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# VANADIUM IN PHOTOSYNTHESIS OF *CHLORELLA FUSCA* AND HIGHER PLANTS

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**The influence of vanadium compounds (vanadate, vanadyl citrate) on photosynthesis in *Chlorella fusca* and in algal and spinach chloroplasts has been investigated. It was found that: 1. At moderately high concentrations (at least 0.1 mM) both vanadate and vanadyl citrate enhance photosynthetic $O_2$ production in intact *C. fusca* cells. At lower V concentration (about 2 $\mu$M) only vanadate stimulates photosynthesis. The increase is dependent on culture conditions and on light intensity. 2. Up to 1 mM V, neither vanadium compound influences PS II activity, either in intact cells or in algal or spinach chloroplasts. 3. The PS I reaction in algal and spinach chloroplasts is maximally enhanced (3-fold) in presence of vanadium (20 $\mu$M). The increase is independent of light intensity. 4. Cr(VI), Mo(VI), and W(VI) (1 mM) stimulate photosynthesis in intact *C. fusca* cells, but do not influence the photosystems of isolated chloroplasts. Vanadium is suggested to act as a redox catalyst in the electron transport from PS II to PS I.**

## Introduction

The transition element vanadium is supposed to be essential for both green plants [1] and animals [2]. Although several approaches were made to explain this on a biochemical basis [3,4], many aspects of the role of vanadium remain unknown to date. For green plants, the function of the metal proved to be rather complex, most investigations having been done only with unicellular green algae. In 1953, Arnon and Wessel [1] were the first to report a stimulatory effect of vanadate on growth and chlorophyll content of *Scenedesmus obliquus*, but not until 20 years later was addition of traces of vanadate generally recommended to obtain maximal biomass in cultures of *Chlorella* and *Scenedesmus* [5]. At this time, the first investigations were started which focused on the biochemical background of the essential function of vanadium in green algae: Meisch and Bielig [6] found that a limited iron chlorosis may be completely overcome in the presence of traces of vanadate, and it could also be demonstrated that vanadium is likely to have two different sites of action in *Chlorella*, the first being located in the biosynthesis of the chlorophylls and the second dealing with photosynthesis [7]. With respect to chlorophyll biosynthesis, vanadium was found to stimulate light-dependent formation of the porphyrin precursor δ-aminolevulinic acid [8,9]. The effect is considered to be catalytic during the δ-aminolevulinic acid transaminase reaction [8, 10]. Since δ-aminolevulinic acid formation is known to be a critical regulatory step in the light-induced plastid development and the greening of plants [11], vanadium has consequently a considerable influence on the structure of the *Chlorella* chloroplast, especially, during iron deficiency [12]. This is accompanied by an increase in the level of some carotenes [13], of cytochrome *f* and of chlorophyll *P*-700 [14].

* Taken in part from the forthcoming doctoral thesis of L.J.M.B.

Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DCIP, 2,6-dichlorophenolindophenol; Tricine, *N*-tris(hydroxymethyl)methylglycine.

Concerning photosynthesis, Warbŭrg et al. [15] reported that the addition of vanadate (as $NaVO_3$) to *Chlorella* suspensions spontaneously increased the photosynthetic $O_2$ production, while vanadyl sulphate had no influence. Arnon [16] found that growth of *Scenedesmus* in the presence of vanadium leads to a higher photosynthetic $O_2$ productivity than found in cells which were cultivated in the absence of the trace element. Therefore two different aspects of V on photosynthesis have to be considered: an immediate effect upon the addition of vanadium to algal cells and a long-term effect on growth after incubation of cultures with vanadium.

Since no definite explanation of these observations had been given, and no further work had been done in this field, the present work deals with the influence of several vanadium compounds on photosynthesis, the investigations being made not only with intact cells of *Chlorella* fusca, but also with chloroplast preparations of both green algae and higher plants.

## Materials and Methods

*Chlorella fusca* strain 211-8b (Collection of Algae, Göttingen) was autotrophically cultivated for 3 days in continuous light (20 $W/m^2$, 1.5% $CO_2$, 28°C) in a liquid medium [6] with Fe(III) citrate in four different ways: in the absence or in the presence of vanadium (0.4 $\mu$M as $NH_4VO_3$), (a) with normal iron supply (18 $\mu$M) and (b) with reduced iron content (1.8 $\mu$M) in order to cause iron stress.

