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LIGHT SCATTERING, CHLOROPHYLL FLUORESCENCE AND STATE OF THE ADENYLATE SYSTEM IN ILLUMINATED SPINACH LEAVES

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Scattering of green light and chlorophyll fluorescence by spinach leaves kept in a stream of air or nitrogen were compared with leaf adenylate levels during illumination with blue, red or far-red light. Energy charge and ATP-ADP ratios exhibited considerable variability in different leaves both in the dark and in the light. Variability is explained by different possible states of the reaction oxidizing triose phosphate or reducing 3-phosphoglycerate. Except when oxygen levels were low, there was an inverse relationship between light scattering and chlorophyll fluorescence during illumination with blue or red light. When CO_2 was added to a stream of CO_2-free air, chlorophyll fluorescence increased, sometimes after a transient decrease, and both light scattering and leaf ATP/ADP ratios decreased. Similar observations were made when air was replaced by nitrogen under blue or high-intensity red light. Under these conditions, over-reduction caused inhibition of electron transport and phosphorylation in chloroplasts. However, when air was replaced by nitrogen during illumination with low-intensity red light or far-red light, light scattering increased instead of decreasing. Under these light conditions, ATP/ADP ratios were maintained in the light. They decreased drastically only after darkening. Although ATP/ADP ratios responded faster than light scattering or the slow secondary decline of chlorophyll fluorescence due to illumination, it appeared that in the steady state, light scattering and chlorophyll fluorescence are useful indicators of the phosphorylation state of the leaf adenylate system at least under aerobic conditions, when chloroplast and extrachloroplast adenylate systems can effectively communicate.

Introduction

Previous work has shown that changes in 9-aminoacridine fluorescence quenching or in scattering of a weak beam of green light by a suspension of chloroplasts can, under certain conditions, indicate changes in the phosphorylation state of the chloroplast adenylate system [1]. In illuminated leaves, 9-aminoacridine cannot be used to monitor the state of the adenylate system because it does not enter intact leaf cells. However, leaves exhibit complex light scattering changes on illumination with red or far-red light [2–6]. Light scattering is decreased by CO_2 [2]. Its response to oxygen depends on light intensity, light quality and composition of the gas phase [2–4,6]. While maintaining different ATP/ADP ratios, the adenylate systems of chloroplasts and cytosol of leaf cells are linked by substrate shuttles [7,8]. Cytosolic and mitochondrial adenylate systems, which also maintain different states of phosphorylation [9], communicate through direct adenylate transfer [10]. Linkage of these systems [8,11,12] makes it possible that light scattering in leaves provides information on the phosphorylation state

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Abbreviation: Chl, chlorophyll.

of adenylates throughout the green tissue of a leaf. In the following, we use direct adenylate measurements, light scattering and chlorophyll fluorescence to obtain information on the state of adenylates in illuminated leaf cells.

Material and Methods

Spinach was grown in the field or, during winter and summer, in the greenhouse under short-day conditions. Light scattering changes of cut leaves were induced by illumination with blue light (half bandwidth from 383 to 557 nm), red light (half bandwidth from 625 to 675 nm), a broad band of red light (half bandwidth from 626 to 743 nm) or far-red light (half bandwidth from 707 to 772 nm) and was measured in transmission at 540 nm as described previously [2]. Chlorophyll fluorescence was measured either at 684 nm or at 740 nm, depending on whether red (filters K 65 and Calflex C from Balzers and RG 610 from Schott) or blue (Corning 9782) light was used for excitation. Suitable filter combinations protected the photomultiplier (used for measuring transmission) and the photodiode (used for measuring fluorescence) against actinic light. The leaves were kept in a closed cuvette which was gassed at a rate of usually 20 l/h. To measure adenylates, disks (diameter 10 mm) were cut from a leaf and gassed with a stream of moist nitrogen or air with and without CO_2 while fluorescence or both light scattering and fluorescence were recorded. Metabolism of the disks was arrested by adding liquid nitrogen during illumination or darkness through a valve to the cuvette. The disks were then removed and ground to a fine powder with 2 ml of frozen perchloric acid (1 M) in a mortar which had been prechilled with liquid nitrogen. Grinding was continued until the mixture thawed. Subsequent procedures were similar to those applied to measure adenylates in chloroplasts [13,14].

Results

Adenylate contents and state of the adenylate system under aerobic and anaerobic conditions

Adenylate contents in leaves were variable. Usually, total adenylates amounted to 150–220

