Quantitative Determination of the Distribution between Two Photosystem II-Mediated Oxidation Reactions in *Oscillatoria chalybea*: A Comparison between Water Oxidation and Hydrogen Peroxide Decomposition

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Dedicated to Professor Wilhelm Menke on the occasion of his 80th birthday

Hydrogen Peroxide, Water Splitting, S-State System, Filamentous Cyanobacterium, Mass Spectrometry

Mass spectrometric analysis reveals that oxygen evolution measured as the consequence of short saturating light flashes in thylakoid preparations of the filamentous cyanobacterium *Oscillatoria chalybea* consists of two portions, one coming from photosynthetic water splitting and one coming from H_2O_2 decomposition. This H_2O_2 decomposition is photosystem II-mediated and it is the S-state system which oxidizes H_2O_2 to give protons and oxygen. Water is neither intermediate nor seems it to be the origin of the reaction. At the high oxygen partial pressure of normal air H_2O_2 production and its decomposition exceeds manyfold the H_2O splitting reaction. H_2O_2 production seems to come from photosystem II but is not necessarily produced on the acceptor side of photosystem II in the sense of a Mehler type reaction. From the reaction rate and the observed labeling density, it is inferred that production and decomposition must take place within the same reaction site which might be for both, the production and decomposition, the S-state system. In this sense, H_2O_2 might be the product of the S-state system and seems somehow associated with S_2 or S_3. Thus, if oxygen evolution is measured as the consequence of short saturating light flashes in an ambient atmosphere of 21% oxygen, mass spectrometry reveals a flash pattern which bears the oxygen label of the ambient atmosphere and which has not much in common with an usual Kok sequence. Such a pattern might start in the first three flashes more or less as a Kok pattern would, but is then in the pattern portion usually characteristic for steady-state oxygen evolution characterized by a periodicity of two indicating that H_2O_2 decomposition requires only two light quanta. At high oxygen partial pressure (*e.g.* in 21% $^{16}O_2$) both reaction portions can be quantitatively determined by labeling the assay with $H_2{}^{18}O$ and measuring the evolved $(^{16}O::^{18}O)/^{18}O_2$ ratio which is representative for water splitting. The measured evolution of $^{16}O_2$ (mass 32) represents H_2O_2 decomposition, if the $^{16}O_2$ portion calculated from the measured mass 34/36 ratio is subtracted. At low oxygen partial pressure the H_2O_2 forming and decomposing reaction is largely suppressed and oxygen evolution from water-splitting prevails. As a hypothesis, H_2O_2 production and its decomposition might be a defective performance of photosystem II at high ambient oxygen partial pressure in these cyanobacteria, perhaps due to the principal absence of two of the extrinsic peptides from photosystem II.

Introduction

We have been able to show that thylakoids and thylakoid particle preparations of the cyanobacterium *Oscillatoria chalybea* exhibit a photosystem II-mediated oxygen-uptake reaction which leads to H_2O_2 formation. This H_2O_2 is decomposed by the S-state system to give protons and oxygen, requiring the accumulation of only two positive charges [1, 2]. This H_2O_2/O_2 cycle takes place simultaneously with the photosynthetic water-splitting reaction [2] in the cyanobacterium and seems to be part of a photosystem II-mediated oxidative process in which the nitrogen storage product cyanophycine (poly-arginyl-aspartate) is used for energy production [3]. It looks as if photosynthetic water splitting as well as H_2O_2 splitting required the presence of a small threshold quantity of oxygen. Thus, under completely anaerobic conditions photosynthetic water splitting did not occur in this cyanobacterium [1]. In the present paper we determine the quantitative repartition of oxygen gas exchange in *Oscillatoria chalybea* between the water-splitting reaction and the "H_2O_2 formation and H_2O_2-splitting reaction" by means of mass spectrometry as well as the oxygen-binding affinity of

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the water-splitting enzyme system in our cyanobacterium.

