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Photosynthetic control, "energy-dependent" quenching of chlorophyll fluorescence and photophosphorylation under influence of tertiary amines

Henrik Laasch and Engelbert Weis
Botanisches Institut der Universität, Universitätsstr. 1, D-4000 Düsseldorf, F.R.G.

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Abstract

The effects of the tertiary amines tetracaine, brucine and dibucaine on photophosphorylation and control of photosynthetic electron transport in isolated chloroplasts of *Spinacia oleracea* were investigated. Tertiary amines inhibited photophosphorylation while the related electron transport decreased to the rates, observed under non-phosphorylating conditions. Light induced quenching of 9-aminoacridine fluorescence and uptake of ^{14}C-labelled methylamine in the thylakoid lumen declined in parallel with photophosphorylation, indicating a decline of the transthylakoid proton gradient. In the presence of ionophoric uncouplers such as nigericin, no effect of tertiary amines on electron transport was seen in a range of concentration where photophosphorylation was inhibited. Under the influence of the tertiary amines tested, pH-dependent feed-back control of photosystem II, as indicated by energy-dependent quenching of chlorophyll fluorescence, was unaffected or even increased in a range of concentration where 9-aminoacridine fluorescence quenching and photophosphorylation were inhibited. The data are discussed with respect to a possible involvement of localized proton flow pathways in energy coupling and feed-back control of electron transport.

Abbreviations; 9-AA – 9-aminoacridine, J_e – flux of photosynthetic electron transport, PC – photosynthetic control, pH_l – H^+ concentration in the thylakoid lumen, pmf – proton motive force, Φ_P – potential quantum yield of photochemistry of photosystem II (with open reaction centers), Q_A – primary quinone-type electron acceptor of photosystem II, q_Q – photochemical quenching of chlorophyll fluorescence, q_E – energy-dependent quenching of chlorophyll fluorescence, q_{AA} – light-induced quenching of 9-amino-acridine fluorescence

Introduction

During a light-induced energization of chloroplasts, protons are pumped across the thylakoid membranes, creating an electrochemical potential difference which is thought to contribute to the driving force of photophosphorylation (Junge and Jackson 1982, Mitchell 1974). The related acidification of the thylakoid lumen is assumed to counteract the proton pumping reactions at the plastoquinone/plastohydroquinone shuttle, thereby controlling the rate of electron donation to PS I (Crofts and Wraigth 1983, Kobayashi et al. 1979, Velthuys 1982). This control mechanism is referred to as photosynthetic control (PC). However, despite this control step downstream to PS II, the built-up of PC is accompanied only by a slight increase of the steady-state level of reduced PS II acceptors, Q_A^-, suggesting a regulation of electron donation by PS II (Weis and Berry 1987; Weis and Lechtenberg 1988; Weis et al. 1987).

There is now ample evidence that PS II can be

regulated by a mechanism of non-photochemical dissipation of absorbed excitation energy and that this regulation is reflected in energy-dependent quenching of Chl *a* fluorescence (Briantais et al. 1979; Horton et al. 1988; Krause and Laasch 1987; Krause et al. 1988; Weis and Berry 1987; Weis and Lechtenberg 1988; Weis et al. 1987). With rising intrathylakoid acidification, photochemically high-efficient PS II centers may be converted to a state, where energy is still trapped but dissipated by a non-radiative, non-photochemical decay (Krause et al. 1988; Weis and Berry 1987; Weis and Lechtenberg 1988; Weis et al. 1987). The potential of this "quenching" mechanism to control the electron transport has been demonstrated for intact plants (Weis and Berry 1987) and isolated chloroplasts (Krause et al. 1988). Both PC and the generation of low-efficiency PS II centers may be regarded as part of a negative feed-back control, mediated via intrathylakoid acidification and adjusting the potential rate of photochemistry to the energy requirement of carbon metabolism. When the steady-state transthylakoid H^+ potential and the intrathylakoid H^+ concentration are relatively low, as during fast ATP-consumption, a release of this feed-back control is expected.

In a previous study, we investigated the relationship between the transthylakoid H^+ gradient, photophosphorylation and PC by means of different uncouplers (Laasch and Weis 1988). Protonophores like nigericin cause uncoupling of photophosphorylation from H^+ translocating reactions concomitant with a release of PC. However, using the tertiary amine 2-butoxy-*n*-(2-diethylaminoethyl)-4-quinolinecarboxyamide, dibucaine, we demonstrated that a "selective" uncoupling of photophosphorylation is possible: dibucaine inhibited photophosphorylation, accompanied by inhibition of light-induced uptake of H^+ into the thylakoid lumen, suggesting a decline in the proton motive force. However, unlike uncoupling with nigericin, dibucaine did not cause a release from PC (Laasch and Weis 1988).

