Interaction of Quaternary Ammonium and Phosphonium Salts with Photosynthetic Membranes

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Distinct concentration ranges of selected quaternary ammonium and phosphonium salts were elaborated to induce stimulatory or inhibitory effects, respectively, on photosynthetic reactions. By means of fluorescence induction measurements 3 different effects of alkylbenzyldimethylammonium chloride (ABDAC; zephirol) in chloroplast preparations from *Pisum sativum* were observed. 60 μM ABDAC produced a strong increase in F_{max} with concurrently improved Kautsky kinetics. Increased ABDAC concentration (500 μM) led to a strong fluorescence quenching – virtually indistinguishable from the conditions following the addition of photosystem II electron acceptors like $K_3Fe[CN]_6$. Further increase of ABDAC to 5 mM provoked a drastic increase in the fluorescence yield together with the complete loss of any detectable kinetics. We suggest a 3-step interaction of ABDAC with the thylakoid membranes of photosynthetic organisms similar to our earlier discussion (Bader and Höper (1993), Z. Naturforsch. **49 c**, 87–94). We examined a series of derivatives with selectively modified side chains, central atoms and counter ions, respectively. Both an alkyl chain of the type ($[-CH_2-]_n$; $n \sim > 10$) and effective polar components are indispensable for the adsorption and intercalation of the molecule onto and into the thylakoid membranes. The benzyl group could be replaced by a methyl residue without any loss of effectiveness; replacement of the central nitrogen (N) by phosphorus (P) and the counter ion Cl by Br did not modify the effects and the results were indistinguishable from the ABDAC effect proper. Shortening of the alkyl chain to $(-CH_2-)_6$ resulted in a less effective interaction of *e.g.* tetraoctylammonium bromide with the photosynthetic membrane. Flash-induced oxygen evolution measurements with selected derivatives (15 μM) substantiated our interpretation of an improved OEC functioning by a substantial lowering of the miss parameter α and the exclusion of a chemical reduction as the standard S-state distribution was not affected. As evidenced by both SDS-PAGE and Western blot analyses the investigated molecules showed a direct interaction. The polypeptide patterns were characterized by a severe shift of the molecular weight components from high (20–67 kDa) to low (< 20 kDa) values.

Introduction

Scientific investigations on the interaction of herbicides have provided important insights into mechanistic details of plant physiological mechanisms not least in the regions of photosystem II and the biosynthesis of pigments (*e.g.* Berard and Pelte, 1999; Böger, 1998; Böger and Sandmann, 1993; Gleiter *et al.*, 1992; Bader and Schmid, 1980). Different ('reverse') approaches dealt with the questions after the effects of specific herbicidal compounds on the physiology of selected plants with the background of developing products of less general phytotoxicity in field studies and for industrial purposes (*e.g.* Zwerger and Pestemer, 2000; Bollich *et al.*, 2000; Bradley *et al.*, 2000). Still today little is known about the effects of fungicides, insecticides or generally biocides on plants, although many of them come into more than close contact with plants, as they are routinely applied as plant protective chemicals *e.g.* in modern agriculture. It should be kept in mind that the integrity of the cells, the cell walls, cutin layers *etc.* represent an effective but by far not perfect protection against exogenous substances. Chemicals might well 'invade' the plant *via* physiological/artificial cell openings like stomata, injuries, cuttings *etc.* An improved knowledge of the detailed effects of insecticides, fungicides, formulation additives *etc.* on photosynthetic membranes is essential for plant

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physiological research and might also be helpful in applied fields *e.g.* with respect to the 'design' of modern plant protective chemicals.

The few existing publications on the effects of quaternary ammonium salts on higher plants, algae and cyanobacteria reported on the inhibition of electron transport reactions in mitochondria from potato (Pireaux *et al.*, 1991), inhibitory effects on both *Chlorella vulgaris* and spinach chloroplasts as evidenced by growth, chlorophyll synthesis and the Hill reaction rates described by Kralova *et al.* (1992), uncoupling of photophosphorylation in chloroplasts (Gross, 1972) and the specific effects of alkylbenzyldimethylammonium chloride (ABDAC, zephirol) on the flash-induced oxygen evolution pattern (Bader, 1989). A positive effect of low concentrations of quaternary ammonium salts on the photosynthetic oxygen evolution was also described by Sersen and Devinsky (1994). Moreover, Bader and Höper (1994) investigated the relaxation kinetics of photosynthetic absorption changes which could be correlated to the oxygen evolution experiments with respect to an improved functioning of photosystem II complexes and/or a decreased transition parameter α (misses).

