Further Evidence for a Functional Relationship between L-Amino Acid Oxidase Activity and Photosynthetic Oxygen Evolution in *Anacystis nidulans*. Effect of Chloride on the Two Reactions

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Anacystis nidulans, O_2 Evolution, L-Amino Acid Oxidase, Chloride

The L-amino acid oxidase from *Anacystis nidulans* is inhibited by cations as well as anions. The inhibition by cations has been previously described (E. K. Pistorius, Eur. J. Biochem. **135,** 217–222 [1983]). We have shown that the order of effectiveness was $M^{3+} > M^{2+} > M^{+}$, when *e.g.* La^{3+}, Ca^{2+} and K^{+} were compared. However, in the concentration range where the monovalent cations inhibited, the inhibition was not entirely due to the cation, but an influence of the anion could also be observed. When monovalent anions were compared as the corresponding sodium salts, the order of effectiveness was $SCN^{-} > NO_3^{-} > Cl^{-}$, $Br^{-} > I^{-} > F^{-} > HCOO^{-} > CH_3COO^{-}$. The inhibition of the L-amino acid oxidase activity by the various salts was strongly influenced by the pH of the reaction mixture. It could be shown that the inhibition by cations increased in the alkaline pH region, while the inhibition by anions increased in the acidic pH region.

Our previous results have also shown that a functional relationship might exist between L-amino acid oxidase activity and photosynthetic O_2 evolution (E. K. Pistorius and H. Voss, Eur. J. Biochem. **126,** 203–209 [1982]). Since the water-splitting complex of photosystem II is affected by a number of anions, although only Cl^{-} and Br^{-} lead to activation of O_2 evolution, we investigated whether a correlation could be obtained between the anion effect on the L-amino acid oxidase and on photosynthetic O_2 evolution. The results show that those anions which have a higher affinity for the enzyme than Cl^{-} or Br^{-}, are especially effective in causing inactivation of the O_2 evolution. Moreover, we show that L-arginine which is a substrate of the L-amino acid oxidase, and Cl^{-} have antagonistic effects on the L-amino acid oxidase reaction and on photosynthetic O_2 evolution. We suggest that this flavoprotein with L-amino acid oxidase activity is modified by Ca^{2+} and Cl^{-} in such a way that it can now interact with Mn^{2+} and catalyze the water-splitting reaction of photosystem II.

Introduction

Much knowledge has recently been accumulated about the polypeptide composition of the photosynthetic O_2 evolving system [1–3]. Especially three polypeptides of approximately 33, 24 and 16 kDa which have been shown to be localized at the inner thylakoid surface in plant chloroplasts have received much attention [1, 4–8]. It has been shown that these three peptides can readily be removed by various washing procedures and that removal of these peptides will lead to loss of the O_2 evolving capacity. These three peptides have a structural rather than a catalytic role and possibly shield the water-splitting complex from other reductants than water. It has been shown that the 33 kDa peptide is essential for preserving Mn in the O_2 evolving system but that it can partially be replaced by high Cl^{-} concentrations, and it has also been reported that Ca^{2+} and Cl^{-} could be substituted for the 24 and 16 kDa peptides [8–13]. These results suggest that the required cofactors for the water-splitting reaction (Mn^{2+}, Ca^{2+} and Cl^{-}) are bound to one (or possibly two) of the intrinsic proteins, but that the protein(s) will loose its cofactors readily in the absence of the smaller peptides. After removal of these peptides the concentration of Cl^{-} and Ca^{2+} required for optimal O_2 evolution is much increased as though these peptides cause an increase in the affinity of the intrinsic protein(s) for its cofactors [11, 12]. Nakatani [14] has suggested that minimally two Mn atoms are required for photosynthetic O_2 evolution and in addition Cl^{-} and Ca^{2+}.

It has been suggested that photosystem II of blue-green algae is essentially similar to that of chloroplasts [15]. Since blue-green algae have a more loosely organized photosynthetic membrane [16], some of the smaller peptides may be lost more readily during

Abbreviations: Enzyme: L-amino acid oxidase or L-amino acid oxygen oxidoreductase (deaminating) E.C. 1.4.3.2.

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preparation, and the requirement for Ca^{2+} (and possibly for Cl^-) can be seen more easily [17]. We have shown that photosynthetically active particles of *Anacystis nidulans* contain a flavoprotein with L-amino acid oxidase activity [18], and we have suggested that this protein might be a component of the water-splitting-complex of photosystem II in *A. nidulans*. In other words, this flavoprotein might have a dual role: acting as an L-amino acid oxidase in the absence of cations and being part of the water-splitting center in the presence of certain cations (Mn^{2+} and Ca^{2+}). Because of the demonstrated importance of Cl^- for the water-splitting complex of chloroplasts, it should be expected that the isolated flavoprotein of *A. nidulans* is also affected by Cl^-.