In this study, we will examine the influence of several amines, different in chemical structure and molecular size, on pH-dependent photosynthetic control and on regulation of PS II, as indicated by "energy-dependent" quenching of Chl fluorescence. Under the influence of dibucaine, light-induced quenching of 9-AA fluorescence and accumulation of ^{14}C-labelled methylamine in thylakoid vesicles was related to photochemical (q_Q) and non-photochemical quenching of Chl fluorescence (q_E). While all tertiary amines studied here, have the potential to lower the driving force of ATP-synthesis, acting in this respect like uncouplers, pH-dependent PC and q_E-quenching of fluorescence were rather insensitive to these substances.

Materials and methods

Chloroplasts were prepared from 5–7 week-old, freshly harvested leaves of *Spinacia oleracea* cv. Monatol. The leaves were illuminated with white light (150 $\mu E\,m^{-2}\,s^{-1}$, Q) for 10–15 min, prior to rupture. The isolation procedure is described in (Laasch and Weis 1988). Until use, intact, isolated chloroplasts were stored at 0°C in the dark. Measurements were carried out at 20°C. The standard reaction medium contained 0.33 M sorbitol, 10 mM KCl, 1 mM ethylenediaminetetraacetic acid, 1 mM $MgCl_2$ and 10 mM N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid, adjusted to pH 7.6 with KOH.

Electron transport

The flux of electron transport (J_e) in intact (type-A, Hall 1972) chloroplasts was measured in standard reaction medium with additions of 4 mM KH_2PO_4 and 2000 U ml^{-1} catalase (EC 1.11.1.6.) and calculated from oxygen evolution. In osmotically ruptured chloroplasts (type-D), 5–20 μM methylviologen, in the presence of 0.5 mM NaN_3 for suppression of catalase activity, served as an electron mediator and J_e was calculated from oxygen consumption. Red actinic light, driving electron transport, was filtered through RG 630 (Schott, FRG) and heat absorbing filters.

9-Aminoacridine fluorescence and methylamine uptake

9-Aminoacridine (9-AA) was added to a concentration of 5 μM. Its fluorescence was excited by light of 400 nm and was detected at 456 nm. Light-induced fluorescence quenching was quantified by a coef-

ficient $q_{AA} = \Delta F/(F - \Delta F)$ (ΔF = quenched fluorescence in the steady-state during illumination; F = maximum fluorescence in the dark), reflecting the ratio of amine bound by chloroplasts over free amine. The measured fluorescence signal was corrected for a small contribution of fluorescence light, emitted by dibucaine (Vanderkooi 1984). Methylamine uptake was assayed simultaneous to 9-AA fluorescence quenching. Methylamine was ^{14}C labelled with a specific activity of 51.8 mCi/mmol, its final concentration was 1 μM. Light-dependent uptake of methylamine in the thylakoid lumen was determined after centrifugation of thylakoids through a layer of silicon oil, as described in Heldt (1980).

Photophosphorylation

Photophosphorylation was measured with type-D chloroplasts, simultaneous with electron transport and 9-AA fluorescence, in a reaction medium which differed from the standard medium by containing 4 mM $MgCl_2$ and 5 mM KH_2PO_4. The irradiance was 2000 $\mu E\,m^{-2}\,s^{-1}$, Q. Phosphorylation was started by addition of 0.5 mM ADP. ATP was determined enzymatically by use of glucose-6-phosphatedehydrogenase (EC 1.1.1.49).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured with type-A (intact) chloroplasts, at room temperature, simultaneous to electron transport and 9-AA fluorescence, using a pulse modulation fluorometer (PAM 100, Walz, FRG) as described in Schreiber (1986). The major components of fluorescence quenching, i.e., energy-dependent (q_E) and photochemical quenching (q_Q), were determined following a modified method of "light-doubling" (Bradbury and Baker 1981; Schreiber et al. 1986). Basal fluorescence (F_o) was induced by weak measuring light pulses ($> 0.5\,\mu E\,m^{-2}\,s^{-1}$, integrated Q). Subsequently, maximum Chl fluorescence in the "dark-state" was obtained during a 2 s, saturating light-pulse of 2500 $\mu E\,m^{-2}\,s^{-1}$ (OG 590, Schott, FRG). Thereafter, steady-state J_e, 9-AA and variable Chl fluorescence (F_v = total fluorescence $- F_o$) were established by continuous illumination. The 2 s light-pulse was then repeated to reveal the maximum variable Chl fluorescence in the steady-state $(F_v)_{ms}$. To achieve a rapid decline of the steady-state fluorescence to a slightly lower, secondary basal fluorescence, F_o' (F_o quenching, see Bilger and Schreiber (1986)), the continuous illumination was switched off in the presence of a weak far-red light, filtered through RG 9 and PAL 720 (Schott, FRG). For calculations of fluorescence quenching parameters, F_v and $(F_v)_{ms}$ were then related to F_o'. The quenching parameters were quantified as $q_E = 1 - (F_v)_{ms}/(F_v)_m$ and $q_Q = 1 - (F_v)/(F_v)_{ms}$. When leaves had been preilluminated prior to the chloroplast isolation, the continuous illumination did not cause significant quenching of fluorescence related to enzymatic phosphorylation of the light harvesting complex (not shown). In the presence of 0.3 μM nigericin, this quenching was < 0.05.

