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Vanadium an essential element for some marine macroalgae

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Abstract. In some marine algae cultivated axenically in the artificial medium ASP6 F2 (pH 8.3) vanadium at 1–100 $\mu g\, l^{-1}$ increases the fresh weight. In the multicellular brown alga *Fucus spiralis* 10 $\mu g\, V\, l^{-1}$ enhances the fresh weight by about 400% while in the green alga *Enteromorpha compressa* the yield is increased by 90%. Red algae do not respond to vanadium. In *Fucus* morphological effects are displayed in more frequent branching and/or broader blades. No significant increase in the chlorophyll content could be demonstrated at the early stage at which these morphological effects first appeared. Later the chlorophyll content increased.

Key words: Algae (marine) – Chlorophyll content algae – *Enteromorpha* – *Fucus* – Growth (algae) – Vanadium.

Introduction

Many marine algae grow poorly in artificial seawaters, which might depend on the lack of some essential element in the medium. Vanadium is one of those trace elements identified in natural seawater, but not included in the ordinarily used artificial seawaters. The concentration in natural seawater is ca. $5 \cdot 10^{-8}$ M. Brown, green as well as red algae show varying contents from 0.3–10 $\mu g\, g^{-1}$ dry weight (Yamamoto et al. 1970). Vanadium could be presumed to play an active role in the metabolism of marine algae, as was previously found for some fresh-water algae belonging to Chlorophyta (Arnon and Wessel 1953; Meisch and Bielig 1975) and Heterocontae (Hesse 1974).

Material and methods

All algal cultures used in the experiments were brought into axenic culture by treatments with antibiotics (Fries 1963), with the exception of *Fucus spiralis* L., which was sterilized by a chlorine solution (Fries 1977). The stock cultures are kept in the artificial seawater ASP6 F2 (Fries 1977). This medium is buffered with Tris · HCl (=Tris(hydroxymethyl)aminomethane hydrochloride) and has a pH of 8.3. The trace elements are chelated by NTA (=nitrilotriacetic acid). The experiments were performed in 100 ml Pyrex flasks containing 25 ml of the same medium and each series contained 5 or 6 parallels. *Enteromorpha compressa* (L.) Grev. was inoculated in the form of one sporeling, about 2 mm long, per flask, while small tufts of the red algae were used. *Fucus* was inoculated by one small thallus tip per flask. *Fucus* was cultivated at 15° C or 17° C, 15 h light, the other species at 17° C, 18 h light per day. The results of *Fucus* and *Enteromorpha* are given as fresh weight and as photos. The dry weight was noted from the other algae.

No special precautions were taken to remove the traces of vanadium which could be supposed to occur in the ingredients of the nutrient medium. NH_4VO_3, Analar British Drug Houses, London, and NH_4VO_3 and vanadium (IV) oxysulphate, Merck, Darmstadt, FRG, were used as vanadium sources. These compounds were solved in redistilled water autoclaved separately and added aseptically to the experimental flasks.

To obtain material enough for chlorophyll determination one-year-old axenic plants were chosen. They had grown out from small inocula in ASP6 F2 without any vanadium addition. These plants were transferred into new flasks with 50 ml medium with 20 $\mu g\, V\, l^{-1}$ and control flasks without any addition. Absorbance of chlorophyll in *Fucus* was measured on a Hitachi 100-20 spectrophotometer after extraction with 90% acetone and the chlorophyll content calculated according to the method described by Jensen (1978) according to the equation $c = \frac{(D_{666} - D_{730}) \cdot V \cdot 10}{890}$ where c=total amount chlorophyll a in mg in volume V in ml, when absorbance at 666 and 730 nm is measured in a 1.0·cm cell.

Results

Among the brown algae, *Fucus spiralis* was available in axenic culture and therefore it was chosen as the first marine subject. Although it has been kept in axenic culture for many years it still keeps its seasonal rhythm and it is very difficult to obtain any regular growth in experiments performed from November to March. In a preliminary experiment during this season, additions of vanadate induced an enhanced shoot formation on the inocula. As Arnon and Wessel