Materials and Methods

Mass spectrometry: The experiments were performed in a modified magnetic sector field mass spectrometer type "Delta" from Finnigan MAT (Bremen, F.R.G.). The experimental set-up with the valve system is described in detail in an earlier paper [4]. All assays were carried out in a homemade measuring cell in which a teflone membrane separated the reaction space from the ionic-source side. The geometry of the cell is described elsewhere [4, 5]. The cell is equipped with a gas-tight lid which permits flushing with different gas mixtures. Signals were recorded on a SE 130-03 BBC Metrawatt three-channel recorder. Light flashes of 8 µsec duration were provided by a Stroboscope (1539 A of General Radio) and usually spaced 300 msec apart. Oxygen partial pressure conditions were established by flushing the assay system with nitrogen and injecting desired quantities of $^{16}O_2$ or $^{18}O_2$ (98% from CEA-Oris, Bureau des Isotopes Stables, Gif-sur-Yvette, France). The oxygen partial pressure in the gas phase was allowed to equilibrate with the liquid assay. It should be noted that the assay is in equilibrium with only a closed gas volume. As the assay is separated, as said above, by a teflone membrane from the evacuated detection system of the mass spectrometer, the assay system constantly looses oxygen and drifts towards anaerobiosis. This time course of oxygen depletion is constantly monitored by measuring the oxygen background of the assay system by simultaneous recording of masses 32 ($^{16}O_2$), 34 ($^{16}O::^{18}O$) and 36 ($^{18}O_2$) in mVolt.

The filamentous cyanobacterium Oscillatoria chalybea was obtained from the algal collection in Göttingen and cultured on different growth media as described earlier [6, 7].

Thylakoid preparations and thylakoid particle preparations were prepared according to Bader *et al.* [6]. The mucoid layer of the cells was digested with glucuronidase (Boehringer, Mannheim) and the cell walls with lysozyme (Sigma) and cellulase (Kinki Yakult, Japan).

Results

If oxygen evolution is measured as the consequence of short saturating light flashes in thylakoid preparations of *Oscillatoria chalybea,* no or only very little oxygen is evolved, if the oxygen partial pressure is below a certain threshold (Fig. 1). In this experiment starting from fairly anaerobic conditions (35 mV $^{16}O_2$), obtained by flushing the system with nitrogen, distinct oxygen background or partial pressure conditions are established by injecting $^{16}O_2$ to give 100 mV background and $^{18}O_2$ to give a background of 37 mV $^{16}O_2$ + 90 mV $^{18}O_2$ and 40 mV $^{16}O_2$ + 137 mV $^{18}O_2$ where the 37 and 40 mV $^{16}O_2$ correspond to the remaining $^{16}O_2$ background after flushing with nitrogen. It should be emphasized that in this experiment the liquid assay system contained only $H_2{}^{16}O$ containing buffer, hence no ^{18}O label in the aqueous system. Oxygen evolution signals are the consequence of 10 flashes and are for the first time given as absolute values. It is seen that rising

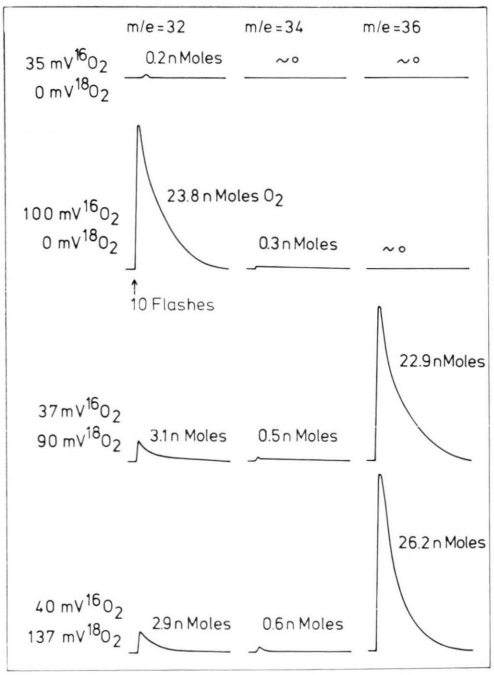


Fig. 1. Mass spectrometric analysis of photosynthetic oxygen evolution measured as masses 32 ($^{16}O_2$), 34 ($^{16}O::^{18}O$) and 36 ($^{18}O_2$) in *Oscillatoria chalybea*. The figure shows the isotope distribution of the oxygen-evolved upon illumination with 10 saturating light flashes spaced 300 msec apart under anaerobic conditions and various low oxygen partial pressures given in mvolt $^{16}O_2$ and $^{18}O_2$ for the recorded signals. Integration of the mass spectrometric signals yielded absolute values of oxygen evolution in nmol. The assay does not contain $H_2{}^{18}O$.