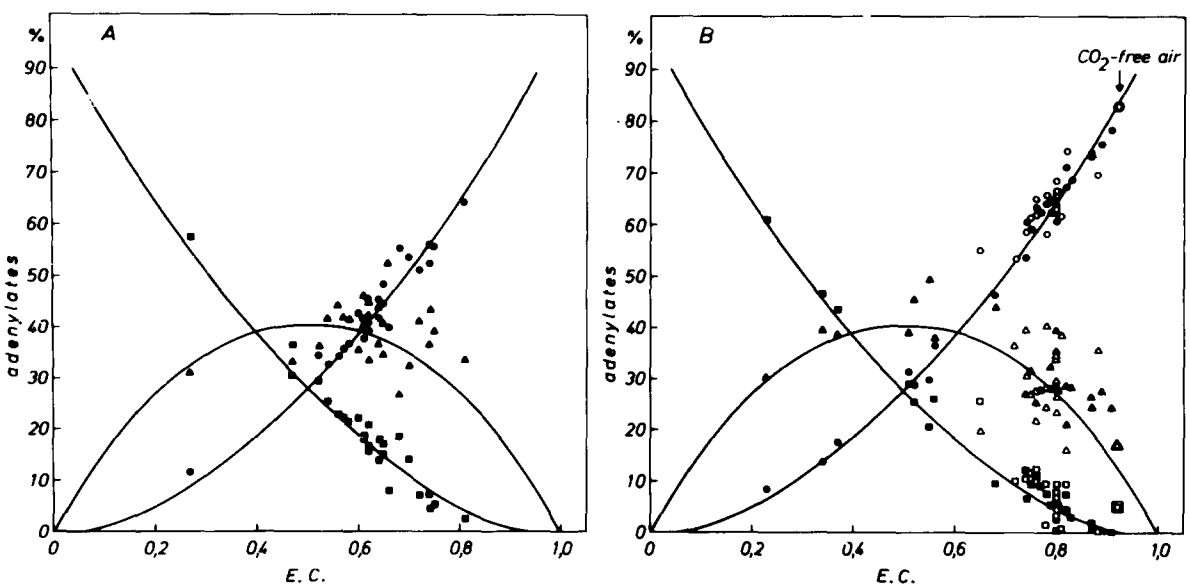

Fig. 1. Adenylate distribution vs. energy charge in darkened spinach leaves (A) and in leaves illuminated with 29 W/m^2 blue light (B, open symbols) or 142 W/m^2 white light (B, closed symbols). Circles: ATP, squares: AMP, triangles: ADP. Lines denote the adenylate distribution that would be expected if only one adenylate pool were to exist in leaves and adenylates of this pool were in adenylate kinase equilibrium. Gas phase was air or, in one experiment (B, energy charge 0.92) CO_2-free air. Note considerable variability of energy charge both in the dark and in the light.

nmol/mg Chl. compared with 40–70 nmol/mg Chl. in aqueously isolated chloroplasts. Fig. 1 shows the energy charge, E.C. = 1/2(2[ATP] + [ADP])/([ATP] + [ADP] + [AMP]) [15], in darkened aerobic leaves (A) and in leaves illuminated with 29 W/m^2 blue light (open symbols) or 142 W/m^2 white light (closed symbols). The blue light is far below and the white light only slightly below light saturation of photosynthesis. Energy charge can vary between a minimum value of zero (AMP the only adenylate) and a maximum value of one (ATP the only adenylate). Solid lines in Fig. 1 show the adenylate distribution calculated for adenylate kinase equilibrium at different E.C. values (assumed equilibrium constant 0.5 [16,17]). The measurements integrate over the energy charge and the adenylate distribution in different leaf cell compartments which are known to be different [8,9]. In view of these differences, the fairly close fitting of the data to the equilibrium state of the adenylate kinase reaction is surprising, as mixing of the adenylate pools of cytosol, chloroplasts and mitochondria by leaf homogenization should introduce deviations from adenylate kinase equilibrium even if adenylates were in equilibrium in vivo. Obviously, deviations introduced by mixing of compartments are not very large.

In the dark, the averaged energy charge of many leaves varied between 0.5 and 0.7, although both lower and higher values were also recorded. Values on the low side were observed especially in the dark immediately after illumination. Under the arbitrary assumption that average phosphate concentrations were 5 mM [18,19], an energy charge of 0.5 corresponds at adenylate kinase equilibrium to a phosphorylation potential [ATP]/[ADP][P$_i$] of about 140 M^{-1} and an E.C. of 0.7 to a phosphorylation potential of 290 M^{-1}.

In illuminated leaves, energy charge varied even more than in darkened leaves (Fig. 1B). Most common were E.C. values between 0.72 and 0.83. Assuming arbitrarily average P$_i$ concentrations of 5 mM, this corresponds to average phosphorylation potentials between 320 and 575 M^{-1}. Even at a low light intensity (blue light), energy charge was within a range of energy charge values observed under illumination with high light intensity (white light).

The scattering of the data in Fig. 1A (dark) and B (light) leaves the momentary impression of chaos. This impression is unjustified. Rather, the data reflect physiological variability.

Both order and variability are shown in Fig. 2. In Fig. 2A, the average ATP level of a leaf rose