The present paper elucidates the significance of structural components of the molecules for the physiological effects by assaying selected derivatives and describes further details of the effects of ABDAC on oxygen evolution and fluorescence characteristics of photosynthetic membranes.

Materials and Methods

Materials

Higher plants: Cell cultures from tomato (*Lycopersicon peruvianum*) were originally obtained from Prof. Dr. H.-P. Mühlbach, Institut für Genetik, Universität Hamburg), cultivated and prepared according to Stöcker *et al.* (1993) and Hüsemann and Barz (1977) in an artificial gas atmosphere enriched with 2% CO_2 (v/v) at room temperature. Measurements of physiological and photosynthetic activity of tobacco cell cultures from this line have been described by Bader and Schüler (1996). Chloroplast preparations from tobacco (*Nicotiana tabacum* var. John William's Broadleaf) were isolated by mechanical homogenization of leaflets, centrifugation and suspension in sucrose containing buffers according to Homann and Schmid (1967).

Cyanobacterium: The filamentous non-heterocystous cyanobacterium *Oscillatoria chalybea* originated from the 'Sammlung von Algenkulturen', Institut für Pflanzenphysiologie, Universität Göttingen (Germany) and was cultivated in an air-conditioned room (26 °C). Details of the cultivation and the preparations have been described (Bader *et al.*, 1983). Protoplast suspensions were obtained by mechanic homogenization of the filaments followed by enzymatic treatments with glucuronidase, cellulase and lysozyme according to Bader *et al.* (1983).

Quaternary ammonium and phosphonium salts (see Fig. 4) were purchased from MERCK-Schuchardt.

Methods

Fluorescence emission was measured in a laboratory-built set-up. The induction light was filtered through a blue plexiglas combined with a SCHOTT BG 28 filter. The emitted fluorescence was detected by means of an EMI photomultiplier Type 9658R under a high tension of 1000–1100 V. Emission curves were recorded on a 5115 storage oscilloscope from Tektronix equipped with a 5A22 N differential amplifier and a 5B12 N dual time base (Bader and Schüler, 1996).

Flash-induced oxygen evolution was measured on the 3-electrode-system laboratory-designed by Schmid and Thibault (1979). The set-up has been built by the 'Société d'Etude et de Construction d'Instruments Astronomiques' in Manosque (France). Flashes from stroboslave 1539A (General Radio) were 5 μs long at an intensity of 600 μE $\times$ $m^{-2} \times s^{-1}$ and fired with a frequency of 3.33 Hz. The signals from oxygen evolution were stored and processed using an Atari Mega ST4 computer with a laboratory-written calculation program (Schulder *et al.*, 1992). Flash amplitudes and/or 250 ms-integrals of the flash-induced oxygen evolution signals were taken as basis for *e.g.* calculation of the respective S-state distributions. The computer calculation program 'VOYONS' (Thibault and Thiéry, 1981; Thiéry, 1991) was used for the mathematical fit of the amplitude values to calculate the S-state dark distributions and the transition probabilities α, β and γ.

Effects on the polypeptide composition of *Oscillatoria chalybea* were investigated and characterized by SDS-Polyacrylamide gel electrophoresis (PAGE) in a 5% collection and a 13% separation gel modified after Laemmli (1970). Prior to electrophoresis, samples from *Oscillatoria chalybea* were incubated with different concentrations of the quaternary ammonium salt ABDAC for 20 h at room temperature in the dark. Electrophoresis was carried out a constant current of 30 mA for 5 h at 4 °C. The gels were stained with Coomassie Brilliant Blue.

Immunoblotting of proteins following the peptide separation by SDS-PAGE was carried out according to the Western Blot analysis described by Renart *et al.* (1979) using a polyclonal monospecific antiserum to the D1 (D2) polypeptide. The separated polypeptides were transferred to nitrocellulose membranes with pressure (2 kg/50 cm^2) for 20 h at room temperature. Specifically bound antibodies were stained by the reaction of peroxidase with hydrogen peroxidase and 4-chloro-1-naphthol from Sigma. Details of our immunological assays have been extensively described (Makewicz *et al.*, 1996; He *et al.*, 1996).

