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**ORIGINAL PAPER**

# Unexpected effect of nickel complexes of some histidine-containing peptides on *Escherichia coli*

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## Abstract

*Binding of metals to peptides and proteins, the formation of metal-peptide complexes, and the ionic interactions are all important phenomena that must carefully be considered in laboratory and field studies. We used four 19-residue peptides and their nickel complexes to prove the effect of peptide conformation on the toxicity level within some Escherichia coli experiments. The results showed that the peptide-Ni complexes had a higher toxicity than the pure peptides. The glycine- and histidine-containing peptides may carry metal ions into the cells, where they can be released by the amino acid residues with high affinity for heavy metal ions. Atomic force microscopy investigation confirmed the structural changes of peptides upon metal binding. The high flexibility of these peptides allows them a free penetration across the cell membrane into the cytoplasm.*

**Keywords:** Ala- and Gly-peptides, metal binding, nickel toxicity, *Escherichia coli*.

## Introduction

Heavy metals have comprehensively toxic effects on cells, mainly as a result of their ability to denature protein molecules [1]. The exposure of living organisms to metal pollution results in mutagenic/genotoxic processes, interference with xenobiotic metabolic pathways, and may also affect glycolysis, the Krebs cycle, oxidative phosphorylation, protein amino acid metabolism as well as carbohydrate and lipid metabolism [2,3].

The Gram-negative bacillus *Escherichia coli* (*E. coli*) became a popular model used for studying the roles of metal stress owing to its duplication time and rapid response to toxicants [2]. Metal-binding peptides on the surface of *E. coli* have been identified as specific transporters only for a few heavy metals such as nickel [3,4].

It is apparent that the metal toxicity can heavily be influenced by environmental conditions. It is also important to understand the mechanisms of microbial tolerance because of the extensive use of some metals and metal compounds as fungicides and disinfectants. Introducing the metal-binding peptides into different root-colonizing bacteria that are engineered for organic degradation would endow them with both metal resistance and metal remediation capabilities.

Regulations of cellular processes following exposure to metal ions have thoroughly been studied [4-7]. However, the molecular mechanisms and underlying responses of cells against various metal ions are not yet completely understood.

Heavy metal ions show, generally, a toxic effect, depending on the concentration, due to the formation of oxygen reactive species (ROS), free radicals, binding at the active centers of enzimes and their inactivation, but also due to conformational changes of physiologically active proteins and peptides. Therefore, this study aims to test (1) the biological activity of

heavy metals (Ni) and peptides on bacteria, and (2) the biological effects on microorganisms (*E. coli*) of $Ni^{2+}$ complexes with four histidine peptides, containing 19 amino acid residues.

## Materials and Methods

*Equipment.* An orbital stirrer GFL 3005 and laboratory glassware were used. A SPM Solver PRO-M AFM (NT-MTD Co. Zelenograd, Moscow, Russia), using a high resolution 'Golden' silicon NSG10/Au/50 cantilever with an Au conductive coating was used. The topographic images were obtained in tapping mode and were repeated on different areas of the same sample.

*Chemical reagents*. All chemicals were of analytic purity (Merck, LabChema-Chemapol, Chinoin, and Chimopar) and all solutions were prepared with redistilled water.

*Biological material*. In the present study, the effect of nickel ions on the growth inhibition of *E. coli* (further called DH5α from a T7 Express Sampler, New England, and BioLabs) was investigated. Pre-cultured *E. coli* was incubated at 37 °C with in liquid medium (0.33 ml LB 2X, 0.33 ml DH5alpha and 0.33 ml $H_2O$) for 12 hours.

The growth rate was determined by monitoring the absorbance at 580 nm by microplate reader in comparison with a blank sample. Those concentrations of metal ions were selected that gave rise approximately 50% growth inhibitions for further experiments.

## Results and Discussion

### Formation of $Ni^{2+}$/peptide complexes

To highlight the role of histidine position in the glycine containing peptide chains, a series of model peptide sequences have been synthesized [8-10].

The primary structures of the investigated peptides, $(GGGGH)_3GGGG$-OH (P19-1), $(GGGH)_3GGGGGGG$-OH (P19-2), $(AAAAH)_3AAAA$-OH (P19-3), $(AAAH)_3AAAAAAA$-OH (P19-4), their synthesis pathway and purification were elsewhere described [8]. Here, we investigated the AFM images of peptide films that differ from one to another (Figure 1). These images suggest various conformational properties of these peptides. Metal binding to various peptides and the conformational changes induced were also investigated by mass spectrometry and circular dichroism spectroscopy [11-14], confirming the role of sequence and the metal ion used.

