Effects of siderophores and amino acids on the growth and photosynthesis of populations of *Chlorella vulgaris* Beijerinck and *Anabaena variabilis* Kützing

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Chlorella vulgaris
Anabaena variabilis
Siderophores
Amino acids
Chlorophyll *a*
Photosynthesis

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Abstract

The object of this paper was to determine whether the presence of the amino acids and siderophores tested is important to the growth and photosynthesis of phytoplanktonic cells cultivated under iron deficiency conditions, and can thus shed light on the function of two groups of natural chelating agents in the aquatic environment. The results obtained indicate that the siderophoric substances and amino acids can modify physiological processes in populations of cells of cyanobacteria and green algae.

1. Introduction

In seawater iron occurs in the form of a stable colloid formed from the hydrated hydroxide $Fe(OH)_3 \times n\ H_2O$. The solubility product (K) of this compound is very low ($K = 10^{-38}$). As a result the accessibility of iron to marine phytoplankton may be limited (Anderson and Morel, 1982). Iron deficiency may be the reason for disturbances in a number of physiological processes taking place in marine organisms (Neilands, 1995). Those biochemical processes depending on the availability of iron and other trace metals are indirectly modified by organic substances exhibiting complexing properties. These substances influence both the level and speciation of dissolved trace metals and thus their availability and toxicity to organisms (Jones, 1970; Baccini, 1983; Kosakowska *et al.*, 1988a,b).

Of the organic chelating agents occurring in the environment, it was amino acids and siderophores whose influence on iron availability were investigated in this study. The level of amino acids in seawater (10 mg dm^{-3}) is sufficient to severally influence speciation of transition series of heavy metals (Jones, 1970).

Compounds from the group of siderophores are less abundant but exhibit stronger complexing properties. They are naturally excreted by some species of bacteria and fungi, certain phytoplanktonic blue-green algae and eukaryotic organisms such as diatoms and pyrrophyta (Murphy *et al.*, 1976; Simpson and Neilands, 1976; Trick *et al.*, 1983; Trick, 1989; Wilhelm and Trick, 1995). Their biosynthesis is regulated precisely by the level of iron in the environment (Neilands, 1995). Literature reports suggest that siderophores complex iron and promote iron transport to the cells which excreted them, thus displaying a specific activity only in relation to the organisms from which they originate.

Murphy *et al.* (1976) have shown that during cyanobacterial blooms, other algae can be completely suppressed owing to the lack of iron, since they are unable to use the iron transported to the cells by siderophores. Bailey and Taub (1980) tested the influence of three hydroxamate siderophores on the growth of green algae cells. Their results corroborate the findings of Murphy and co-workers. They concluded that not all algae are inhibited by siderophores and not all siderophores affect algal growth to a similar extent. However, no mechanism for this phenomenon was suggested.

The purpose of this paper was to determine whether the presence of selected amino acids and siderophores is important to the growth and photosynthesis of algae and cyanobacteria cultivated under iron-deficient culture conditions. We present our findings from two algae – *Chlorella vulgaris* and *Anabaena variabilis* – in order to shed light on the function of the two groups of natural complexing agents in the aquatic environment.

2. Materials and methods

An axenic culture of the green alga *Chlorella vulgaris* Beijerinck (A1–76) was isolated from the Baltic Sea (Institute of Oceanology PAS, Sopot) and a non-axenic culture of *Anabaena variabilis* Kützing (C–122) was obtained from the Culture Collection of Autotrophic Organisms (Prague) and utilised in this study.

To reduce iron contamination from the culture glassware, all flasks were repeatedly rinsed with 3N NaOH, then 3N HCl and finally, deionised distilled water.

The algae were grown on Bristol's mineral medium (Starr, 1964) depleted of trace metals (Armstrong and Van Baalen, 1979; Stauber and Florence, 1985). Weighed portions of individual salts were dissolved in deionised distilled water. The solution obtained was sterilised by filtration on Sartorius 0.2 mm membrane filters. The medium was passed through a column of Chelex–100 resin in Na^+ form (Bio-Rad Laboratories) to remove traces of di- and trivalent metals (Davey *et al.*, 1970).