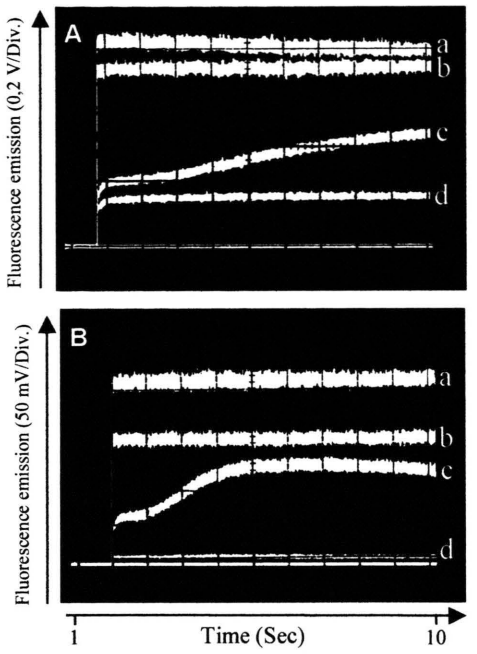

Fig. 1. Original oscilloscope recordings of the fluorescence induction curves in photosynthesis.
A) Chloroplasts from higher plants (*Nicotiana tabacum*). Effect of ABDAC, $K_3[Fe(CN)]_6$ and sodium dithionite on the fluorescence emission. a) 5.4×10^{-3} M ABDAC. b) crystals of sodium dithionite. c) control. d) 5.4×10^{-4} M ABDAC; same trace: 5×10^{-3} M $K_3[Fe(CN)]_6$.
B) Cell cultures from higher plants (*Lycopersicon peruvianum*). Effect of ABDAC on the fluorescence emission. a) 5.4×10^{-3} M ABDAC. b) 2.7×10^{-3} M ABDAC. c) control assay without ABDAC. d) cell-free buffer control with 5.4×10^{-3} M ABDAC.

Results and Discussion

Quaternary ammonium salt biocides can exert positive (stimulatory) effects on photosynthetic oxygen evolution and on absorption change characteristics of photosynthetic membranes under specific conditions and at relatively low concentrations (Bader, 1989; Sersen and Devinsky, 1994; Bader and Höper, 1994). The fact that the interaction of alkylbenzyldimethylammonium chloride (ABDAC or zephirol) with *e.g.* chloroplasts from higher plants might show complex effects (positive or negative – depending on the concentration) could be substantiated by measurements of the fluorescence emission. Fig. 1A shows the fluorescence induction in chloroplasts from *Nicotiana tabacum*. In the presence of 5.4 mM ABDAC no Kautsky effect was observed after short dark adaptation and F_{max} was nearly identical or even higher (Fig. 1A curve a) as in the presence of the standard chemical reductant sodium dithionite (Fig. 1A curve b). (From a strictly chemical point of vue a substantial reducing capacity of ABDAC can be virtually excluded and might at best be discussed for the benzyl group within the molecular structure.) Surprisingly, a concentration of 0.54 mM ABDAC yielded the same degree of fluorescence quenching as did 5 mM potassium hexacyano-III-ferrate (Fig. 1A curve d), a standard electron acceptor well-known to quench fluorescence by oxidizing the reduced photosystem II acceptor Q. The effect was principally reproduced using cell cultures from higher plants (Fig. 1B; tomato, *Lycopersicon peruvianum*). Generally, we observed that the fluorescence quenching was less pronounced what may be seen in context with a different accessibility of the photosynthetic membranes in whole plant cells in comparison to chloroplast preparations. On the other hand we observed a much stronger enhancement of F_m at high ABDAC concentrations of 10^{-2} M (Fig. 2).

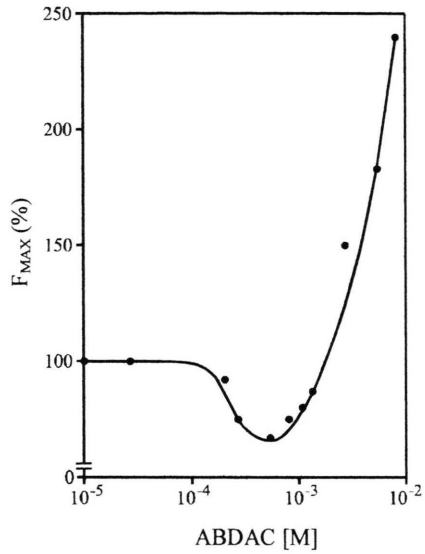

Fig. 2. Concentration dependence of the ABDAC-mediated modulation of the fluorescence emission maximum in a higher plant cell culture from tomato (*Lycopersicon peruvianum*). Measuring conditions as in Fig 1.

