Bull. Environ. Contam. Toxicol. (1988) 40:532–538

Bulletin of Environmental Contamination and Toxicology

Effect of Amino Acids on the Toxicity of Heavy Metals to Phytoplankton

Alicja Kosakowska, Leonard Falkowski,* and Jolanta Lewandowska

Institute of Oceanology, Polish Academy of Sciences,
ul.Powstańców Warszawy 55, 81-967 Sopot, Poland

Sea water contains free amino acids in concentrations ranging from 20 to 100 μg dm^{-3} (Andrews and Williams 1971). The most abundant representatives of this group of compounds in the surface water were found to be glycine, serine and ornithine. The amino acids are utilized in the phytoplankton cells as the source of nitrogen as well as carbon in the case of slow rate of photosynthesis (Paul 1985).

Heavy metals distribution in the sea water can be indispensable or delitorious for the organisms living in that environment depending on their concentration and speciation (Florence 1982; Morel and Hudson 1985). The bioavailability as well as toxicity of the elements are strongly influenced by the organic matter dissolved in water. Saunders (1957) postulated, that the interaction between organic matter and trace elements results significant ecological consequences.

The effect of a number of organic compounds, including amino acids, on the toxicity of heavy metals was tested (Fogg and Westlake 1955; Jones 1970; Baccini 1983), but there were no reports elucidating compounds representing various types of structure.

This paper deals with the effect of amino acids representing various structure groups on the toxicity of copper, cadmium and mercury against to *Chlorella vulgaris* and *Anabeana variabilis*.

MATERIALS AND METHODS

Axenic strains of *Anabaena variabilis* obtained from the Moscow University Collection and *Chlorella vulgaris* (A1-76) isolated from the Baltic water, were chosen as the tests organisms. The algae were grown on sterile

---

*Correspondence and reprint requests

synthetic Bristol's medium (Starr 1964) with the following modifications: ammonium nitrate (5 x 10^{-4}M) was applied instead of sodium nitrate, no Na_2EDTA was added and the concentration of ferric ions was 10^{-8}M.

The medium was inoculated to achieve the concentration of chlorophyll a 3 x 10^{-5}mg cm^{-3} with the Cyanophyta cells or 10^5 cm^{-3} of Chlorophyta cells and the cultures were incubated 7 days at 28° ± 2°C and at continnous illumination of 6000 lux.

This cycle was repeated after addition of water solutions of amino acids and salts of heavy metals to the appropriate samples. The final concentrations in the analyzed cultures of glycine, alanine, methionine, valine, glutamic acid, aspartic acid, glutamine, asparagine, proline and histidine were 2.5 x 10^{-5}M, whereas serine, tyrosine, cysteine, phenylalanine and tryptophan were 5 x 10^{-6}M because of their lower solubility.

Copper was added as $CuSO_4$ x 5 H_2O to a concentration of 7.9x10^{-7}M and cadmium as $CdCl_2$ x 2 1/2 H_2O to a concentration of 3.5 x 10^{-6}M in both cultures, whereas mercury as $HgCl_2$ to a concentration of 9.8x10^{-8}M in Anabaena variabilis and 7.4 x 10^{-8}M in Chlorella vulgaris cultures.

The flasks were rinsed with hydrochloric acid and washed several times with redistilled water. The adsorption of heavy metal ions on the glass surface and their real concentrations in the cultures were not measured because of the comparative mode of celculation of the results.

The effect of the amino acids tested on the toxicity of heavy metals to the phytoplankton cells was determined as the ratio of the amount of chlorophyll a in the tested and control (without heavy metals) samples. The chlorophyll a in the cultures was measured by means of the modified Strickland and Parsons (1972) method. All the experiments were repeated no less than three times.

RESULTS AND DISCUSSION

The results of the effect of the amino acids on the toxicity of copper, cadmium and mercury to Anabaena variabilis and Chlorella vulgaris are presented in Tables 1-2.

The amino acids dissolved in the medium in concentrations below 2.5 x 10^{-5}M did not cause any substantial increase in the concentration of chlorophyll *a*, which was no more than 10% higher in the samples containing the amino acids compared with the control group.

Addition of copper to the cultures at concentration 7.9×10^{-7}M caused 80 and 50% inhibition of the growth of the blue-green and green algae, respectively. In the presence of amino acids the toxicity of copper to the phytoplankton cells was significantly modified. In the presence of cysteine, aspartic acid and tyrosine, complete disappearance of the toxic effect was observed in the both cultures, with asparagine and tryptophan in the cultures of *Cyanophyta*, and glycine in those of *Chlorophyta*. Significant increase of the biomass was observed also in the presence of the remaining amino acids; only alanine and phenylalanine did not modify the toxicity of copper to the green algae cells.

Table 1. The effect of amino acids on the toxicity of copper, cadmium and mercury to *Chlorella vulgaris*.