oxygen partial pressure from 35 to 100 mV $^{16}O_2$ restores oxygen evolution from 0.2 nmol to 23.8 nmol. This value is apparently exclusively due to the H_2O_2-splitting reaction described earlier, as $^{18}O_2$ in the gas phase shifts the observed O_2 signal from mass 32 to mass 36 (compare Fig. 3 in ref. [1]). If the observed mass 32 signal under 100 mV $^{16}O_2$ background was due to water splitting, addition of $^{18}O_2$ to the gas phase should give the same peak as before at mass 32. What is observed is competition between $^{16}O_2$ and $^{18}O_2$ which demonstrates an O_2-uptake reaction prior to the observed O_2 evolution. It should be noted again that no substantial mass 34-signal is observed, which clearly excludes that the uptake reaction leads to $H_2{}^{18}O$ as an intermediate. If the observed $^{16}O_2$ signal is plotted against the O_2-partial pressure (in the experiment of Fig. 1 these values are 35 mV, 37 mV, 40 mV and 100 mV), the dependence of the oxygen signal on the oxygen partial pressure would yield apparently a sigmoidal shaped curve or a curve which starts on the abscissa at around 35 mV O_2. On the other hand, the *quasi* linearity of the $^{16}O_2$ signal in dependence on the $^{16}O_2$ background shown in Fig. 1 demonstrates that the assay system really measures only one reaction, namely the H_2O_2/O_2 reaction. A contained water-splitting portion of the gas exchange contributes under the conditions shown with only 1/10 to the overall exchange (Figs. 2 and 3). The contribution of the water-splitting reaction in the preparation used is determined by introducing, in addition to the label of the gas phase, $H_2{}^{18}O$ into the liquid phase. Already a low label of $H_2{}^{18}O$ (25% in Fig. 2 and 28% in Fig. 3) permits to separate the contribution of the water-splitting reaction from that of H_2O_2 splitting. According to mechanistic reasons described in detail earlier [1, 4] the theoretical isotope distribution belonging to the water-splitting reaction, with a labeling density of 25% $H_2{}^{18}O$ in the assay will be 6.52% mass 36 ($^{18}O_2$) with a high label, namely 37.5% in mass 34 ($^{16}O::{}^{18}O$). The corresponding quantity of unlabeled O_2-evolved is 56.25% ($^{16}O_2$). Thus, for example measurement of the mass 32:34 ratio in the assay with all oxygen in the gas phase being $^{18}O_2$ permits the quantitative evaluation of the water-splitting reaction in Fig. 2 and 3. The theoretical ratio of masses 32:34 is 1.5 for Fig. 2. However, only a ratio of 1.3 is observed which represents 86% of the theoretical value. On

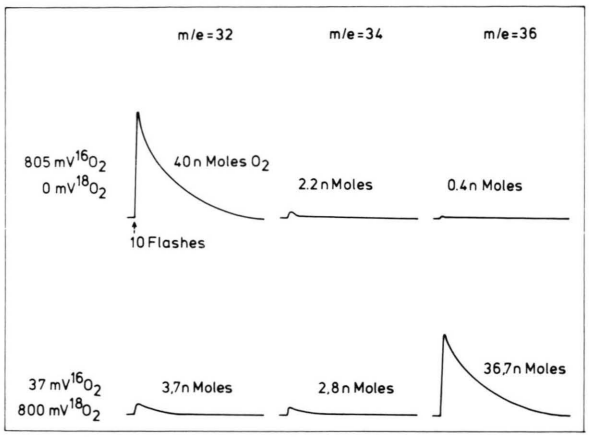


Fig. 2. Photosynthetic oxygen evolution measured as the consequence of 10 short-saturating light flashes in *Oscillatoria chalybea*. The reaction assay contained 25% $H_2{}^{18}O$. After flushing the measuring cell with N_2 an artificial gas atmosphere was installed with only $^{16}O_2$ and no $^{18}O_2$ or as indicated with $^{18}O_2$ and a low $^{16}O_2$ content (in mVolt).