Materials and Methods

Anacystis nidulans (Synechococcus leopoliensis) B 1402-1 was obtained from the Sammlung von Algenkulturen, Universität Göttingen. The growth of cells was the same as reported previously [18], and the French press particles were also prepared as described previously (particles A of [18]). O_2 uptake and O_2 evolution were measured with a Gilson Oxygraph [18]. The composition of the reaction mixtures for both assays is given in the legends to the tables and figures.

The L-amino acid oxidase from *A. nidulans* was purified as described previously [19]. We have used side fractions of the last column of the purification, and these had specific activities of 12 to 36 μmol O_2 taken up × mg protein^{-1} × min^{-1} (with L-arginine as substrate, in air, in absence of catalase at 20 °C). The enzyme was dialyzed against 0.02 M Hepes-NaOH, pH 7, before use.

NH_4^+ determination: After 15 min reaction time in the oxygraph the reaction was stopped by adding H_2SO_4 (300 μmol H_2SO_4 were added to 1.5 ml of the reaction mixture). The sample was centrifuged and in an aliquot of the supernatant which was neutralized with NaOH, NH_4^+ was determined enzymatically by measuring the decrease of the absorbance of NADPH in the presence of excess α-ketoglutarate and glutamate dehydrogenase [20]. When ferricyanide was present in the reaction mixture, sodium ascorbate (10 μmol) was added to the reaction mixture for the NH_4^+ test to destroy excess ferricyanide.

In previous publications we have always used L-arginine-HCl which was adjusted with NaOH to the pH of the reaction mixture. In this paper we used L-arginine (free base) which was adjusted to the pH of the reaction mixture either with HCl (L-Arg-HCl) or with a solution of Hepes (L-Arg-Hepes), *e.g.* a solution of 0.1 M L-Arg (free base) contained at pH 7 to 7.2 in addition 0.09 M HCl or 0.3 M Hepes, respectively.

Results and Discussion

Inhibition by various anions and cations of L-*arginine oxidation catalyzed by the purified* L-*amino acid oxidase*

We had previously shown that the oxidative deamination of L-arginine catalyzed by the purified L-amino acid oxidase of *A. nidulans* was inhibited by various cations and that the order of effectiveness was $M^{3+} > M^{2+} > M^+$, when *e.g.* La^{3+}, Ca^{2+} and K^+ were compared [18]. The divalent cations could roughly be divided into two groups: the alkali earth metals with lower affinity for the enzyme and the transition metals, like *e.g.* Mn^{2+} and Zn^{2+}, with higher affinity for the enzyme [19]. The experiments which we show here, indicated that in the higher concentration range where the monovalent cations inhibited, the anion as well as the cation contributed to the inhibition of L-arginine oxidation.

In all our previously published experiments, L-arginine-HCl (L-Arg-HCl) was used as substrate. When we realized that anions, such as Cl^-, also had an effect on the activity of the enzyme, we started to use L-arginine (free base) adjusted with Hepes to the pH of the reaction mixture (L-Arg-Hepes).

The results of the present studies are necessarily complex. Table I shows the relative effectiveness as inhibitors of chloride and acetate with a variety of counter ions. The data is given in the form of the salt concentration required for 50% inhibition. The table shows that a substantial difference between chloride and acetate could not be demonstrated with the di- and trivalent cations which are effective inhibitors themselves at low concentrations, but with the monovalent cations which inhibited only at higher concentrations, the chloride was about two to three times as inhibitory as the acetate. A comparison of the effect of various anions added as the corresponding sodium salts is given in Table II. If the halogen ions were compared at pH 7, the order of effectiveness was Cl^-, $Br^- > I^- > F^-$. NO_3^- was more effective than Cl^-, and SCN^- was most effective. Formate

Table I. Inhibition of L-amino acid oxidase by various salts: Comparison of mono-, di- and trivalent cations as the corresponding chloride or acetate.
Oxidative deamination of L-Arg (measured as O_2 uptake) catalyzed by the purified L-amino acid oxidase from *A. nidulans* was measured as described under Materials and Methods. The O_2 uptake was 34 μmol O_2 taken up × mg protein $^{-1}$ × min^{-1} in the absence of added salts. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 16 mM L-Arg-Hepes, pH 7, L-amino acid oxidase (2.7 μg protein), and the salts as indicated.