Consequently, we investigated a wide concentration range and found that even 3 distinct effects have to be distinguished in the case of chloroplast preparations (pea, *Pisum sativum*). For the results depicted in Fig. 3 we chose conditions with specifically unpronounced Kautsky kinetics of the control assay and observed a substantial improvement

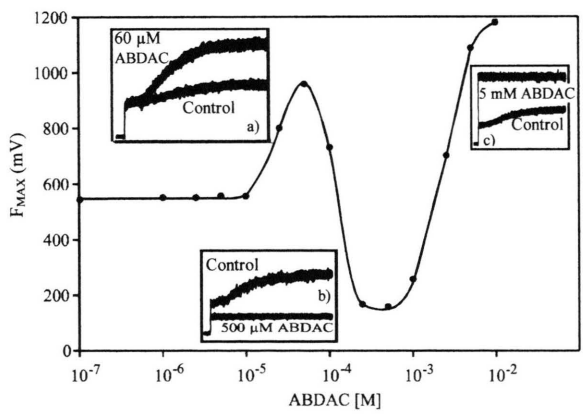

Fig. 3. Concentration dependence of the ABDAC-mediated modulation of the fluorescence emission maximum in a chloroplast preparation from pea (*Pisum sativum*). The insets show in addition 3 selected original tracings of the fluorescence emission curves at the respective ABDAC-concentation illustrating the ABDAC effect on the Kautsky kinetics.

of the kinetics *together with* an increase in the fluorescence yield at low concentrations of ABDAC ($< 10^{-4}$ M). Thus, the interaction of the chemical with the photosynthetic membranes might consist of one out of three different reactions depending on the respective concentration and these can be seen in context with the earlier given interpretations (Bader and Höper, 1994). From that point of view the initial adsorption of the molecules onto the membrane *via* the polar components appears to readily affect the functioning of the photosynthetic membranes and this might reflect the kinetics of inset (a) in Fig. 3. During a second phase of action the penetration of the alkyl chain into the inner lipophilic membrane regions results in a defined conformational change of the membranes and thus to an improved electron flow from the reduced PS II into PS I and this consequently results in the well-known quenching of the fluorescence yield (Fig. 3 inset b). In fact, with this concentration the original observation of an ABDAC-dependent improvement of the OEC-functioning had been described (Bader, 1989; Bader and Höper, 1993). At high concentrations, however, the continued and extended intercalation of ABDAC molecules might lead to a structural disturbance or even a separation of essential parts of the respective membrane so that the electron flow is impaired. The effect then consists of an increased fluorescence emission – the resulting structural modification leads to the same high fluorescence yield without any Kautsky effect (Fig. 3 inset c) as do high concentrations of photosystem II specific electron transport inhibitors like DCMU! In any case, no efficient re-oxidation of the reduced photosystem II acceptor Q can occur, no matter what the effect is based on – an electron transport inhibition, a highly concentrated chemical reductant like sodium dithionite or a 'destructive' modification of the photosynthetic membranes by ABDAC.

From the complexity of the results presented thus far we were led to deal with the question which structural component(s) of the molecule might be preponderently responsible for the described effects and which components appear to be dispensable without a loss of efficiency. Therefore, we selected a number of distinct but comparable chemicals with respect to specific structural specificities (length of the alkyl chain, counter ion, presence or absence of a benzyl group, modifica-

tion of nitrogen as central atom) and scrutinized the effects of the chemicals depicted in Fig. 4. The relevant modifications of the derivatives can be summarized as follows: BTMAC (derivative 1) contains no alkyl chain at all and in BTMAH (derivative 2) chloride is replaced by hydroxide as an additional modification. HDTMAC (derivative 3) contains an identical alkyl chain as ABDAC does but the benzyl group is omitted – identical to HDTMAB (derivative 5) what is a bromide instead of a chloride. The significance of BTBAC (derivative 4) consists of 3 relatively short (instead of 1 long) alkyl chain(s). TOAB (derivative 6) contains four intermediary alkyl chains and the central atom in TBHDPB (derivative 7) consists of phosphorus instead of nitrogen.