The dependence of Chl fluorescence on the pH of the chloroplast suspension medium was studied in the presence of 0.5 μM nigericin and 10 mM Na-acetate. The chloroplasts were preincubated for 3 min at various pH and subsequently illuminated with a 4 s light-pulse. Since there was no effect on $(F_v)_m$ at pH > 7, a coefficient for pH-induced quenching was defined as $q_H = 1 - (F_v)_{mn}/(F_v)_{m7}$, where $(F_v)_{mn}$ is the maximum fluorescence at the pH adjusted.

Results

The effects of nigericin and of the tertiary amine tetracaine on photophosphorylation, phosphorylating electron transport and quenching of 9-AA fluorescence (q_{AA}) are shown in Fig. 1. Nigericin inhibited photophorphorylation in correlation with q_{AA}. At concentrations, where photophosphorylation is completely inhibited, electron transport measured in the presence of ADP and P_i was stimulated to its maximum rate. This reflects a release from PC as expected during "classical" uncoupling (McCarty 1977).

Photophosphorylation and q_{AA} were also inhibited by tetracaine. However, as reported for dibucaine (Laasch and Weis 1988), the inhibition was not accompanied by stimulation of electron transport. Instead, the flux of electron transport was lowered to the level observed under non-

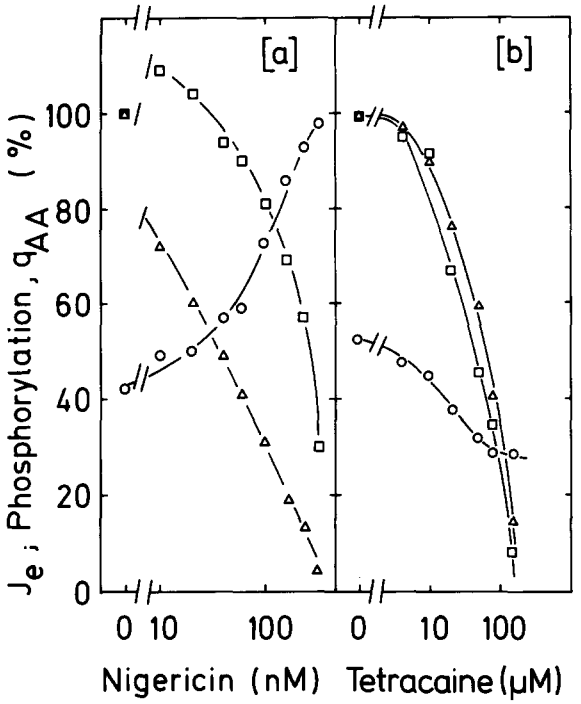

Fig. 1. Effects of nigericin (a) and tetracaine (b) on steady-state q_{AA} (△), phosphorylating electron transport (○) and photophosphorylation (□) in type-D chloroplasts. Electron transport was mediated by methylviologen. The irradiance was 2000 μE m^{-2} s^{-1}. 100% activity corresponds to a) 263 and (b) 318 μmol ATP synthesized mg^{-1} Chl h^{-1}. 100% q_{AA} is equal to (a) 0.91 and (b) 0.96.

phosphorylating conditions (absence of ADP). Addition of nigericin to tetracaine-treated chloroplasts caused a stimulation of J_e to the rate observed in the presence of nigericin alone. This indicates that in a range of concentration where tetracaine inhibited phosphorylation, PC of electron transport was maintained and electron transfer was not inhibited *per se*. Insofar, tetracine resembles an energy transfer inhibitor.

However, different to what is expected for energy transfer inhibitors, tetracaine and other amines such as dibucaine, cause a decline of the proton motive force, as indicated by the next experiment. Although the mechanism leading to light-induced quenching of 9-AA fluorescence is still a matter of discussion (Kraayenhof and Arents 1977; Schuldiner et al. 1972; Van et al. 1987), we used the 9-AA approach to follow the "energy status" of the thylakoid membrane. Additionally, the light-induced accumulation of ^{14}C-labelled methylamine was probed to indicate the uptake of protons into the thylakoid lumen (Rottenberg et al. 1972). This, in comparison to tetracaine and dibucaine, small amine molecule is assumed to distribute between the thylakoid lumen and the suspension medium according to the H^+ concentration in the two compartments (Rottenberg et al. 1972). In Fig. 2, the effect of dibucaine on the accumulation of ^{14}C-labelled methylamine and 9-AA (calculated from fluorescence quenching) by thylakoid vesicles are compared. Both, methylamine and 9-AA accumulation, are expressed as the ratio between amine accumulated in the thylakoid vesicles and free amine in the medium. With rising concentration of dibucaine, 9-AA as well as methylamine uptake were inhibited in parallel. A similar result was obtained when thylakoid membranes were gradually uncoupled with nigericin. This indicates that both, methylamine and 9-AA accumulation are inhibited by dibucaine, and that the decline of q_{AA} is not based on a specific interaction of tertiary amines with 9-AA.