Additions	50% Inhibition at [mM]
NaCl	102
$Na(OOCCH_3)$	270
KCl	149
$K(OOCCH_3)$	384
NH_4Cl	89
$NH_4(OOCCH_3)$	233
$(CH_3)_4NCl$	84
$(CH_3)_4N(OOCCH_3)$	153
$CaCl_2$	1.6
$Ca(OOCCH_3)_2$	2.0
$MnCl_2$	0.16
$Mn(OOCCH_3)_2$	0.17
$ZnCl_2$	0.14
$Zn(OOCCH_3)_2$	0.14
$LaCl_3$	0.11
$La(OOCCH_3)_3$	0.14

Table II. Inhibition of L-amino acid oxidase by various salts: Comparison of the effect of anions.
Conditions as for Table I.

Additions	50% Inhibition at [M]
NaSCN	0.02
$NaNO_3$	0.05
NaCl	0.10
NaBr	0.10
NaI	0.13
NaF	0.24
Na(OOCH)	0.23
$Na(OOCCH_3)$	0.27
Na_2SO_4	0.14

and acetate were approximately equivalent to F^-. It should be pointed out that both anions which were more inhibitory than Cl^-, contained a nitrogen atom.

The pH profile of the L-amino acid oxidase activity is given in Fig. 1, together with the pH profile of the photosynthetic O_2 evolution in Anacystis particles

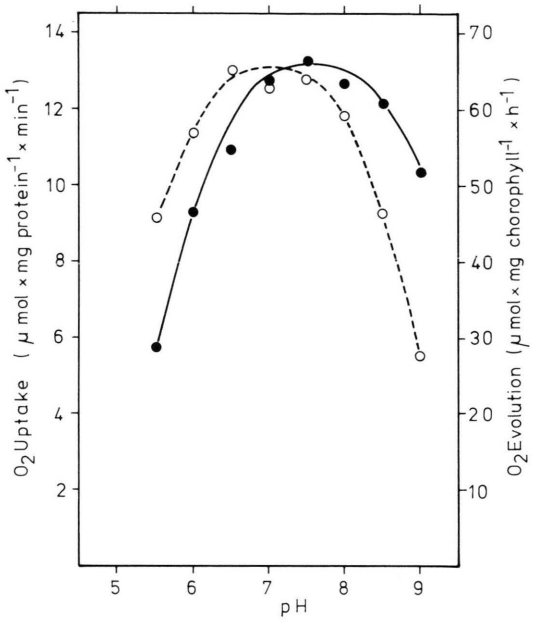

Fig. 1. pH profile of the oxidative deamination of L-arginine catalyzed by the purified L-amino acid oxidase and pH profile of photosynthetic O_2 evolution in presence of $CaCl_2$ catalyzed by Anacystis particles.
Oxidative deamination of L-Arg (measured as O_2 uptake) (●——●——●——●) and photosynthetic O_2 evolution (○---○----○) were measured as described unter Materials and Methods. For the oxidative deamination reaction of L-Arg, the reaction mixture contained in a total volume of 1.85 ml: 54 mM buffer, 16 mM L-Arg, and L-amino acid oxidase (2.7 μg protein). For the photosynthetic O_2 evolution, the reaction mixture contained in a total volume of 1.85 ml: 54 mM buffer, 5 mM potassium ferricyanide, 54 mM $CaCl_2$, and Anacystis particles containing 34 μg chlorophyll. As buffers were used: Mes-NaOH for pH 5.5 to 6.5, Hepes-NaOH for pH 7.0 and 7.5 and Tricine-NaOH for pH 8.0 to 9.0. L-Arg was adjusted to the corresponding pH values with Mes (pH 5.5 to 6.5) or with Hepes (pH 7.0 to 9.0).

supplemented with $CaCl_2$ (discussed later). Fig. 2 shows the inhibition of the L-amino acid oxidase by a selected variety of salts, as a function of pH. In general, both cation and anion were inhibitory, and the cation inhibition predominated in the alkaline pH range, whereas the anion inhibition was more prominent in the acidic pH region. The measurements with $CaCl_2$ and $MnCl_2$ in Fig. 2D give an impression of the involvement of an ionizable group with a pK of approximately 7. This suggests a direct interaction of the cation with an ionizable group on the enzyme, since the substrate, L-arginine, has no pK at this point.