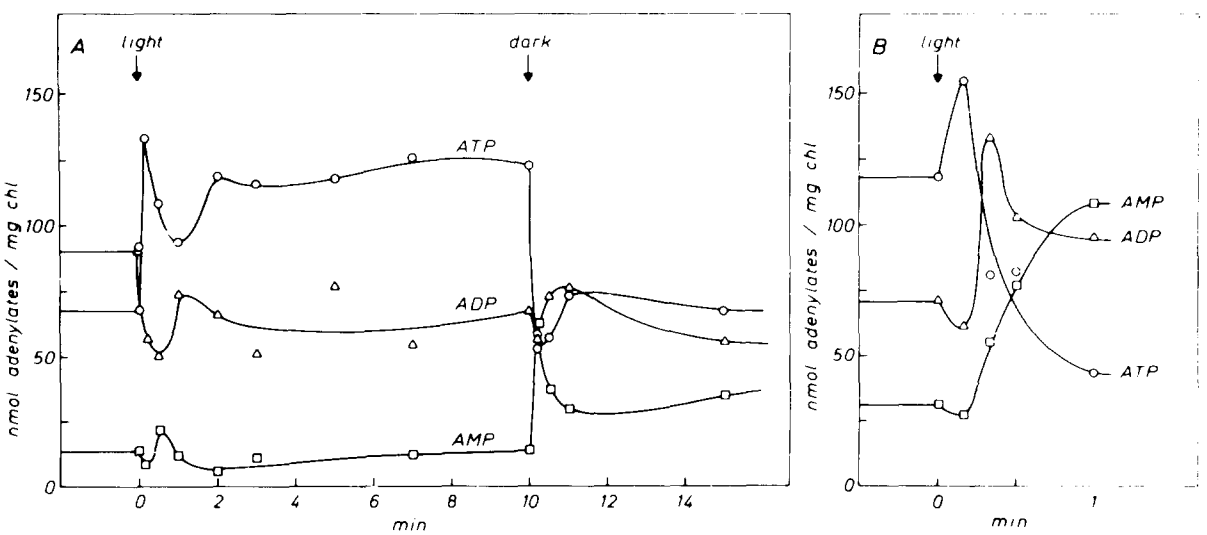

Fig. 2. Adenylate levels in two different spinach leaves (A and B) in darkness and during illumination with 142 W/m^2 white light in air. At the indicated times, individual disks from the two leaves were frozen in liquid nitrogen, while darkness or illumination was maintained. Adenylates were extracted from the disks and measured.

sharply on illumination, reached a maximum after 10 s in the light, exhibited a transient decrease and then remained at a level clearly above the previous dark level. Darkening caused a rapid decrease in ATP. Changes in ADP lagely mirrored those of ATP. AMP levels were low except after darkening. The rapid increase of AMP on darkening has been observed before in chloroplasts [20]. It suggests the existence of an unknown photosynthetic reaction generating AMP.

Fig. 2B shows for another leaf essentially the same kinetic response that is apparent in Fig. 2A. However, in this leaf the dark level of ATP was much higher than that in the leaf of Fig. 2A. After the initial light-dependent increase in ATP which was also observed in Fig. 2A, there was a large decrease far below the original dark level which was absent in Fig. 2A. The kinetic response of ADP was similar in general to that seen in Fig. 2A, but in contrast to the experiment of Fig. 2A, the level of ADP was higher in the light than in the dark. The AMP response observed in the leaf of Fig. 2B was different from that seen in Fig. 2A. It is dictated by the adenylate kinase reaction which, close to its equilibrium state, requires a high AMP concentration when ATP levels are low.

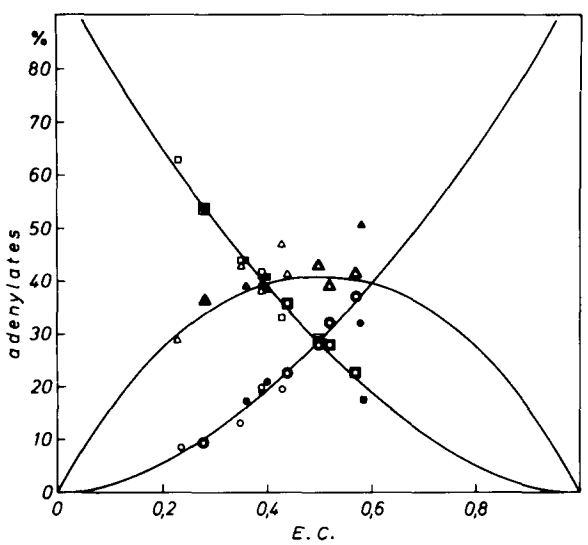

Fig. 3. Adenylate distribution vs. energy charge in leaves kept in a stream of nitrogen in the dark (closed symbols) or under illumination with 29 W/m^2 blue light (open symbols, doubled) or 142 W/m^2 white light (open symbols). Note differences from energy charge in air which was shown in Fig. 1.

Fig. 3 shows energy charge in leaves gassed with nitrogen. Again comparison of the experimental data with the theoretical curves shows that adenylates were not far from adenylate kinase equilibrium. The high energy charge values commonly observed in aerobic leaves in the dark (Fig. 1A and B) were absent under anaerobiosis. The maximum energy charge observed under nitrogen in the dark was 0.6 and more commonly values below 0.4 were calculated. This corresponded to ATP/ADP ratios of about 0.5 as compared to ATP/ADP ratios close to 1 in air. Low energy charge values were also observed by Sellami [21] in darkened anaerobic wheat leaves. In contrast to Sellami's observations, however, illumination of anaerobic spinach leaves with white light capable of exciting Photosystem II did not raise energy charge to the high values which were the rule in illuminated aerobic leaves, although increases above dark values were often observed. Maximum energy charge values observed in the light under anaerobiosis were below 0.6 as compared with values as high as 0.92 in the presence of CO_2-free air (Fig. 1B). Even in the presence of CO_2 which stimulates electron flow, ATP/ADP ratios were very low in anaerobic leaves [22]. Oxygen was required to increase ATP/ADP ratios. In anaerobic chloroplast suspensions, illumination was incapable of raising chloroplast ATP/ADP ratios [23].

