Effect of Vanadate and Iron Stress on the Pigment Composition of *Chlorella fusca*

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The pigment composition of *Chlorella fusca* has been investigated in the absence and in presence of vanadate and during iron stress.

1. Fe-deficiency generally decreases algal pigment content without change of the ratio chlorophyll a/b.
2. A twofold vanadate induced increase of the chlorophylls is accompanied by a similar enhancement of several xanthophylls, while lutein remains unaffected.
3. Vanadate stimulates the formation of β-carotene up to 5 fold, the effect being less obvious during iron stress.

Introduction

The physiological role of vanadium in plants has been extensively investigated with the unicellular green algae *Chlorella* and *Scenedesmus*, where a stimulatory effect of the trace metal on growth and chlorophyll formation [1–3] and on photosynthesis [4] has been detected. The metal was found to be involved in the light dependent biosynthesis of the porphyrin precursor δ-aminolevulinic acid [5, 6]. On the other hand, green algae which became chlorotic during iron stress, showed normal growth and chlorophyll formation after addition of vanadate [2], this effect being paralleled by a normalization of the structural organization of the iron stressed chloroplast [7]. The composition of the algal carotenoids, however, had not been so far investigated in connection with vanadate. Since carotenoids are important components of the photosynthetic membrane, with functions not only as photoprotectors of the chlorophylls against bleaching [8, 9], but also as antennae pigments and as direct participants in the mechanisms of photosynthesis [10, 11], their distribution and composition in *Chlorella* during iron deficiency and vanadate treatment should be of interest in order to obtain further information about the regulation of carotenoid biosynthesis in plants.

Material and Methods

Chlorella fusca, strain 211-8 b (Collection of Algae, Göttingen) was autotrophically cultivated in the nutrient medium of Arnon and Wessel [1] as described earlier [2]. Iron was always offered as Fe(III)-citrate (0.1 and 1.0 mg Fe/l = 1.8×10^{-6} and 1.8×10^{-5} M respectively), vanadium was added as NH_4VO_3 (20 µg V/l = 4×10^{-7} M). The algae were cultivated with different iron supply in the absence and in presence of vanadate for 3 days in continuous fluorescent white light (15000 lux = 22 W/m²), then collected by centrifugation, the washed cells being used for pigment analysis.

Dry weight of algae and chlorophyll content were estimated as described elsewhere [2]. The pigments were extracted from the cells with hot methanol and the carotenoids were separated by TLC on $CaCO_3$/MgO plates and measured spectrophotometrically either in ethanol or in chloroform according to Hager and Meyer-Berthenrath [12].

Results and Discussion

As known from earlier studies [2], normal growth of *C. fusca* is achieved in liquid media with iron as Fe(III)-citrate (1 mg Fe/l). When the metal is offered as ferric chloride, which readily hydrolyzes to insoluble $(FeOOH)_x$ in neutral solution, iron deficiency is induced in the algae [2]. In the present study, the same symptoms of iron stress, *e.g.* reduction of growth and cell volume, chlorosis, arose when only one tenth of the normal Fe-supply (0.1 mg Fe/l as citrate) was offered to the algal medium. In this case, both, dry weight and chlorophyll content were decreased to about 50%. After addition of vanadate (4×10^{-7} M), however, the iron deficiency was completely overcome: Dry weight and chlorophyll con-

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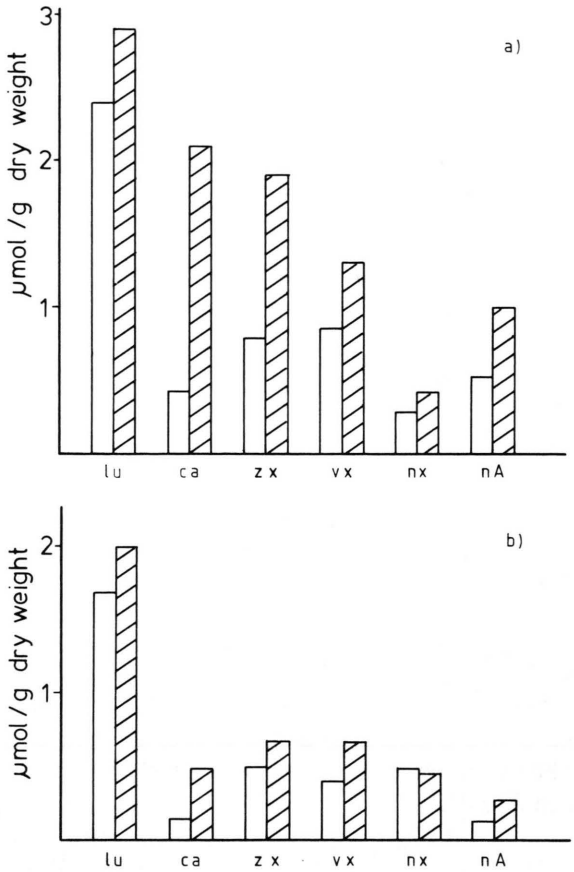

