (curves 1 and 1') or at 15°C (curves 2 and 2') Curves 1' and 2', leaves were 'pre-activated' (1 s red light, 70 $W \cdot m^{-2}$) 10 s before giving the light pulse Light pulses were indicated by downward arrows

15°C leaves (Fig. 5a). These data support the suggestion that the extent of field gradients which appear in the light under pseudo-steady-state conditions is closely linked to the extent of spill-over of absorbed energy from closed PS II centres ot PS I.

Fig. 5b shows absorbance changes following a 1.5 ms light pulse.As PS II centres largely remain open during such short illumination (as concluded from fluorescence data; not shown), only a small fraction of the absorbed energy will be spilled over from PS II to PS I. Under such conditions, there appeared to be no significant difference in the signal amplitude between the two types of leave. Furthermore, in dark-adapted leaves the dark decay was unchanged, indicating that the overall proton conductivity of the thylakoid membrane is not affected by the temperature pretreatment. If the leaves were 'preactivated' by a short illumination (1 s red light; 70 $W \cdot m^{-2}$), the dark decay was accelerated. Different mechanisms have been proposed to explain such 'light activation' of the dark-decay (see Ref. 20; but see also Ref. 24), but the common factor in these explanations is the assumption that the acceleration is related to the light-triggered preactivation of the thylakoid ATPase. This sort of activation appeared to be increased in 35°C leaves, where the half-time of the decay was shortened from 280 to 80 ms.

Slow light-scattering and fluorescence changes

The above suggestion that the light-induced electrochemical gradient across the thylakoid membrane is favoured in 35°C leaves is further supported by a different approach. In the experiment shown in Fig. 6, leaves were illuminated for 30 s with red light (70 $W \cdot m^{-2}$) in CO_2-free air at room temperature. Under such conditions ATP- and NADPH-consumption will be low, and Q will be largely reduced. Then, the slow decline in fluorescence (for a review, see Ref. 17) as well as concomitant light-scattering changes [21] are mainly connected to the light-driven acidification of the thylakoid space. In leaves the extent of light-scattering changes correlates well with the light-induced increase in the stromal ATP/ADP ratio [21]. The figure shows that in 35°C leaves both signals were more pronounced than in 15°C leaves. This again indicates that under conditions

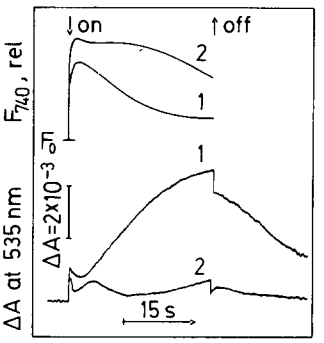


Fig 6 Fluorescence at 740 nm and apparent absorbance changes at 535 nm ('light scattering') in leaves, measured simultaneously at room temperature during illumination with red light (70 W m^{-2}) The leaves were preincubated for 4 h in the dark at 35°C (curves 1) or at 15°C (curves 2), then rapidly put into the measuring cuvette, and gassed for 1 min with CO_2-free air before measuring.

where the non-cyclic electron transport from water to NADPH is limited, the energization of thylakoid membranes, most likely mediated by a cyclic reaction around PS I, is favoured in 35°C leaves.

It appeared that preadaptation to high temperature results in an increase in the photosynthetic capacity. The steady-state rate of photosynthetic CO_2-uptake by leaves was measured at room temperature (red light, 70 $W \cdot m^{-2}$; 500 ppm CO_2 in air) as described previously [29]. In a typical experiment, the rate was 110 μmol $CO_2 \cdot dm^{-2} \cdot h^{-1}$ in 15°C leaves and 155 μmol CO_2 in leaves preadapted to 35°C (data not shown).

Discussion

There is evidence that it is the phosphorylation of the LHC protein which controls the distribution of excitation energy between the two photosystems during a State 1–State 2 transition in leaves [30]. The data presented here indicate that the operation of this control system depends on the leaf temperature. Changes in the initial distribution of energy are seen during State 1-State 2 transitions at both low and at high temperatures. Such changes may be accomplished by changes in the connection between the LHC protein and the two photosystems. Spill-over, however, requires direct interaction between PS II and neighbouring PS I complexes. From these data it appears that the extent