Light scattering, chlorophyll fluorescence and adenylate levels

In a previous publication [1], evidence has been presented that the light-induced proton gradient in spinach chloroplasts, which is believed to be a dominanat part of the driving force for chloroplast ATP synthesis, gives rise to increased light scattering by the chloroplasts. Under specified conditions, quenching of 9-aminoacridine fluorescence, another indicator of the state of the chloroplast proton gradient [24–26], or light scattering can be used to indicate the phosphorylation state of chloroplast adenylates under steady-state conditions [1]. Chlorophyll fluorescence is dominated both by the energy state of the thylakoid system by the redox state of electron-transport chain [27–31]. Fig. 4 shows a simultaneous recording of light scattering (535 nm) and chlorophyll fluorescence of a leaf. On illumination with 90

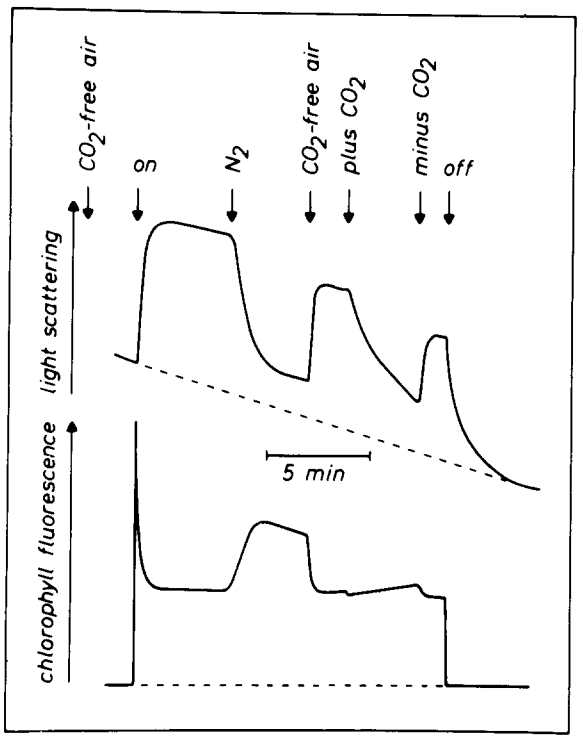

Fig. 4. Simultaneous recording of light scattering and chlorophyll fluorescence of a spinach leaf illuminated with 90 W/m^2 red light (half bandwidth: 626–675 nm) as influenced by CO_2 or anaerobiosis. Rate of gas flow was 30 l/h.

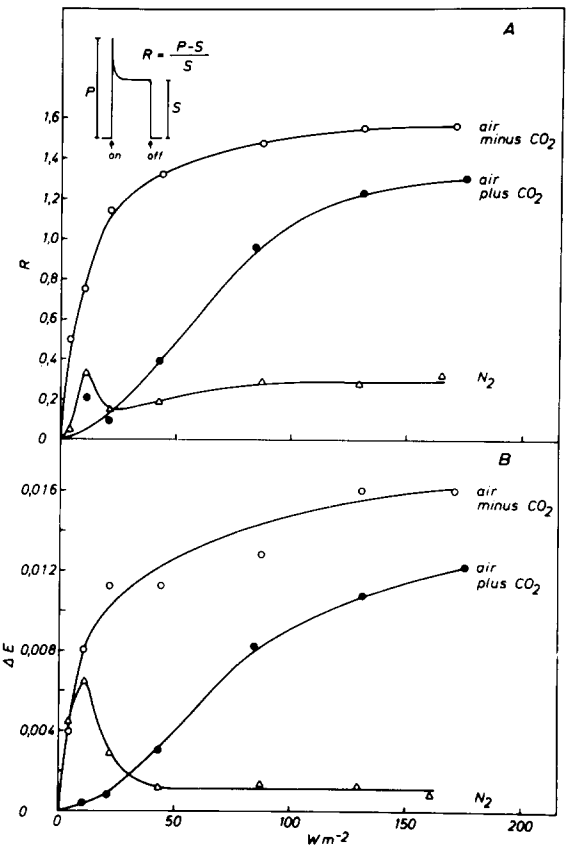

Fig. 5. Normalized chlorophyll fluorescence R (A) and light scattering (B) by a spinach leaf as a function of the intensity of actinic illumination with red light (half bandwidth: 626–675 nm) in CO_2-free air, air or nitrogen. The inset in A defines R. Dark times between illumination periods were 3 min; illumination periods 4 min; values taken after 4 min illumination.

W/m^2 red light (half bandwidth 625–675 nm) in a stream of CO_2-free air, light scattering increased rapidly, while chlorophyll fluorescence decreased after an initial increase. The response of chlorophyll fluorescence to illumination is known at the Kautsky phenomenon [32]. In the steady state, a high scattering level was maintained together with a low level of chlorophyll fluorescence. When CO_2-free air was replaced by nitrogen, light scattering decreased and chlorophyll fluorescence increased. These responses were caused by over-reduction of the electron-transport chain [6]. Readmission of CO_2-free air reversed these changes. During photosynthesis, when CO_2 was present and ATP was consumed, light scattering was decreased and chlorophyll fluorescence increased.

