Effect of Cyanide on the S_2- and S_3-State in the Filamentous Cyanobacterium *Oscillatoria chalybea*

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Dedicated to Professor Wolfgang Kowallik on the occasion of his 60th birthday

Filamentous Cyanobacterium, Water-Splitting, S-States

Low concentrations of cyanide affect the flash-induced oxygen evolution pattern in thylakoid preparations of the filamentous cyanobacterium *Oscillatoria chalybea*. At a cyanide concentration of 2×10^{-6} M the effect lies exclusively on the S_2- and S_3-state whereas the subsequent flash yields and the steady state are barely affected, showing that the reaction center is not touched by the reagent. The influence of the chemical is characterized by the fact that at basic pH (7.5–8.5) the deactivation mode of S_2 and S_3 is changed, showing that the cyanide-ion is the reactant. Whereas in the control the deactivation of S_2 yields as usual S_1 and S_{-1}, the deactivation pattern in the presence of cyanide shows that S_2 deactivates – without giving S_1 –, to a more reduced state, probably S_0. In the flash pattern the two signal amplitudes of the first two flashes are lowered to zero in the presence of 2×10^{-6} M cyanide and become uptake signals at higher cyanide concentrations. It is seen that in the presence of cyanide S_2 and to a lesser extent S_3 in the *Oscillatoria* system apparently react with oxygen. In tobacco thylakoids no effect of cyanide on the flash pattern itself is observed, even at higher concentrations (*e.g.* 10^{-4} M), although the signal amplitudes of the entire pattern are affected at such concentrations. Photosystem II of cyanobacteria is characterized by the absence of the two extrinsic 16 kDa and 23 kDa peptides. As shown by mass spectrometry inside-out vesicles from tobacco thylakoids, in which these two extrinsic peptides have been removed by NaCl-washing, exhibit simultaneously flash-induced O_2-evolution and an O_2-uptake which both require the presence of Cl^- and Ca^{2+} ions. Both the evolution signal and the uptake signal are DCMU sensitive and are inhibited by KCN. An amperometrically measured flash pattern of inside-out tobacco vesicles shows, if the assay is supplemented with Cl^- ions, the usual flash pattern known from the literature. The experiments seem to indicate that even in the washed inside-out condition the S-state system of higher plants is much less sensitive to ambient oxygen than in the phylogenetically older system of the filamentous cyanobacterium *Oscillatoria chalybea*.

Introduction

In former publications it has been shown that if oxygen evolution is measured in thylakoids of the filamentous cyanobacterium *Oscillatoria chalybea* as the consequence of short saturating light flashes a substantial amperometric signal is observed under the first flash [1] which was correlated with metastable S_3. As a general observation life-times of all other S-states appeared to be also longer than in higher plants. In early greening stages of etioplasts of oat Franck and Schmid have observed the same O_2-patterns as in *Oscillatoria* [2]. In the following we were able to show that O_2-evolution measured by means of mass spectrometry in the ambient atmosphere of air (21% O_2) always consisted of two portions, one coming from the splitting of the water molecule and the other from the decomposition of H_2O_2 which apparently was formed *and* decomposed by the S-state system [3, 4]. From the experimentally not always very clearly observable periodicity of two it was concluded that two light quanta were necessary for the decomposition of H_2O_2 and that S_2 was the decomposing S-state [4]. We interpret this peculiar O_2-metabolism in *Oscillatoria chalybea* as being correlated with the principal absence of the two extrinsic peptides of 23 kDa and 16 kDa from cyanobacterial photosystem II [4]. Hence, what was observed as a defect reaction of photosystem II in higher plant prepara-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, (2[N-morpholino]ethane sulfonic acid).

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tions for example in NaCl-washed inside-out vesicles, lacking the two extrinsic peptides, and in which H_2O_2 can serve as an electron donor to photosystem II [5, 6] seems to be a principle reaction in photosynthetic O_2-evolution in cyanobacteria [4]. In the following we describe the effect of cyanide on S_2 and S_3 in thylakoid preparations of *Oscillatoria chalybea*.

Materials and Methods

The filamentous cyanobacterium Oscillatoria chalybea was obtained from the algal collection in Göttingen (Germany) and cultured on nitrate as the sole nitrogen source in the medium as described earlier [7].

