Interaction of Cu(II) ions with FadA and P1 proteins from Fusobacterium nucleatum

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Many studies indicate a link between colorectal cancer and *Fusobacterium nucleatum*. The exact mechanism leading to the development of this disease is constantly being researched by scientists from various groups. One of the hypotheses assumes the ability of the *Fusobacterium nucleatum* in the presence of transition metal ions e.g. copper(II) and endogenous oxidants to generate reactive oxygen species (ROS). It is known that the accumulation of ROS in the body can result in inflammation states, during which elevated levels of copper(II) ions is also observed. This strong correlation between ROS and copper(II) ions as well as *Fusobacterium nucleatum* and colorectal cancer has become the starting point for research conducted in this dissertation.

As a part of the presented work, the coordination properties of protein fragments (FadA and P1) from *Fusobacterium nucleatum* with copper(II) ions were characterized. To achieve this aim, a various spectroscopic methods such as: UV-Vis, CD, EPR and NMR were used.

In addition, the theoretical calculations confirmed and supported the proposed coordination models for complexes with fragments of FadA protein. An equally important was to determine the stoichiometry and stability constants of the copper(II) complexes in aqueous solution.

In order to implement this assumption, potentiometric titration was performed. In addition, mass spectrometry (MS) was selected to confirm the stoichiometry of the formed complexes. The logK* values were also calculated, which allowed for comparison of the interaction force between individual complexes with the same coordination mode.

It was also important to examine whether selected ligands, as well as their complexes with copper(II) ions are capable of generating reactive oxygen species (ROS). Spectroscopic methods (UV-Vis and luminescence) with appropriate selective compounds were used for ROS detection. In addition, for selected compounds, to identification of radicals and to the assessment of plasmid DNA damage gel electrophoresis was used.

The final stage of the study was to determine the ability to generate ROS by the entire bacterial cell (*Fusobacterium nucleatum*), and also checking if free radicals are formed outside or inside the bacterial cell.

The conducted tests showed that the tested ligands are capable of forming with copper(II) ions both mononuclear (with a molar ratio of metal to ligand 1:1 and 1:2) as well as polynuclear complexes in an aqueous solution. The carboxyl group from the glutamic acid side chain was not involved in copper(II) ion binding. However, it was found that the position of the histidyl residue affects the direction of the metal ion binding process. In addition, all ligands containing two histidyl residues in the amino acid sequence form complexes with high prevalence at pH 5.5, characterized by similar stability. The exception is the complex Cu(II)-**Ac-FGEH^{4}EH^{6}GRDYKNGWE-NH$_2$** for which the logK* value was about an order of magnitude higher compared to all other complexes. Most likely, the stabilization of this complex is affected by the presence of a tryptophan residue and the resulting cation-π interaction as well as interactions between the rings of the tryptophan and histidine residue.

It has been observed that the efficiency of hydroxyl radical generation is related to the number of histidyl residues in the amino acid sequences. The binding of copper(II) ion by ligands containing one histidyl residue stimulates to the formation of larger amounts of this radical as compared to free ligands. In turn, in the case of complexes with ligands containing two or three histidyl residues in amino acid sequences, this reaction proceeds with lower efficiency compared to free ligands.

Preliminary tests carried out on the entire bacterial cell have proved that in the presence of copper(II) ions and hydrogen peroxide, this bacterium is capable of generating free radicals. In addition, it was found that the radicals are formed outside the bacterial cell, which may partially confirm the thesis about the involvement of surface proteins in the process of radical formation.

This research is a great contribution and enrichment of knowledge in the field of bioinorganic chemistry. In addition, the interdisciplinary nature of the research allows the association of *Fusobacterium nucleatum* with colorectal cancer and proposing a new mechanism of colon cancer.