Fig. 5 shows these effects as a function of the intensity of illumination with a beam of red light. It is apparent that light scattering by a leaf (Fig. 5B) responds to illumination and to differences in the composition of the gas phase in a fashion that is very similar to that of the response of the variable part of chlorophyll fluorescence R (for definition of R, see the kinetics of chlorophyll fluorescence by a leaf in the inset of Fig. 5A). In the presence of 21% O_2 and in the absence of CO_2, both light scattering and R increased hyperbolically with light intensity. The half-maximum response of light scattering and of R was at about 10W/m^2 red light. In the presence of CO_2, both light scattering and R were decreased, and the response curve to light intensity became sigmoidal. The extent of suppression of light scattering and R by CO_2 varied within rather large limits in differ-

ent leaves (not shown). Factors were the opening state of the stomata, capacity for photosynthesis and apparently also the physiological state of the leaf. Under nitrogen, there was again a general similarity in the response of R and light scattering to illumination. The amplitude of the responses of different leaves kept in a stream of nitrogen under red light varied within rather wide limits. It is tempting to relate this variability to the variation in energy charge depicted in Fig. 3. Still, at high light intensities, changes of light scattering and R were in nitrogen almost always small compared with the changes observed in air. Similarly, energy charge during illumination in nitrogen was almost always lower than energy charge in air (cf. Fig. 3 with Fig. 1).

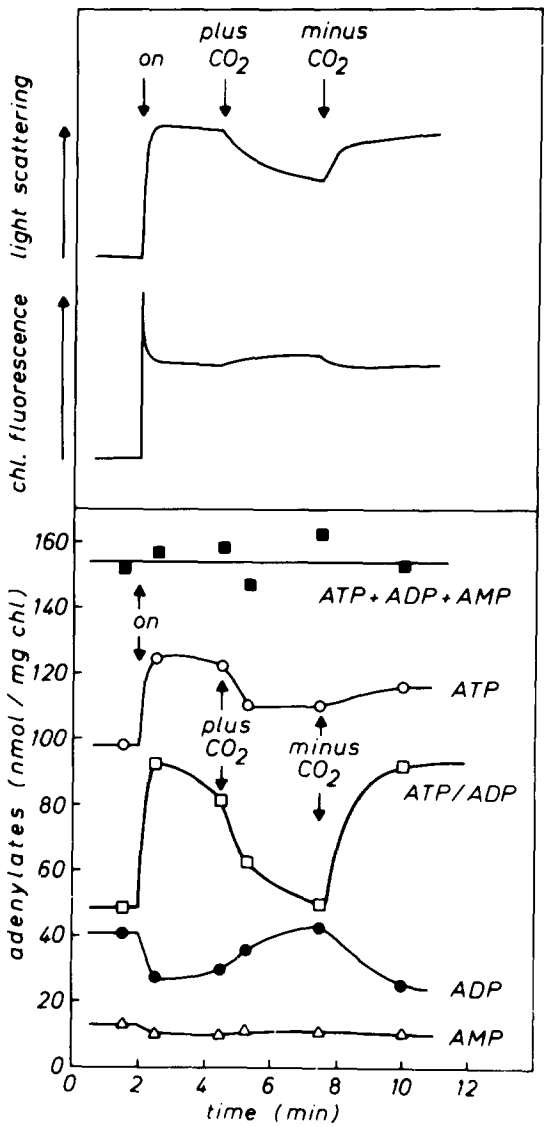

Fig. 6. Simultaneous recording of light scattering and chlorophyll fluorescence of a spinach leaf as compared with leaf adenylate levels. The leaf was first gassed with CO_2-free air. 380 ppm CO_2 were added to and withdrawn from the gas stream as indicated. Illumination with 80 W/m^2 red light (half bandwidth: 626–675 nm).

Fig. 6 shows that when CO_2 decreased light scattering and increased chlorophyll fluorescence, ATP levels also decreased and ADP levels increased in a leaf illuminated with red light in the presence of 21% oxygen. Withdrawal of CO_2 resulted in increased light scattering and decreased fluorescence, and these responses were accompanied by an increase in ATP and a decrease in ADP.

Changes in the levels of adenylates in an illuminated leaf during the transition from air to nitrogen and vice versa are shown in Fig. 7. After an increase on illumination (not shown), ATP

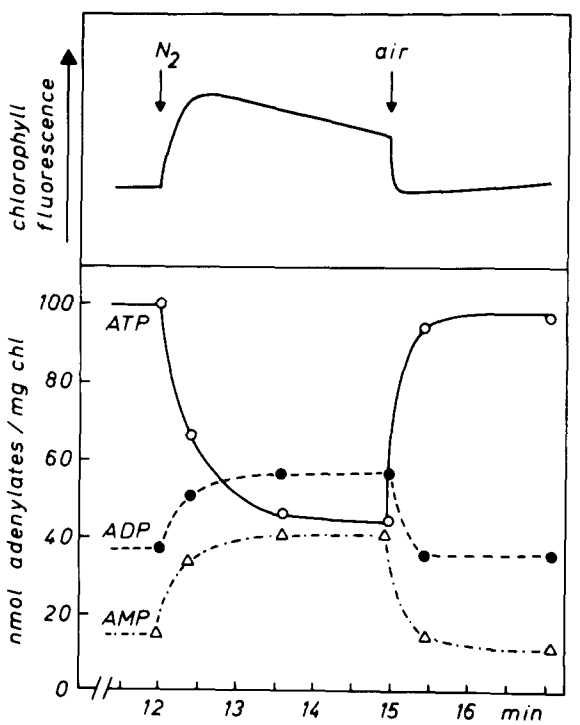

Fig. 7. Chlorophyll fluorescence and leaf adenylate levels during a transition from air to nitrogen and vice versa. Illumination with 29 W/m^2 blue light (half bandwidth: 383–557 nm).

levels decreased when air was replaced by nitrogen and ADP and AMP levels increased. Readmission of air restored the original ATP levels, and ADP and AMP levels declined again. Chlorophyll fluorescence which was simultaneously recorded increased under nitrogen and then exhibited a slow decrease. The original low fluorescence level was rapidly reestablished when nitrogen was replaced by air. Light scattering was not recorded in this experiment, but the relationship between light scattering and chlorophyll fluorescence is shown in Figs. 4 and 5.