Thylakoid preparations of Oscillatoria chalybea were prepared according to Bader *et al.* [1]. The procedure required the digestion of the mucoid layer on *Oscillatoria* cells with glucuronidase (Boehringer, Mannheim) and a cell wall digestion with lysozyme (Sigma) and cellulase (Kinki Yakoult, Japan).

Tobacco chloroplasts were prepared from Nicotiana tabacum var. John William's Broadleaf (JWB) according to Homann and Schmid [8].

The reaction mixture contained in a total of 2 ml tobacco chloroplasts corresponding to 70 µg chlorophyll and thylakoids of *Oscillatoria* corresponding to 40 µg chlorophyll. The assays, were if not stated otherwise, carried out in 30 mM KCl and 60 mM Tricine-NaOH (pH 7).

Inside-out vesicles of tobacco thylakoids were prepared according to Åkerlund [9]. They were obtained from tobacco thylakoids by distribution in an aqueous 2-phase system between dextrane/polyethylene glycole after disruption of the thylakoids in a French press.

Mass spectrometry was carried out with a modified magnetic sector field mass spectrometer type "Delta" from Finnigan Mat (Bremen, Germany), which is an isotope ratio mass spectrometer equipped with a two directional focussing device "Nier type I" [3]. The experimental set-up with the valve system and the geometry of the home-made measuring cell have been described earlier [3].

Amperometric measurements of O_2-evolution and -uptake have been carried out with the "Three Electrode System" described by Schmid and Thibault [10] which permits the simultaneous measurement of oxygen evolution and uptake induced by short saturating light flashes.

Saturating light flashes of 8 µs duration were obtained from a Xenon flash lamp (Stroboscope 1539 A of General Radio). The time between flashes was 300 msec. $^{18}O_2$ (98%) was from CEA-Oris, Bureau des Isotopes Stables, Gif-sur-Yvette, France.

Results

Fig. 1 shows photosynthetic oxygen evolution measured polarographically with our "Three Electrode Device" described earlier [10] in the absence and presence of 2×10^{-6} M KCN. It is clearly seen that the control sequence shows an O_2-signal under the first flash with an amplitude somewhat higher as that seen under the following second flash. This is the usual sequence seen with *Oscillatoria chalybea* preparations, which as should be mentioned here again, yields perfect fits when compared within the frame of the Kok model with the theoretical sequence built from the first four signal amplitudes and the shape factors α, β and γ [1]. Addition of 2×10^{-6} M KCN to the assay, which under control conditions gives such a se-

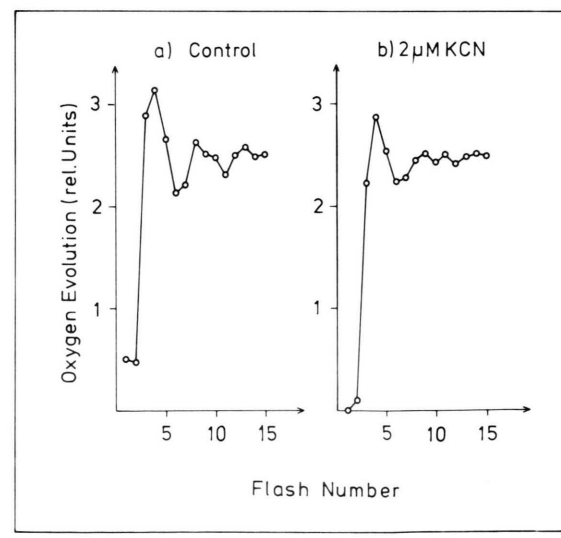

Fig. 1. Oxygen evolution measured as the consequence of a train of saturating light flashes spaced 300 ms apart in thylakoids of *Oscillatoria chalybea*. Dark adaptation 30 min, pH of the assay 7.5. a. Control assay without addition; b. assay in the presence of 2×10^{-6} M KCN.