In the medium thus prepared, the iron concentration was determined spectrophotometrically with aid of bathophenanthroline (Smith *et al.*, 1952), and was found to be equal to 1.2×10^{-7} M.

Aqueous solutions of the following organic substances were used in the experiments: schizokinen, retro-(Et)-arthrobactin (obtained from Prof. A. Chimiak and Dr. M. Milewska, Department of Organic Chemistry, Technical University of Gdańsk), rhodotorulic acid, L-cysteine and L-aspartic acid (Aldrich). Concentrations of siderophores and amino acids in the medium were 2×10^{-6}M (molar ratio of chelator: added Fe $\cong$ 10).

The inocula for *Chlorella vulgaris* and *Anabaena variabilis* were 1×10^5 cells cm^{-3} and 3×10^{-5} mg cm^{-3} of chlorophyll *a* respectively. Cultures without organic compounds served as control samples. The cultures were incubated under continuous illumination at an intensity of 6000 lux and a temperature of 25 ± 1°C. Each variant of the experiment was repeated at least nine times.

After 7 days the cultures were filtered through Whatman GF/C filters and the chlorophyll *a* content was measured according to the modified Strickland and Parsons method (Strickland and Parsons, 1972).

In the experiments involving carbon–14 incorporation, *Chlorella* and *Anabaena* cultures were treated with amino acids and siderophores of the same concentration as described above. The algae cultivated without the test compounds were the control samples. The procedure was adapted from Strickland and Parsons (1972). Carbon–14 (as $Na^{14}HCO_3$) was added to the suspension of cells at a final activity of 1.85×10^{-3} MBq cm^{-3}. After 4 h incubation the cells were filtered through Sartorius 0.45 mm membrane filters, washed three times with sterile medium, dried and analysed for activity in a liquid scintillation counter (Lind and Campbell, 1969).

Statistical evaluation of the results attained was based on the Student t-test. The testing hypothesis was carried out at a significance level of $\alpha = 0.01$.

3. Results

The results of experiments on the influence of siderophores and amino acids on the chlorophyll *a* concentration in cells of *C. vulgaris* and *A. variabilis* are presented in Tab. 1.

Table 1. Concentration of chlorophyll *a* in populations of *Chlorella vulgaris* and *Anabaena variabilis* cultivated under iron-deficient conditions in the presence of selected siderophores and amino acids; bold figures indicate the values for spiked samples, which differ significantly from the results of a control based on Student's t-test at $\alpha = 0.01$; number of replicates = 9

Organic substances tested [2 mmol dm^{-3}]	Chlorophyll *a* concentration [mg m^{-3} ± SD]	
	C. vulgaris	*A. variabilis*
control	452 ± 55	316 ± 37
L-cysteine	512 ± 46	327 ± 34
L-aspartic acid	**598 ± 33**	348 ± 45
rhodotorulic acid	**1193 ± 66**	**616 ± 23**
retro-(Et)-arthrobactin	**1294 ± 54**	358 ± 57
schizokinen	**1171 ± 77**	**469 ± 42**

It follows from the experiments that the presence of L-aspartic acid, schizokinen, rhodotorulic acid or retro-(Et)-arthrobactin in the medium resulted in an increase in chlorophyll *a* content in the population of *C. vulgaris* cells by 30–180% in comparison to the control sample. In the case of the culture of blue-green algae *A. variabilis*, the presence of schizokinen and rhodotorulic acid caused an increase in the amount of chlorophyll *a* by 50% and 90% respectively, in comparison with the control sample. In the presence of the remaining tested compounds, the changes in chlorophyll *a* level in comparison with the control sample were statistically insignificant.