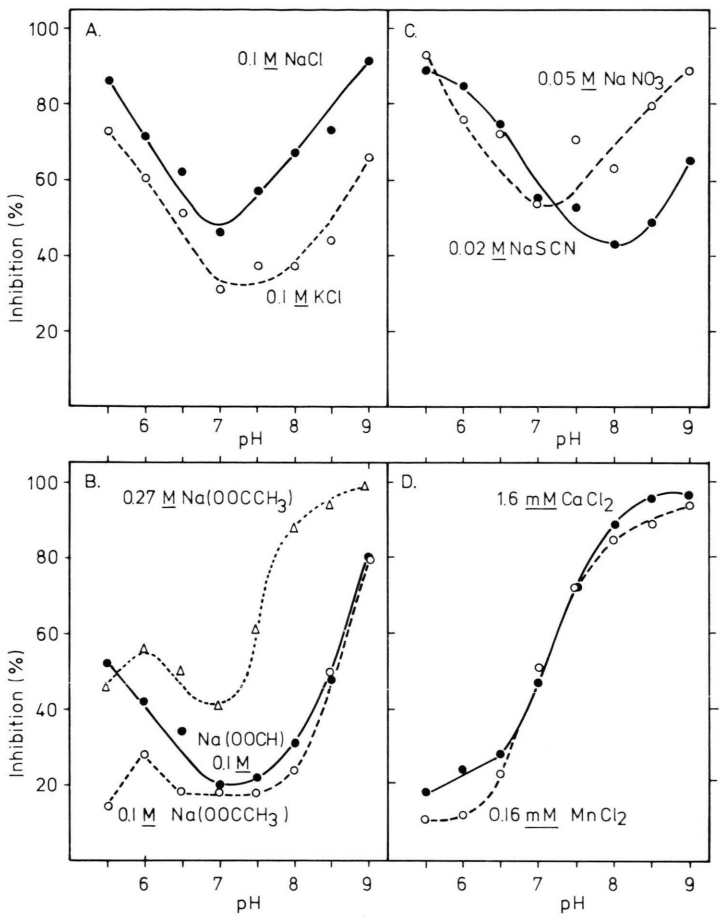

Fig. 2. pH profile of the inhibition of the L-amino acid oxidase activity by various salts. Oxidative deamination of L-Arg (measured as O_2 uptake) was measured as described under Materials and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM buffer, 16 mM L-Arg, L-amino acid oxidase (2.7 μg protein), and the salts as indicated. The buffers used were the same as in Fig. 1, and L-Arg was also adjusted to the corresponding pH valued as in Fig. 1. When $CaCl_2$ and $MnCl_2$ were tested, Hepps-NaOH was used instead of Tricine-NaOH, since Tricine binds polyvalent cations.

Effect of anions on photosynthetic O_2 evolution

The early work of Warburg and Lüttgens [21] indicated that maximal rates of electron transport in chloroplasts could only be attained if chloride ions were available. Stimulatory effects with added Br^- and NO_3^- have also been observed [21–23]. However, Sinclair [24] has recently reported that anions such as NO_3^-, CH_3COO^- or SO_4^{2-} would replace functional Cl^- and cause an inhibition of photosynthetic O_2 evolution. Nakatani [13] has shown that only Cl^- and Br^- can activate O_2 evolution, while no activation was achieved with anions such as NO_3^-, HCO_3^- or SO_4^{2-}. The flavoprotein with L-amino acid oxidase activity which we believe to be part of the water-splitting-complex of photosystem II in *A. nidulans*, is inhibited by all of these anions (as shown in Table II). Since Cl^- (or Br^-) is specifically required for activation of the O_2 evolution, it might be expected that those anions which have a higher inhibitory effect and therefore a higher affinity for the enzyme than Cl^- should be most inhibitory for O_2 evolution, presumably by replacing Cl^-.

The experiments here reported have been done with French press particles of *A. nidulans* as described previously [18]. These particles were prepared in the presence of $CaCl_2$ but then suspended in buffer without $CaCl_2$. The particles still contain sufficient Mn^{2+}, but only residual amounts of Ca^{2+} and Cl^-. They require addition of $CaCl_2$ to the reaction mixture for optimal O_2 evolution activity. NaCl at high concentrations can also lead to stimulation of the O_2 evolution activity, but Ca^{2+} is required to obtain maximal activity (see also [13]). However, high concentrations of $CaCl_2$ become inhibitory (Fig. 3). The pH profile for the O_2 evolution in the presence of $CaCl_2$ is given in Fig. 1.