quence, abolishes the signal under the first and second flash fully (Fig. 1b). Hence, KCN reacts in this preparation with the O_2-evolving system by interaction with the S_3- and S_2-state. With this concentration and at pH 7.5 no substantial effect on the amplitudes of the subsequent flash yields is observed which means that the reaction centers are not damaged by the chemical. A shortening of the life time of S_3 and S_2 with this KCN concentration seems to be at first glance an interpretational possibility, hence an action in the sense of an ADRY reagent [11]. Higher concentrations of KCN ($10^{-5}-10^{-4}$ M) lead under the first two flashes to a substantial O_2-uptake, without affecting the remainder of the sequence too much (Fig. 2a, b). This can mean that after interaction of the oxygen-evolving complex with KCN, S_2 or S_3 or both interact after dark adaptation with oxygen before a similar pattern as usual is produced (Fig. 2b). A careful scrutiny of the effect of the reaction pH itself on the deactivation, together with that of KCN at the respective pH, shows that in the absence of KCN deactivation of the S-states is generally faster at the basic pH 8.5 than at an acid pH (pH 6.5) (Fig. 3a and 3b). At pH 6.5 (acid pH) the deactivation of the S_2- and S_3-state is practically not affected by the presence of 2.5 μM KCN (Fig. 3) but at the basic pH of 8.5 the S-state deactivation with particular respect to the S_2- and S_3-state is slower in the presence of 2.5 μM KCN than in its absence (Fig. 3a). Generally it is observed that S_2 is deacti-

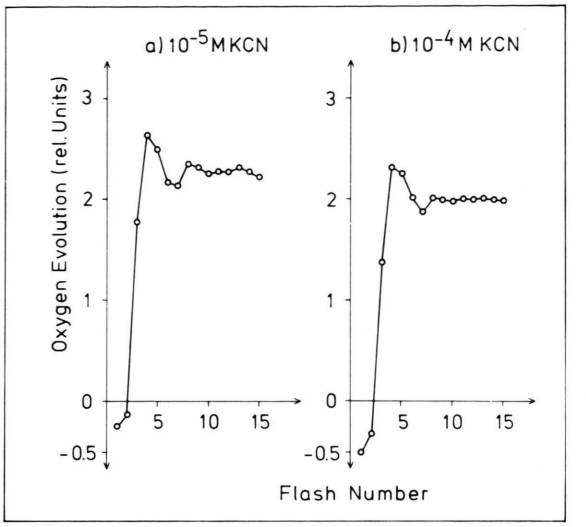

Fig. 2. Oxygen evolution pattern measured with thylakoids of *Oscillatoria chalybea* as described for Fig. 1. a. In the presence of 10^{-5} M KCN; b. in the presence of 10^{-4} M KCN.

vated either to S_1 or S_{-1} [12]. However, at both pH values shown in Fig. 3 it is seen that Y_3 hence S_1 stays constant which means that in the presence of KCN S_2 does not deactivate to give S_1 but deactivates to a more reduced S-state (probably S_0). From the pH dependence of the KCN effect on the first two flash signals it appears that the CN^- ion is the reactive agent (Fig. 3). The potential site of

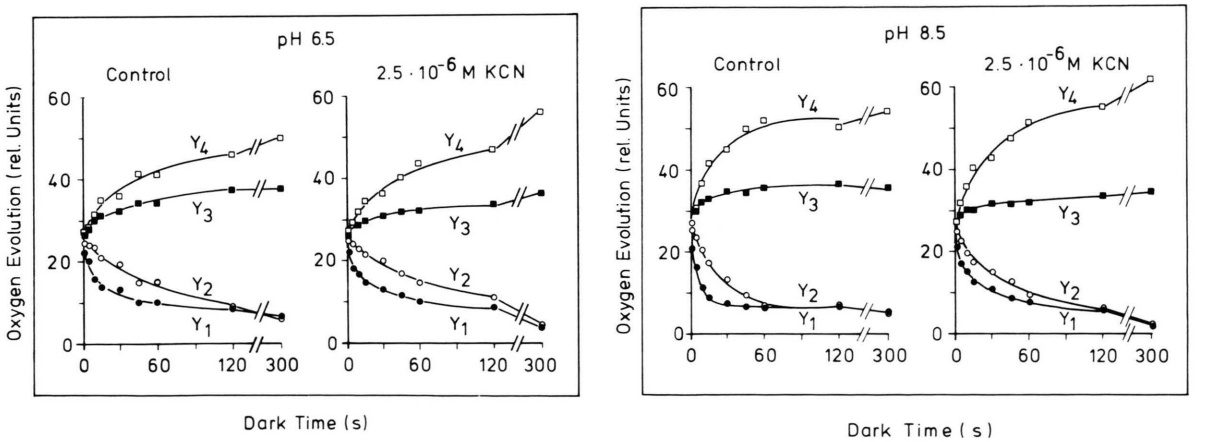

Fig. 3. Deactivation of S-states measured as the amperometrically measured oxygen yield of flash numbers (Y_1, Y_2, Y_3, Y_4) in dependence on the dark time between the flash sequences in thylakoids of *Oscillatoria chalybea;* left drawing control experiment without additions; right drawing assay in the presence of 2.5×10^{-6} M KCN. a. Assay at pH 6.5; b. assay at pH 8.5.