The first approach was the analysis of the fluorescence emission in chloroplasts from *Nicotiana tabacum* in the absence or in the presence of the selected derivatives (Table I line a). The concentration of the respective chemical was taken from the experiment shown in Fig. 1 where an identical

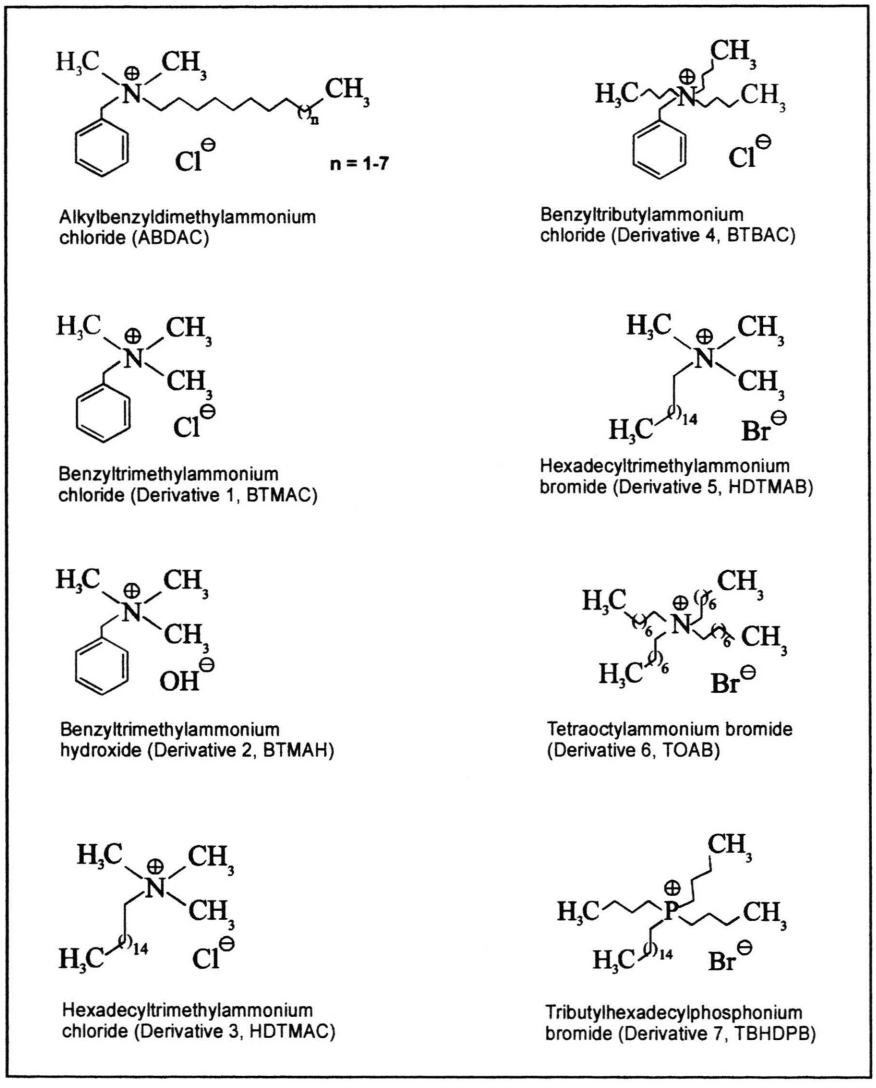

Fig. 4. Chemical structures of alkylbenzyldimethylammonium chloride (ABDAC) and selected chemicals (derivatives 1–7) with specific molecular modifications. See text for further details.

Table I. Effect of ABDAC and derivatives 1–7 on the fluorescence emission in a chloroplast preparation from a higher plant (tobacco; *Nicotiana tabacum*) and protoplast suspensions from a cyanobacterium (*Oscillatoria chalybea*). The table reflects both the *quenching* of the fluorescence emission induced by relatively low concentrations of the respective chemical (5.4×10^{-4} M) and the *increase* of F_{max} observed in the presence of high concentrations (2.7×10^{-3} M).

Assay	ABDAC	1 BTMAC	2 BTMAH	3 HDTMAC	4 BTBAC	5 HDTMAB	6 TOAB	7 TBHDPB
a) *Nicotiana tabacum* Control (= 100) Additions: 5.4×10^{-4} M	37	102	101	49	102	49	59	38
b) *Oscillatoria chalybea* Control (= 100) Additions: 2.7×10^{-3} M	356	101	99	295	99	294	135	360