Amino acid added	Chlorophyll *a* % of the control sample* ± SD			
	without heavy metals	Cu 7.9×10^{-7}M	Cd 3.5×10^{-6}M	Hg 7.4×10^{-8}M
control	100 ± 7.0	54 ± 6.0	23 ± 3.0	54 ± 8.0
GLY	104 ± 6.0	99 ± 7.0	29 ± 2.0	63 ± 9.0
ALA	100 ± 5.0	52 ±10.0	23 ± 5.0	77 ± 6.0
VAL	100 ± 4.0	74 ± 3.0	28 ± 4.5	71 ± 4.0
SER	99 ± 6.0	78 ± 6.0	28 ± 5.0	71 ± 6.0
CYS SH	99 ± 8.0	109 ± 6.0	43 ± 5.0	120 ±11.0
MET	103 ± 3.0	83 ± 8.0	27 ± 6.0	81 ± 5.0
GLU-OH	98 ± 6.0	81 ± 3.0	26 ± 2.0	71 ± 6.0
ASP-OH	103 ± 4.0	104 ± 9.0	31 ± 2.0	72 ± 3.0
$GLU-NH_2$	101 ± 7.0	78 ± 6.0	27 ± 4.0	75 ± 5.0
$ASP-NH_2$	106 ±10.0	74 ± 4.0	28 ± 6.0	70 ± 5.0
ARG	112 ±16.0	70 ± 2.5	25 ± 3.0	69 ± 4.0
HIS	111 ± 5.0	76 ± 2.5	25 ± 5.0	78 ± 5.0
PHE	109 ±10.0	56 ± 8.0	25 ± 3.0	68 ± 4.5
TYR	101 ± 5.0	89 ± 3.0	30 ± 2.5	67 ± 5.0
TRY	100 ± 7.5	66 ± 4.0	26 ± 5.0	72 ± 6.0
PRO	109 ± 6.0	83 ± 7.0	31 ± 3.0	74 ± 6.0

*control: the sample without heavy metals and amino acid

--- differ significantly from the sample with heavy metal alone based on a Student's t test, $\alpha = 0.01$. The number of replicates = 9.

Cadmium at concentration 3.5×10^{-6}M caused 80% inhibition of the growth of both algae species. Of the amino acids tested only cysteine substantially decreased its toxic effect. The effect of the remaining amino acids was weak or not detectable.

In experiments with cultures of *Chlorella vulgaris* grown in the presence of mercury at a concentration of 7.4×10^{-8} M, cysteine was also the only amino acid that fully cancelled its toxic effect. In the case of *Anabaena variabilis* cultures, a substantial decrease in the toxicity of mercury was observed for glutamine, cysteine and methionine.

Table 2. The effect of amino acids on the toxicity of copper, cadmium and mercury to *Anabaena variabilis*.

Amino acid added	Chlorophyll a % of the control sample* ± SD			
	without heavy metals	Cu 7.9×10^{-7}M	Cd 3.5×10^{-6}M	Hg 9.8×10^{-8}M
control	100 ± 6.5	20 ± 4.0	21 ± 5.0	19 ± 4.0
GLY	100 ± 2.0	51 ± 4.0	27 ± 4.0	30 ± 4.0
ALA	104 ± 3.5	28 ± 6.0	26 ± 4.0	32 ± 5.0
VAL	101 ± 3.0	62 ± 6.0	26 ± 2.0	28 ± 2.0
SER	109 ± 2.0	80 ± 5.5	25 ± 2.0	29 ± 5.0
CYS SH	110 ± 4.0	116 ± 8.0	51 ± 5.5	91 ± 6.0
MET	115 ±10.0	79 ± 7.0	33 ± 3.0	61 ± 7.5
GLU-OH	111 ± 5.0	69 ± 8.0	26 ± 2.0	32 ± 4.0
ASP-OH	101 ± 4.0	94 ± 8.0	28 ± 2.0	30 ± 3.5
$GLU-NH_2$	106 ± 2.0	72 ± 7.0	22 ± 2.0	93 ± 6.0
$ASP-NH_2$	110 ± 7.0	93 ± 6.0	24 ± 5.0	46 ± 5.0
ARG	105 ± 4.5	46 ± 6.0	25 ± 2.0	29 ± 3.0
HIS	97 ± 4.0	81 ± 8.0	24 ± 4.0	35 ± 4.5
PHE	107 ± 3.5	26 ± 5.0	27 ± 4.0	29 ± 5.0
TYR	108 ± 7.0	103 ± 8.0	33 ± 3.0	30 ± 4.0
TRY	111 ± 5.5	89 ±10.0	26 ± 3.5	30 ± 3.0
PRO	116 ±10.0	41 ± 6.0	29 ± 3.5	30 ± 2.0

*control: the sample without heavy metals and amino acid

-- differ significantly from the sample with heavy metal alone based on a Student's t test, α = 0.01. The number of replicates = 9.

Reports on the toxicity of heavy metals against phytoplankton cells and its ecological significance have frequently appeared in the literature. Studies on inhibition of the growth of various cultures of *Cyanophyta* and *Chlorophyta* caused by the addition salts of copper, cadmium and mercury were discussed in papers given by Rosko and Rachlin (1977), Reuter et al. (1979), Cain et al. (1980).