of spill-over changes during a State 1–State 2 transition strongly varies with temperature. In principle, the effect of high temperature on energy distribution in leaves described here (Fig. 3) resembles the effect of a gradual decrease in the cation concentration on energy distribution in isolated membranes [14,15]: in the presence of high concentration of charge screening cations, isolated membranes are well stacked and the extent of spill-over is low. In this case, protein phosphorylation only induces a change in the initial distribution of energy. At lower cation level, when electrostatic screening is weaker, phosphorylation is assumed to induce a greater lateral randomization of PS I and PS II complexes, so that the probability of spill-over is increased in addition to a change in the initial distribution of energy. Exposure to a high temperature could influence the lateral segregation of the two photosystems into different domains of the membranes in a similar way as the gradual decrease in the cation level. It seems likely that in leaves incubated at high temperatures randomization of the two photosystems is faciliated, so that protein phosphorylation brings about a greater connection between PS II and PS II complexes and related spill-over of energy. In contrast, when the leaves are preincubted at high temperature, PS II remains well separated from PS I, similar as is the case at high level of charge-screening cations. This could explain. why in State 2, where the protein kinase is assumed to be active, the probability of direct energy transfer from PS II to PS I remains small in 15°C leaves (Fig. 3). However, further studies are needed to clarify whether this interpretation sufficiently describes the effect of temperature on energy distribution in leaves.

Fernyhough and co-workers [33] ruled out the ATP/ADP ratio as being a main intrinsic factor in regulating the protein kinase activity, as they demonstrated with isolated chloroplasts that LHC phosphorylation is at a maximum just under conditions where ATP-consumption is high. Alternatively, it is proposed that the protein-kinase is activated by reduced electron-transport carriers between PS II and PS I, possibly PQ (e.g., Ref. 37). On the other hand, the data reported here show that at high temperature the dark state resembles a State 2, indicating that protein phosphorylation can also occur in the dark when the PQ pool in leaves is largely oxidized (as was seen from fluorescence data; not shown). Further studies appear to be necessary to clarify whether or not the redox state of PQ interferes with the state regulation in leaves.

In the experiments shown in Figs. 5–7 the electrochemical gradient across the thylakoid membrane produced by the photosynthetic electron transport is related to the distribution of excitation energy between the two photosysystems. Leaves have been preadapted to different temperatures to manipulate experimentally the energy distribution. Obviously, the electrochemical gradient connected to the non-cyclic electron transport is only slightly affected by energy distribution. Rather, it is the energy transduction potential produced by a cyclic electron transport around PS I which is under the control of energy distribution. When NADP is largely reduced, electrons will be delivered from reduced ferredoxin to a cyclic pathway [34]. However, the efficiency of the cyclic reaction around PS I should depend on the PS II actıvitiy, relative to that of PS I, and on the overall redox-poise of the components involved in this reaction. If the absorbed energy is redistributed in favour of PS I, the photochemical activity of this photosystem is enhanced, while that of the water-splitting system declines. Therefore, overreduction of the electron-transport chain should be avoided, and the electron transport around PS I and related 'energization' of the membrane can take place more efficiently. This is indeed seen in leaves where the energy distribution ıs changed in favour of PS I, and the data here support the concept proposed by Allen and Bennett [7] and Allen (Ref. 36; see also Ref. 33) that the balance between the cyclic and the non-cyclic pathway for electrons is under the control of energy distribution.

Cyclic electron transport and related 'cyclic' photophosphorylation is suggested to compensate ATP deficiency during CO_2 fixing conditions [34,35]. If the ATP/ADP ratio in the stroma is too low, phosphorylation by phosphoglyceric acid cannot proceed. As a consequence, NADPH-consumption declines and ferredoxin becomes largely reduced. Under such conditions, redistribution of the absorbed energy in favour of PS I, at the expense of PS II excitation, would serve to stimu-

late cyclic electron transport and photophosphorylation and, therefore, would help to reestablish NADPH consumption.

It appears likely that the overall regulation of energy distribution has a function in maintaining the balance between photosynthetic electron transport, photophosphorylation and energy-consumping processes (such as the Calvin cycle) during variations in the environmental conditions. From these data it is seen that the two mechanisms to regulate energy distribution, 'initial' distribution of energy between PS II and PS I and spill-over of energy from PS II to PS I show differential sensitivity to environmental parameters such as light and temperature. Further attention should be focussed on the question whether differential sensitivity reflects different physiological functions of these mechanisms: changes in the initial distribution of energy could have a function in optimizing the quantum efficiency of photosynthesis, especially during variations in the light quality, while resonance energy transfer from PS II to PS I, which most efficiently occurs from closed PS II centers to attached PS I complexes, might preferentially serve to regulate the cyclic electron transport and photophosphorylation.

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