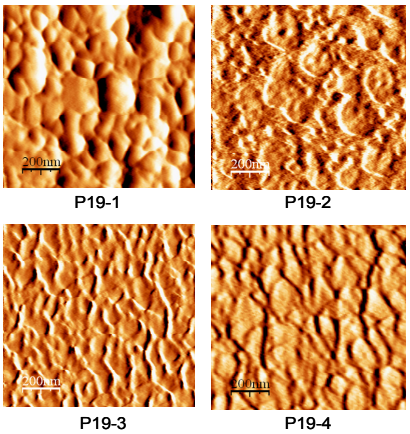


**Figure 1.** AFM micrographs of glycin- and alanin-peptides with histidine

Even if P19-1 peptide is an isomer of P19-2 peptide, the P19-1 peptide film showed globular features, whereas P19-2 peptide one displayed a vacuolar structure. Alanin-peptides

films (P19-3 and P19-4) evidenced some fibrillar structures on the surface, with length between 50 and 300 nm.

The morphology of films formed with peptides P19-1 and P19-3 complexes with $Ni^{2+}$ was significantly different from one to another and also from that of pure peptides (Figure 2).

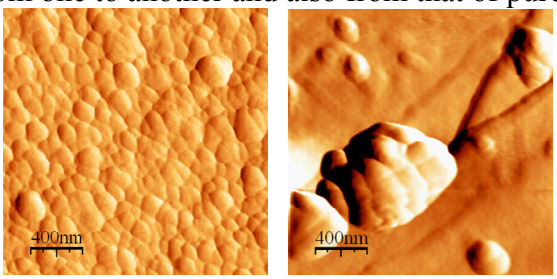


**Figure 2.** AFM micrographs of peptide P19-1:Ni and P19-3:Ni complexes

As Figure 2 shows, the surface of P19-1:Ni film presented a dense packing of globular formations of 50 – 200 nm size, while P19-3:Ni generated a film with less nanoparticles and unusual sizes and shapes (two central particles, higher than 1 μm, surrounded by globular formations with a diameter of 100 – 300 nm). At the same time, very low values for roughness and height characterized P19-3:Ni film, $R_a$ = 5.4 nm and $h_a$ = 2.6 nm, as compared to the films produced by the free peptide ($R_a$ = 121.3 nm and $h_a$ = 46.3 nm).

**Biological activity**

Heavy metals toxicity is strongly influenced by the environmental conditions, whereas the microorganisms have tolerance mechanisms or detoxification [1,7]. Many of the detoxification mechanisms are not unique to microorganisms that grow on metal-contaminated environment [15,16].

Depending on the origin of bacterial strain and incubation time, the absorbance of peptides sample, peptide-metal salts or metal complexes were found to be very different. However, the ratio of the obtained values remained approximately constant. Thus, although the average absorbance decreased, P19-2 had a tendency to inhibit bacterial growth, and P19-4 had a strong incentive effect in this experiment. The absorbance of control suspension of *E. coli* was 0.924 ± 0.004, while P19-1 and P19-2 had the following values: 0.941 ± 0.029, and 0.873 ± 0.112, respectively (Figure 3).

By adding metal ions in solution (1:1 peptide: metal molar ratio), the results were modified to increase the toxicity in comparison with pure peptides, but each peptide modulate the toxicity of different form of such complexes. The $Ni^{2+}$-peptide complexes treatment of the environment culture resulted in the decreasing of the absorbance down to 0.598 ± 0.048, and 0.564 ± 0.005, respectively, which showed a weak action of glycine-peptide in the presence of toxic $Ni^{2+}$ ions. By assigning a percentage value of 100% to the control containing *E. coli* without peptides or metal ions, the following values for the investigated samples were obtained: P19-1, 101.8% P19-2, 94.5%, P19-1 + $Ni^{2+}$, 64.7%, P19-2 + $Ni^{2+}$, 61.0%. The absorbance of the suspension of *E. coli* treated with $Ni^{2+}$ chloride (1 mM) was only 0.621 ± 0.144, representing a reduction of bacterial growth to 67.2%.

Thus, P19-1 peptide (1 mM) did not influence the biological cycle of bacteria, but showed a light tendency to increase the toxicity of $Ni^{2+}$ ions. The treatment with P19-1-$Ni^{2+}$ complexes resulted in decreasing of absorbance less significant in comparison with $Ni^{2+}$ treatment. P19-2 peptide reduced the bacterial mass to 94.5%, while the P19-2-$Ni^{2+}$ complex to 61.0%.