Photosynthetically active particles of *C. fusca* were isolated according to Senger and Mell [17]. Chloroplasts from market spinach were prepared as described elsewhere [18]. Chlorophyll was estimated spectrophotometrically [6].

Photosynthetic $O_2$ production of intact *C. fusca* cells was monitored polarographically with a Clark electrode (Oxygen monitor YSI-53) in 0.1% $NaHCO_3$ (28°C) during illumination with red light ($\lambda > 620$ nm). Light intensity was determined with a YSI Kettering radiometer 65 A.

PS II activity of intact cells was measured using *p*-benzoquinone [17], while the PS II reaction of chloroplasts was investigated using either *p*-benzoquinone or ferricyanide. The PS I reaction of chloroplast preparations was determined polarographically as $O_2$ uptake during the light-dependent reduction of methyl viologen with either DCIP [19] or DAD [20] as electron donors.

L-(+)-Ascorbic acid, DCIP, and the metal compounds were obtained from E. Merck (Darmstadt). Tricine was purchased from Sigma (Munich), DCMU and methyl viologen from Serva (Heidelberg) and DAD from EGA-Chemie (Steinheim).

Cr(III) glycine was prepared according to Küntzel and Dröscher [21]. Stock solutions of VO citrate were made by adding equimolar amounts of $VOSO_4 \cdot 5H_2O$ and trisodium citrate to the appropriate buffer solution.

## Results

### *Photosynthesis in intact C. fusca cells*

From earlier observations it is known that the culture conditions (iron supply, V treatment) exert a striking influence on the development of the photosynthetic apparatus of *C. fusca* [12]. The algae were therefore cultivated for 3 days in the four different ways mentioned in the methods. These cells were then tested for their photosynthetic productivity. Cell suspensions (2 ml samples in 0.1% $NaHCO_3$, containing about 15 $\mu$g chlorophyll) were transferred to the polarographic cell, and the $O_2$ production was monitored during illumination with red light (light saturation) for 5 min, vanadate (as $NaVO_3$) in increasing concentrations ($10^{-1}$–$10^3$ $\mu$M) was added and the $O_2$ output was measured for another 5 min. The relative increase of $O_2$ productivity (calculated as $\mu$mol $O_2$/mg chlorophyll per h) in presence of vanadium is shown in Fig. 1.

Fig. 1 demonstrates that vanadate generally stimulates algal photosynthesis in a wide molar range. The characteristics show a maximum at 2 $\mu$M V (up to 70% increase); with higher V concentration, the effect drops, while above 100 $\mu$M V a steep increase can be observed. This effect, however, is dependent on the conditions of precultivation. Highest values were observed with cells grown with V during iron deficiency.

Similar tests were performed with vanadyl citrate as V(IV) compound (Fig. 2), because insoluble $VO(OH)_2$ precipitates at physiological pH from uncomplexed VO salts such as $VOSO_4$. In contrast to the investigations with vanadate, it may be noted that

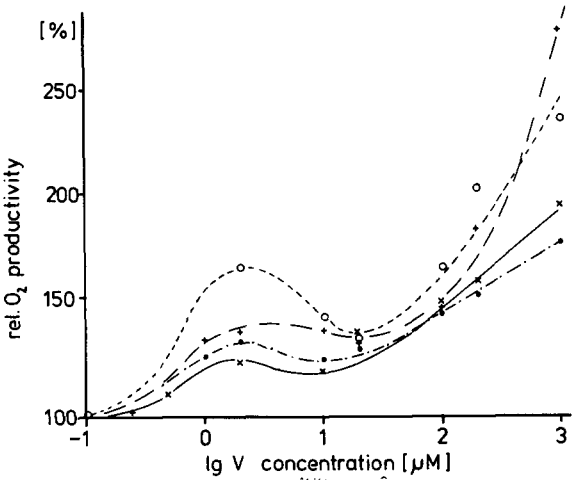


Fig. 1. Influence of $NaVO_3$ on photosynthesis of *C. fusca* cultivated under various conditions. Indicated is the V-induced, relative $O_2$ productivity as percentage of the controls. Photosynthetic $O_2$ production was measured with algal suspensions (2 ml, 15 μg chlorophyll) in 0.1% $NaHCO_3$ at 28°C and under red light (over 620 nm, light saturation). Culture conditions: x, normal Fe supply (18 μM); +, normal Fe supply + V (0.4 μM); •, iron deficiency (1.8 μM); ○, iron deficiency + V (0.4 μM).