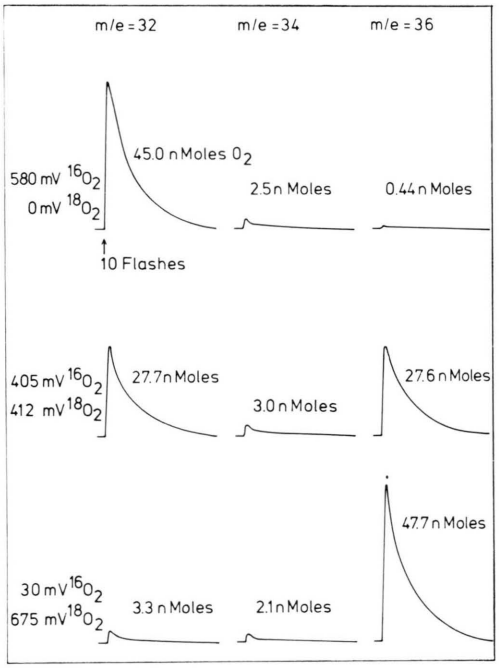


Fig. 3. Photosynthetic oxygen evolution as the consequence of 10 light flashes in *Oscillatoria chalybea*. The assay contained 28% $H_2{}^{18}O$. After flushing the measuring cell with N_2 artificial gas atmosphere conditions were installed. The figure represents monoisotopic conditions with virtually 100% $^{18}O_2$ and 100% $^{16}O_2$ respectively together with the exactly mixed condition of 50% $^{16}O_2$/ 50% $^{18}O_2$.

the other hand the ratio of masses 34:36 in assays with only $^{16}O_2$ (1/1 $^{16}O_2$) in the gas phase have all the tendency to be very close to the theoretical value of 6 (Fig. 2). This might indicate interference of $^{18}O_2$ *with the water-splitting reaction* probably in the sense of the earlier described indication of an isotope effect, observed in context with the anaerobiosis effect [1]. It was seen, as in Fig. 1 here, that in the absence of oxygen no oxygen-evolving reaction whatsoever was taking place [1, 4]. This anaerobiosis was overcome by the presence of a small quantity of O_2 which probably had to be bound first, before O_2 evolution *i.e.* water splitting could occur, since each O_2 evolution signal was preceded by a small O_2 uptake (Fig. 2 in ref. [1]). The extent of the here observed preponderance of the H_2O_2-splitting reaction over the water-splitting reaction depends on growth conditions and on the stage of growth of the cyanobacterium. As stated earlier, both reactions yield O_2 and protons [1] which means that splitting of one equivalent water and one equivalent H_2O_2 yields a stoichiometric O_2 ratio of 1:2. Thus, conditions have been chosen and described earlier in which both reaction levels, simultaneously determined, give 50% water splitting and 50% H_2O_2 splitting (Fig. 3 in ref. [1]).

In a system with $H_2{}^{18}O$ in the aqueous assay measurements of the mass peaks 34 and 36 permit the separation of contributions due to the water-splitting reaction. Fig. 4 shows the photosynthetic water-splitting reaction starting from anaerobic conditions in dependence on the $^{16}O_2$-partial pressure. The plot shows a curve in which mass 34 O_2 evolution starts at around 20 mV partial pressure (which is close to zero) but which in contrast to the experiment in Fig. 2 becomes at very low partial pressure (100 mV) practically independent on O_2-partial pressure. In this case a very small amount of oxygen is apparently necessary in order to initiate the reaction. We think that the action consists in inducing a conformational change or a change in redox potential in the water-splitting enzyme. In the case of the H_2O_2/O_2 cycle ($^{16}O_2$ *i.e.* mass 32 in Fig. 4) the O_2 dependency of the evolution reaction measures the O_2 affinity of the uptake enzyme which leads to H_2O_2 formation. Both reactions clearly belong to photosystem II as shown amongst others by the fact that Tris-washing abolishes O_2 evolution as a whole in this cyanobacterium (Table I).