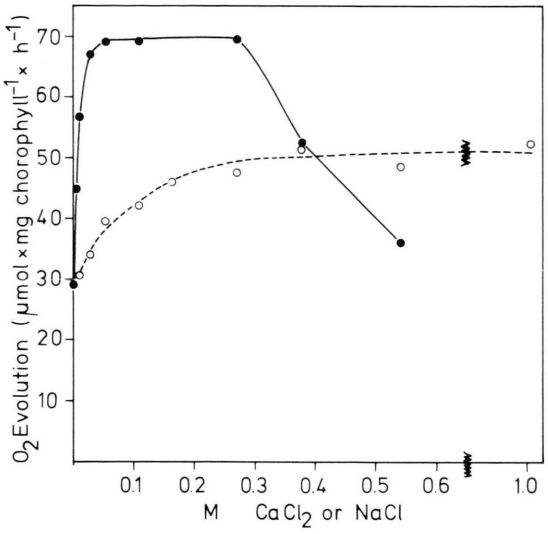

Fig. 3. Stimulation of photosynthetic O_2 evolution in *Anacystis* particles by $CaCl_2$ or NaCl. Photosynthetic O_2 evolution was measured as described under Materials and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 5 mM potassium ferricyanide, Anacystis particles containing 34 µg chlorophyll, and $CaCl_2$ (●——●——●) or NaCl (○---○---○) as indicated in the Fig.

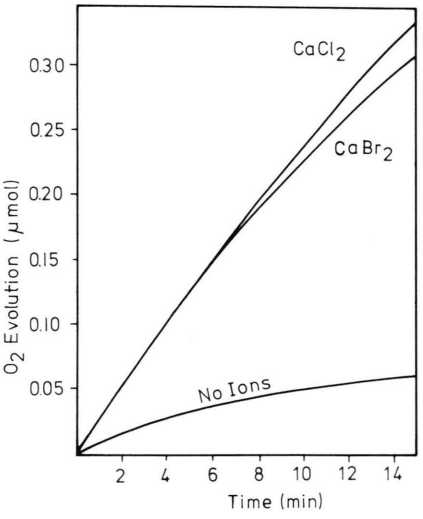

Fig. 4. Stimulation of photosynthetic O_2 evolution in *Anacystis* particles by $CaCl_2$ or $CaBr_2$. Photosynthetic O_2 evolution was measured as described under Materials and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 5 mM potassium ferricyanide, Anacystis particles containing 23 µg chlorophyll, and 54 mM $CaCl_2$ or 54 mM $CaBr_2$ was added when indicated.

When no $CaCl_2$ was added to the Anacystis particles, the O_2 evolution was very small (Fig. 4). Activation could be obtained with $CaCl_2$ or $CaBr_2$. Both anions are very similar in Pauling radii (1.81 and 1.95 Å, respectively) and have practically identical inhibitory effects on the L-amino acid oxidase activity (see Table II). All other tested anions (except Cl^- and Br^-) inhibited photosynthetic O_2 evolution. Fig. 5 shows the effect of adding sodium salts of various anions to the O_2 evolving Anacystis preparation which had been activated by $CaCl_2$. We used NaCl as a control for a possible effect of Na^+ in replacing Ca^{2+}. As can be seen in Fig. 5, NaCl (the same was true for NaBr, not shown) had only a very minor effect in reducing the O_2 evolution in the presence of $CaCl_2$. Therefore the inhibition which was observed with the other sodium salts ($Na(OOCCH_3)$, $NaNO_3$ and NaSCN), could entirely be attributed to the corresponding anions. The effect of increasing concentrations of $NaNO_3$ is shown in Fig. 6. In general, it

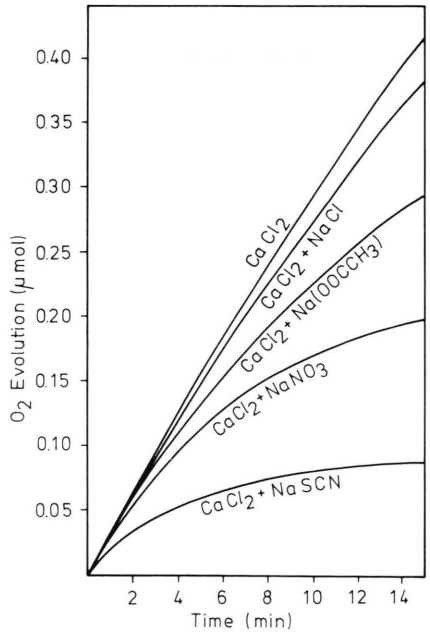

Fig. 5. Influence of various anions on photosynthetic O_2 evolution in *Anacystis* particles in presence of $CaCl_2$. Photosynthetic O_2 evolution was measured as described under Materials and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 5 mM potassium ferricyanide, 54 mM $CaCl_2$, and Anacystis particles containing 31 µg chlorophyll. In addition were added: 54 mM NaCl, 54 mM $Na(OOCCH_3)$, 54 mM $NaNO_3$, or 11 mM NaSCN, when indicated.