low concentrations (1–10 μM) of VO citrate do not enhance photosynthesis. A positive effect of the VO complex can be observed only at a higher molarity (over 100 μM V). Iron-deficient cells show a slight increase in photosynthesis only when cultivated in presence of V, while maximal enhancement (more than 200%) was measured with cells which were cultivated with both normal iron supply and V. Iron stressed cells cultivated without V, however, generally failed to respond to the addition of the vanadyl compound.

To investigate the light dependence of the observed vanadium effect on algal photosynthesis, *C. fusca* grown in the absence of V (normal iron supply) was prepared for $O_2$ measurements as above. The intensity of the actinic red light was varied from 5 to 88 W/m² and V (1 μM or 1 mM) was added either as $NaVO_3$ or as VO citrate (Fig. 3).

The V-induced increase in photosynthetic $O_2$ production is dependent not only on V concentration, but also on light intensity. At the high V concentration of 1 mM, $NaVO_3$ and VO citrate yield nearly the same increase (max. 120% at 88 W/m²), while at 1 μM V, only vanadate enhances photosyn-

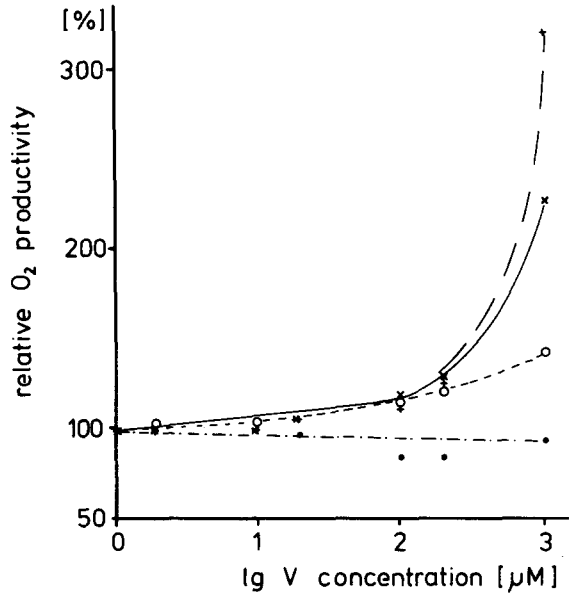


Fig. 2. Influence of VO citrate on photosynthesis of *C. fusca*, cultivated under various conditions. Indicated is the vanadium-induced, relative $O_2$ productivity as percentage of the controls. For illustrations of symbols and conditions see Fig. 1.

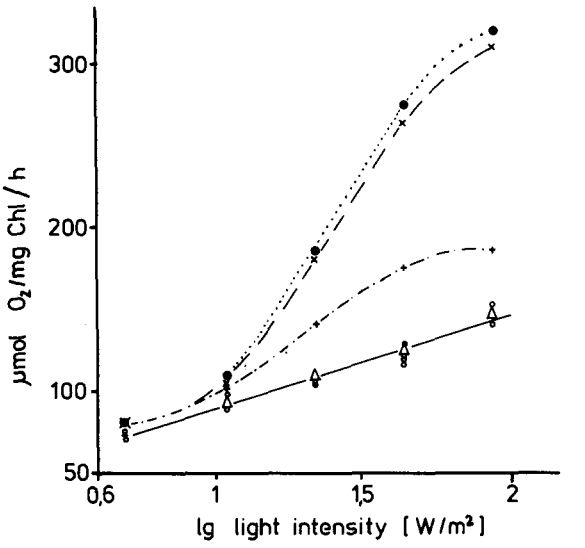


Fig. 3. Influence of light intensity on photosynthesis of *C. fusca* with and without addition of vanadium compounds. Measurements were performed with cells grown in the absence of vanadium as indicated in Fig. 1. ○, without V; △, VO citrate (1 μM); +, $NaVO_3$ (1 μM); •, VO citrate (1 mM); x, $NaVO_3$ (1 mM).

thesis to a certain extent (maximum 50% increase at 44 W/m$^2$).