If oxygen evolution is measured as the consequence of short saturating light flashes, the amount of oxygen plotted as a function of the flash number of the sequence does not yield the usual Kok pattern (Fig. 5). The sequence starts out somewhat as a Kok sequence would do and yields at higher flash numbers, especially in the region where the Kok sequence usually shows steady-state oxygen evolution, an oscillation behaviour with a periodicity of two (Fig. 5). The cumulated oxygen yield in dependence on the number of flashes is shown for comparison in Fig. 5. A regular Kok pattern, however, is observed, if the buffer system of the assay is labeled with $H_2{}^{18}O$ and the flash yield of oxygen is recorded for $^{18}O_2$ (Fig. 3 in ref. [4]) or mass 34 ($^{16}O::{}^{18}O$).

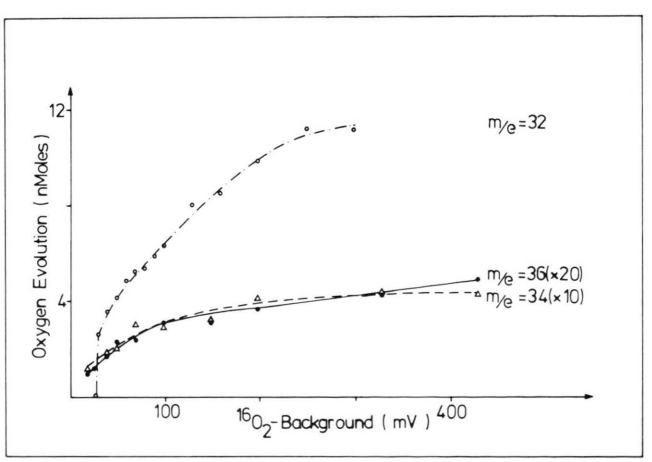


Fig. 4. Dependence of photosynthetic oxygen evolution on the $^{16}O_2$ background of the analyzing system. The reaction assay contained 50% $H_2{}^{18}O$. Under such conditions signals of masses 34 and 36 belong exclusively to the water cleavage reaction, whereas the mass 32 signal reflects a mixture (varying with the oxygen content of the assay) of water oxidation and the H_2O_2 decomposition.

Table I. The influence of Tris treatment on oxygen evolution measured by means of mass spectrometry in the filamentous cyanobacterium *Oscillatoria chalybea*. The signals were induced by a single as well as by 10 short-saturating light flashes spaced 300 msec apart and recorded in parallel as $^{16}O_2$ (m/e = 32), $^{16}O::^{18}O$ (m/e = 34) and $^{18}O_2$ (m/e = 36). The oxygen evolution was measured in dependence on the incubation time in Tris buffer (0.8 M, pH 8) prior to the mass spectrometric analysis. After Tris buffer treatment the preparation was washed once with 0.06 M tricine/0.03 M KCl (pH 7.5) and resuspended in the same buffer. Values are given in nmol oxygen evolution of the respective isotope. Negative values refer to oxygen uptake.

	Control		1 h Tris		2 h Tris		2¾ h Tris	
	1 flash	10 flashes	1 flash	10 flashes	1 flash	10 flashes	1 flash	10 flashes
m/e = 32	4.2	50	4.6	47.8	0.2	21.4	−0.4	−0.8
m/e = 34	0.14	1.3	0.08	1.64	≈0	0.68	−0.12	−0.16
m/e = 36	4.2	48	6.0	56.8	≈0	21.2	−0.3	−1.8

