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Alkylbenzyldimethylammonium chloride, a stabilizer of the S-state system in the filamentous cyanobacterium *Oscillatoria chalybea*

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Alkylbenzyldimethylammonium chloride (ABDAC) affects the oxygen-evolution pattern induced by a train of short saturating light flashes in the filamentous cyanobacterium *Oscillatoria chalybea*. In particular, the compound largely improves the oscillation of the sequence, i.e., the damping of the oscillation of the oxygen amplitudes is strongly reduced. The ratio of the oxygen amplitudes Y_{max}/Y_{min} which specifically is always low with *Oscillatoria chalybea* is increased from average values around 1.4 to about 4 (even 5–6 in extreme cases). A mathematical fit shows that the reduced damping of the sequence can be attributed to an important decrease of the miss parameter. It appears that addition of ABDAC leads to a significant deceleration of the deactivation kinetics of the oxygen yields. Our experiments show that in the presence of the compound the life-time of the S-states is increased.

Photosynthetic oxygen evolution under short saturating light flashes produces a specific and damped oscillation pattern with a periodicity of four [1,2]. The interpretation of this pattern is described by the Kok model [2]. A typical pattern of the manyfold studied green alga *Chlorella* shows no or only very little oxygen evolution under the first flash, a little more under the second flash and maximal flash yield under the third flash. If oxygen is measured as the consequence of short saturating flashes with the filamentous cyanobacterium *Oscillatoria chalybea*, a pattern distinct from that of *Chlorella* is observed. There is always a substantial amperometric signal observed under the first flash with maximal flash yield usually being observed under the fourth flash [3]. The damping of the sequence is always very strong in comparison to the one observed with sequences of chloroplasts from higher plants or of *Chlorella*. Normally a third maximum can hardly be recognized. The positive amperometric signal under the first flash measured even after extensive dark adaptation means existence of metastable S_3, an interpretation which is, according to the coherent Kok model, barely acceptable. Aware of papers by Åkerlund [4], and Berg and Seibert [5] we tested whether our signal was artefactually produced on the electrode surface in the presence of our cyanobacterial thylakoids by a substance which had incidentally the same half-wave potential as oxygen. Schröder and Åkerlund [4,6] had shown that NaCl-washed inside-out thylakoids of spinach loose the two extrinsic 16 kDa- and 23 kDa-peptides and showed an H_2O_2-caused amperometric signal under the first flash. This signal was enhanced by the addition of H_2O_2 and abolished by catalase [4,5]. In our experimental system neither led the addition of H_2O_2 to an enhancement nor that of catalase to an abolition of the amperometric signal under the first flash [7]. The addition of catalase, even high amounts, however, seemed to have a beneficial effect on the flash sequence produced with our cyanobacterial thylakoids in the sense that the miss parameter, i.e., the damping of the sequence appeared appreciably reduced. In the course of these studies we realized that a chemical component serving as an enzyme stabilizer contained in the purchased catalase preparation was causing the observed phenomenon. This compound was alkylbenzyldimethylammonium chloride (zephirol).

Thylakoid preparations of the filamentous cyanobacterium *O. chalybea* were prepared as described earlier [3,8].

Amperometric measurements of oxygen evolution as the consequence of short saturating light flashes were carried out with the three-electrode system described by Schmid and Thibault [9]. Light flashes were produced by a stroboscope (1539 A General Radio).

Abbreviation: ABDAC, alkylbenzyldimethylammonium chloride (zephirol).

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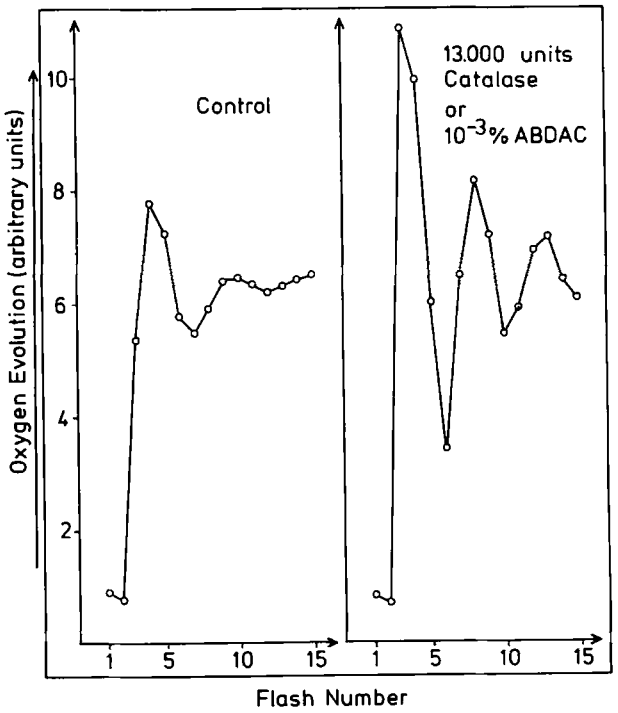

Fig. 1. Effect of catalase preparation on the flash-induced oxygen evolution pattern of *Oscillatoria chalybea*. The reduction assays were preincubated in the light for 15 min before being put on the electrode and allowed to sedimentate for 15 min. Dark adaptation time, 15 min.