From former studies [26] it is known that *C. fusca* which has been grown in the presence of trace amounts of chromium (as Cr(III) glycine or $K_2Cr_2O_7$) shows a small (20%) enhancement in photosynthesis. Thus we were interested to know whether other transition elements related to V, e.g., Cr, Mo, W, Mn, are able to enhance algal photosynthesis in a similar manner. Photosynthetic $O_2$ production in *C. fusca* was monitored as above (light saturation) after addition of 1 mM of Cr ($K_2Cr_2O_7$), Mo [$(NH_4)_6Mo_7O_{24}$], W ($Na_2WO_4$), and of Mn ($KMnO_4$), respectively. Except for Mn, all other elements were found to stimulate the $O_2$ output, and in the order of effectiveness, the following sequence could be established: V(V) > Cr(VI) > Mo(VI) > W(VI) > Mn(VII) = 0. Vanadate proved to be the most effective of the metals tested; dichromate and molybdate enhanced photosynthesis to about 50% and 25%, respectively, while tungstate was only slightly stimulatory (8% increase).

The Hill reaction was measured with intact *C. fusca* cells. *p*-Benzoquinone was used as a Hill reagent, since it is known to penetrate the cell wall and the chloroplast membrane easily [17]. In the molar range 1 to 100 μM, neither V(V) ($NaVO_3$) nor V(IV) (VO citrate) was found to enhance the Hill reaction significantly. At higher V concentration, however, inhibition occurs (25% at 1 mM V).

*Photosynthesis in C. fusca chloroplasts*

In order to define the site of action of vanadium in photosynthesis, we isolated photosynthetically active chloroplast particles from *C. fusca*. In these preparations, both PS I and PS II activity were tested in connection with the trace element.

Table I shows that PS II (assayed with either *p*-benzoquinone of ferricyanide as electron acceptors) is not influenced by $NaVO_3$ or VO citrate over a wide concentration range ($10^{-1}$–$10^3$ μM); $10^4$ μM V inhibits the reaction. When PS I activity was measured, only $NaVO_3$ was added, since vanadate is readily reduced to V(IV) in presence of ascorbate (Fig. 4).

Fig. 4 shows that V remarkably enhances the PS I activity of *C. fusca* chloroplasts. Maximal effects were observed with about $10^2$ μM V, the extent of stimulation being dependent on culture conditions (see Materials and Methods) of the algal cells from which the chloroplast particles had been prepared. A maximal 2.8-fold increase of PS I activity can be obtained with

TABLE I

INFLUENCE OF VANADIUM ON THE PS II REACTION IN CHLOROPLAST PARTICLES OF CHLORELLA FUSCA

The PS II assay contained (2 ml): chloroplasts (15 μg chlorophyll), 2 μmol *p*-benzoquinone, 20 μmol NaCl, 20 μmol KCl, 5 μmol $MgCl_2$, 20 nmol EDTA, 40 μmol Tricine, pH 7.5, 0.68 mmol sucrose and the vanadium compounds as indicated; the reaction was carried out at 15°C under actinic red light (greater than 620 nm, light saturation). All values are percentages of the V-free controls.

| V compound added | PS II activity after addition of V (μM) | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 | 1 | 10 | 100 | 1000 | 10000 |
| $NaVO_3$ | 97 | 99 | 99 | 98 | 95 | 51 |
| VO citrate | 96 | 90 | 103 | 102 | 101 | 0 |