Using type-A (intact) chloroplasts, we compared the effects of nigericin and the tertiary amine dibucaine on J_e, 9-AA and Chl fluorescence quenching

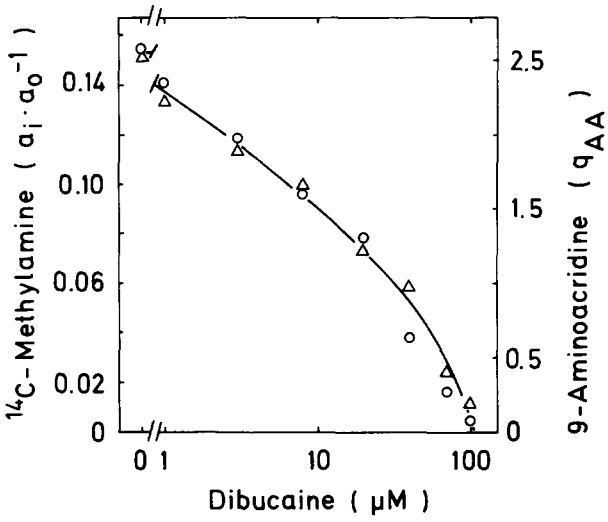

Fig. 2. Light-induced binding of 9AA and ^{14}C-methylamine in type-D chloroplasts, under constant light (600 μE m^{-2} s^{-1}), in dependence of the concentration of dibucaine. The concentration of ^{14}C-methylamine was 1 μM, that of 9AA, 5 μM. Binding of 9AA (△) is expressed in terms of q_{AA} ($=\Delta F/(F - \Delta F)$) and binding of ^{14}C-methylamine (○), in analogy, as the ratio of bound (a^i) over free (a_o) methylamine.

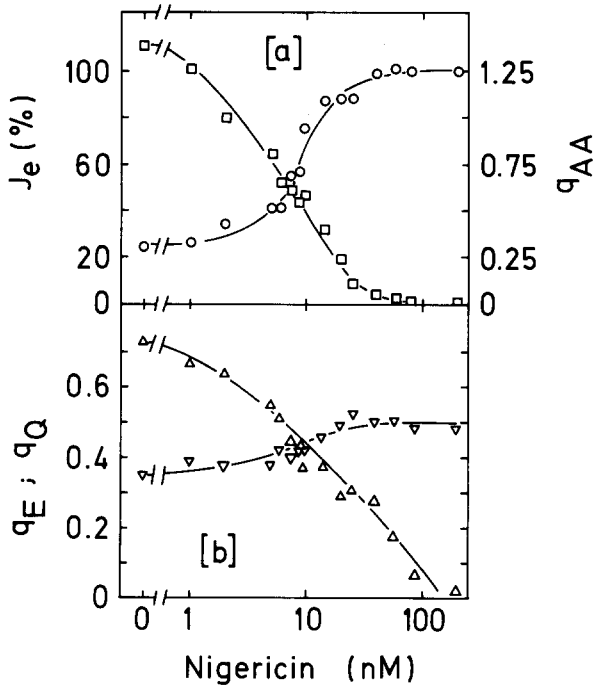

Fig. 3. Effects of the concentration of nigericin on (a) q_{AA} (□), J_e (○) and (b) on q_Q (▽) and q_E (△), under constant light ($75\,\mu E\,m^{-2}\,s^{-1}$) in type-A chloroplasts. Electron transport was mediated by NO_2^- reduction and was measured as O_2 evolution. 100% J_e corresponded to 95 μmol O_2 evolved mg^{-1} Chl h^{-1}.