Assay conditions are essentially as described earlier [8]. Thylakoid particles equivalent to 70 μg Chl were suspended in 0.06 M Tricine buffer containing 0.03 M KCl (pH 7.5).

Catalase was purchased from Boehringer. The catalase preparation from Boehringer contains as an enzyme stabilizer alkylbenzyldimethylammonium chloride.

Alkylbenzyldimethylammonium chloride was purchased from Merck-Schuchardt, Alkyl means a product mixture containing a $-C_8-C_{18}$ residue.

As described earlier, catalase addition did not affect the amperometric signal under the first flash of an oxygen evolution pattern of *O. chalybea* [7]. Fig. 1 shows the control measurement with thylakoid preparations from this filamentous cyanobacterium as the consequence of a train of short saturating light flashes. As was demonstrated earlier, the signal seen under the first flash is neither due to a photoinhibition of a respiratory phenomenon [10], nor is it produced by leaked-out hydrogen peroxide [5], since catalase did not remove the signal nor did addition of hydrogen peroxide increase it. However, Fig. 1 clearly shows an effect of our catalase suspension on the oxygen evolution pattern of *Oscillatoria*. If high amounts of the enzyme were applied to the thylakoid preparation, the corresponding pattern appeared substantially changed. The maximum signal of the sequence shifted from the fourth to the third flash connected with a strong increase in oxygen yield. Correspondingly, the minimum shifted from the 7th to the 6th flash in connection with a strong decrease of O_2-evolution. The oscillation of the entire sequence is much more pronounced after catalase addition, which means that the damping was consistently lowered by the addition of catalase. Damping of a normal *Oscillatoria* sequence is mainly caused by a high amount of misses. Usually such a sequence is characterized by 30% misses or more and only 2–3% double hits [3]. Fig. 1 suggests that this particularly high miss parameter is reduced. At first we attributed the effect of Fig. 1 to catalase itself but became soon aware that an ingredient of the purchased catalase preparation induced the observed phenomenon, as the enzyme suspension had lost this property after being dialyzed (results not shown). This compound was shown to be alkylbenzyldimethylammonium chloride and is added by the producer to such catalase suspensions as a stabilizing agent. The chemical is supposed to impair growth of microorganisms in the suspension in general and the liberation of proteases from these microorganisms as it is supposed to have a surface-active effect on the cell membrane of microorganisms (information from Boehringer). Addition of the pure, commercially purchased chemical in the suitable concentration was shown to induce exactly the same effect on the flash sequence of *Oscillatoria* as shown in Fig. 1 for the catalase suspension. It appears that, if the assay is preincubated with ABDAC in the light for 15 min, a 10-times lower ABDAC concentration is sufficient to produce the equivalent effect observed in the dark. Calculation of the S-state distribution via a mathematical fit of the sequence in the Kok-model supported the interpretation of Fig. 1, namely a decrease of misses. Tables I and II show the dark distribution of S-states together with the transition probabilities

TABLE I

Determination of the S-state population in dark-adapted thylakoid preparations of Oscillatoria chalybea calculated from a mathematical fit in the 4-state Kok model

Dark adaptation, 15 min; ABDAC concentration in the assay, 10^{-3} %.

Sequence	Values for dark distribution (%)				Transition probabilities		Relative quadratic deviation Δ (%)
	S_0	S_1	S_2	S_3	misses α	double hits γ	
Control	58.3	47.2	−10.3	4.7	33.7	7.5	1.4
ABDAC	33.1	68.0	−3.7	2.7	18.8	3.2	1.16

TABLE II

Determination of the S-state population in dark-adapted thylakoid preparations of Oscillatoria chalybea calculated from a mathematical fit in a 5-state Kok model

Dark adaptation, 15 min; ABDAC concentration in the assay, 10^{-3} %.

Sequence	Values for dark distribution (%)					Transition probabilities		Relative quadratic deviation Δ (%)
	S_{-1}	S_0	S_1	S_2	S_3	misses α	double hits γ	
Control	9.8	46.6	44.8	−4.8	3.6	31.4	4.9	0.97
ABDAC	6.0	31.2	61.9	−1.5	2.4	17.6	2.1	0.7