action might be cytochrome b_{559} which due to the principal absence of the extrinsic 23 kDa and 16 kDa peptides might be accessible to cyanide inhibition. As we have not been able to detect in *Oscillatoria chalybea* catalase, the haem protein cytochrome b_{559} which under certain conditions catalyses catalase-like reactions might be the candidate. This view would agree with a 10 year-old report by Packham *et al.* who observed a cyanide action on the water-splitting process although these authors could not observe a cyanide inhibited photooxidation of cytochrome b_{559} in their preparation from *Pisum sativum* [13]. It looks as if in the presence of cyanide electrons originating from water-splitting (or H_2O_2 decomposition) are given back to oxygen. Otherwise, the assumption that catalase or a cyanide-sensitive reaction, equivalent to catalase action occurring in the thylakoid lumen, would also fully explain the experimental observation. In this case in the presence of KCN, H_2O_2 production but no decomposition would occur. This is what is shown by mass spectrometry. Thylakoid preparations of *Oscillatoria chalybea* evolve as the consequence of short saturating flashes oxygen, which originates besides from the usual water-splitting reaction from a flash-induced O_2-uptake by the S-state system, leading to H_2O_2 formation which then is decomposed by the S-state system to give O_2 [4, 14]. This reaction can be detected by mass spectrometry if O_2 of the ambient gas atmosphere is supplemented or substituted by $^{18}O_2$ (Fig. 4b). The experiment clearly shows that the O_2-evolution signals under the two conditions shown in Fig. 4a and 4b are diminished in the presence of KCN. The observation in Fig. 4c, namely that the mass 36 signal under two flashes is considerably more affected than the signal caused by a single turnover flash, points to the fact that cyanide affects above all the reactivity of the S_2-state. At high cyanide concentrations such as 10^{-3} M the cyanide effect seems to be much less specific [4]. No O_2-evolution remains at 10^{-3} M cyanide and the uptake observed corresponds numerically to the former evolution signal, hence evolution seems to be inverted to a fast uptake [4].

In higher plants, *i.e.* in tobacco chloroplasts KCN does not affect the flash pattern nor signal amplitudes, if concentrations in the range of 10^{-6}–10^{-5} M are used (Fig. 5a). Higher concentrations affect only the signal amplitudes but not the oscil-

