EFFECT OF SOME PEPTIDES AND THEIR METAL COMPLEXES ON WHEAT GERMINATION

EFECTUL UNOR PEPTIDE ŞI COMPLECŞI METALICI AI ACESTORA ASUPRA GERMINAȚIEI GRÂULUI

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Abstract. *Recently, the antitoxic effect of glutathione, a well-known tripeptide against heavy and radioactive metal ions was proved in wheat germination experiments. This work aims at presenting new results concerning some other peptides, such as tetraglycine and histidine-containing ones. The biological activity of various metal ions as well as their complexes with peptides was followed using lots of 50 seed samples of wheat. The concentrations of treatment solutions ranged from 10^{-4} M to 10^{-2} M. After a 7 day period of germination in the presence of the investigated compounds, the wheat plantlets were cut from the seeds and their height and weight measured. Higher concentrated solutions of mercury, silver, copper and other heavy metal ions exhibited highly inhibitory activity on the wheat germination, while the less concentrated solutions, below 10^{-3} M, had a moderate effect. The peptides P9 and P10 proved to be toxic by their selves, whereas their complexes with silver and mercury reduced the metal toxicity. Toxicity risk of both newly synthesized peptides and heavy metal ions was discussed.*

Key words: metal-peptide complex, peptide toxicity, wheat, germination.

Rezumat. *Recent, efectul antitoxic al glutationului asupra ionilor metalelor grele şi radioactive a fost demonstrat în experimente de germinație a grâului. Lucrarea prezintă noi rezultate obținute cu alte peptide, cum ar fi tetraglicina şi unele cu resturi de histidină. Activitatea biologică a mai multor ioni metalici, precum şi a complecşilor acestora cu peptidele a fost studiată folosind loturi de câte 50 semințe de grâu. Concentrația soluțiilor de tratament a variat de la 10^{-4} M la 10^{-2} M. După 7 zile, plantulele au fost tăiate, determinându-se înălțimea şi greutatea. Soluțiile cu concentrații relativ mari de mercur, argint, cupru, etc. au avut o acțiune inhibitoare asupra germinației grâului, iar concentrații mai mici de 10^{-3} M au avut o influență moderată. Peptidele P9 şi P10 au prezentat toxicitate proprie, iar complecşii lor cu argint şi mercur au redus toxicitatea. Potențialul de toxicitate al peptidelor nou sintetizate, dar şi a ionilor metalelor grele a fost, de asemenea, discutat.*

Cuvinte cheie: complecşi peptido-metalici, toxicitate peptide, grâu, germinație.

INTRODUCTION

Very low concentrations of micro-elements are either toxic, or stimulants, whereas they proved to be very harmful at high concentrations due to the formation of reactive oxygen species or their interaction with cellular proteins [5]. Metal ions interact with peptides and proteins and affect the conformation and hence their activity. The investigation of the relationship between proteins and metals led to the emergence of a new science, namely *Metallomics* within the Life Science area [12,7].

Therefore, the purpose of this paper is the synthesis of peptides with high affinity toward heavy metal ions, as well as metal-peptide complexes, their characterization by mass spectrometry and circular dichroism and testing their biological activity. Since germination experiments are simple, inexpensive, rapid and spectacular, as well as easily to be performed [1-3,6,8,10], the action of the known substances or newly synthesized ones on living organisms can be tested using wheat seeds being germinated. We show here only a few results of the measurements due to reduced printing space. It was found that cysteine-containing peptides may play an important role in decreasing the heavy metal toxicity.

MATERIALS AND METHODS

Instrument. Spectrophotometric measurements were made with a spectrophotometer UV/VIS model Libbra S35 PC with 1-cm quartz cells. Mass spectra of peptides and their metal complexes were performed at the University Konstanz, Germany, with a mass spectrometer Esquire 3000Plus (Bremen, Germany). Circular dichroism studies for the interaction of these compounds and proteins and were made on a JASCO-715 spectropolarimeter, in quartz cuvettes of 0.5 mm. Studies of atomic force microscopy were performed with a microscope SPM Solver PRO-M AFM (NT-MTD Co., Zelenograd, Moscow, Russia). AFM images were obtained at a resolution of 256 × 256 pixels at the 10 μm × 10 μm and 40 μm × 40 μm.

Reagents. Tetraglycine (EGA Chemie, Steinheim, Germany), glutathione, cystine, N-acetyl cysteine (Merck), cadmium acetate, sodium and copper sulfate (Chimopar Bucharest) were used. The synthesis of the two peptides with high binding capacity of heavy metal ions, P9 and P10, with the sequences Cys-His-Gln-Tyr-His-His-Asn-Glu-Arg, and Arg-Cys-His-Gln-Tyr-His-His-Asn-Arg-Glu was described previously [4,9].

Biological material. Samples of wheat (*Triticum aestivum*), Henika variety, were purchased from Agricultural Research Station Suceava.