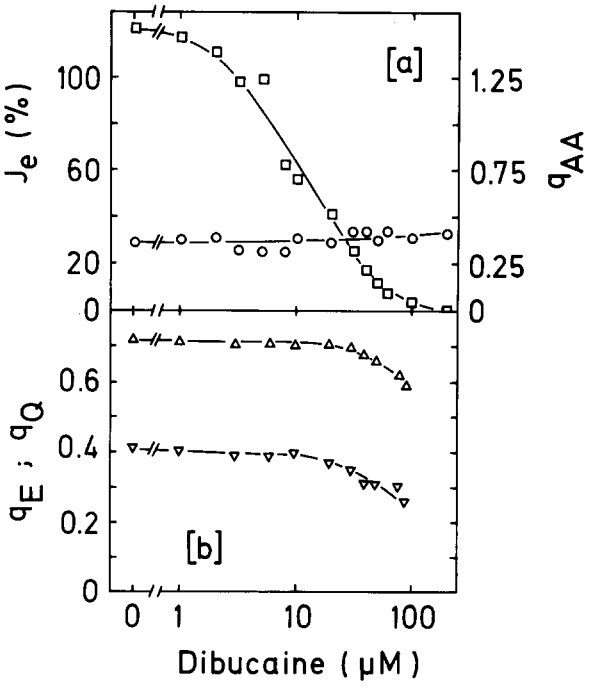

Fig. 4. Effects of the concentration of dibucaine on (a) q_{AA} (□), J_e (○) and (b) q_Q (▽) and q_E (△)) in the presence of constant light flux density. Experimental conditions as in Fig. 3. 100% J_e correspond to 105 μmol O_2 evolved mg^{-1} Chl h^{-1}.

under non-phosphorylating conditions. Similar to what has been observed in type-D chloroplasts (Fig. 1), J_e increased as q_{AA} and q_E decreased with rising concentrations of nigericin (Fig. 3(a)). Maximum J_e was obtained at concentrations where q_{AA} was close to zero. Similar observations have been made with gramicidin D (see also Fig. 6). The incoupler-induced stimulation of electron flux was accompanied by a slight, but significant increase in the coefficient for Q_A-dependent Chl fluorescence quenching, q_Q (Fig. 3(b)). This indicates a decline in the steady-state level of Q_A^-, as may be expected when control of electron transport downstream ofQ_A is released. The uncoupler effect on the reduction level of Q_A may partially be compensated by a simultaneous release in control of PS II activity (Weis and Berry 1987).

Different to the effect of nigericin, control of electron transport and q_E-quenching under non-phosphorylating conditions were not or only slightly affected by dibucaine in a range of concentration where complete decline of q_{AA} (Fig. 4) and photophosphorylation occurred (not shown). Again, addition of nigericin caused a stimulation of electron transport to its maximal rate, independent on the concentration of dibucaine present. The data suggest, that not only the flux control of electron transport (PC), but also the q_E-related control of PS II remains unaffected by dibucaine up to a concentration of 100 μM.

In Fig. 5, quenching of variable Chl fluorescence in type-A chloroplasts is related to the pH of the suspension medium. It was demonstrated earlier, that reversible fluorescence quenching occurs when the pH of the chloroplast suspension medium was sufficiently low. This quenching was assumed to be equivalent to q_E-quenching caused by light-induced acidification of the thylakoid lumen (Briantais et al. 1979). Here, 50% quenching of the variable fluorescence was obtained at pH 4.6. The "titration curve" of fluorescence quenching was not affected by 50 μM dibucaine.

When q_E, determined in experiments as shown in Figs. 3 and 4, was plotted against q_{AA}, relatively

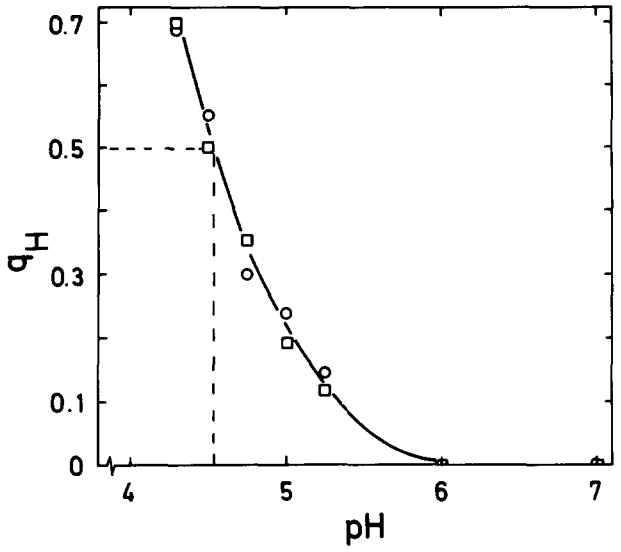

Fig. 5. Dependency of Chl fluorescence quenching in type-A chloroplasts in absence (○) and presence (□) of 50 μM dibucaine, on the pH in the thylakoid lumen, as imposed by the pH of the chloroplast suspension medium in the presence of 10 mM Na-acetate and 0.5 μM nigericin. The chloroplasts were incubated at the indicated pH for 3 min prior to the determination of fluorescence quenching. q_H was defined as $1 - (F_v)_{mn}/(F_v)_{m7}$, where n is the indicated pH.