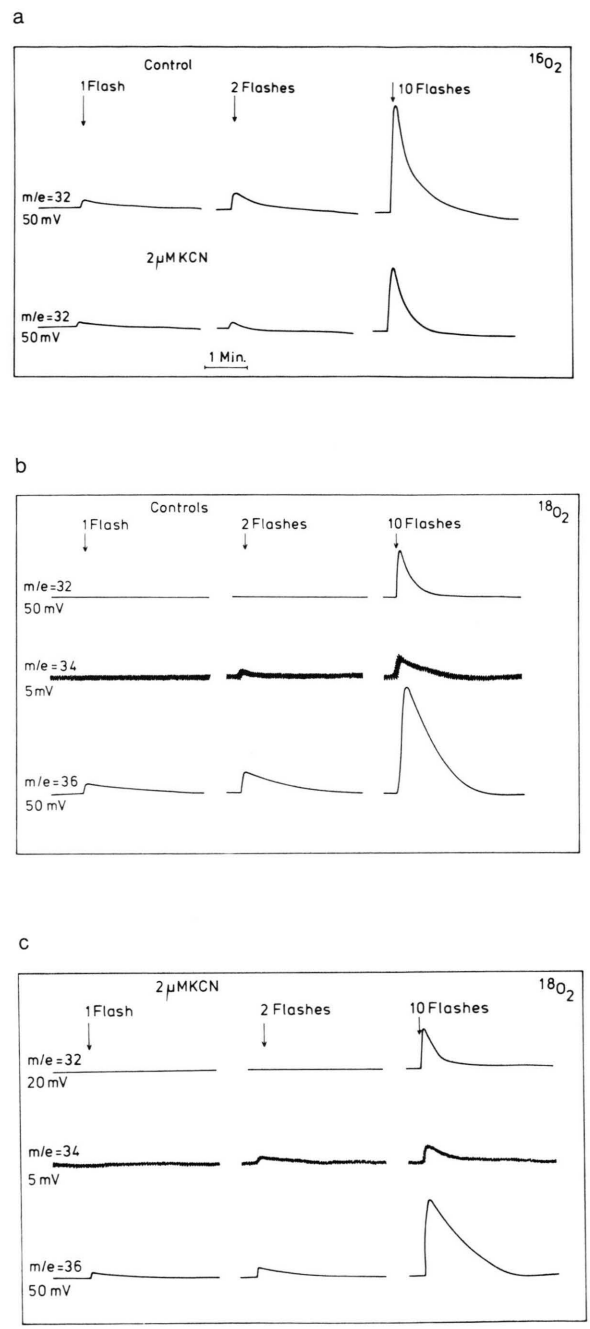

Fig. 4. Oxygen evolution measured in thylakoids of *Oscillatoria chalybea* by mass spectrometry as the consequence of 1, 2 and 10 flashes. a. Without label in the gas atmosphere, measured as $^{16}O_2$ (mass 32); upper recording control, recording at bottom in the presence of 2×10^{-6} M KCN. b and c. Labeling of the gas atmosphere with $^{18}O_2$; simultaneous recording of masses 32 ($^{16}O_2$); 34 ($^{16}O^{18}O$) and 36 ($^{18}O^{18}O$); b. in the absence and c. in the presence of 2×10^{-6} M KCN.

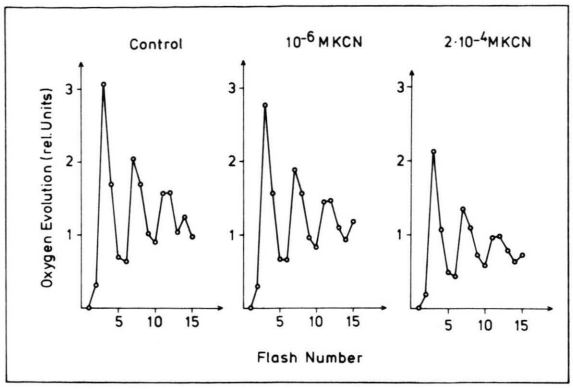

Fig. 5. Oxygen evolution measured as the consequence of train of saturating light flashes spaced 300 msec apart in stroma-freed chloroplasts of *Nicotiana tabacum* var. John William's Broadleaf. Dark adaptation 20 min, pH of the assay 7.5. a. Control. b. Assay in the presence of 10^{-6} M KCN. c. Assay in the presence of 2×10^{-4} M KCN.