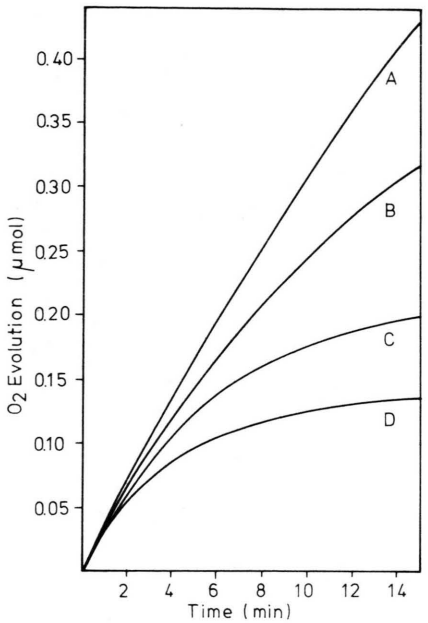

Fig. 6. Influence of increasing $NaNO_3$ concentrations on photosynthetic O_2 evolution in *Anacystis* particles in presence of $CaCl_2$.
Conditions were the same as in Fig. 5. In addition to 54 mM $CaCl_2$ were added:
A: no further addition; B: 11 mM $NaNO_3$; C: 54 mM $NaNO_3$ and D: 108 mM $NaNO_3$.

can be said that except for Cl^- and Br^- which have a clear and rather specific stimulatory effect on the photosynthetic O_2 evolution, the other anions tested were inhibitory for O_2 evolution as well as for L-amino acid oxidase activity.

Relationship between the effect of L-*arginine and chloride on the oxidative deamination reaction and on photosynthetic O_2 evolution*

We have been puzzled about the fact that we did not see an inhibition of the photosynthetic O_2 evolution by substrates of the L-amino acid oxidase, like *e.g.* L-arginine. Since L-arginine and Ca^{2+} are partly competitive in the oxidative deamination reaction [19], it would – on the other hand – be expected that the photosynthetic O_2 evolution is inhibited by L-arginine at very low concentrations of Ca^{2+} if this flavoprotein with L-amino acid oxidase activity is actually part of the water-splitting complex. However, this could normally not be observed [18], although there was a small effect of arginine on photosynthetic O_2 evolution under flash light illumination [25]. Since we had always used L-arginine-HCl as substrate in our previous experiments, we can now attribute the absence of an L-arginine effect to the presence of Cl^- in the added L-arginine solution. Cl^- would inhibit the oxidative deamination of L-arginine but would stimulate photosynthetic O_2 evolution. This becomes even more complicated, since cationic amino acids could assist in Cl^- binding through Coulombic interactions and thereby increase the Cl^- concentration at the water oxydation site. This has recently been discussed by Andersson *et al.* [12] who suggested that one of the functions of the 23 kDa peptide which has a high percentage of basic amino acids, might be that those basic amino acids in the peptide help to concentrate Cl^- at the reaction center.

The antagonistic effects of L-arginine and Cl^- on the oxidative deamination reaction and on photosynthetic O_2 evolution in *Anacystis* French press particles are shown in Table III. The oxidative deamination is measured as NH_4^+ production from L-arginine. These experiments were done with *Anacystis* particles in the light with ferricyanide as electron acceptor and in the absence of added $CaCl_2$.

The results show that addition of L-arginine resulted in NH_4^+ production which was higher with L-arginine-Hepes than with L-arginine-HCl as expected because of the inhibitory effect of added Cl^- on the

Table III. Effect of amino acids on photosynthetic O_2 evolution and NH_4^+ production catalyzed by *Anacystis* particles in the light and presence of ferricyanide.
Oxidative deamination of L-Arg (measured as NH_4^+ production) and photosynthetic O_2 evolution were measured as described under Materials and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 5 mM potassium ferricyanide, Anacystis particles containing 75 μg chlorophyll, and the various amino acids (49 mM) as indicated. The reaction time was 15 min. The conditions used were not optimal for photosynthesis, since larger amounts of particles were necessary to get adequate NH_4^+ determinations.