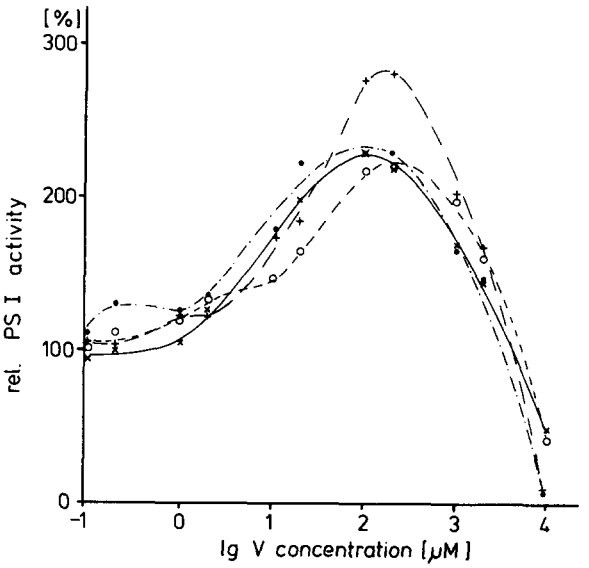


Fig. 4. The effect of vanadium on the PS I activity of *C. fusca* chloroplasts as indicated by the vanadium-induced relative $O_2$ uptake as percentage of the controls. The reaction mixture contained (2 ml): chloroplasts (15 μg chlorophyll); 0.1 mmol potassium phosphate, pH 6.5; 10 μmol potassium ascorbate; 0.4 μmol methyl viologen; 0.2 μmol DCIP; 0.2 μmol $NaN_3$; 5 nmol DCMU and varying concentrations of vanadium, added as $NaVO_3$. The assays were performed at 15°C in red light (over 620 nm, light saturation). For illustrations of symbols and culture conditions see Fig. 1.

0.2 mM V in chloroplasts derived from organisms grown in the presence of V and normally supplied with Fe. In all cases, high V concentrations (over 2 mM) inhibit the reaction.

Similar tests with 0.1 mM of Cr(III), Cr (VI), Mo-(VI), W(VI), and Mn(VII), respectively (compounds used as above) revealed no positive influence of the metals on either PS II or PS I activity in isolated chloroplasts.

In contrast to the V effect on photosynthesis in intact cells, vanadate was found to stimulate the PS I reaction of chloroplast particles independent of light intensity: In the range 5 W/m$^2$ to light saturation (over 100 W/m$^2$) and in presence of 0.1 mM V, a fairly constant increase of 140–150% can be observed.

*Photosynthesis in spinach chloroplasts*

Since most investigations concerning the function of vanadium in green plants had been performed with unicellular green algae, no clear answer can be given as to whether this trace metal is also essential for higher plants. Preliminary studies have shown that vanadate stimulates photosynthesis in higher plants (*Elodea, Lemna*) in a similar manner as with *C. fusca* (Meisch and Becker, unpublished results). Chloroplasts from spinach which are easy to prepare were therefore chosen to check the influence of V on photosynthesis of higher plants.

PS II activity of spinach chloroplasts was investigated in connection with either vanadate or VO citrate as above. Both V compounds, in the molar range 1–10$^2$ μM do not enhance the PS II reaction. Higher V concentrations decrease the photoreaction, the VO compound being more inhibitory than vanadate (64 and 17% inhibition, respectively with 1 mM V).

Investigation of the PS I activity in spinach chloroplasts revealed a considerable enhancement by V with an optimum (320% increase) at about 20 μM V (Fig. 5). The effect can also be demonstrated in the presence of uncouplers of photophosphorylation such as ammonia (250% increase with 1 mM of both V and $NH_4^+$ compared to 178% with V and 91% with $NH_4^+$, respectively). When DAD is used instead of DCIP as an electron donor, a similar V effect can be observed, thereby demonstrating that the same V effect occurs within the different chemical systems used to stimulate photosynthetic electron transport by PS I.