Treatment solutions. Various solutions with concentrations ranging from 10^{-4} M to 10^{-2} M were prepared. The solutions of glutathione had the following concentrations: $3 \cdot 10^{-3}$ M, $5 \cdot 10^{-3}$ M and 10^{-2} M, respectively.

Germination test. Four lots, each consisting of 100 seeds were taken and germinated on filter paper at 20 ºC. The first count took place at 3 days (germination energy, EG) and the second one at 7 days (germination rate, GR).

Wheat growth. The 10^{-4} M - 10^{-2} M solutions of substances being investigated from the biological point of view were prepared. Lots of 50 seeds in three replicates, were treated with 5 mL of treatment solution or distilled water (control). Treatment lasted for one hour; afterwards the seeds were arranged uniformly in Petri dishes, on

double filter paper with the treatment solutions. The seeds were regularly wetted with 5 mL of redistilled water. After 7 days, the plantlets were cut from the seeds, measured and weighed (height H, in cm and mass m, in grams). Due to the reduced amounts of available peptides, volumes of only 1 ml of 10^{-3} M for lots of 10 seeds were used.

Statistics. The results were processed using the Tukey test [11].

RESULTS AND DISCUSSIONS

All the investigated peptides showed binding properties of metal ions, leading to metal-peptide complexes highlighted by mass spectrometry. For example, P10, with 1377.5 molecular weight showed characteristic signals at m/z 276.7 (5+); 345.6 (4+), 460.2 (3+) and 689.7 (2+), while its mixture with silver nitrate in 2:1 molar ratio led to the formation of complexes with one to three silver ions bound to a single molecule of P10 (fig. 1). P10 showed the highest binding capacity toward silver ions, followed by that for nickel and copper ones. The lead and cadmium ions were weakly bound by peptides.

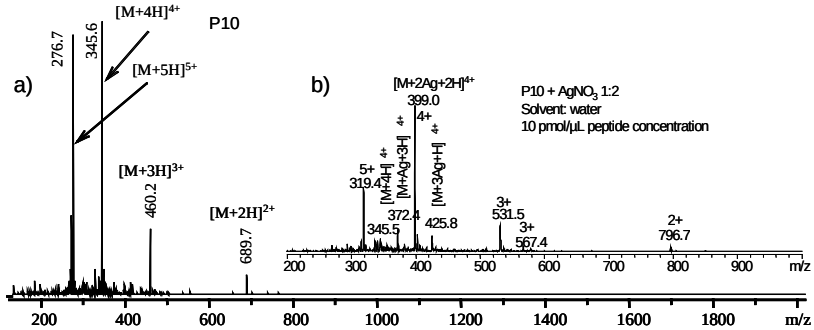

Fig. 1. Mass spectra of P10 peptide (a) and its complexes with silver ions (b).

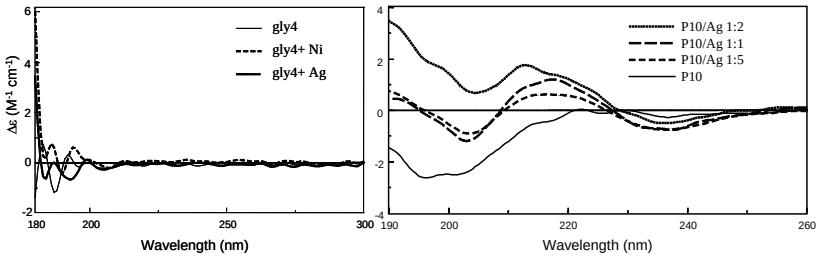

Fig. 2. CD spectra of tetraglycine, P10 peptide and their metal complexes.

Tetraglycine bound copper ions although contains only one pair of free amino and carboxylate groups. The oligomerization of these peptides under the action of metal ions, especially copper was also found. Metallic ions altered the secondary structure of peptides (fig. 2) and led to the formation of molecular aggregates as revealed by atomic force microscopy (fig. 3 and fig. 4).

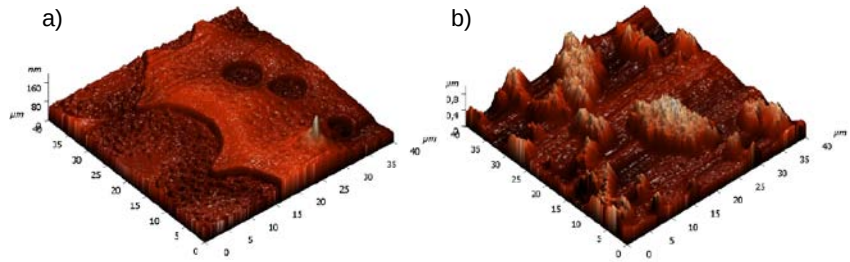

Fig. 3. AFM images of glutathione (1 mg/mL) and its complex with Cu^{2+} ions (b).