The results of experiments on the influence of siderophores and amino acids on the rate of incorporation of carbon–14 into the cells of *C. vulgaris* and *A. variabilis*, incubated in an iron deficient medium, are presented in Tab. 2.

The experiments show that the addition of L-cysteine, L-aspartic acid, rhodotorulic acid, retro-(Et)-arthrobactin or schizokinen to the medium resulted in an increase in carbon–14 incorporation into the cells of *A. variabilis* by 40–50% on average in comparison to the control group. In the case of *C. vulgaris*, none of the test substances, with the exception of rhodotorulic acid, influenced the process of photosynthesis. In the presence of rhodotorulic

Table 2. The rate of carbon–14 incorporation into *Chlorella vulgaris* and *Anabaena variabilis* cells cultivated in under iron-deficient conditions in the presence of selected siderophores and amino acids; bold figures indicate the values for spiked samples, which differ significantly from the results of a control based on Student's t-test at $\alpha = 0.01$; number of replicates = 9

Organic substances tested [2 mmol dm^{-3}]	Radioactivity of cells [CPM ± SD] × 10^2	
	C. vulgaris	*A. variabilis*
control	975 ± 44	1007 ± 19
L-cysteine	1018 ± 51	**1446 ± 29**
L-aspartic acid	1041 ± 19	**1421 ± 30**
rhodotorulic acid	**684 ± 53**	**1466 ± 62**
retro-(Et)-arthrobactin	1114 ± 10	**1507 ± 55**
schizokinen	986 ± 55	**1420 ± 82**

acid, even a 30% reduction in the incorporation of carbon–14 into the cells of the green alga was observed.

The increase in the pre-incubation time with this chelator to 24 h resulted in the elimination of the inhibitory effect of rhodotorulic acid (activity equal to 98 ± 3% of the control samples).

4. Discussion and conclusion

The results obtained indicate that substances with a siderophoric character stimulate the growth of *Chlorella vulgaris* to a much larger extent than that of *Anabaena variabilis* when monitored by chlorophyll *a* concentration. These results were obtained when the algae were incubated in an iron-deficient medium and harvested for chlorophyll *a* measurements after seven days of incubation.

In contrast, the rate of carbon–14 incorporation into *Chlorella vulgaris* was inhibited in the presence of rhodotorulic acid, but no significant effects were recorded in the presence of L-cysteine, retro-(Et)-arthrobactin or schizokinen when compared to photosynthesis in the control cultures. Literature reports indicate that many species of algae, both green and blue-green, are inhibited by siderophores. For example, desferioxamine inhibited *Ankistrodesmus* sp., *Seleneastrum capricornutum, Scenedesmus basiliensis, Anacystis* sp. and *Anabaena* sp. (Murphy *et al.*, 1976; Bailey and Taub, 1980).

In the case of *Anabaena variabilis* a clear increase in the rate of carbon–14 incorporation was observed for all the substances tested.

The effects of the test substances on the two physiological processes examined, *i.e.* the growth and photosynthesis of green and blue-green algae are depicted in Figs. 1, 2.