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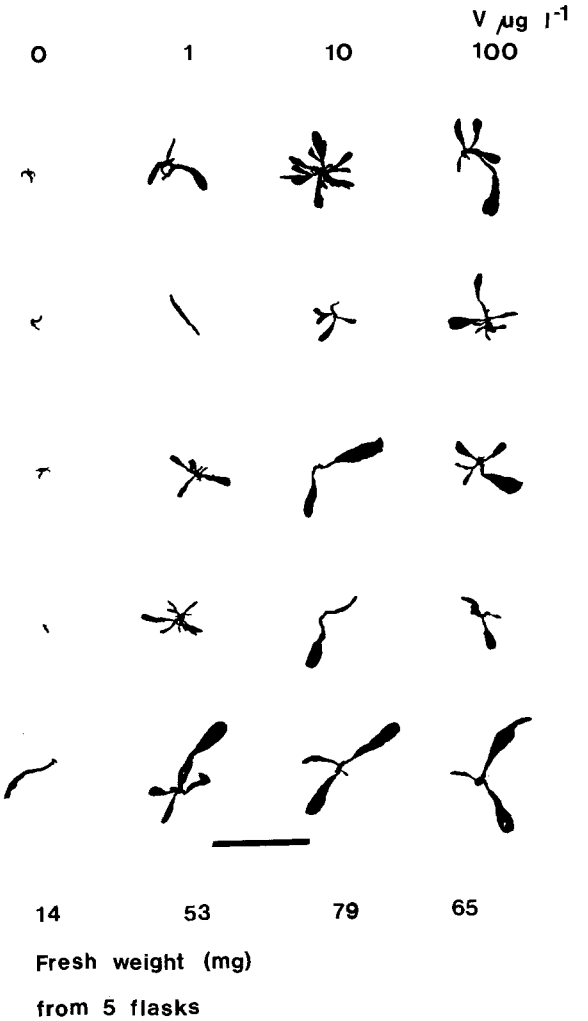

Fig. 1. Growth of *Fucus spiralis* with vanadium, 1–100 µg l^{-1}, added as NH_4VO_3. All inocula in a horizontal row originated from the same plant. Fresh weight given as total weight of the five plants from one series. Experimental time 53 d (November–December). 15° C, 15 h light. The bar corresponds to 2 cm

(1953) found in their experiments with the fresh-water alga *Scenedesmus obliquus* that 1–100 µg l^{-1} was the range for positive effects of V, this range was chosen for additions of $NH_4VO_3 \cdot 5H_2O$ to *Fucus spiralis*. The effects on the morphology were striking, as can be seen in Fig. 1. The fresh weight was also strongly influenced; 10 µg l^{-1} ($2 \cdot 10^{-7}$ g-at l^{-1}) increased growth more than 5 times while 100 µg was less favorable. Growth and morphology of *Fucus* is strongly influenced by phenylacetic acid and p-hydroxyphenylacetic acid (10^{-5}–10^{-7} M) during its growth period, April to August (Fries 1977), but even with these additions growth is very irregular during the winter. Figure 2 shows the result of an experiment performed during the winter with an addition of 10 µg V l^{-1}. Addition of V induced growth in most inocula and growth was further enhanced by the highest concentration of phenylacetic acid, whereas p-hydroxyphenylacetic acid did not have any effect. To confirm that the results in fact depended upon vanadium and not on some impurity in the salt used, ammonium vanadate from two different suppliers, as well as vanadium (IV) oxysulphate, were tested as vanadium sources. All of them gave the same result.

To investigate whether vanadium effects are common among marine algae, *Enteromorpha compressa* was chosen as a representative of the green seaweeds. In this alga additions of 1–100 µg V l^{-1} increased the fresh weight. The maximum effect was displayed at 10 µg l^{-1} with an increase of about 90% but the morphology was not so strongly changed as in *Fucus* (Fig. 3).

Many red algae have a lower content of vanadium in their thalli than brown and green algae, an exception being *Polysiphonia urceolata* (Dillw.) Grev. (Yamamoto et al. 1970). Therefore, this species was investigated together with *Goniotrichum alsidii* (Zanard.) Howe and *Nemalion helminthoides* (Vell. in With.) Batt. which were also available in axenic cultures. No morphological changes or increases of growth measured as dry weight could be observed. A slight decrease of the dry weight was noted in *Goniotrichum* and *Nemalion* at an addition of 100 µg V l^{-1}. *Nemalion* was also cultivated in another artificial medium (Harrison et al. 1980) which I have found more suitable for this species, but no positive effect of vanadium could be obtained with either medium. *Nemalion* was also cultivated under varied light conditions and/or day lengths, but no response to vanadium was observed.