latory pattern (Fig. 5b). Fig. 5 shows the usual flash pattern of oxygen evolution in tobacco chloroplasts, and that in the presence of 10^{-6} M and 2×10^{-4} M KCN. No specific effect on oxygen evolution under a particular flash number is seen (Fig. 5). It should be noted that the oxygen evolution pattern observed in higher plant chloroplasts such as in tobacco chloroplasts is not dependent on a certain level of ambient O_2 nor is the mass spectrometrically measured evolution in its isotopic distribution dependent on the O_2 of the ambient gas phase in the assay (Tables I and II in ref. [4]). No flash-induced uptake phenomenon is measured in such chloroplast preparations. Under the assumption that the water-splitting complex in *Oscillatoria chalybea,* due to the absence of the two extrinsic 16 and 23 kDa peptides, interacts directly with/or is accessible to O_2, H_2O_2 and KCN [4, 14], NaCl-washed inside-out vesicles from tobacco thylakoids should exhibit properties comparable to those of normal *Oscillatoria* thylakoids. Inside-out vesicles from tobacco thylakoids, if tested in the mass spectrometer in the presence of $^{18}O_2$ and tested in a KCl-Tricine buffer, exhibit a flash-induced uptake at mass 36 which is DCMU-sensitive (Fig. 6). This preparation yields when measured amperometrically in KCl-Tricine buffer, a fully normal O_2-evolution pattern with nearly optimal amplitudes. If to this assay 10^{-6} M KCN is added, no effect at all is observed in the mass spectrometric assay neither on the evolution signal at mass 32 nor on that of the uptake signal at mass 36 (experiments not shown). Only high KCN concentrations from 10^{-4} onward lead to an effect on the evolution signal but apparently to a much lesser extent on the $^{18}O_2$-uptake signal (Fig. 6b). This might mean that the mere absence of the two extrinsic peptides in photosystem II of *Oscillatoria chalybea* is not the exclusive reason for the accessibility and interaction of cyanide with the S-state system. Mass spectrometry permits to demonstrate the direct relationship between the O_2-evolution and the O_2-uptake signal shown in Fig. 6a. If NaCl-washed inside-out vesicles of tobacco thylakoids are spun down and suspended in Tricine-MES buffer, hence in the absence of chloride ions, a reaction mixture which contains no $H_2{}^{18}O$ in the assay but just $^{18}O_2$-label in the O_2-containing gas phase (in equilibrium with the assay) gives in the mass spectrometric assay no evolution signal. Both masses 32 and 36 are observed as uptake signals measured as the consequence of 90 flashes (Fig. 7a). This uptake is sensitive to 2×10^{-6} M DCMU and is inhibited by 1 mM KCN in the assay (Fig. 7b). If the assay is supplemented with cal-

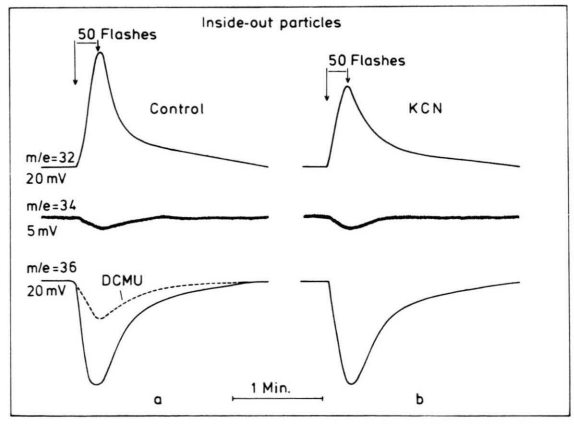

Fig. 6. Oxygen gas exchange measured by mass spectrometry as the consequence of 50 saturating flashes in inside-out vesicles from thylakoids of *N. tabacum* var. John William's Broadleaf washed with NaCl in order to remove the two extrinsic peptides (23 kDa and 16 kDa). Labeling of the gas atmosphere with $^{18}O_2$; the assay contains only a $H_2{}^{16}O$ containing buffer system. a. Control; recording of $^{16}O_2$ (mass 32) evolution, $^{16}O^{18}O$ (mass 34) uptake and $^{18}O_2$ (mass 36) uptake; b. assay in the presence of 1.7 mM KCN.

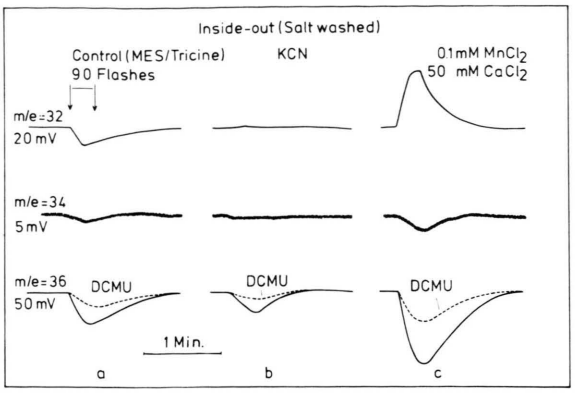

Fig. 7. Oxygen gas exchange measured by mass spectrometry as the consequence of 90 saturating light flashes in NaCl-washed inside-out vesicles from thylakoids of *N. tabacum* var. John William's Broadleaf suspended in a *chloride-free* Tricine-MES buffer system. The gas atmosphere over the assay was labeled with $^{18}O_2$. a. Only uptake signals are observed at masses 32 ($^{16}O_2$) and masses 36 ($^{18}O_2$); b. these uptake signals are sensitive to 10^{-3} M KCN; c. addition of 0.1 mM $MnCl_2$ and 50 mM $CaCl_2$ transforms the mass 32-uptake signal into an evolution signal and enhances the mass 36-uptake signal.

cium and manganese chloride, hence with Ca^{2+}, Mn^{2+} and Cl^- ions, a mass 32 evolution signal appears and the 36 uptake signal is also enhanced (Fig. 7c), hence both signals are dependent on Ca^{2+} and Cl^- ions and are sensitive to DCMU.