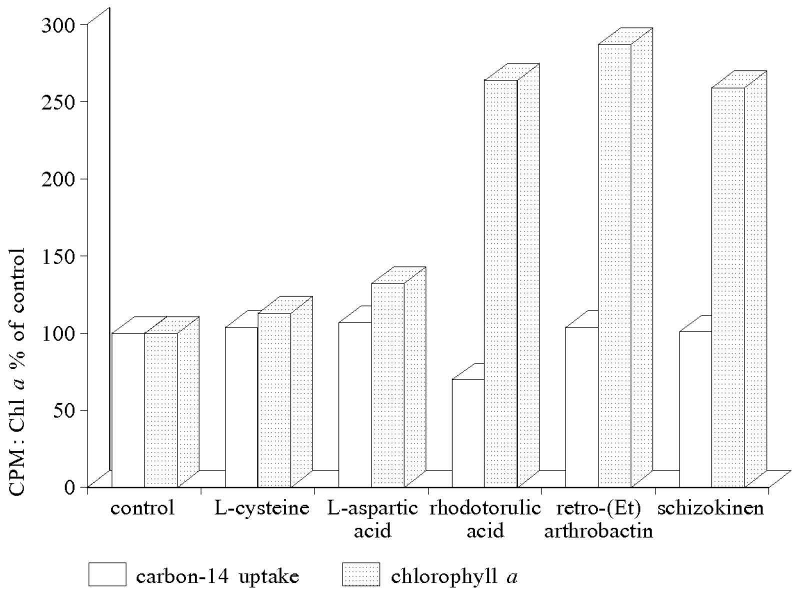

Fig. 1. Comparison of the rate of carbon–14 uptake with chlorophyll *a* concentration in a population of *Chlorella vulgaris* incubated in the presence of selected chelators

The results of chlorophyll *a* measurements indicate that the test substances had a different influence on the two species of algae. In the case of *Chlorella vulgaris* (green algae) a clear increase in chlorophyll *a* occurred, while the cyanobacterium *Anabaena variabilis* responded with increased chlorophyll *a* only in the case of selected siderophores (Fig. 3).

This might have been caused by two different mechanisms. *Chlorella vulgaris* might have utilised the added substances as agents promoting iron transport. This would indicate that green algae are capable of excreting siderophore-like substances, and that the chemical structure of these substances is similar enough to the substances tested for them to be utilised in iron transport to the cells. Another possibility is that in the course of the seven-day-long incubation *Chlorella vulgaris* developed an iron utilisation mechanism. The availability of iron was a growth-limiting factor in the experiments. Under such conditions algae are known to excrete substances promoting iron transport to cells (Simpson and Neilands, 1976; Murphy *et al.*, 1976; Armstrong and Van Baalen, 1979; Trick *et al.*, 1983).

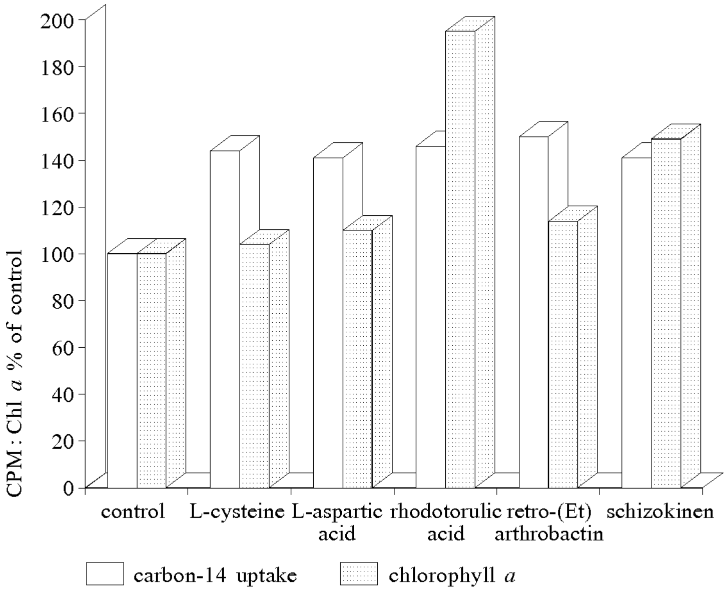

Fig. 2. Comparison of the rate of carbon–14 uptake with chlorophyll *a* concentration in a population of *Anabaena variabilis* incubated in the presence of selected chelators