α (misses) and γ (double hits). It is clearly seen that the miss parameter α which is always particularly high for *Oscillatoria* preparations is decreased to nearly one half (reduced from over 30% to 17–18%, which corresponds to the values reported in the literature for, e.g., *Chlorella* or higher plant chloroplasts). This result is the same regardless whether the traditional 4-state Kok model or a 5-state Kok model including a higher reduced state S_{-1} was used for the mathematical fit of the *Oscillatoria* sequence. In either case the S-state distribution is qualitatively identical and goes hand in hand with a strong decrease in the number of misses upon ABDAC addition. Moreover, ABDAC apparently has a stabilizing effect on the water-splitting enzyme, as it improves not only the functioning of the oxygen-evolving apparatus by reducing the number of misses, but clearly also increases the life-times of all S-states. Fig. 2 shows that ABDAC significantly decelerates the deactivation kinetics of the flash-induced oxygen yields. This effect is particularly obvious for the flash yields under the first and second flash of a sequence being equivalent to the S_3- and S_2-state. This means that the life-time of these oxidation steps, which is anyhow long in *Oscillatoria chalybea* when compared to other organisms or preparations, is further increased by ABDAC. The prolonged dark period necessary for the stable dark distributionof the S-states under these conditions can be particularly well demonstrated by plotting the Y_6-deactivation kinetics with Y_6 being the minimum oxygen yield of the sequence against time (Fig. 3). In the presence of ABDAC the flash-induced oxygen yield under the 6th flash still decreases, even after dark intervals of 15 min between the sequences. Thus, it looks as if ABDAC is able to penetrate the membranes of thylakoids from *Oscillatoria*. This penetration might be facilitated by conformational changes induced by light.

The present paper shows that ABDAC interacts with the oxygen-evolving complex improving the efficiency of the flash-induced water oxidation essentially by in-

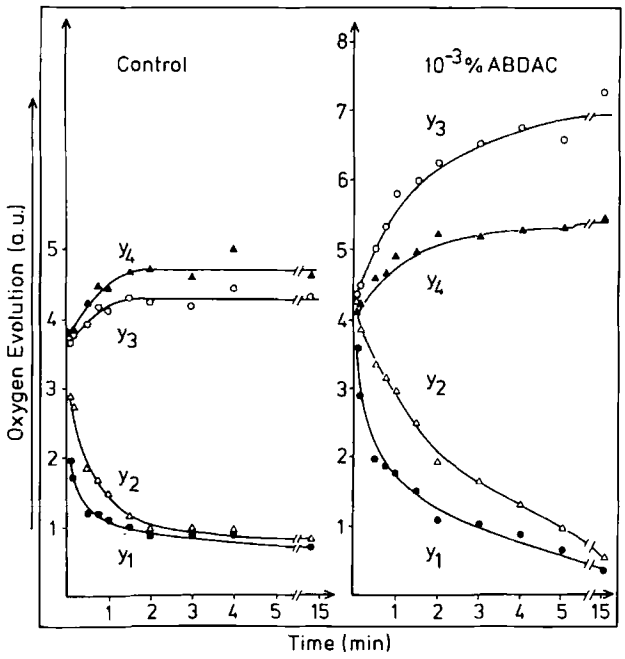

Fig. 2. Dependence of oxygen amplitudes and deactivation kinetics of the first four flashes of a sequence on the dark intervals between sequences; experiment without and with addition of ABDAC (10^{-3} %).

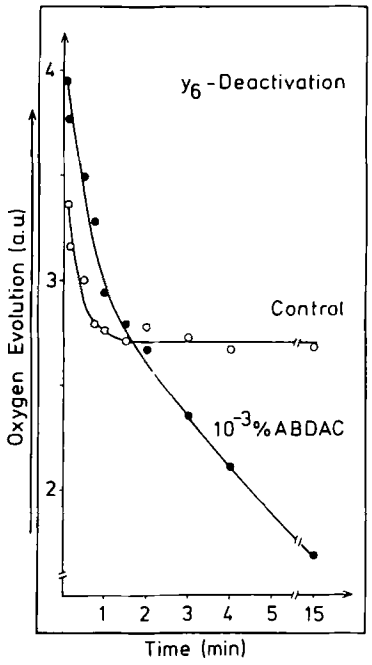

Fig. 3. Dependence of the oxygen amplitudes and the deactivation kinetics of Y_6 (being the minimum of the flash pattern) on the dark intervals between the sequences. Experiment without and with addition of ABDAC.

creasing the percentage of successful photoevents and the stability of the respective oxidation steps.

It should be emphasized that ABDAC does not affect the two positive signal amplitudes of the first two flashes at all (Fig. 1). The effect of ABDAC on the relative dark composition of the S-state distribution is, as already seen in one glance from the shape of the sequence (the maximum appears under the third flash as usual), characterized by a decrease of the S_0- and an increase of the S_1-condition (Tables I and II). The effect of ABDAC by which the miss parameter appears reduced or by which the efficiency of the photosynthetic apparatus is increased, clearly leads interpretation in the direction of a stabilizing effect of all S-states. The effect of ABDAC is clearly distinct from effects of ammonia and substituted amines on the oxygen-evolving complex as reported by Beck and Brudvig [14,15] or Förster and Junge [16] and does not represent for example an interference of the amine ABDAC with a particular S-state such as the rapid binding of NH_3 to the S_2-state described by Velthuys [17]. It appears that the life-time of all S-states is increased (Fig. 2); hence, the effect is clearly opposite to that of an ADRY-reagent such as NH_2OH or CCCP which accelerates the deactivation kinetics of the system [17–19]. This in turn could mean that ABDAC exerts its effect as an effector molecule which induces a conformational change of the protein–lipid structure of the photosynthetic apparatus.

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