Fig. 1. Pigment composition of *Chlorella fusca*, grown in the absence (□) and in presence (▨) of vanadate. a) Cultures with normal Fe-supply (1 mg Fe/l); b) iron deficient cultures (0.1 mg Fe/l). lu = lutein, ca = β-carotene, zx = zeaxanthin, vx = violaxanthin, nx = neoxanthin, nA = neoxanthin neo A.

tent of the algae increased more than twofold (Table I). With respect to the carotenoids, it is known that their composition changes in different strains of *C. fusca* (syn. *C. pyrenoidosa*): Besides β-carotene and traces of α-carotene and antheraxanthin, *C. fusca* 211-8 b contains large amounts of lutein and as minor components the xanthophylls violaxanthin, zeaxanthin, neoxanthin, and neoxanthin neo A [11].

In the present study we investigated whether iron stress and vanadate treatment which have well established effects on chlorophyll biosynthesis [2] may have also consequences for the development and composition of the algal carotenoids.

After 3 days growth under the conditions mentioned above, the carotenoids of *C. fusca* were separated and quantitatively determined (Fig. 1). Fig. 1 shows that in the absence of vanadate, iron deficiency substantially lowers the whole carotenoid content of *C. fusca*: β-carotene is decreased to one third, violaxanthin and zeaxanthin to about one half, while lutein is less influenced. In addition, the relation neoxanthin/neoxanthin neo A is altered from 0.5 to 4, the total amount of the isomers being also decreased during iron stress. In the presence of vanadate, however, the situation remarkably changes: The known effect of the trace metal on chlorophyll formation (about twofold increase, see Table I) is paralleled by a similar increase of most of the xanthophylls, while the lutein content is not significantly altered by vanadate. On the other hand, β-carotene formation is enhanced up to 5 fold in presence of vanadate, the effect being more marked in cultures with normal iron supply.

The overall effect of iron stress and vanadate treatment of the carotenoids of *C. fusca* is shown in Table I. During iron stress, vanadate enhances the carotenoids to about 40%, while in cultures with normal Fe-supply, a more than 80% increase can be observed. Table I shows also that the ratio xanthophylls to carotene (x/c) is substantially lowered by vanadate, while the ratio chlorophyll a/b remains fairly constant.

Increased β-carotene levels and lower values *x/c* are normally observed when shade-type chloroplasts are exposed to high light intensities. They turn then to sun-type chloroplasts which develop higher values of the ratio chlorophyll a/b and also a higher value for the ratio chlorophylls to carotenoids (a +b/x + c) [13]. We therefore conclude that vanadate does not induce a sun-type chloroplast in the algae, the V-effect thus being due to metabolic implications other than light adaptation.

Table I. Content and ratios of carotenoids and chlorophylls in *Chlorella fusca* (µmol pigment/g dry weight), grown for 3 days in the absence and in presence of vanadate and under iron stress. (a, b = chlorophyll a and b, x = xanthophylls, c = β-carotene).

	Control *		Iron stress **	
	−V	+V	−V	+V
chlorophylls	19.4	42.9	10.8	24.1
carotenoids	5.3	9.6	3.3	4.6
a/b	4.5	4.6	4.1	4.0
a + b/x + c	3.7	4.5	3.3	5.2
x/c	11.6	3.6	22.6	8.4

* 1 mg Fe/l, ** 0.1 mg Fe/l.

The results presented above may be discussed in connection with the regulation of carotenoid biosynthesis in green algae. Since it is known that vanadate stimulates light-dependently the chlorophyll biosynthesis of green algae on the δ-aminolevulinic acid level [5], it may be that carotenogenesis in *C. fusca* is photocontrolled by an intermediate of chlorophyll biosynthesis. For carotenoid containing mycobacteria, protoporphyrin-IX has been proposed to be the photoreceptor for photoinduction of carotenogenesis [14, 15], but with respect to green plants, the problem is still unresolved [16]. On the other hand, it is known that in presence of vanadate, *C. fusca* is not only increased in chlorophyll P 700 and in cytochrome f content [17], but shows also a higher photosynthetic activity, these effects being closely connected with photosystem I (PS I) [4]. Since a large β-carotene pool is associated with the antenna pigments of PS I [18], there could be a special need for this carotenoid, when vanadate induces a higher activity of PS I. This is quite consistent with our observation that besides β-carotene, only those xanthophylls are increased which are biogenetic relatives to β-carotene, while lutein which is considered to derive from a branched pathway [19], is only slightly influenced by vanadate.

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