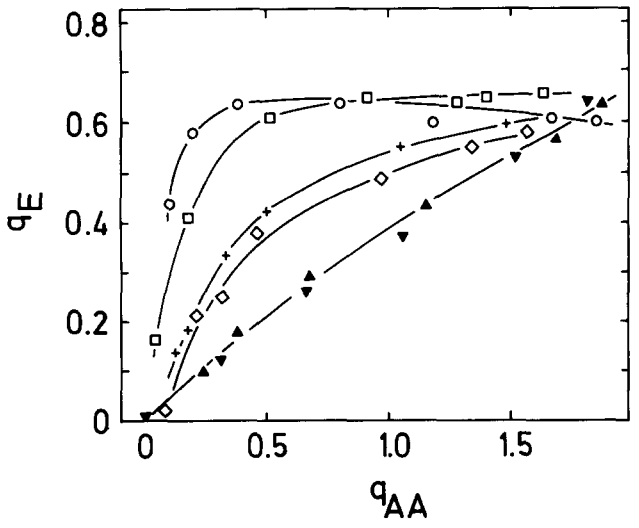

Fig. 6. Relationship of q_E-quenching and q_{AA} in type-A chloroplasts under constant irradiance (75 $\mu E\,m^{-2}s^{-1}$), in the presence of increasing concentrations of nigericin (▲), gramicidin D (▼), NH_4Cl (◇), tryptamin (+), dibucaine (○) and brucine (□). Electron transport was mediated by NO_2^- reduction.

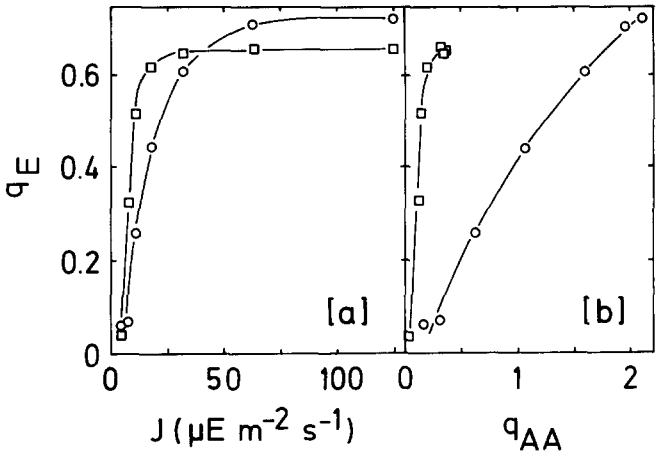

Fig. 7. Dependence of q_E-quenching in absence (○) and presence (□) of 50 μM dibucaine, on light flux density (a) and q_{AA} (b), as varied by light flux. q_{AA} was assayed simultaneous to q_E-quenching.

strong correlation was obtained when nigericin or gramicidin D were used as uncouplers. In contrast, a large change in q_{AA} was related to only small changes in q_E when dibucaine or 10,11-dimethoxystrychnin (brucine) were used (Fig. 6). It indicates that in the presence of tertiary amines, q_E-quenching is almost independent on "thylakoid energization", as indicated by q_{AA} or methylamine uptake. When ammoniumchloride or tryptamine were used, a relationship between q_E and q_{AA}, intermediary between those obtained with ionophoric uncouplers or tertiary amines, was obtained (see also Laasch and Weis (1988)).

The light responses of q_E and q_{AA} as measured in the presence or absence of dibucaine are pictured in Fig. 7. We observed a significant increase of the initial slope of the light response of q_E when dibucaine was present (Fig. 7(a)). It suggests that the amine may even enhance the quantum yield of q_E formation. A rather parallel increase in q_E and q_{AA} was observed with untreated chloroplasts with increasing light flux (Fig. 7(b)). In the presence of dibucaine, however, q_{AA} remained low, while q_E increased to a high level.

In the steady state, the apparent photochemical yield is partly controlled by the level of Q_A reduction: PS II centers associated with Q_A^- are incapable to undergo photochemical energy conversion. Since photochemical quenching of Chl fluore-

scence, q_Q, indicates the proportion of excitation energy at PS II consumed by "active" centers, we can calculate the potential photochemical yield of PS II, Φ_P, for different steady-state conditions following the equation: $\Phi_P = J_e/I\ q_Q$ (J_e, rate of electron transport, calculated from O_2 evolution; I, incident light flux). A rigorous treatment of this approach, allowing the analysis of the steady-state regulation of PS II, has been presented recently (Weis and Berry 1987).

