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Influence of organic compounds on the toxicity of copper and cadmium to algae cells

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Abstract

The effect of siderophores, cysteine and Na_2EDTA in cultures of *Chlorella vulgaris* and *Anabaena variabilis* containing copper or cadmium, on the chlorophyll-*a* content, as well as on the rate of carbon fixation, has been investigated. Experiments on copper accumulation in *Chlorella vulgaris* cells grown in the presence of organic compounds have been also carried out. The siderophores, cysteine and Na_2EDTA reduced the toxicity of copper and cadmium to axenic strains of algae and natural phytoplankton. No correlation between the toxic effect of copper and its bioavailability was observed.

Introduction

Organic matter considerably influences speciation of heavy metals in sea water and in consequences, affects their biogeochemical behaviour. It has been generally assumed that the activity of free metal ions determinates their bioavailability, as well as toxicity (SUNDA and GUILLARD 1976, SUNDA and LEWIS 1978, JACKSON and MORGAN 1978, DAVIES 1978, CANTERFORD and CANTERFORD 1980, RABSCH and ELBRÄCHTER 1980, MOREL and MOREL-LAURENS 1983, SUNDA and FERGUSON 1983, RABSCH et al. 1984). The ecological consequences of the formation of metal ion complexes with organic matter have been postulated in a number of papers, but further evidence is still required (SAUNDER 1957, RAI et al. 1981, MOREL and HUDSON 1985). The present paper deals with the effects of sideophores on algal cultures containing copper and cadmium. Due to the alkalinity of sea water and the presence of free oxygen highly insoluble forms of iron are abundant. Siderophores are the low molecular compounds excreted by living cells, which faciliate the uptake of iron from insoluble forms, making these available to microorganisms and plants (NEILANDS 1973, CHIMIAK 1984). Siderophores have been identified in marine algae, as well as in sea water, but their ecological role is still doubtful (MURPHY et al. 1976, MC COY et al. 1978, ARMSTRONG and VAN BAALEN 1979, MC KNIGHT and MOREL 1979, 1980, ENG–WILMOT and MARTIN 1979, 1980, ANDERSON and MOREL 1982, TRICK et al. 1983, MOREL and MOREL–LAURENS 1983, SUNDA and FERGUSON 1983). In our experiments we also included Na_2EDTA, a commonly used chelating agent, and cysteine, a strong copper and cadmium detoxifying compound (KOSAKOWSKA et al. 1987).

Materials and methods

The tested compounds were: rhodotorulic acid, avaitin B, retro-(Et)-schizokinen, retro-(Et)-arthrobactin (Department of Organic Chemistry, Technical University of Gdansk), desferal (Ciba-Geigy Pharmaceuticals), dihydroxybenzoic acid (DHB, Aldrich), cysteine (Sigma), and the disodium salt of ethylenediaminotetraacetic acid (Na_2EDTA, POCH). All chemicals used were of analytical grade.

For the laboratory experiments axenic strains of *Chlorella vulgaris* (A1–76) isolated from Baltic water and *Anabaena variabilis*, obtained from the Moscow University Collection were used. In addition, samples of natural phytoplankton, in which species of the *Bacillariophyceae* were the dominant, were taken from the Gulf of Gdansk.

The algae were grown in modified Bristol's sterile synthetic medium with ammonium nitrate (5.10^{-4} mol) instead of sodium nitrate. Trace metals, as well as Na_2EDTA were excluded from the medium. The solution of nutrients in redestilled water was passed through Chelex–100 Na^+ (Bio-Rad, DAVEY et al. 1970). The medium was inoculated with *Chlorella vulgaris* to obtain 10^5 cells per cm^3 or with *Anabaena variabilis* to a chlorophyll *a* concentration of 3.10^{-5} mg cm^{-3}. The cultures were incubated for 7 days at 6000 lux. In order to determine the effects of organic compounds and copper or cadmium on the chlorophyll-*a* content, the tested chelators and metals were added to appropriate samples and the cycle of incubation was repeated. Copper was added in form of $CuSO_4$ (8.10^{-7} mol·dm^{-3}), cadmium as $CdCl_2$ ($3:5.10^{-6}$ mol·dm^{-3}). The siderophores (except DHB and desferal) were dissolved in a minimal amount of dimethylformamid and diluted with sterile deionized water to a concentration of 2.10^{-4} mol·dm^{-3}. Desferal and cysteine were dissolved in water only. DHB was neutralized with $NaHCO_3$ and diluted as above. The final concentration of the organic compounds tested was 4.10^{-4} mol·dm^{-3}. After 7 days, the cultures were passed through Whatmann GF/C filters and the chlorophyll-*a* content measured according to SHOAF and LIUM (1976).