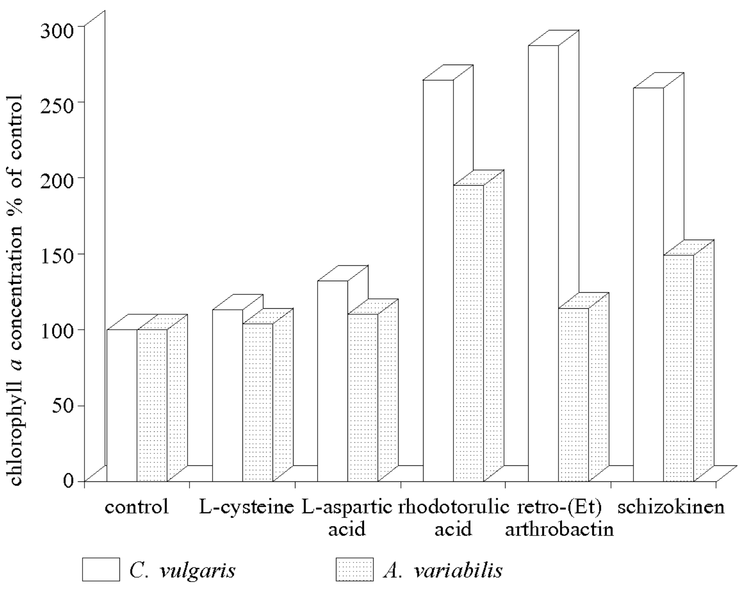

Fig. 3. Comparison of chlorophyll *a* concentration in populations of *Chlorella vulgaris* and *Anabaena variabilis* cultivated in the presence of selected chelators

Both mechanisms can occur simultaneously. In the case of *Anabaena variabilis* (blue-green algae) the stimulation is much less evident, which would indicate that these two growth stimulation mechanisms are less pronounced.

The results of carbon–14 uptake seem to contradict those recorded with chlorophyll *a* (Fig. 4).

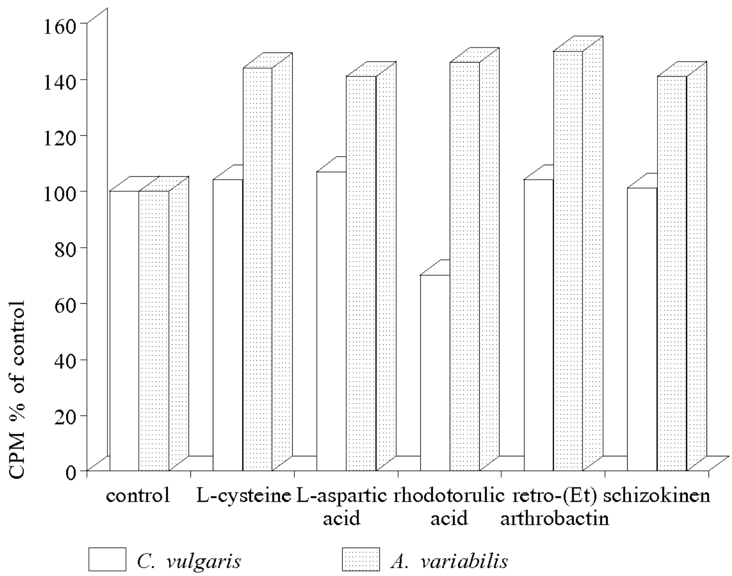

Fig. 4. Comparison of the rate of carbon–14 uptake in populations of *Chlorella vulgaris* and *Anabaena variabilis* cultivated in the presence of selected chelators

Anabaena variabilis exhibited increased incorporation of carbon–14 when siderophores and amino acids were added to the culture medium. This might have been caused by the algae using the added substances as a source of carbon. Blue-green algae are known to make use of external organic substances as a source of carbon (Danforth, 1962; Fogg *et al.*, 1973). Increased carbon–14 incorporation in the presence of amino acids might support this explanation.

No carbon–14 was incorporated when siderophores and amino acids are added to the culture medium of *Chlorella vulgaris*. This would indicate that the substances were not utilised as a carbon source. Of the two mechanisms explaining the increase in chlorophyll *a*, the adaptation of algae to an iron-deficient medium seems to be supported by the results of carbon–14 incorporation. The direct utilisation of siderophores would have resulted in an instant increase in carbon–14 incorporation, an event which was not recorded.

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