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

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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 x 10^{-6} and 1.8 x 10^{-5} M respectively), vanadium was added as NH₄VO₃ (20 μg V/l = 4 x 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 CaCO₃/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)₂ 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 x 10^{-7} M), however, the iron deficiency was completely overcome: Dry weight and chlorophyll con-
Fig. 1 shows that in the absence of vanadate, iron deficiency substantially lowers the whole carotenoid content of *C. fusca*: α-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, α-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 α-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 = α-carotene).

<table>
<thead>
<tr>
<th></th>
<th>Control *</th>
<th>Iron stress **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−V</td>
<td>+V</td>
</tr>
<tr>
<td>chlorophylls</td>
<td>19.4</td>
<td>42.9</td>
</tr>
<tr>
<td>carotenoids</td>
<td>5.3</td>
<td>9.6</td>
</tr>
<tr>
<td>a/b</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>a + b/x + c</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>x/c</td>
<td>11.6</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* 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.