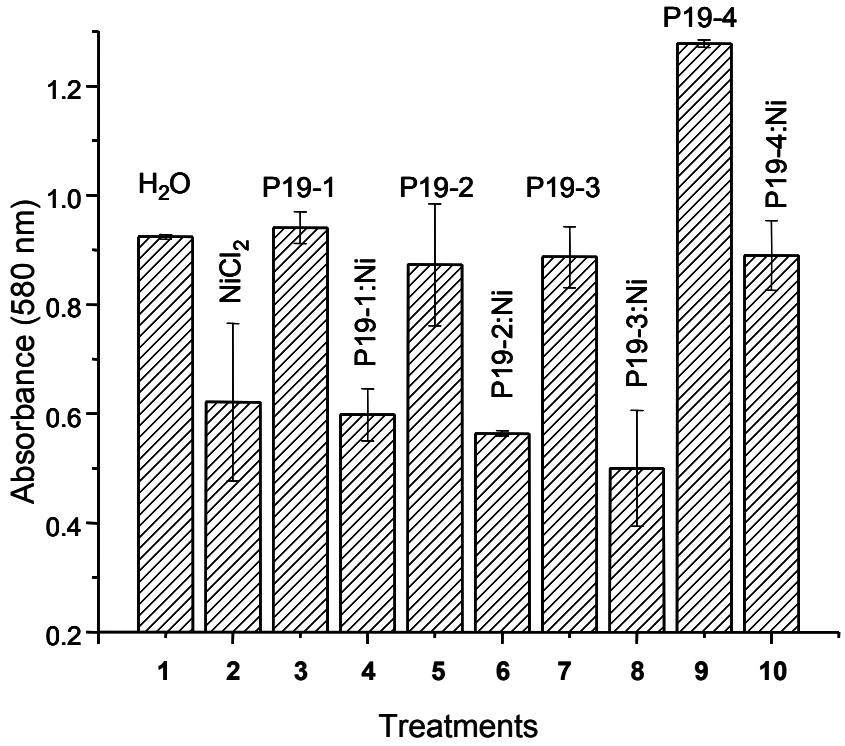


**Figure 3.** The effect of different peptide-Ni complexes on *Escherichia coli* growing

Since mass spectrometry investigations showed that $Ni^{2+}$ ions bind to the Gly-peptides, one may conclude that the complex with $Ni^{2+}$ ions did not significantly change the bacterial growth. The reduction in the absorbance of P19-2-$Ni^{2+}$ complex was assigned to biological activity of P19-2 peptide. Thus, by subtracting the contribution of peptide we obtained a 64.6% contribution for P19-2-$Ni^{2+}$ complex, so close to the value obtained for P19-1-$Ni^{2+}$ complex, and 64.7% and that of the ions $Ni^{2+}$ (67.2%). Although differences between the values obtained are less significant, the $Ni^{2+}$ complexes of both peptides showed a clear trend of increasing the toxicity of $Ni^{2+}$ ion by Gly-peptides. We explain these observations in that glycine- and histidine-containing peptides may carry metal ions into the cells, where they can be released by the amino acid residues with high affinity for heavy metal ions. High flexibility of these peptides allows them free penetration through the membrane (where α-helix conformations are favored) in the cytoplasm (where they form random coil structures). On the other hand, it was highlighted the high toxicity of $Ni^{2+}$ ions at 10 mM concentrations, but moderate, at 1 mM concentrations, which promotes the study of the toxicity of these ions.

## Conclusion

The phenomenon of microbial interaction with heavy metals in the presence of structurally different peptides allows no simple explanation. The reduction of metal toxicity may occur by the formation of complexes between metal ions and the peptides. Extracellular complexation followed by the peptide complex transport across the cell membrane is probably an example of gratuitous resistance.

The results showed that the peptide-Ni complexes had a higher toxicity than the pure peptides. The glycine- and histidine-containing peptides may carry metal ions into the cells, where they can be released by the amino acid residues with high affinity for heavy metal ions. High flexibility of these peptides allows them free penetration through the membrane in the cytoplasm.

Finally, consideration of the microbe-metal-peptide interactions is clearly of a more general interest to ecologists as it draws attention to the sorts of interactions that may occur in natural habitats between microbes and other nutrients.

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