Chlorophyll a was measured in *Fucus* plants grown with and without added V. No significant differences in chlorophyll content could be demonstrated in plants after 18 d. When new measurements were made after 45 d the situation had changed. The chlorophyll a content had increased in all vanadium-treated plants, in some plants by more than 50% (Table 1). In the determinations made after 18 d much more algal material was extracted than in the later experiments, so even small differences should have been observed.

Discussion

With this investigation the influence of vanadium on algal growth has been broadened to include not only unicellular fresh-water algae but also a brown seaweed, *Fucus spiralis*, and a green alga, *Enteromorpha compressa*. In experiments with unicellular green algae, Meisch and coworkers (1977, 1978) found that V increased the dry weights with an optimum at pH 7, and at higher concentrations also increased chloro-

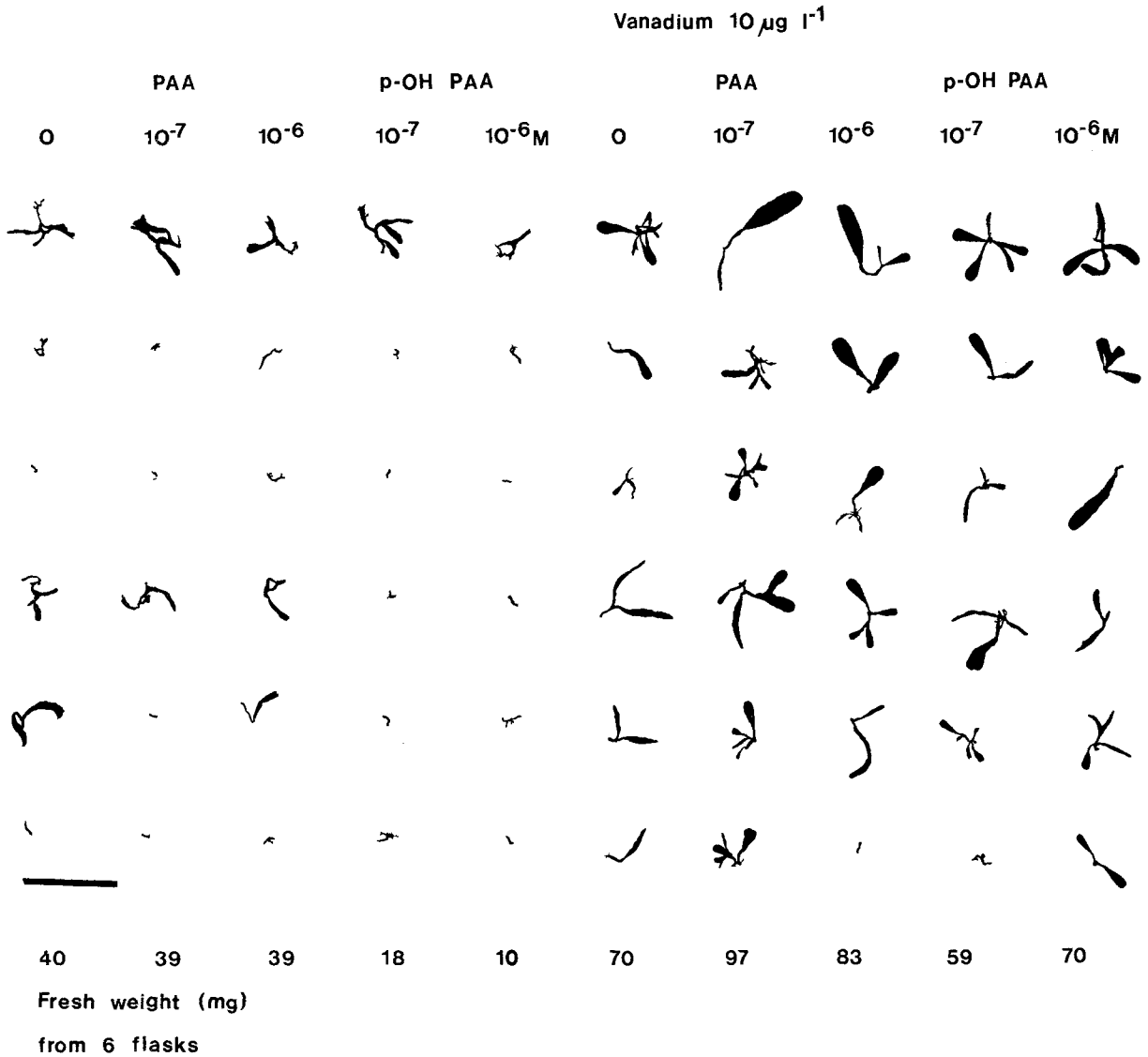

Fig. 2. Effects of vanadium, 10 µg l^{-1} on *Fucus spiralis* in combination with phenylacetic acid and p-hydroxyphenylacetic acid. All inocula in a horizontal row originated from the same plant. Fresh weight given as total weight of the 6 plants in one series. Experimental time 53 d (January–February). 17° C, 15 h light. The bar corresponds to 2 cm