fluorescence quenching had been shown both for ABDAC and $K_3[Fe(CN)]_6$. An additional control was carried out again to substantiate the effect from ABDAC itself. Table I line a summarizes the results and shows unequivocally that the length of the alkyl chain plays the essential role. Both derivatives 3 (HDTMAC) and 5 (HDTMAB) carry an equally long C-chain as does ABDAC and provoke an about identical lowering of the fluorescence. As these two derivatives are different with respect to their counter ion (Cl^-/Br^-) we conclude that this molecular component does not play any significant role in the function. Specifically interesting was the effect of derivative 7 (TBHDPB) on the fluorescence emission as it was identical to the one exerted by ABDAC. This chemical contains a similarly long alkyl chain; it contains, however, both a different central atom (P^+ instead of N^+) and a different counter ion (Br^- instead of Cl^-). Thus, none of these two structural components appears to be essential for the effect on the fluorescence of chloroplasts. Possibly, derivative 6 (TOAB) shows an intermediary effect although the significance of the difference cannot be derived from Table I line a alone. However, lack of any fluorescence quenching effect can be clearly inferred from the table for derivatives 1 (BTMAC) and 2 (BTMAH) and none of these components contains any alkyl chain. A similar lack of effect was observed with derivative 4 (BTBAC) what suggests that butyl groups do not represent sufficiently long C-chains for an effective interaction with the photosynthetic membrane.

In further experiments we tried to substantiate these observations and interpretations and analyzed the same derivatives in fluorescence experiments with the filamentous cyanobacterium *Oscillatoria chalybea*. Moreover, we chose concentrations (2.7×10^{-3} M) which had been shown to be maximally effective in enhancing the fluorescence (compare Fig. 1). Line b in Table I shows that the evaluations of derivatives 1–7 derived from line a can be directly brought forward from the fluorescence quenching assays and that the choice of the organism (cyanobacterium or higher plant) does not principally affect the conclusion. Again, derivatives 3 (HDTMAC) and 5 (HDTMAB) (both containing an alkyl chain of 16 C-atoms) exert an identical effect – similar to the one with ABDAC. Derivative 7 (TBHDPB) is identical to ABDAC in its function and derivative 6 again has a slight tendency to an intermediary extent of effect. Even at high concentrations derivatives 1 (BTMAC), 2 (BTMAH) and 4 (BTBAC) did not show any chemical-dependent increase in the maximum fluorescence in comparison to the respective control. As all of these 3 chemicals contain a benzyl group, we might conclude again that a benzyl group is absolutely dispensable for an effective interaction of the respective molecule with the photosynthetic membranes. This result furthers the interpretation that a chemical reduction of *e.g.* photosystem II components by the biocides can be excluded (*vide supra*), because from a chemical point of view just the benzyl group represents by far the most prominent (and only) part of the molecule with respect to a weak (if any) reducing activity.

The effective chemicals (ABDAC, derivatives 3, 5, 6 and 7) induce stable conformational changes within the photosynthetic membranes which do not require the continued presence of the com-

pounds. Assays which had been incubated in the presence of the respective chemical for 15 min and thoroughly washed prior to the measurements yielded results identical to the ones observed in the presence of the salts (results not shown). Thus, the salt-induced modified structure of the photosynthetic membranes which leads to the improved electron flow from PS II as evidenced by the strong fluorescence quenching is stable even in the absence of (excess) chemicals. We assume that the molecules which are directly involved in the interaction are steadily inserted into the hydrophobic parts of the membranes so that an 'invariable' conformation is attained.

The effect on the flash-induced oxygen evolution was analyzed with derivative 7 (TBHDPB) as an example (Fig. 5). The modification of the typical oxygen evolution pattern of *Oscillatoria chalybea* was similar to the one known from ABDAC (Bader, 1989). Again, we conclude that both the central atom and the counter ion of the salts appear to be less important, as derivative 7 is a bromide and contains phosphorus instead of nitrogen as central atom in comparison to the chloride ABDAC. With this derivative we observed all the specificities of the effect of ABDAC on the oxygen flash patterns including the shifts of both Y_{max} and Y_{min} together with an improved oscillation. Mathematical fit of the amplitude values (Thiéry, 1991; Thibault and Thiéry, 1981) shows that the contribution of the miss parameter α is strongly reduced (from about 25% to 12%) by the addition of TBHDPB (Table II). Accordingly, the values for the successes (β) increase, whereas all other parameters remain virtually unaffected. In order to substantiate our interpretation that the detergent-induced effect is distinct from reduction we ran another control experiment in the presence of 50 μM NH_2OH. The effect of this chemical on flash patterns is well-known and consists of the shift of Y_{max} to at least Y_5 and the substantial population of over-reduced states S_{-1} or S_{-2} (Bouges, 1971; Förster and Junge, 1987; Hanssum and Renger, 1987; Messinger *et al.*, 1991). Table II lists the calculations for *Oscillatoria chalybea* under our conditions and shows that in fact only the fit in the 6-state model including the S_{-2} state yields a reasonable value for Δ_q(%). Both the control as the TBHDPB-assays could easily be fitted with the 4-state model and not even the assumption of S_{-1} (5-state model) did improve the quality of the fit. Thus, the effect of TBHDPB cannot be interpreted in terms of reduction. Detailed analyses of the flash patterns with particular respect to Y_1 and Y_2 revealed a specific effect on these two flash signals (Fig. 6). To a varying extent and depending on the added derivative the first two signals of a sequence were enhanced and both the rise time and the relaxation time were accelerated. Largely identical effects have recently been discussed in detail for exogenously added inorganic salts and far red irradiation (Spiegel and Bader, 2001). Strikingly, also this effect is clearest seen for derivative 7 (TBHDPB) which has been shown to be as effective as ABDAC and completely absent for derivative 4 (BTBAC) which has been shown to be ineffective also with respect to the other parameters described thus far. The described results give further evidence to our interpretation that differences in the flash yields and the time constants of Y_1 and Y_2 in particular are defined by the conformation of the photosynthetic membranes so that they are modified by many types of conformational changes and cannot be taken as proof for the involvement of principally different reactions.