Discussion

The water-oxidizing enzyme system in the filamentous cyanobacterium *Oscillatoria chalybea* is characterized by a number of property differences when compared to that of higher plants. First, it appears that the life time of all redox states of the enzyme is higher than in higher plants which means that the decay kinetics of all redox states under normal conditions (*e.g.* room temperature *etc.*) is distinctly slower. The life time of the S-states in the enzyme system of higher plants is comparable to that of *Oscillatoria* only at low temperatures [16, 17] or in an early assembly stage which is an early greening stage when for example during greening of oat etioplasts the photosynthetic apparatus develops [2]. The properties of the phylogenetically old system of the filamentous cyanobacterium *Oscillatoria chalybea* are comparable to those of the early chloroplast developmental state above all with respect to the reactivity of the S_3-state. It appears that in both cases the S_3-state excels with an extremely low reactivity [15]. Only part of this redox state decays slowly in the dark to give S_2 and S_0 whereas a big part of it remains for a long time as metastable S_3 in the system [3]. Obviously no interaction with oxygen of the ambient oxygen occurs. The observed break in the decay kinetics after 30 sec suggests the blocking of the state in a conformational situation (Fig. 3b). Different appears the reactivity of the S_2-state. Its deactivation determines eventually the S-state distribution of a dark-adapted sample. The S_2-state is obviously a very reactive state in *Oscillatoria* as well as in the greening oat system [4, 15]. Thus, S_2 in the *Oscillatoria* condition obviously interacts with oxygen of the ambient atmosphere [4, 14] and S_2 is also the most reactive state towards reagents such as hydroxylamine in etiochloroplasts of oat [15]. Recently Messinger and Renger [16], and Messinger *et al.* [17] have shown that this difference in reactivity between S_3 and S_2 is fully transferable to the higher plant system where reactions take place in a completely different time scale region. In the present paper we are able to show that the *Oscillatoria* enzyme system interacts in a most specific way with cyanide. The specific effect lies on S_2 and S_3 (Fig. 1). An effect of cyanide on the water-splitting process has also been reported for inside-out vesicles from higher plants many years ago by Packham *et al.* [13]. At this point it should be pointed out that the S-state system exhibits typical enzyme system properties. Thus in *Oscillatoria* deactivation of the S-states is clearly increased by increasing the pH (Fig. 3a and b). Clearly, protein conformation plays the decisive role in the observed S-state reactivity. Within this pH effect 2×10^{-6} M CN^- interacts with the S-state system by slowing down the fast portion of the decay kinetics of S_3 and slowing down the deactivation of S_2 (Fig. 3). The reagent apparently interacts specifically with S_2 and also with S_3 as in a flash pattern only the first and second flash signal are affected without touching the remainder of the sequence significantly (Fig. 1b). It looks as if the reagent modifies the redox condition of the dark-adapted enzyme system as the comparison between flash sequences of cyanide-treated and control thylakoids shows (Fig. 1a and b): The control pattern (Fig. 1a) corresponds to an ideal S-state