In the experiments on copper accumulation in *Chlorella* cells, the concentration of copper in the culture medium was 100 µg dm^{-3}. Some of the filters were taken for chlorophyll-*a* measurements, the remaining were dried at 60° C and the biomass determined gravimetrically. The dried filters, as well as filtrates of cultures medium 70 cm^3 were transferred to teflon vessels and mineralized, first with 1 cm^3 of concentrated HNO_3 and after heating to white fumes, with 1 cm^{-3} of HNO_3–$HClO_4$ 1:1 mixture. The acids were evaporated, the residue dissolved in 25 cm^3 of 0.01 molar HCl and the copper concentration determined by means of AAS (Instrumentation Laboratory).

To determine the incorporation of carbon-14 from $NaH^{14}CO_3$ into the algal cells, solutions of the tested organic compounds, heavy metal salts and inoculum of algae cells were added to media as described above and preincubated for 24 hrs. 0.2 cm^3 of labelled sodium bicarbonate solution (5 µCi cm^{-3}) (STRICKLAND and PARSONS 1960) was added to 20 cm^3 of the cultures. After 4 hrs incubation the cells were filtered through Sartorius 0.45 µm membrane filters and their activity measured in a Beckmann liquid scintillation counter (LIND and CAMPBELL 1969).

All the experiments were repeated at least three times.

Results

The effect of chelators on chlorophyll-*a* content in cultures of *Chlorella vulgaris* and *Anabaena variabilis* containing copper or cadmium is shown in Table 1.

All compounds examined reduced the toxicity of copper against *Chlorella vulgaris*. Only desferal did not reveal statistically significant effects. Rhodotorulic acid, cysteine and Na_2EDTA added to the cultures of *Chlorella vulgaris* containing cadmium as well as to the cultures of *Anabaena variabilis* containing copper or cadmium, increased significantly the chlorophyll-*a* content, whereas the remaining compounds had no effects. The influence of the organic compounds and copper or cadmium on the rate of carbon-14 incorporation into the algal cells is shown in Table 2.

Table 1

Chlorophyll-a content in cultures of *Chlorella vulgaris* and *Anabaena variabilis* containing chelators and copper or cadmium

| Chelator | Content of chlorophyll-a mg $\cdot$ dm^3 $\pm$ SD | | | |
| | Copper concentration $8 \cdot 10^{-7}$ mol dm^{-3} | | Cadmium concentration $3.5 \cdot 10^{-6}$ mol dm^{-3} | |
	Chlorella vulgaris	*Anabaena variabilis*	*Chlorella vulgaris*	*Anabaena variabilis*
without chelator	0.28±0.02	0.08±0.01	0.39±0.02	0.10±0.01
Rhodotorulic acid	**0.86**±0.25	**0.25**±0.02	**0.70**±0.07	**0.12**±0.01
Retro-(Et)-nor-schizokinen	**0.43**±0.02	0.13±0.01	0.42±0.02	0.12±0.01
Avaitin B	**0.44**±0.03	0.14±0.01	0.41±0.01	0.12±0.01
Retro-(Et)-arthrobactin	**0.45**±0.03	0.13±0.01	0.38±0.02	0.10±0.03
Desferal	0.41±0.10	N.D.	N.D.	N.D.
DHB	**0.60**±0.17	N.D.	N.D.	N.D.
Cysteine	**0.81**±0.08	**0.41**±0.03	**0.55**±0.04	**0.20**±0.01
Na$_2$EDTA	**0.51**±0.04	**0.34**±0.02	**0.63**±0.04	**0.37**±0.03