When a broad band of red light including far-red light (half bandwidth 626–743 nm) is used as a source of actinic illumination instead of red light as in Fig. 5, the response curves for light scattering in air are shifted to higher light intensities and the response curves for nitrogen to lower intensities

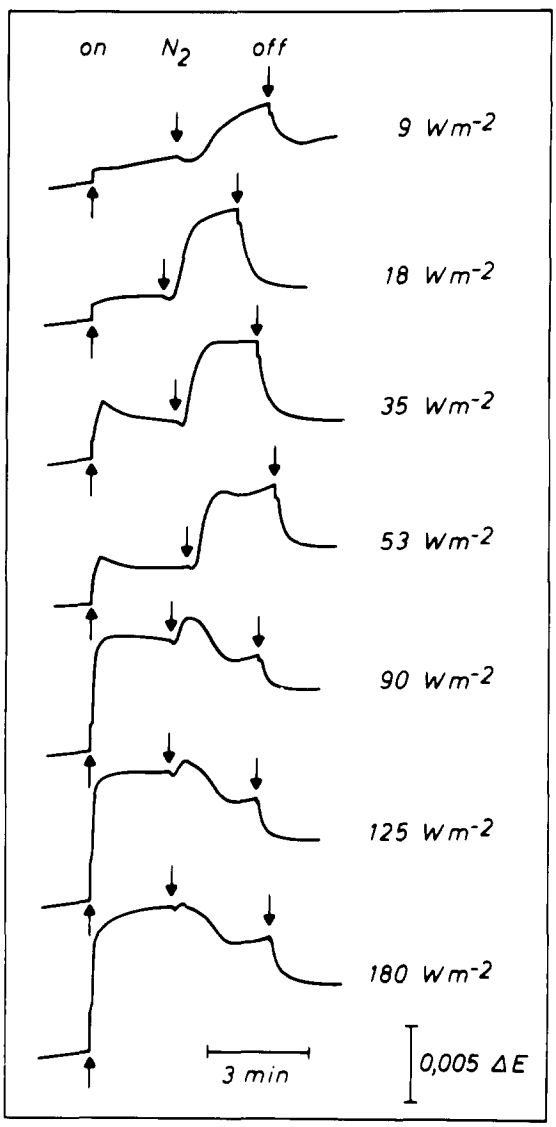

Fig. 8. Light scattering of a spinach leaf illuminated with different intensities of a broad band of red light including also far-red light (half bandwidth: 628–743 nm). Where indicated, the stream of air was replaced by nitrogen. This caused reduction in the oxygen content of the leaf cuvette which slowly became anaerobic.

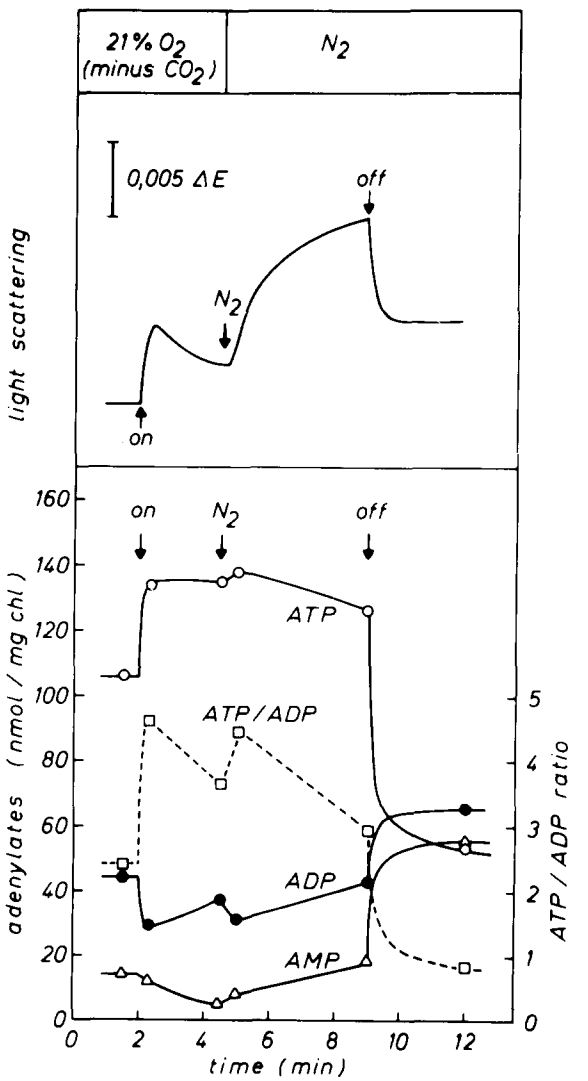

Fig. 9. Light scattering of a spinach leaf illuminated with 18 W/m^2 red plus far-red light (half bandwidth: 628–743 nm) in air and nitrogen and corresponding leaf adenylate levels.