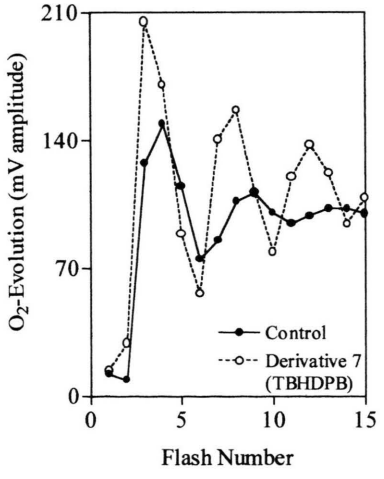

Fig. 5. Flash-induced oxygen evolution in the cyanobacterium *Oscillatoria chalybea* in the absence and in the presence of tributylhexadecylphosphonium bromide (15 μM derivative 7; TBHDPB). Flashes were 5 μs long and fired at a frequency of 3.33 Hz following a dark adaptation of 10 min.

Our conclusion that the ammonium and phosphonium salts directly interact with photosynthetic membranes and affect structural and conformational specificities was sustained by analyses of the

Table II. Effect of tributylhexadecylphosphonium bromide (TBHDPB; derivative 7) on the S-state distribution and on the transition probabilities of flash-induced oxygen evolution. For comparison the known effect of hydroxylamine (Bouges, 1971) has also been considered and measured under identical conditions.

Assay	Model	S_{-2}	S_{-1}	S_0	S_1	S_2	S_3	α	ß	γ	Δ_q(%)
Control amplitudes	4-states	–	–	36.7	59.8	0	3.5	26.7	68.3	5.0	1.28
	5-states	–	9.5	33.9	53.7	0	2.9	24.4	73.4	2.2	0.98
Derivative 7 (TBHDPB)	4-states	–	–	34.6	59.2	3.1	3.1	12.8	84.2	3.0	1.85
(15 µM)	5-states	–	7.0	32.6	53.5	4.0	2.9	11.4	86.5	2.1	0.85
Hydroxylamine	4-states	–	–	25.4	23.7	24.0	26.9	20.6	79.4	0	14.11
(50 µM)	5-states	–	0	23.5	23.4	24.9	28.2	11.3	88.7	0	14.74
	6-states	48.7	0	13.4	12.5	12.4	13.0	25.5	72.8	1.7	0.98

Mathematical fit of the photosynthetic oxygen evolution amplitudes in *Oscillatoria chalybea* using the computer simulation program developed by Thibault and Thiéry (1981) and Thiéry (1991). The program was run with 120 iterations under the assumption of 4, 5 or 6 S-states, respectively. α – misses, β – successes, γ – double hits. The quality of the fit can be derived from the mean quadratic deviation values Δ_q given in %.

