The physicochemical characterization of redox-active copper-peptide systems. The implications for the free radical mechanism of carcinogenesis

mgr Paulina Katarzyna Walencik
Supervisor: dr hab. Alina Bieńko, Prof. UWr
Co-Supervisor: dr Maciej Witwicki

The main aim of the research summarized in this dissertation was to correlate thermodynamic stability and structure of Cu^{II} and Cu^{I} complexes with their redox activity in biological systems. The investigations involved nine various peptide ligands, designed to structurally mimic the active fragments of two bacterial proteins FomA and Aim1. Both proteins are expressed in anaerobic bacteria *Fusobacterium nucleatum* and their interaction with copper ions have been reported as the potential risk of the surge in the intracellular oxygen metabolism and oxidative stress. The adopted strategy allowed to obtain and characterize a number of compounds that imitated the metalloproteins redox-active centres. The employed peptide ligands represented histydyl and methionyl structural motifs -$H(X)_nH$- and -$H(X)_nM$- (where H, M, and X are histidine, methionine and any amino acid residue, respectively), which are highly affinitive towards copper ions.

In order to accomplish the main objective of this work, the structural and electrochemical characterization of peptide complexes with Cu^{II} and Cu^{I} ions were carried out using a vast array of experimental methods. The Cu^{I} coordination to peptide ligands and the stoichiometry of the formed complexes were determined by the use of nuclear magnetic resonance (NMR) spectroscopy and more precisely with the ^{1}H NMR and ^{1}H-^{1}H TOCSY experiments. For the estimation of the thermodynamic stability, the values of conditional dissociation constants were obtained from the competitive titration with two different chelating agents.

The equilibrium binding of Cu^{II} ions to peptides were characterized by overall and stepwise stability constants derived from potentiometric titrations experiments. The binding mode of Cu^{II} in the formed complexes was studied in depth with the use of complementary spectroscopic techniques: UV-Vis absorption, circular dichroism (CD) and electron paramagnetic resonance (EPR) spectroscopy.

The electrochemical activity of Cu^{II}- and Cu^{I}-peptide systems was analyzed by means of cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. In addition, for some of the complexes, the reactivity experiments towards dioxygen O_2 and ascorbic acid HAsc were performed.

The correlation of electrochemical, spectroscopic and potentiometric data allowed to identify how the structure of the complexes and the presence of individual donor moieties can affect the redox activity of metallic centre and its reduction potential. For the majority of studied systems,

a quasi-reversible redox conversion Cu^{II}-peptide + e^- ⇄ Cu^{I}-peptide accompanied by the formation of the electrochemical intermediate species was revealed.

It was proven that the binding of Cu^{II} and Cu^{I} ions to the *bis*-His moiety allows for the unhindered oscillation of metallic centre between the +I and +II oxidation states. The equilibrium process in which water molecules are exchanged by amide groups within Cu^{II} aqua ion coordination sphere caused the decrease in reduction potential and slowed down the electron transfer. It was also shown that the stability of +II oxidation state underwent an increase with the number of bounded amide nitrogens.

In spite of the Cu^{I} preferences towards the sulphur moieties, the mixed structural motifs -$H(X)_nM$- were found to be inferior to -$H(X)_nH$- in binding Cu^{I}. Moreover, the simultaneous coordination of Cu^{I} to imidazole and thioether moieties, observed for the -$H(X)_nM$- ligands was found to inhibit the redox conversion.

For the examination of peptide complexes reactivity towards O_2 and ascorbic acid (HAsc), a new concept of the radical generation in biological systems emerged. It was demonstrated the role of HAsc cannot be limited only to the initiation of the metallic centre redox cycle. Instead, this compound, together with O_2, might generate reactive radicals able to cause oxidative damages. In addition, the His residue was identified as the moiety most susceptible to oxidative damages.

These studies on both Cu^{II} and Cu^{I} systems provided a valuable contribution to the general knowledge in the field of coordination chemistry, bioinorganic chemistry, and electrochemistry of peptide complexes. The unique character of this dissertation is also emphasized by the fact that the presented research was mainly focused on the redox cycle of copper ions, that can be considered as the primary cause of radicals generation and prooxidative action in various biological systems. Based on the obtained data the mechanism of Cu^{II}-peptide+ e^- ⇄ Cu^{I}-peptide conversion was described for all the complexes formed at pH= 7.50, which represents the physiological conditions. Furthermore, the studies allowed to identify which of the structural factors have the strongest impact on the redox and prooxidative activity.