(cf. Fig. 5 with data in Ref. 2). Fig. 8 shows light scattering by a spinach leaf illuminated with different intensities of a broad band of red light during a change from air to nitrogen. While nitrogen suppressed light scattering at high intensities as in Fig. 4, it stimulated scattering at low intensities although oxygen-dependent ATP formation is inhibited in nitrogen [23]. It was of interest to measure the response of leaf adenylates when scattering was increased rather than decreased by nitrogen as in the experiment of Fig. 7. Fig. 9 shows that illumination in air with 18 W/m^2 red plus far-red light increased ATP and decreased ADP and AMP levels. Although nitrogen is expected to interfere seriously with extrachloroplast ATP production, it did not greatly affect the overall leaf ATP content. The ATP/ADP ratio even exhibited a transient increase during the transition from air to nitrogen. This is in marked contrast to the experiment of Fig. 7, where anaerobiosis produced a drastic decrease in the overall ATP/ADP ratio during illumination with short-wavelength red light. As should be expected, darkening in nitrogen caused a drastic decrease in ATP and in the ATP/ADP ratio below the values measured in the dark before illumination when the gas phase was air.

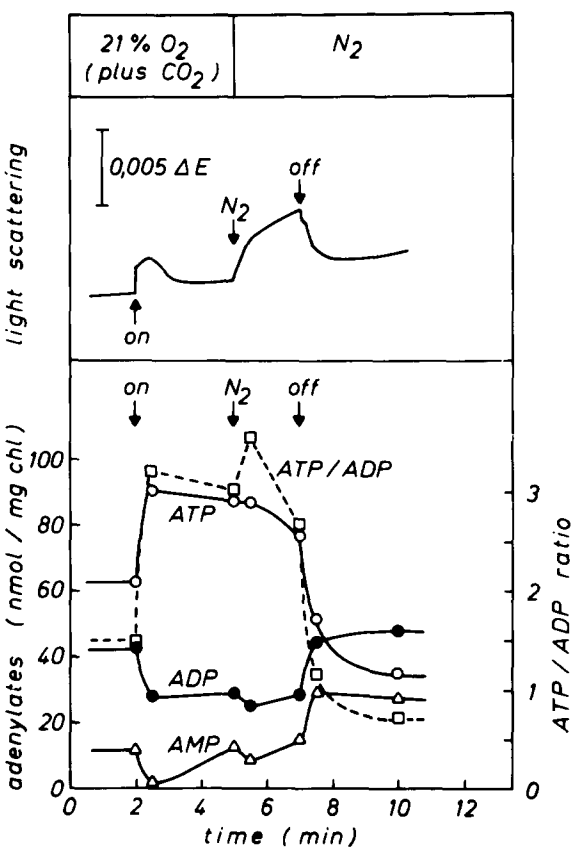

Fig. 10. Light scattering of a spinach leaf illuminated with 150 W/m^2 far-red light (half bandwidth: 707–772 nm) in CO_2-free air and nitrogen and corresponding leaf adenylate levels.

In a similar experiment, a high intensity of far-red light (half bandwidth 707–772 nm) was used to illuminate a spinach leaf in a stream of CO_2-free air and nitrogen (Fig. 10). Far-red light produces only a transient scattering response in air but promotes light scattering in nitrogen [2]. Illumination in CO_2-free air increased the overall leaf ATP level and the ATP/ADP ratio. Nitrogen increased light scattering and failed to cause much decrease in energy charge. As in Fig. 9, there was even a transient increase in the ATP/ADP ratio. Darkening then produced the usual low energy charge seen under anaerobic conditions.

Discussion

This work contains two main messages. One is that within limitations that can be defined, light scattering and chlorophyll fluorescence of illuminated leaves not only indicate thylakoid energization [2,3] but can also be used as indicators of the state of the leaf adenylate system, and the other that this state can considerably vary in apparently healthy leaves both in the dark (Fig. 1A) and in the light (Fig. 1B). Previous work has shown that the reaction responsible for carbon reduction in photosynthesis

$$PGA + ATP + NADPH + H^+ \rightarrow DHAP + ADP + P_i + NADP$$

has considerable freedom of adjustment. Different combinations of ATP/ADP ratios, of NADPH/NADP ratios and of 3-phosphoglycerate/dihydroxyacetone phosphate (PGA/DHAP ratios) can assure efficient carbon flux in this reaction [13]. Although the chloroplast ratio of ATP to ADP is, in the light, a simple function of ATP production vs. consumption, complexity is introduced by different possible states of regulation between linear, cyclic and pseudocyclic electron

fluxes [6,33] and probably also by different permeability properties of the thylakoid membrane which may vary with the physiological state of the leaves. Indications of such differences in permeability are different decay times of light-induces scattering after darkening (data not shown). Coupling between electron flow and phosphorylation and regulation of electron fluxes thus govern ATP production. Consumption is influenced not only by the availability of CO_2 but also by regulation within the carbon cycle and by photorespiration. This complexity finds expression in the different states of leaf adenylates as shown for two photosynthesizing leaves in Fig. 2A and B and for a large number of individual leaf experiments in Fig. 1B.

An indicator function of light scattering for the state of the adenylate system of illuminated intact chloroplasts has been deduced from experiments comparing light scattering, quenching of 9-aminoacridine fluorescence and levels of chloroplast adenylates [1]. Also, conditions have been defined under which light scattering and the phosphorylation state of chloroplast adenylates do not show parallel behavior. Expecially the experiments shown in Figs. 6 and 7 suggest that both light scattering and chlorophyll fluorescence also have indicator function for the adenylate systems of illuminated leaf cells. They demonstrate that in an illuminated leaf, ATP levels decrease and ADP levels increase when either photosynthesis is permitted in the presence of oxygen (Fig. 6) or air is replaced by nitrogen under illumination with blue light which is capable of exciting Photosystems II and I (Fig. 7). Under both conditions, chlorophyll fluorescence is increased (when CO_2 is added, often after a transient decrease, see Fig. 4) and light scattering decreased.