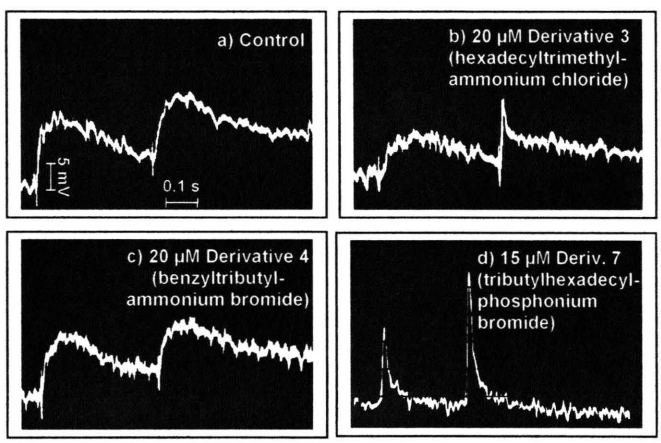

Fig. 6. Effect of selected derivatives on the flash-induced oxygen evolution in *Oscillatoria chalybea*. The figure shows the first flash amplitudes Y_1 and Y_2 at an extended time scale. Conditions as in Fig. 5.

polypeptide composition of the respective membranes. SDS-polyacrylamide gel electrophoresis (PAGE) was performed with protoplast preparations from *Oscillatoria chalybea* in the absence and in the presence of ABDAC in different concentrations. PAGE turned out problematic with several cyanobacteria not least because of their high amount of exopolysaccharides what makes a good resolution somewhat problematic. Ultrasonic treament and an additional lysozyme pre-incubation tended to improve the quality of the gel. The high protein concentration in the kDa region between 50 and 67 is largely reduced and 'dissolved' by the treatment (results not shown). Moreover, we observed the 3 strongly stained bands at > 17 kDa which might reflect the α and β subunits of the phycobilisomes with MW between 17 and 22 kDa (Sidler, 1994). In PAGE analyses the effect of the ammonium salt consisted of a dramatic loss of high molecular weight components together with an increase of low molecular weight bands in the region of < 17 kDa. This is seen in a densitogram using the 530 nm absorption in correlation to the mobility of the peptides (Fig. 7). From the derived quantities we conclude that the effect consists of conformational changes of membranes leading to the facilitated separation of complexes. Most probably even the quarternary structure of the polypeptides is affected (*e.g.* separation of subunits) as the maximum of components is found in the lowest regions of the 13% gel. SDS-PAGE in the presence of inorganic salt derivatives 1–7 yielded identical results as both the fluorescence and oxygen evolution measurements (results not shown).

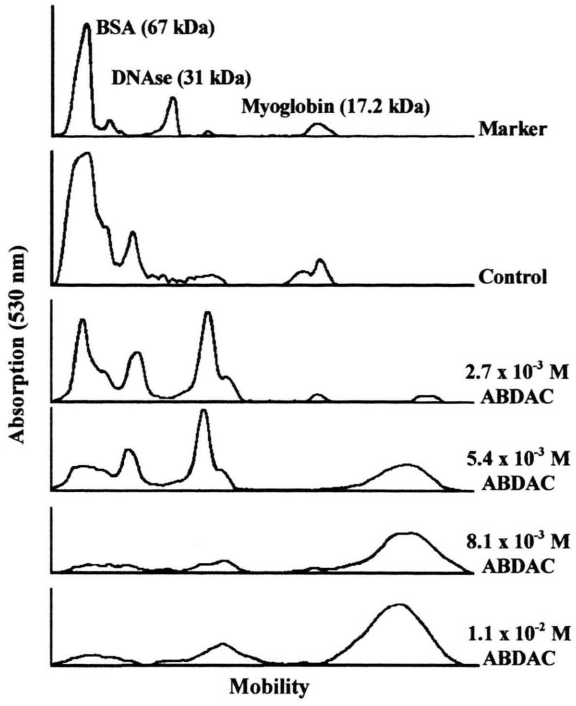

Fig. 7. Densitogram of the gel lanes containing markers, controls and selected ABDAC concentrations from SDS PAGE analysis (13%, incubation time 20 h, room temperature) of protoplasts from *Oscillatoria chalybea*.

As an example for the effects of ABDAC on specific polypetides we carried out Western blot analysis with polyclonal monospecific antisera to the D1 and the D2 protein, respectively (results not shown). The presence of both proteins in the *Oscillatoria* preparations could be demonstrated together with the stained band at about 67 kDa. The observation of these bands with monospecific antisera to D1 (or D2) corroborates the interpretation of a dimerization of these essential photosystem-II proteins in this region of the gel. ABDAC completely abolished the respective bands. Identical results were obtained with the chromoproteids of the phycobilisomes phycocyanin and allophycocyanin (results not shown). Thus, the ammonium salt does not only induce conformational structures of the membranes but also affects single polypeptides in a way that either their tertiary or quaternary structure is modified or essential structural components of the peptides are significantly changed preventing even the highly specific antigen-antibody interaction.

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