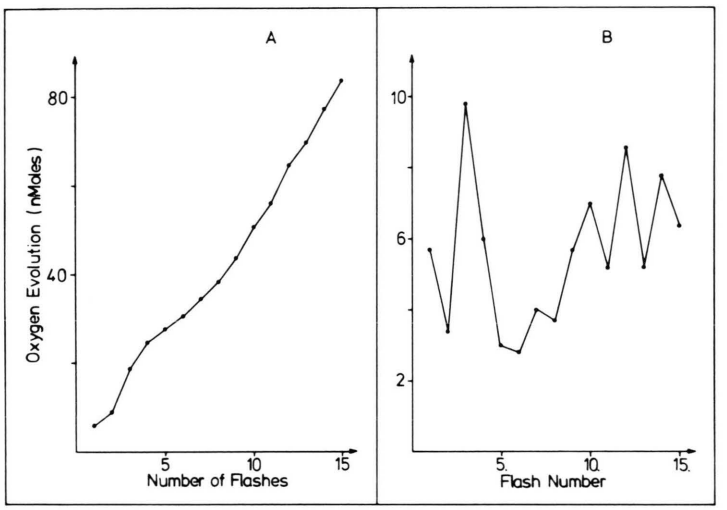


Fig. 5. Mass spectrometric analysis of photosynthetic oxygen evolution measured as mass 32 in *Oscillatoria chalybea* by a train of short-saturating light flashes in an ambient atmosphere of 7% oxygen. The figure shows the accumulated signals in dependence on the number of flashes (A) as well as the single flash resolutions of the oxygen amplitudes (B).

Discussion

The literature describes very carefully under which conditions H_2O_2 can act as electron donor to photosystem II preparations in higher plants, but not much is known about its formation in the region of photosystem II. Work by Åkerlund and co-workers [8] shows that in NaCl washed "inside-out particles"of spinach in which the two extrinsic 16 and 23 kDa peptides have been washed away, H_2O_2 functions as an electron donor to photosystem II. The authors think that it is the removal of these two proteins which creates the accessability of H_2O_2 as an electron donor to photosystem II. This interpretation suites us in the sense that the photosystem II complex of cyanobacteria lacks these two extrinsic peptides by principle. Our work shows that H_2O_2 formation *and* its decomposition are an inherent property of photosystem II and O_2 metabolism in our filamentous cyanobacterium [1–3]. In fact we were able to show that thylakoids and particle preparations of the filamentous cyanobacterium *Oscillatoria chalybea* exhibit a photosystem II-mediated O_2-uptake reaction which leads to H_2O_2 formation [1]. The H_2O_2-formed is decomposed by the S-state system in a reaction which needs only two light quanta and yields protons, electrons and oxygen [1]. It is apparently under conditions of chloride deficiency when H_2O_2 becomes an electron donor to photosystem II of higher plants as work by Berg and Seibert [9] and Sandunsky and Yocum [10] shows. As cyanobacteria lack these two extrinsic peptides which re-

gulate in photosystem II of higher plants the binding and binding affinity of chloride and calcium ions, it appears that observations on H_2O_2 oxidation made under calcium ion and chloride deficiency in higher plants are directly, that is under natural conditions, applicable to cyanobacteria. Cyanobacteria seem to live somehow under chronical chloride and calcium deficiency, since they bind these essential ions with much lower affinity than higher plants. May be due to this property their photosystem II owns under natural conditions after all the faculty to oxidize H_2O_2.

From the methodological point of view oxygen gas exchange in *Oscillatoria chalybea* preparations measured by polarography and mass spectrometry provides some very interesting insights. If oxygen evolution in *Oscillatoria chalybea* is measured polarographically as the consequence of short saturating light flashes by means of the large surface electrode developed by Schmid and Thibault [11], a sequence is observed which can be analyzed according to the Kok model [6, 12]. The sequence appears different from the usual *Chlorella* sequence of the literature in that a substantial O_2 signal is observed under the first flash with maximal flash yield under the fourth flash [6]. Mathematical analysis of such a sequence in the four state Kok model yields a better fit than a *Chlorella* sequence [6]. Hence, this method essentially measures O_2 evolution due to photosynthetic water-splitting reaction describable within the Kok model [12]. However, when oxygen evolution in *Oscillatoria chalybea* as the consequence of short saturating light flashes is measured in a normal ambient gas atmosphere of 21% O_2, 79% N_2, in equilibrium with the assay, by means of mass spectrometry a Kok sequence is only observed, if the assay system is labeled with $H_2{}^{18}O$ and if mass 34 ($^{16}O::^{18}O$) or mass 36 ($^{18}O_2$) is taken into account ([4] and Fig. 4). If $^{16}O_2$ (*i.e.* mass 32) is the background species that is the species which makes up the O_2-partial pressure of the surrounding gas phase (in equilibrium with the liquid assay system), no such sequence is observed. The sequence then observed has nothing in common with a "Kok sequence" (Fig. 5).