distribution in the sense of the 4-state Kok model comprising ≈ 36% S_0, ≈ 40% S_1, ≈ 11% S_2 and ≈ 13% S_3 (see Table II in ref. [1]) whereas the pattern obtained after cyanide treatment (Fig. 1 b) corresponds to much more reduced conditions in the sense of the 5-state model and comprises up to ≈ 30% S_{-1}, ≈ 30% S_0, ≈ 40% S_1 having no S_2- and S_3-state anymore in the system after a prolonged dark adaptation. Cyanide apparently removes the metastability from the S_3-state, which according to what has been said above might be due to a change in the conformation of the enzyme system. At higher cyanide concentrations the effect is certainly less specific but nevertheless most informative (Fig. 2a and b). At the high concentration of 10^{-4} M, cyanide again affects in particular the S_2- and S_3-state but affects the remainder of the sequence and steady state O_2-evolution as well, showing that centers are modified and/or destroyed. In both cases S_2 and to a lesser extent S_3 react with O_2 (Fig. 2a and b) leading to the observed uptake signals. As was discussed above, cyanide seems to modify the conformational condition of the enzyme and in this strongly modified enzyme condition the S_2 and S_3 redox state interact severely with O_2, the main reactivity however, being associated with S_2. Concerning the similarity of the observed interaction, it might suggest that the redox condition itself of S_2 and S_3 might also be very similar or comparable. The interaction of CN^- might consist in a direct interference with the reactive manganese. The reason of this changed reactivity and accessibility of the cyanobacterial photosystem II towards different reagents and to ambient O_2 might be the absence of the two extrinsic peptides 23 kDa and 16 kDa from the structural complex. In the higher plant system these peptides regulate amongst others binding of the essential chloride ions and of Ca^{2+} to the enzyme complex. Absence of these peptides means amongst others decrease in binding affinity to chloride. Nevertheless, the cyanobacterial system for example that of many *Oscillatoria* species does not necessarily suffer from chloride deficiency as the natural habitat of the organisms is often that of salt water impregnated sand layers of sea shores in which damped light intensities and low oxygen concentrations prevail [18]. It looks as if under natural experimental and environmental conditions the water-splitting enzyme or the oxygen-evolving complex of *Oscillatoria* lives under a situation of chronicle chloride deficiency in which the enzyme interacts with oxygen, as the water-splitting site is not protected from O_2 attack by the extrinsic peptides as in the higher plant system. In other words the system is under the actual conditions defective or leaky, the main problem being the interaction with O_2, which would have its parallel in the oxygen sensitivity of nitrogenase or ribulose 1,5-bisphosphate carboxylase. For the circumvention of this inherent problem cyanobacteria have apparently developed different solutions. Thus, a *Synechococcus* species which is supposed to lack the two extrinsic peptides (23 kDa and 16 kDa) notwithstanding does not show any interaction of the S-state system with oxygen (Pistorius, Bader, and Schmid, in preparation).

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[1] K. P. Bader, P. Thibault, and G. H. Schmid, Z. Naturforsch. **38c,** 778 (1983).
[2] F. Franck and G. H. Schmid, Z. Naturforsch. **39c,** 1091 (1984).
[3] K. P. Bader, P. Thibault, and G. H. Schmid. Biochim. Biophys. Acta **893,** 564 (1987).
[4] K. P. Bader and G. H. Schmid, Biochim. Biophys. Acta **936,** 179 (1988).
[5] W. P. Schröder and H. E. Åkerlund, Biochim. Biophys. Acta **848,** 359 (1986).
[6] S. P. Berg and M. Seibert, Photosynthesis Res. **13,** 3 (1987).
[7] K. P. Bader and G. H. Schmid, Arch. Microbiol. **127,** 33 (1980).
[8] P. Homann and G. H. Schmid, Plant Physiol. **42,** 1619 (1967).
[9] H. E. Åkerlund, C. Jansson, and B. Andersson, Biochim. Biophys. Acta **681,** 1 (1980).
[10] G. H. Schmid and P. Thibault, Z. Naturforsch. **34c,** 414 (1979).
[11] G. Renger, Biochim. Biophys. Acta **25b,** 428 (1972).
[12] P. Thibault, Thèse d'État presentée à l'Université d'Aix-Marseille II, Faculté des Sciences de Luminy, le 7 septembre 1987. Titre: Contribution a l'étude des proprietés de l'emission photosynthétique d'oxygène. Recherche d'un modèle coherent.
[13] N. K. Packham, R. W. Mansfield, and J. Barber, Biochim. Biophys. Acta **681,** 538 (1982).
[14] K. P. Bader and G. H. Schmid, Z. Naturforsch. **45c,** 757 (1990).
[15] F. Franck and G. H. Schmid, Biochim. Biophys. Acta **977,** 215 (1989).
[16] J. Messinger and G. Renger, FEBS Lett. **227,** 141 (1990).
[17] J. Messinger, U. Wacker, and G. Renger, Biochemistry **30,** 7852–7862 (1991).
[18] M. Villebrandt, W. E. Krumbein, and L. J. Stal, Abstract Book of the 15th International Symposium on Nitrogen Fixation with Non-Legumes, p. 27, Florence, Italy 1990.