Several authors also stressed the significance of the forms of the metals in the sea environment upon their biological properties, especially the influence of organic matter (Saunders 1957; Jones 1970; Sunda and Lewis 1978; Florence 1982; Morel and Hudson 1985).

The results given in the present paper reveal that amino acids affect the process of detoxification of heavy metals in the sea environment.

Amino acids, peptides and proteins are abundant in the sea environment. They are excreted by various organisms or formed as a result of degradation of the organic matter (Fogg and Westlake 1955; Fogg 1966; Mc Knight and Morel 1979). The concentration of amino acids in the sea environment was established as 20 to 100 μg dm^3 (Andrews and Williams 1971).

Fogg and Westlake (1955) indicated the biological importance of the interaction of extracellular polypeptides with heavy metals. Formation of complexes of amino acids with ions of heavy metals in the sea environment was postulated by Jones (1970).Jackson and Morgan(1978) performed a theoretical analysis of the observations reported on the trace metal-chelator interactions and phytoplankton growth in sea-water media.

Our results revealed the ability of amino acids representing various structural groups to decrease the toxicity of ions of copper, cadmium and mercury.Of the heavy metals tested, copper was the most sensitive to the presence of particular amino acids in algae cultures. Only alanine and phenyloalanine did not cause a decrease in toxicity of this element to tested algae cultures.

The biological activity of mercury was less dramatically changed in the presence of the amino acids compared with that of copper, however, all the tested compounds caused decrease of toxicity of that metal to the plankton cells.

The effect of amino acids on the toxicity of cadmium was the weakest as compared with copper and mercury. Among the tested compounds the most active was cysteine.

These results correspond well with the known ability of heavy metal ions to form complexes with amino acids. However, analysis of the influence of various acids on particular metals did not reveal any strict correlation between the biological effect and chemical affinity.

Amino acids have a greater influence on the toxicity of copper and mercury to Anabaena cells compared with Chlorella vulgaris.

The above results indicate that the interaction of amino acids with ions of copper, cadmium and mercury affect their biological properties, which can result in ecological consequences.

Acknowledgements. The authors acknowledge the financial support of the Polish Academy of Sciences MR.I.15 project.

REFERENCES

Andrews P, Williams PJLeB (1971) Heterotrophic utilization of dissolved organic compounds in the sea.III. Measurement of the oxidation rates and concentrations of glucose and amino acids in sea water. J Mar Biol Assoc UK 51:11-125

Baccini P (1983) Vom Stoffhaushalt aquatischer Ökosysteme zur Frage nach der chemischen Bindung.Fresenius Z Anal Chem 316:575-581

Cain JR, Paschal DC, Hayden CM (1980) Toxicity and bioaccumulation of cadmium in the colonial greenalgae, Scenedesmus obliquus. Arch Environ Contam Toxicol 9:9-16

Florence TM (1982) The speciation of trace elements in waters. Talanta 29:345-364

Fogg GE (1966) The extracellular products of algae. Oceanogr Mar Biol Ann Rev 4:195-212

Fogg GE, Westlake DF (1955) The importance of extracellular products of algae in fresh water. Verh Int Ver Limnol 12:219-232

Jackson GA, Morgan JJ (1978) Trace metal-chelator interactions and phytoplankton growth in sea-water media: Theoretical analysis and comparison with reported observations. Limnol Oceanogr 23:268-282

Jones GE (1970) Metal organic complexes formed by marine bacteria. In: Hood DW (ed) Organic matter in natural waters. Inst Mar Sci Alaska Occas Pub 1:301-319

McKnight DM, Morel FM (1979) Release of weak and strong copper-complexing agents by algae. Limnol Oceanogr 24:823-837

Morel FMM, Hudson RJM (1985) The geobiological cycle of trace elements in aquatic systems: redfield revisited. In: Stunm W (ed) Chemical processes in lakes. Wiley Interscience, New York, pp.251-281

Paul HJ (1985) Uptake of organic nitrogen. In: Carpenter EJ, Capone DG (ed) Nitrogen in the marine environment, Academic Press, New York, p.275-308

Rosko JJ, Rachlin JW (1977) The effect of cadmium, copper, mercury, zinc and lead on cell division, growth and chlorophyll *a* content of the chlorophyta *Chlorella vulgaris*. Bull Torrey Bot Club 104:226-233

Rueter JG, McCarthy JJ, Carpenter EJ (1979) The toxic effect of copper on *Oscillatoria* (Trichodesmium). Limnol Oceanogr 24:558-562

Saunders GW (1957) Interrelationships of dissolved organic matter and phytoplankton. Bot Rev 23:389-410

Starr RC (1964) The culture collection of algae Indiana University. Amer J Bot 51:1013-1044

Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. 2nd ed Bull Fish Res Bd Can 167

Sunda WG, Lewis JAM (1979) Effect of complexation by natural organic ligand on the toxicity of copper to a unicellular algae, *Monochrysis lutheri*. Limnol Oceanogr 23:870-876

Received February 21, 1987; accepted July 21,1987.