Amplitudes of this type of O_2 evolution are particularly sensitive to O_2-partial pressure in the sense that lowering O_2-partial pressure strongly diminishes the extent of this type of O_2 evolution (Fig. 1, 2 and 4). The question arises why this effect is not seen on the electrode. Our current explanation is that on our bare electrode system only particles in intimate contact with the electrode surface are measured. Due to the oxygen consumption of the electrode at the high polarization voltage used, these particles are in a *quasi* anaerobic condition which after sufficient sedimentation, keeps the system in the region of an O_2 background where O_2 evolution due to H_2O_2 splitting is practically inexistent. Moreover, O_2 evolution due to water splitting is a very fast reaction in which oxygen after the charge accumulation in four flashes, which requires $\approx$ 1.5 msec, appears in approximately 1 msec (Schulder, Bader and Schmid, in preparation) thus outranging the signal which would occur due to H_2O_2 splitting. Although the metabolic provenance of H_2O_2 is not entirely clear, it should be noted that it is certain that H_2O_2 formation is the consequence of an O_2-uptake reaction which after all explains why the fast O_2-evolution reaction due to H_2O_2 decomposition by the S-state system (measured as the consequence of short saturating light flashes) occurs with a certain time delay in comparison to O_2 evolution due to water splitting (Figs. 2 and 3 in ref. [1]).

The metabolic provenance of H_2O_2 is still under investigation. From the data obtained here and reported earlier [1, 2] only two possibilities have to be considered. First H_2O_2 production is due to an oxidative process mediated by a photosystem II-associated enzyme reaction [3]. This enzyme must be topographically very close to the S-state system in order to justify the observed evolution speed (see Fig. 3 in ref. [1]). As judged from the extent of the reaction, the enzyme could be part of the reaction center, as the reaction is observed with particle preparations that exhibit essentially only photosystem II activity [1, 13], and Tris-treatment abolishes both reactions namely O_2 evolution due to water splitting as well as that due to H_2O_2 splitting (Table I). The second possibility to be taken into account is that the H_2O_2-forming enzyme or enzyme system, although associated with the photosystem II particle preparations, has nothing to do with photosystem II proper but is in such a close structural relationship to the S-state system that the diffusion distance for H_2O_2 is short enough as to permit the reaction rates observed [1, 15]. In this case only the H_2O_2 decomposition

would be photosystem II-mediated and production would come from elsewhere. In the frame of this possibility H_2O_2 production could be thought to come from the alternative respiratory pathway demonstrated in thylakoid preparations of *Oscillatoria chalybea* [2]. Our experiments support the first possibility. Thus, if, as we think, H_2O_2 production and its decomposition are inherent properties of photosystem II in these filamentous cyanobacteria, oxygen necessary for the H_2O_2 formation could as a possibility come from photosynthetic water splitting, if oxygen partial pressure is low, whereas at the high oxygen partial pressure of normal air, O_2 coming from the O_2 equilibrium between air and the aqueous phase is taken up. It is interesting to be aware of the fact that under conditions of labeling the assay system with $H_2{}^{18}O$ the $^{16}O::^{18}O$ and $^{18}O::^{18}O$ produced by photosynthetic water splitting [1, 4] would yield H_2O_2 with mixed oxygen label ($H-{}^{16}O-{}^{18}O-H$) and a fully labelled ($H-{}^{18}O-{}^{18}O-H$) molecule species, if the O_2-uptake reaction was for example due to a classic oxidase type reaction. The H_2O_2-decomposing mechanism which we have demonstrated to occur in *Oscillatoria chalybea* [1], namely the decomposition by the S-state system yielding only protons and O_2 would quantitatively yield back the O_2 species with masses 34 and 36 which photosynthetic water splitting had produced before. This is to say that mass spectrometry would completely overlook the reaction sequence in which O_2 produced by water splitting is taken up by an oxidase type reaction yielding H_2O_2 which only *then* would be decomposed by the S-state system.