In Fig. 8, Φ_p-values calculated from data shown in Fig. 4 are plotted versus q_{AA}. In the absence of uncouplers or amines (high q_{AA}), Φ_p was usually 60% lower than the maximum value, obtained in the presence of 0.5 μM nigericin, when q_{AA} was close to zero. A strong correlation of Φ_p and q_{AA} was observed when nigericin served as an uncoupler. However, when q_{AA} was inhibited by dibucaine, Φ_P remained relatively low. Addition of 0.3 μM nigericin to samples, already containing dibucaine, always revealed the maximum Φ_P values, as obtained in the presence of nigericin alone. When brucine was used instead of dibucaine, Φ_P was even less affected than by dibucaine (Fig. 8). The data show that tertiary amines do not affect pH-dependent regulation of PS II.

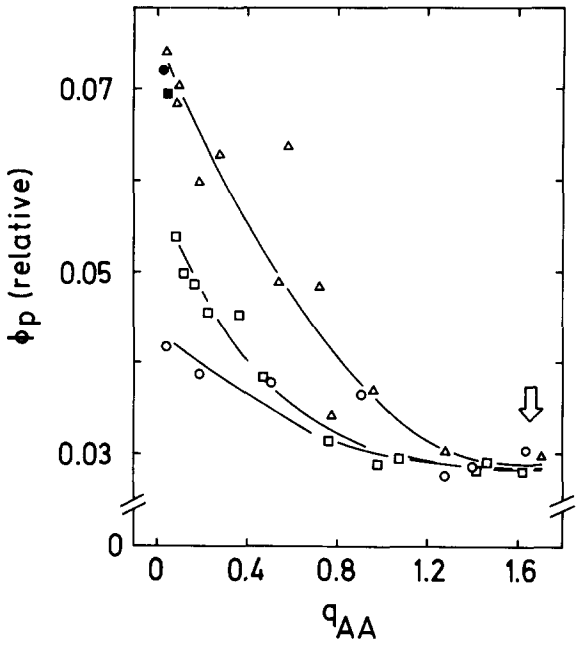

Fig. 8. Dependency of Φ_P, the steady-state quantum yield of PS II centers with oxidized q_A, on q_{AA}, in the presence of different concentrations of nigericin (△), dibucaine (□) and brucine (○) under constant light (75 $\mu E\,m^{-2}\,s^{-1}$) in type-A chloroplasts. Φ_P was calculated from J_e, the incident light flux J_L, and q_Q-quenching, following the equation: $\Phi_P = J_e/J_L \cdot q_Q$. J_e is given in relative units. Control values without additions of uncouplers are indicated by a downward arrow. Maximum additions were 0.5 μM nigericin, 100 μM dibucaine and 1 mM brucine. Values of Φ_P in presence of 0.5 μM nigericin additionally to 100 μM dibucaine (■) and 1 mM brucine (●) are shown.

Discussion

In the present study, we used the fluorecent amine 9-AA as an indicator for the light-induced "energization" of thylakoid vesicles. In dependence of the energization, 9-AA is trapped by the vesicles and thereby its fluorescence is quenched. As alternative hypotheses for explanation of 9-AA fluorescence quenching, i) an accumulation of protonated amine in the thylakoid lumen, reflecting different H^+ concentrations inside and outside the thylakoid vesicles (Schuldiner et al. 1972) and ii) an attraction of positively charged amine by negative charges on the surface of energized membranes (Kraayenhof and Arents 1977) have been discussed. Independent on the underlying mechanism, a strong empirical correlation between 9-AA fluorescence quenching and the phosphate potential (Strotman and Lohse 1988) supports the suggestion that 9-AA indicates a proton potential driving ATP-synthesis.

To exclude possible artifacts of the 9-AA signal due to direct interactions between 9-AA and tertiary amines, we compared the data from the 9-AA approach with that of ^{14}C-methylamine uptake by thylakoid vesicles. According to Portis and McCarty (1976) and Rottenberg et al. (1972) small hydrophilic amine molecules do not directly interact with constituent parts of the membranes. Their uptake into thylakoid vesicles is thought to be determined by internal and external proton concentrations, as indicated by the equation: $[R\text{-}NH_3^+]_{in}/[R\text{-}NH_3^+]_{out} = [H^+]_{in}/[H^+]_{out}$ (Portis and McCarty 1976; Rottenberg et al. 1972).

The similarities in the response to dibucaine, of q_{AA} and ^{14}C-methylamine uptake (Fig. 2) indicate that under the experimental conditions used here, specific artifacts related to the 9-AA approach are of minor importance. Nevertheless, the data

presented may not rule out the possibility that 9-AA fluorescence quenching as well as ^{14}C-methylamine uptake, are infact no direct indicators for the formation of a proton gradient between the bulk aqueous phases in and outside the thylakoid membranes. They may rather be indicators of an energetized state of the membrane, which is produced during the build-up of ΔpH and released either by ATP-synthase activity or by influence of dibucaine.