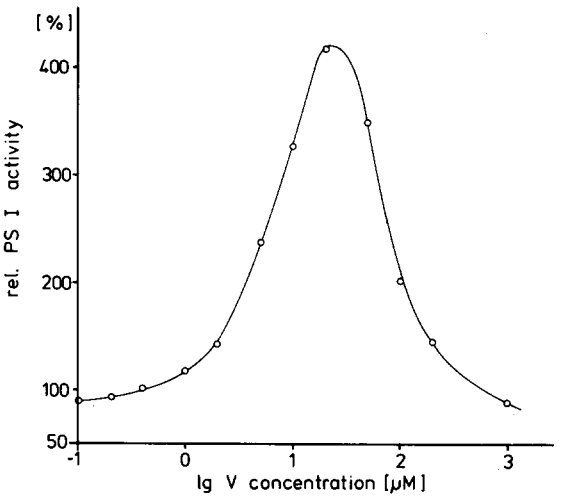


Fig. 5. The effect of vanadium on the PS I activity of spinach chloroplasts. Indicated is the vanadium-induced relative $O_2$ uptake as percentage of the controls. The reaction mixture contained (2 ml): chloroplasts (15 μg chlorophyll); 2 μmol potassium phosphate, pH 7.4; 70 μmol NaCl; 10 μmol $MgCl_2$; 0.4 μmol methyl viologen; 0.4 μmol DCIP; 0.2 μmol $NaN_3$; 40 nmol DCMU; 10 μmol potassium ascorbate and varying concentrations of vanadium, added as $NaVO_3$.

Similar investigations with the other transition elements (Cr, Mo, W, Mn) showed that they do not enhance the activity of either of the two photosystems in spinach chloroplasts.

## Discussion

The trace element vanadium is able to act as a potent accelerator of photosynthesis in both green algae and higher plants. Two different effects have to be discussed. The first is closely connected with the growth conditions of the organisms, where traces of vanadate promote the development of the photosynthetic structures within the chloroplasts, paralleled by an increase of a number of photosynthetically active pigments [13,14]. This long-term effect is combined with a higher rate of photosynthesis in plants grown in the presence of V as was discovered by Arnon [16]. A second V effect is the spontaneous response of intact cells or isolated chloroplasts to the addition of V compounds by an increased rate of $O_2$ production (intact cells) or a higher electron flow via PS I (chloroplasts). Warburg et al. [15], who first demon-

strated that vanadate spontaneously enhances the photosynthetic $O_2$ production of intact *Chlorella* cells, did not explain this phenomenon. On the other hand, vanadyl sulphate, which he had also tested, proved to be inactive.

In our tests to show vanadium effects of *C. fusca* cells, we find maximal response after addition of 50–100 $\mu$g V/l (approx. 1–2 $\mu$M), the very amount of V which is known to have optimal influence on algal growth [7] and also a V concentration which naturally occurs in living plants [22]. In this range of V concentration, the vanadyl compound proved to be ineffective, the observation being consistent with the findings of Warburg et al. [15] who treated the algae with 2 $\mu$M $VOSO_4$. Higher V concentrations (over 0.2 mM) show an even stronger enhancement of the $O_2$ output, $VO^{2+}$ being as effective as $VO_3^-$. The lack of the V effect with low concentrations of vanadyl ions may be due to a slower uptake of V into the cell or vanadyl complexation in the cytoplasm.

As known from the studies of Rosen et al. [23], vanadyl and vanadate ions can act as artificial electron donors or acceptors in photosynthesis of isolated chloroplasts, when applied in high concentration (0.03–0.4 M). We therefore suggest that the effect of vanadium on photosynthesis at the lower V concentration could be physiological, while with increased V supply the natural system is superimposed by an artificial donor/acceptor operation of $VO^{2+}/VO_3^-$.

The effect of V on the PS I reactions of isolated chloroplasts did not show any light dependence, as observed with intact *C. fusca* cells. This suggests that the uptake of V into the cell which is known to be energy dependent [24], accounts for this different behaviour.

With respect to the effect of V in PS I, we have to discuss the point of impact of the metal during the light mediated electron flow in this system. Uncoupling of photophosphorylation by V can be excluded, because the V effect is also observed in the presence of known uncouplers like ammonia.

The oxidation of vanadyl ions to vanadate has a standard potential of about 1 volt [25], and the reaction occurs according to the following equation:

$$VO^{2+} + 2\,H_2O \rightleftharpoons VO_3^- + 4\,H^+ + e^-$$

Since four protons are involved, the potential at pH 7 may be lowered to 400–0 mV, depending on the ratio $VO_3^-/VO^{2+}$. This leads to the suggestion that the effect of vanadium is based on a reversible change between its tetra- and pentavalent states in the electron transport chain from PS II to PS I. This points to an exclusive role of vanadium in photosynthesis compared to the other transition metals tested, since the latter failed to influence the photosystems in isolated chloroplasts.

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