We think that the provenance of the H_2O_2 is the remaining interesting point. In this context it seems certain that the producing reaction belongs somehow to the immediate vicinity of the photosystem II reaction center. But here is should be clarified whether the H_2O_2 formation originates from a reaction which results from the enzymic bifunctionality of a photosystem II protein (see work by Pistorius and co-workers [13]) or whether H_2O_2 or a peroxide is a partially oxidized intermediate of water oxidation [16, 17]. If the second possibility applied exclusively, only the S_3-state could be associated with H_2O_2 or a peroxide. A hint in this direction could be the positive signal under the first flash in a polarographically measured flash pattern. This, however, does somehow not fit observations on H_2O_2 decomposition measured by mass spectrometry which exhibits a periodicity of two (Fig. 5), hence requires two light quanta, a fact which not only results from our experiments but also from those published by Mano and co-workers [18].

The observations made in Figs. 2 and 3 with the extreme situation of the reaction distribution between water splitting and H_2O_2 decomposition almost forces oneself to think that the measured photosystem II-mediated O_2-gas exchange is the product of a reaction in which O_2 of the gas atmosphere joins photosynthetically formed oxygen for H_2O_2 formation.

Thus, two reactions would have to be considered.

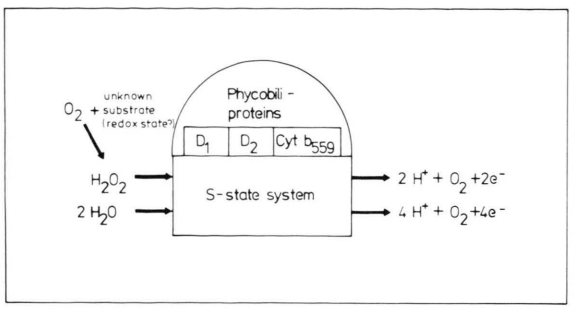
$$\text{I.}\quad H_2O \xrightarrow[\text{system}]{\text{S-state}} O_2 + 4H^+ + 4e^-;$$

$$\text{II.}\quad \text{a substrate (unknown)} + O_2\left(\begin{array}{l}\text{from the atmosphere}\\ \text{+ that from the photosynthetic}\\ \text{water splitting}\end{array}\right) \rightarrow$$

$$H_2O_2 \xrightarrow[\text{system}]{\text{S-state}} O_2 + 2H^+ + 2e^-.$$

Due to the observed effect of anaerobic conditions (Fig. 1) especially on the second reaction, it has been clearly demonstrated that at low oxygen partial pressure in the assay system photosynthetic water splitting regulates the "oxidase reaction", which under these conditions clearly depends only on the extent of the water-splitting reaction. In this region of O_2-partial pressure the oxidase reaction is tandem-arranged to the water splitting whereas at high O_2-partial pressure the reaction *competes* with the water-splitting reaction within the S-state system itself or at least by the circumstance that the S-state system is used for H_2O_2 decomposition. According to a hypothesis of Pistorius and Gau [13] a 36 kDa protein belonging to the reaction center complex of photosystem II in the coccal cyanobacterium *Anacystis nidulans* exhibits under certain conditions the properties of an L-amino acid oxidase with a remarkably high turn-over. If this hypothesis is applied, reactions I and II could belong to the same structural complex, which might suite interpretations of a large part of the observations made, concerning reaction speed and isotope distributions [1, 2, 4].

Scheme:



However, as H_2O_2 formation and decomposition occur with high rates not only in the entire organism [2] or in thylakoid preparations [14] but also in photosystem II particle preparations [1] in assays with no external mediator or substrate, the high oxygen concentration of the ambient atmosphere (air) is the only serious and probably decisive cause for the observed reaction. The latter might simply be a defective performance of photosystem II in the cyanobacterium at high oxygen concentrations, perhaps due to the simpler structure of photosystem II which lacks two of the extrinsic peptides.

Acknowledgement

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