When light becomes saturating, the pH in the bulk phase of thylakoid vesicles (pH_l) presumably declines to values between 5 and 4 (Heldt and Sauer 1971). Only under this condition, strong q_E-quenching and PC occurs (Weis and Lechtenberg 1988; Weis et al. 1987). Following the pH titration of Chl fluorescence quenching (Fig. 5), significant quenching is expected to occur below pH 5. The maintenance of q_E-quenching in the presence of dibucaine may point out that despite of the decline of q_{AA} and ^{14}C-methylamine uptake, the high proton concentrations necessary for induction of q_E quenching are maintained in the thylakoid lumen. The opposite effect was shown by Oxborough and Horton (1987). They demonstrated that q_E-quenching may be inhibited by antimycin A at concentrations where the proton gradient, as measured by 9-AA fluorescence, was not inhibited. Apparently, only "classical" ionophoric uncouplers like nigericin, inhibit "membrane energization" in a non-selective way, by decreasing both, the driving force of ATP-synthesis and the proton potential controlling q_E-quenching. This rises the question how the protons driving ATP-synthesis and "energy-dependent" control mechanisms are related to each other.

Specific inhibition of photophosphorylation under maintenance of PC and q_E-quenching may be explained by either of the following mechanisms: i) inhibition of the phosphorylating reaction itself without acceleration of H^+ efflux (energy transfer inhibition), ii) amine-induced discharge of the bulk-to-bulk electrochemical proton potential difference with a related inhibition of photophosphorylation, but conservation of a "local" H^+ potential in charge of control mechanisms, and iii) selective discharge of a local H^+ potential involved in ATP synthesis without direct effect on the bulk-to-bulk H^+ potential difference.

The first mechanism may be ruled out as direct inhibition of the ATP synthase should be accompanied by an increase, and not be a decrease of q_{AA} and methylamine uptake, as observed (Figs. 1 and 2). Rather, the presented data may be explained by assuming that protons pumped into the thylakoid lumen may enter domains which are unequivalent in function. It has been argued frequently that the distribution of protons within the lumen of energized thylakoids is not necessarily homogenous (for review see Ferguson (1985)). According to this view, the bulk phase is separated from the electrostatic boundary layer at the membrane surface. Surface exposed protein groups tend to enlarge the boundary layer, thereby creating electrostatic 'pockets' and sequestered proton domains (Plesner and Michaeli 1974). The proton exchange between local domains and the bulk phase may to a certain degree, be restricted (Laszlo et al. 1984).

A functional proton domain may be created by a specific effect of tertiary amines on the sites of pH-dependent control at the cytochrome *b/f* complex and at PS II: Tertiary amines may hinder the release of H^+ from the sites of proton pumping. As a result, the creation of a pmf would be inhibited while accumulation of H^+ in the vicinity of proton pumping reactions and of control mechanisms, respectively, would even be stimulated. This agrees with the observation that the formation of q_E-quenching in low light was even enhanced by tertiary amines.

It has also been proposed that energy transduction at the ATP-synthase complex is mediated by a local proton potential. Concepts of direct proton cycling between redox components and the ATP synthesizing complexes have been developed recently (Kraayenhof et al. 1986; Laszlo et al. 1984). Even an intra-membrane proton transmission has been postulated (Laszlo et al. 1984). In these concepts, the bulk ΔpH is a side product of a short-range proton cycling, rather than the primary driving force of phosphorylation. Rottenberg (1983), working with mitochondria, based his analysis of energy transduction on the idea that phosphorylation is forced by an electrochemical bulk-to-bulk gradient (Mitchell 1974), but extended this concept by assuming that H^+ are transmitted from the bulk phase to the coupling factor via a local "proton channel". He suggested that substances like "general anesthetics" selectively discharge these "proton channels", thereby inhibiting phosphorylation without inducing a

collapse of the bulk ΔpH. While ionophoric uncouplers probably discharge all H^+ gradients and domains in a non-specific way, complex amines may equilibrate with the different phases more selectively and may even affect the H^+ exchange between these phases. Further discussion of dibucaine effects on ATP-synthesis is presented elsewhere (Laasch and Weis 1988).

Ammoniumchloride is supposed to give rise to an increase of pH_l. As expected, it thereby reduces q_{AA} and the ATP/e_z ratio (Giersch 1981) and, to a certain degree, causes a release of pH-dependent q_E-quenching (Fig. 6). However, ammoniumchloride is not an efficient uncoupler. In isolated thylakoids, it required concentrations > 4 mM for a complete inhibition of q_{AA} and inhibition of photophosphorylation (data not shown). In contrast, the tertiary amines studied inhibited q_{AA} and ATP-synthesis at concentrations, 20 to 200 times lower than those required for NH_4Cl. This points to a more specific effect of tertiary amines on proton exchange and energy transduction. Although the mechanism of action of tertiary amines it still far from clear and needs further examination, it is yet evident that their use provides a tool for investigation of energy transduction and electron transport control in chloroplast membranes, as well as more generally, of amine effects on biomembranes.

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