The content of chlorophyll-a (mg $\cdot$ dm^{-3}) in control samples without heavy metal and chelator was 0.56±0.06 in *Chlorella vulgaris* cultures and 0.37±0.05 in *Anabaena variabilis* cultures, respectively. The concentration of chelator was $4 \cdot 10^{-6}$ mol dm^{-3}. Cultures were grown for 7 days in Bristol's medium passed through Chelex-100, at 28°C, $6 \cdot 10^3$ lux. The bold figures differ significantly from the results for samples with heavy metal alone based on a Student's t-test at α=0.01. Number of replicates =5 N.D.: not determined

Table 2

Carbon-14 incorporation into *Chlorella vulgaris* and *Anabaena variabilis* cells in cultures containing chelators and copper or cadmium.

| Chelator | cpm X 10^3 in sample $\pm$ SD | | | |
| | Copper concentration $(2.5 \cdot 10^{-1}$ µg $\cdot$ cm$^{-3})$ | | Cadmium concentration $(2 \cdot 10^{-1}$ µg $\cdot$ cm$^{-3})$ | |
	Chlorella vulgaris	*Anabaena variabilis*	*Chlorella vulgaris*	*Anabaena variabilis*
without	46.8± 1.40	10.8± 0.03	46.8± 0.94	17.1±0.51
Rhodotorulic acid	**104**.4± 4.17	**100**.8±18.14	37.2± 0.74	**50**.4±2.52
Desferal	**67**.2± 3.36	**96**.3±20.22	34.8± 0.69	**45**.0±2.25
DHB	46.8± 1.87	**90**.9±25.45	45.6± 3.19	18.9±0.57
Na$_2$EDTA	**187**.2±46.80	**82**.8± 7.45	**189**.0±18.96	**84**.6±5.07

The radioactivity (CPM X 10^3) of control samples incubated without heavy metal and chelator was 120±7.2 in *Chlorella vulgaris* cultures and 90±4.5 in *Anabaena variabilis* cultures. Concentration of chelator was $4 \cdot 10^{-6}$M. The cells were incubated in Bristol's medium passed through Chelex-100, incubation period 4 hours, time of preincubation with metal and chelator – 24 hours, carbon-14 added as NaH^{14}CO$_3$ to give a final activity of $5 \cdot 10^{-2}$µCi cm^{-3}. Bold figures differ significantly from the results for sample with heavy metal alone based on Student's t test at α=0.01. Number of replicates = 5

Table 3
The incorporation of carbon-14 into natural phytoplankton in samples of seawater containing copper and chelator

Copper ($\mu g\ cm^{-3}$)	Chelator ($4 \cdot 10^{-6}\ mol\ dm^{-3}$	cpm X 10^3 in sample $\pm$ SD
without copper	without chelator	22.07±0.17
	Rhodotorulic acid	20.75±0.21
	DHB	21.85±1.09
	Na$_2$EDTA	20.75±0.83
0.05	without chelator	11.47±0.23
	Rhodotorulic acid	**20.31±1.22**
	DHB	9.93±0.29
	Na$_2$EDTA	**20.31±0.22**
0.10	without chelator	7.95±0.08
	Rhodotorulic acid	**17.66±1.06**
	DHB	7.06±0.21
	Na$_2$EDTA	**19.43±0.97**

Time of incubation 4h, carbon-14 added as $NaH^{14}CO_3$-5 $\cdot$ $10^{-2}\mu Ci\ cm^{-3}$, dominant species belonged to the *Bacillariophycae*, chlorophyll *a* content 3 x 10^{-3} mg $\cdot$ dm^{-3}. Bold figures differ significantly from the results for samples with heavy metal alone based on a Student's *t* test at α=0.01. Number of replicates = 3

Table 4
The effect of chelators on the distribution of copper in cultures of *Chlorella vulgaris*

Sample No	Medium	Content of	
		Chlorophyll-*a* (mg dm^{-3})	Biomass (mg d.w. dm^{-3})
1	Control	0.6 ±0.1	127± 9
2	Cu(0.1 $\mu g\ cm^{-3}$)	0.3 ±0.1	122±17
3	Cu + rhodotorulic acid	1.2 ±0.3	157±11
4	Cu + desferal	0.45±0.1	55±15
5	Cu + DHB	0.75±0.2	135±14
6	Cu + cysteine	0.9 ±0.1	105± 9