When far-red light is also a significant component of actinic illumination, suppression of light scattering by nitrogen is observed only at fairly high light intensities [2]. At low intensities, nitrogen stimulates light scattering (Fig. 8). Furthermore, far-red light given alone is not very active in promoting light scattering in the presence of oxygen [2], presumably because electron flow to oxygen drains electrons from the cyclic electron-transport pathway. In the presence of nitrogen, light scattering is increased by far-red light (Fig. 10). Thus, light scattering changes produced by a broad beam of low-intensity red light or by high-intensity far-red light in air and nitrogen are opposite to those produced by high-intensity red light (both short wavelength and broad beam) or by blue light. These differences can be used to test the concept that changes in light scattering can reflect changes in the phosphorylation state of leaf adenylates. Figs. 9 and 10 show that the dramatic decrease in ATP/ADP ratios produced by the transition from air to nitrogen under blue illumination in Fig. 7 was not observed during illumination with a broad beam of red light (18 W/m^2) or high-intensity far-red light (150 W/m^2). Rather, there was a further increase in ATP/ADP. That this increase was not maintained during prolonged anaerobic illumination does not indicate a decrease in chloroplast energization but rather a decrease in energization of other leaf compartments. Adenylate measurements in leaves integrate over several adenylate pools of leaf cells. Anaerobiosis makes oxidative phosphorylation impossible. Thus, under anaerobiosis extrachloroplast ATP requirements must largely be met by chloroplast ATP production. Although it is known that chloroplast adenylate pools can communicate, linkage involves complex shuttle transfers of metabolites such as dihydroxyacetone phosphate and 3-phosphoglycerate and dicarboxylates [7,8]. It is doubtful whether these shuttle transfers can function efficiently under nitrogen, as functioning requires oxidation of extrachloroplast pyridine nucleotides. Thus, under nitrogen, the high-phosphorylation state of the adenylate system indicated by increased light scattering must be expected to be confined largely to the chloroplasts. It is therefore not surprising that the average ATP level of a leaf decreases somewhat during prolonged exposure to far-red light under nitrogen. Figs. 9 and 10 therefore not only do not contradict, but actually confirm the conclusions drawn from Figs. 6 and 7 that light scattering is capable of indicating the state of adenylates in chloroplasts and, as far as communication of different adenylate systems is possible, also in green leaf cells. Since Fig. 5 shows a close correlation between light scattering and variable fluorescence R, fluores-

cence may also be used to record changes in the phosphorylation state of leaf adenylates in the light.

However, there are conditions under which a good correlation between increased light scattering, decreased chlorophyll fluorescence and a high phosphorylation state of the leaf adenylate system cannot be expected to exist. At low oxygen concentrations, an increase in chlorophyll fluorescence may not indicate a decrease in ATP but rather increased reduction of the electron-transport chain. Usually, changes in light scattering and in slow chlorophyll fluorescence are antiparallel, but at low oxygen concentrations they may parallel each other (data not shown).

Fig. 2 shows that on illumination ATP levels of a leaf rise to a maximum which is followed by a dip of various magnitude. High ATP levels are observed after about 15 s illumination. The rise in light scattering and the secondary decrease in chlorophyll fluorescence (Kautsky effect) are often, though not always, slower than the rise in ATP. Moreover, their half-times depend on the previous history of the leaves. After long peroids in the dark, light scattering by a leaf may increase and chlorophyll fluorescence decrease very slowly after its fast initial rise, while gas-exchange measurements indicate fast onset of photosynthesis (data not shown).

Several reasons appear to be responsible for the failure of light scattering and chlorophyll fluorescence to properly assess the state of the adenylate system in the dark/light transition. One is that on illumination initially a membrane potential is known to be mainly responsible for ATP formation in the chloroplasts [34]. It decreases as the proton gradient increases. In the steady state, the proton gradient is believed to carry the main burden of chloroplast ATP synthesis.

Furthermore, in chloroplasts, quenching of 9-aminoacridine fluorescence, which indicates formation of the proton gradient, and the secondary decline of chlorophyll fluorescence, which is related to the increase in light scattering, show comparable kinetics only after repeated light/dark cycles, not on a first illumination after a long dark exposure [1]. This indicates that the translation of a proton gradient into a light scattering or a fluorescence signal is a complex phenomenon that merits further exploration.

Finally, in the light/dark transition, changes in adenylate pools are faster (Fig. 2A) than the reversal of the light-induced light scattering change (Fig. 4). Naturally, the decline in chlorophyll fluorescence is very fast.

Thus, during dark/light and light/dark transitions, light scattering and chlorophyll fluorescence are unreliable as indicators of changes in the phophorylation state of leaf adenylates. At the present state of information, their usefulness is limited to changes in steady-state situations in air (changes in light intensity and composition of the gas atmosphere during continued illumination). Under nitrogen, light scattering appears to indicate mainly the phosphorylation state of chloroplast adenylates. Even so, the use of scattering and fluorescence measurements gives easy access to information on the energy state of green cells that otherwise is very difficult to obtain.

Acknowledgments

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