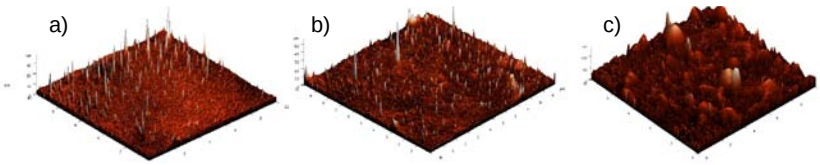

Fig. 4. AFM images of a) P10, b) P10 complex with copper ions (1:10 molar ratio) and c) Cu-P10 complex in the presence of sodium dodecylsulfate (1:10:10 molar ratio).

Atomic force microscopy (AFM) can be used to directly observe the conformational changes of proteins and peptides, showing their interaction with their environment, also including the formation of metal ion assemblies. Thus, glutathione formed a thin film (fig.3, a), whereas its complex with copper produced microscopical formations with heights of several hundred nanometer (fig. 3, b). On the contrary, P10 formed an area which became coarse after treatment with copper ions (fig. 4). On adding a surfactant substance (SDS), with the role of dispersion the peptidic molecules (fig. 4, c), the formation of large nanostructures was observed (fig. 4, c). Glutathione has a powerful protective action against heavy metal ions, reducing virtually all toxicity of copper, silver, nickel ions. Other peptides with cysteine or cystine had the same effect.

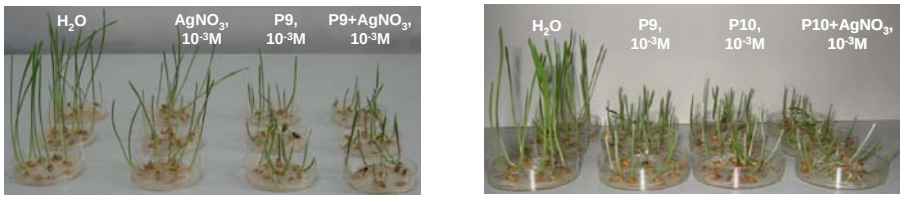

Fig. 5. Effect of silver ions, P9 peptide and P9-silver complex (left), that of P9 and P10 peptides, and P10-silver complex (right) on wheat germination

Silver ions inhibit the germination of wheat (fig. 5) and the cysteine-containing peptides P9 and P10 strongly inhibit this process. The binding of silver ions on both peptides resulted in the reduction of their toxicity, possibly due to silver-induced conformational changes. P9 and P10 decreased germination

parameters by about 50%, the length of radicles being more strongly influenced by the silver ions and these two peptides.

Table 1

The effect of Gly4 peptide and its complexes with metals in wheat germination experiments (Concentration, $4x10^{-3}$ M).

Treatment	The total height of plantlets in the lot (H, cm)	Mean height of plantlets (h, cm)	Mass of plantlets in the lot (m, g)	Average mass of plantlets (m, mg)
Control, H_2O	449.5±17.1	9.7±0.5	2.7±0.1	57.0+3.8
Gly4	354.4±14.8	8.1±0.3	2.7±0.2	48.3±2.6
Gly4+Hg^{2+}	386.7±24.2	8.1±0.2	2.4±0.3	49.0±2.9
Gly4+Cu^{2+}	101.8±17.6	2.8±0.1	0.9±0.2	23.7±2.2
Gly4+Ag^{2+}	250.0±20.4	5.5±0.2	1.6±0.1	35.7±1.1
Gly4+Pb^{2+}	419.9±27.5	8.8±0.5	2.6±0.3	54.3±3.4
D	73.5	1.5	0.5	1.1

A significant difference between tetraglycine activity and the control was found, as well as its activity and that of the complex with copper and silver ions (table 1). Interesting is that the lead ions decreased inhibitory effect of tetraglycine, while mercury ions seem to lose their toxicity in the presence of Gly4.

The toxicity of heavy metal ions is related to the formation of reactive oxygen species and the formation of complexes with biologically active molecules, such as certain enzymes, peptides, nucleic acids, etc. This paper showed that metal-protein interaction is much deeper and that conformational changes of proteins and peptides may also play an important role in the toxicity of metal-protein complexes. Binding of metal ions at different chain polypeptides is different, depending on the type of metal, concentration, pH, etc.

CONCLUSIONS

The newly synthesized peptides as well as the known ones were characterized and their biological activity was demonstrated in wheat germination experiments. MS, CD and AFM techniques revealed the binding of metal ions on peptides and proteins as well as their structural changes. The newly synthesized peptides, P9 and P10, proved to be rather toxic. However, they reduced the toxicity of heavy metal ions. Though simple in structure, tetraglycine significantly affected the wheat germination and modulate the toxicity of heavy metal ions being investigated.

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