Additions	O_2 Evolution	NH_4^+ Production
	μmol × mg chlorophyll^{-1} × h^{-1}	
None	8.5	0
L-Arg-Hepes	2.7	22.1
L-Arg-HCl	8.7	17.4
D-Arg-Hepes	13.0	0
D-Arg-HCl	15.6	0
L-Ala	7.1	0
L-Citrulline	7.7	0

L-amino acid oxidase activity. However, the O_2 evolution was not at all reduced in the presence of L-arginine-HCl compared to the O_2 evolved in the absence of L-arginine-HCl (compare line 1 with line 3 of Table III). This indicated that the O_2 uptake (due to the oxidative deamination reaction) was balanced by the increase in O_2 evolution (due to the added Cl^-). The table also shows that L-arginine-Hepes caused a reduction of the net amount of O_2 evolved, though the effect was not as large as the maximum that could be calculated from the NH_4^+ value. The stoichiometry of the oxidative deamination reaction is 1 μmol NH_4^+ formed per 0.5 μmol O_2 taken up, since the particles contained catalase-like activity. D-arginine had a stimulatory effect on O_2 evolution though it gave no NH_4^+, since it is not a substrate for the enzyme (but an inhibitor of L-arginine oxidation). The stimulatory effect of D-arginine can be explained by the assistence of basic amino acids in Cl^- binding (see above). Dublicate experiments showed some fluctuation in the O_2 values because of the inaccuracy of the measurements of low levels of O_2 uptake or evolution, but the results shown are typical results of many experiments.

Since the Cl^- concentration cannot be increased without also increasing the cation concentration, the antagonistic effects of various salts on the two reactions (oxidative deamination and photosynthetic O_2 evolution) are always the sum of the anion and cation effect (especially when monovalent ions, such as NaCl, were used). Table IV shows the response of the photosynthetic O_2 evolution to increasing amounts of $CaCl_2$ and NaCl and the decrease in NH_4^+ production from L-arginine that accompanied the addition of the activating ions. Clearly, when the O_2 evolution was fully activated, the L-amino acid oxidase activity disappeared.

Table IV. Antagonistic effect of $CaCl_2$ and NaCl on photosynthetic O_2 evolution and NH_4^+ production catalyzed by *Anacystis* particles in the light and presence of ferricyanide. Oxidative deamination of L-Arg (measured as NH_4^+ production) and photosynthetic O_2 evolution were measured as described under Material and Methods. The reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, 49 mM L-Arg-Hepes, pH 7, 5 mM potassium ferricyanide, Anacystis particles containing 68 μg chlorophyll, and $CaCl_2$ or NaCl as indicated. The reaction time was 15 min. The conditions were not optimal for photosynthesis, since larger amounts of particles were necessary to get adequate NH_4^+ determinations.

Additions	O_2 Evolution	NH_4^+ Production
	μmol × mg chlorophyll^{-1} × h^{-1}	
None	0	46.1
0.5 mM $CaCl_2$	1.4	46.1
1 mM $CaCl_2$	2.6	49.1
3 mM $CaCl_2$	14.6	17.3
5 mM $CaCl_2$	26.6	2.9
11 mM $CaCl_2$	26.1	0
27 mM $CaCl_2$	31.1	0
54 mM $CaCl_2$	35.4	0
11 mM NaCl	1.7	46.5
27 mM NaCl	4.1	46.5
54 mM NaCl	8.2	31.6
108 mM NaCl	21.5	11.9
162 mM NaCl	23.9	1.6
270 mM NaCl	25.1	0
411 mM NaCl	25.3	0

Conclusions

The data presented in this paper represent an extension of our previous studies, all of which suggest that a flavoprotein with L-amino acid oxidase activity found in *A. nidulans* has many of the properties which one would expect of one of the proteins in the water-splitting complex of photosystem II in *A. nidulans* [18, 26]. It is our opinion that this flavoprotein is modified by Cl^- and Ca^{2+} in such a way that it can now interact with Mn^{2+} and catalyze the water-splitting reaction of photosystem II. However, it is unclear at the present time what the chemical nature of this modification is. The results of the experiments presented here show that the flavoprotein is not only modified by cations as previously reported [18, 19] but also by anions. The amino acid oxidase activity is suppressed by all tested cations and anions while only specific ions (Mn^{2+}, Ca^{2+} and Cl^-) will lead to activation of the photosynthetic O_2 evolution. As shown here, these ions can fairly easily be removed by the other ions (see also [18]), especially by those ions for which the flavoprotein has a higher affinity than for Ca^{2+} or Cl^-. This is in good agreement with results recently reported on photosystem II of plants. Those experiments seem to indicate that the water-splitting complex has a fairly weak affinity for its required cofactors and that several smaller peptides help to concentrate or stabilize those ions at the water-splitting center [8]. An involvement of both negatively charged and positively charged groups in maintaining the functional integrity of the site of water oxidation has also been suggested by Johnson *et al.* [27].