Sample No	Content of copper		
	particulate ($\mu g\ dm^{-3}$)	dissolved ($\mu g\ dm^{-3}$)	per unit of cells ($\mu g\ mg^{-1}$ d.w.)
1	9± 3.7	7.3±3.7	0.07±0.03
2	87± 7.0	14.5±6.5	0.73±0.20
3	85± 2.1	9.2±2.7	0.64±0.05
4	93± 7.6	10.2±2.7	1.77±0.45
5	88± 2.4	11.2±3.3	0.65±0.09
6	87±10.0	10.6±2.3	0.85±0.05

Control – the samples without copper and chelator, concentration of chelator was $4 \cdot 10^{-6}$ mol dm^{-3}, cultures were grown for 7 days in Bristol's medium passed through Chelex-100, at 28°C, $6 \cdot 10^3$ lux. Number of replicates = 5

In cultures containing copper, the addition of rhodotorulic acid or Na$_2$EDTA increased the rate of carbon-14 incorporation in both strains of the algae, whereas DHB was active only to *Anabaena variabilis* cells. The rate of photosynthesis in cultures containing cadmium increased in both strains of algae after addition of Na$_2$EDTA, whereas rhodotorulic acid and desferal increased the rate of carbon-14 incorporation only in *Anabaena variabilis* cells.

In the experiments with natural phytoplankton, rhodotorulic acid and Na$_2$EDTA increased the rate of carbon-14 incorporation in samples containing 0.05 and 0.1 µg cm^{-3} of copper (Table 3).

The results of pilot experiments on copper accumulation in *Chlorella vulgaris* cells in cultures containing chelators are shown in Table 4.

The concentration of 100 µg copper per dm^3 caused 50 % decrease of the chlorophyll-*a* content, whereas the amount of biomass was not significantly affected. In cultures to which copper had been added, about 90 % of the metal was found in particulate matter. The concentration of copper in the algae cells cultivated in the presence of chelators was in the same range as in samples to which only metal was added, except for those containing desferal in which the copper concentration was higher.

Discussion

The effects of siderophores on the properties of heavy metals were observed previously and the ecological implications of these interactions were considered (ARMSTRONG and VAN BAALEN 1979, MC KNIGHT and MOREL 1979, 1980, ANDERSON and MOREL 1982, MOREL and MOREL–LAURENS 1983).

Our experiments show that the siderophores, as well as cysteine and Na$_2$EDTA reduce the toxicity of copper and cadmium to axenic strains of algae and natural phytoplankton samples. A broader spectrum of organic chelators affected the chlorophyll-*a* content, as well as rate of carbon-14 incorporation in cultures containing copper as compared to cadmium. The lower complexing ability of cadmium is generally accepted in literature. In the present, long time experiments with cultures containing cadmium, in which chlorophyll-*a* content was measured, both *Chlorophyta* and *Cyanophyta* strains were significantly affected as a result of addition of organic chelators. In contrast, the increase of rate of carbon-14 fixation in cultures containing heavy metal and organic chelators was more pronounced in *Anabaena variabilis* cells than in *Chlorella vulgaris* cells.

MOREL and MOREL–LAURENS (1983) postulated that a high concentration of magnesium and calcium ions in sea water should minimize the interaction of siderophores with copper. The results of carbon-14 incorporation in natural phytoplankton in sea water samples containing copper and siderophores contradict this thesis. We assume the possibility of biological consequences of heavy metal-siderophore interaction in natural environments, however, high concentration of both components applied in the experiments should be taken in consideration.

Results of copper accumulation by the algae in cultures containing organic chelators did not reveal any correlation between the heavy metal content in the algal cells and the toxic effects observed. About 90 % of the copper added to the cultures was found in *Chlorella vulgaris* cells in samples containing copper only, in which inhibition of algae growth was observed, as well as in those samples to which organic compounds were added and increase of chlorophyll-*a* content was determined. This contradiction with the literature data may be caused by a high concentration of copper in the medium (RABSCH et al. 1984). Our conclusions need further evidence, using lower concentrations of heavy metals and more precise measurements of their contents inside the cells.

Acknowledgements

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