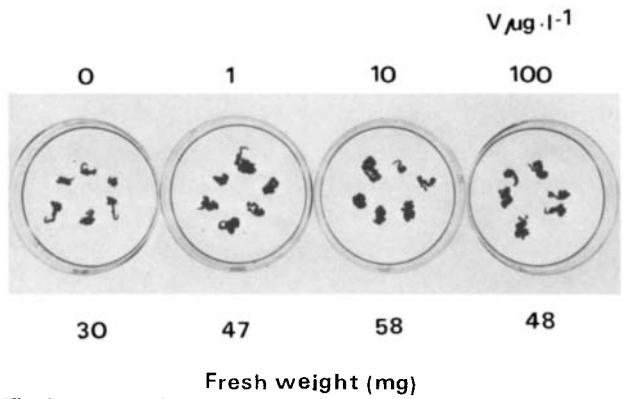

Fig. 3. Growth of *Enteromorpha compressa* with additions of vanadium, 1, 10 and 100 µg l^{-1}. The six plants from the same series are photographed in a 5 cm Petri dish. 17° C, 18 h light. Incubation time 42 d (January–February)

phyll synthesis, with an optimum at pH 7–8.5. In the brown and green seaweeds investigated, 1–100 µg l^{-1} induced a strong increase in fresh weight which was especially striking in *Fucus*. The enhancement of the chlorophyll seems not to be the primary reaction to the vanadium addition as the small inocula develop and show morphological differences before a measurable increase in chlorophyll content could be observed.

None of the red algae showed any positive response at 1–100 µg V l^{-1}. In *Chlorella* and *Scenedesmus* the reactions were shown to be correlated to the light intensity (Meish and Bielig 1975). Although day length and/or light intensity were varied in experiments with *Nemalion multifidum* vanadium did not increase growth. Only a slight decrease was observed

Table 1. Determination of chlorophyll a in *Fucus* plants, axenically cultivated with or without 20 µg V l^{-1}, given as NH_4VO_3. Algal material extracted in 90% aceton. When grown out *Fucus* plants were used, two flasks with as equal plants as possible were kept together from the start and one of them was given vanadium. These pairs are kept together in the table

Time of vanadium treatments in days	Type of algal material used	Vanadium addition	Amount of	
			Fucus used (mg FW)	Chl (mg g^{-1} FW)
18	Complete plants with developed blades	0	189	0.6097
		V	214	0.6165
		0	242	0.6165
		V	204	0.6900
	More tube-like thalli	0	146	0.8927
		V	139	0.8904
45	Uniform blades cut off from identical plants	0	57	0.6629
		V	57	0.8674
		0	82	0.3933
		V	82	0.5933
	Plants developed from small inocula in −V and +V[a]	0	17	0.3955
		V	31	0.5573
	V added to grown out plants	0	11.5	0.6427
		V	13.5	0.9931

[a] Material from an experiment similar to that in Fig. 1

with 100 µg V l^{-1}. A connection between chlorophyll synthesis and vanadium effects seems less likely, as it would imply a difference in the pathways of chlorophyll synthesis in brown and red algae. However, the vanadium content of seaweeds varies with the season (Yamamoto et al. 1970), which might point to a changing demand during the development of the plants. The most suitable season for experiments to demonstrate a measurable demand for vanadium might thus easily be missed. Hence some caution must be exercised when drawing conclusions from negative results.

It is still unknown in what oxidation state vanadium is assimilated in the algal cells. Most experiments with fresh water algae are performed at pH-values around 6 where vanadium presumably is cationic, being a strong oxidant up to pH 7 (Macara 1980). Natural seawater has a pH of 7.9–8.3 and in ASP6 F2 pH is kept constant at 8.3 by Tris·HCl during the experiments. In the sea water, V is normally present as vanadate. This could point to a different role in marine than in fresh-water algae. In series containing V, practically all *Fucus* inocula started development, in contrast to those in series without any additions. Whether this indicates an essential role of vanadium in metabolism or a special function in cell division remains an open question.

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