So far our evidence for the involvement of the flavoprotein with L-amino acid oxidase activity in photosynthetic O_2 evolution is only indirect and is mainly based on the mirror image effect of certain ions on the two activities: oxidative deamination of certain L-amino acids and photosynthetic O_2 evolution. Both activities have been shown to be present in thylakoid preparations of *A. nidulans* [18]. Recently Satoh *et al.* [3] have isolated a highly purified photosystem II complex from *Synechococcus sp.* and named this complex the "water-plastoquinone oxidoreductase" in analogy to the "NADH-ubiquinone oxidoreductase" in the mitochondrial respiratory chain. We eventually hope to isolate an equally highly purified photosystem II complex from *A. nidulans* and to show that such a complex still contains the L-amino acid oxidase activity. This would then imply that such a complex contains flavin.

Acknowledgements

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[1] N. Murata, M. Miyao, and T. Kuwabara, in The Oxygen Evolving System of Photosynthesis (Y. Inoue, N. Murata, A. R. Crofts, G. Renger, Govindjee, and K. Satoh, eds.), pp. 213–222, Academic Press, Inc., Tokyo, Orlando, San Diego, San Francisco, New York, London, Toronto, Montreal, Sydney, São Paulo 1983.
[2] M. Yuasa, T.-A. Ono, and Y. Inoue, Photobiochem. Photobiophys. **7,** 257–266 (1984).
[3] K. Satoh, T. Ohno, and S. Katoh, FEBS Lett. **180,** 326–330 (1985).
[4] Y. Yamamoto, M. Doi, N. Tamura, and M. Nishimura, FEBS Lett. **133,** 265–268 (1981).
[5] H.-E. Åkerlund, C. Jansson, and B. Andersson, Biochim. Biophys. Acta **681,** 1–10 (1982).
[6] B. L. Møller and P. B. Høj, Carlsberg Res. Commun. **48,** 161–185 (1983).
[7] B. Andersson, C. Larsson, C. Jansson, U. Ljungberg, and H.-E. Åkerlund, Biochim. Biophys. Acta **766,** 21–28 (1984).
[8] N. Murata and M. Miyao, Trends Biochem. Sci. **10,** 122–124 (1985).
[9] M. Miyao and N. Murata, FEBS Lett. **170,** 350–354 (1984).
[10] M. Miyao and N. Murata, FEBS Lett. **168,** 118–120 (1984).
[11] D. F. Ghanotakis, J. N. Topper, G. T. Babcock, and C. F. Yocum, FEBS Lett. **170,** 169–173 (1984).
[12] B. Andersson, C. Critchley, I. J. Ryrie, C. Jansson, C. Larsson, and J. M. Anderson, FEBS Lett. **168,** 113–117 (1984).
[13] H. Y. Nakatani, Biochim. Biophys. Res. Commun. **120,** 299–304 (1984).
[14] H. Y. Nakatani, Biochim. Biophys. Res. Commun. **121,** 626–633 (1984).
[15] E. H. Evans, R. England, and J. Manwaring, in Photosynthetic Prokaryotes: Cell Differentiation and Function (G. C. Papageorgiou and L. Packer, eds.), pp. 175–183, Elsevier Sci. Publ. Co., Inc., Amsterdam 1983.
[16] D. W. Krogmann, in Photosynthesis I, Encyclopedia of Plant Physiology, New Series (A. Trebst and M. Avron, eds.), **Vol. 5,** pp. 625–636, Springer-Verlag, Berlin 1977.
[17] J. J. Brand, FEBS Lett. **103,** 114–117 (1979).
[18] E. K. Pistorius and H. Voss, Eur. J. Biochem. **126,** 203–209 (1982).
[19] E. K. Pistorius and H. Voss, Biochim. Biophys. Acta **611,** 227–240 (1980).
[20] F. DaFonseca-Wollheim, Z. Klin. Chem. Klin. Biochem. **11,** 421–426 (1973).
[21] O. Warburg and W. Lüttgens, Naturwissenschaften **32,** 301 (1949).
[22] G. Hind, H. Y. Nakatani, and S. Izawa, Biochim. Biophys. Acta **172,** 277–289 (1969).
[23] P. O. Sandusky and C. F. Yocum, Biochim. Biophys. Acta **766,** 603–611 (1984).
[24] J. Sinclair, Biochim. Biophys. Acta **764,** 247–252 (1984).
[25] E. K. Pistorius and G. H. Schmid, Z. Naturforsch. **39c,** 1097–1103 (1984).
[26] E. K. Pistorius, Eur. J. Biochem. **135,** 217–222 (1983).
[27] J. D. Johnson, V. R. Pfister, and P. H. Homann, Biochim. Biophys. Acta **723,** 256–265 (1983).