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DI PARMA

DEPARTMENT OF CHEMISTRY,
LIFE SCIENCES AND
ENVIRONMENTAL SUSTAINABILITY

Parma, 19/11/2020

To the Scientific Council of the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences of Poland

Dear Sirs,

I am delighted to provide here with my review and comments on the Doctoral thesis of Mrs Karolina Bossak-Ahmad, submitted to the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences and a partial fulfillment for obtaining the doctoral degree in exact and natural sciences. The graduate research has been performed under the supervision of prof. dr hab. Wojciech Bal, and dr Tomasz Frączyk as the auxiliary supervisor.

The thesis is entitled “Ternary complex formation of His2 peptides in the presence of Cu(II) ions. A quantitative Analysis of selected human and yeast peptides”. The thesis is composed by the sections: summary, introduction, aim of the thesis, list of five peer reviewed papers included in the thesis, five chapters which summarize the research presented in the selected papers, discussion, conclusions, and reprints of the five selected papers included in the thesis. Overall, the thesis is very well organized, scholarly written, and very well presented in terms of stylistic devices.

The summary presents clearly in one page the big picture related to the scientific context of Mrs Bossak-Ahmad’s research. Also, the summary anticipates the main results of these studies, making upfront clear to the reader the impact of these findings in the area of bioinorganic chemistry.

The introduction contains several subsections. In the first main subsection, elements of the biological chemistry of copper are presented. The subsection starts describing copper proteins, then the focus is moved on homeostasis and shuttling of copper outside the cells. Attention is paid on the different molecules that transport and store copper, especially proteins and peptides bearing

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histidine residues. As for the latter types of copper binding ligands, oligopeptides which have the His residue in either position 2 or 3 are treated in great detail. Since the studies of Mrs Bossak-Ahmad are focused on copper(II), the biochemistry of the metal in this oxidation state is principally treated. In-cell transport of Cu(I) is also briefly described.

In the following subsections of the introduction the candidate extends her description of some aspects of the bioinorganic chemistry of copper by presenting three oligopeptides which correspond to the main copper chelators whose interest has triggered her investigations (namely the α-factor, the GHK human wound factor and the AAH short peptide). Coligands of copper complexes that operate metal binding in ternary complexes with peptides are also described (urocanic acid, Human serum albumin, and hCTR1).

Chapter 4 describes the studies aimed at clarifying the structural nature of the adduct between Cu(II) and the N-term binding site of the pheromone α-factor of yeast, and its stability. The studies were performed by potentiometric titrations, CD and UV-Vis titrations, and EPR. On one hand, the stability of the adduct of Cu(II) at the WGW site was determined making clear that this site may be metalated at the pH of yeast cultures. On the other hand, the studies presented in this chapter show that co-ligands such as imidazole or glycine act as enhancers in terms of stability of the adduct between Cu(II) and the heptapeptide model of α factor.

Chapter 5 is central in the research presented in this thesis. The most important aspect of the studies reported in chapter 5 is the use of tripeptides as models of natural Cu(II)-binding peptides. The tripeptides and the techniques used to study their Cu(II) binding capabilities were chosen very wisely. Also, the presentation of the results is very clear and the discussion takes the reader by the hand toward an uncontroversial and solid interpretation. Perhaps, the most important result relates with the stability and kinetics of Cu(II) exchange of the copper adduct with AHH, which presents features typical of peptides with His in position 3 (ATCUN) and copper exchange rates typical of peptides which bear His in position 2.

The studies presented in chapter 6 are a follow-up of those presented in chapter 5, although possibly more interesting in the perspective to clarify the role that peptides bearing a His residue in

either position 2 or 3, or both, may have in extracellular Cu(II) transport. Here, the presence of a third imidazole ligand (being it simple imidazole or a His-containing protein such as HSA) is demonstrated to prompt the coordination Cu(II)/AHH to change from a 4N to 3N+1N mode. Also, the latter coordination environment enhances the stability of Cu(II) complexation, taking it to the same stability of the Cu(II) adduct with HSA. The implications that these findings have in the elucidation of extracellular copper trafficking have been clearly pinpointed later in the discussion section.

Chapter 7 presents a reinvestigation of the affinity of human serum albumin for Cu(II). That is a fundamental preliminary information that has to be available for a correct interpretation of the results of studies that aim at clarifying whether or not the N-term site does compete with other His containing peptides for copper binding, or rather they together form ternary species.

Chapter 8 investigates cis-urocanic acid as a potential physiological copper chelator that may form ternary complexes with His-containing peptides. Also in this system, it has been found that the formation of the ternary complex between cis-urocanic acid, GHK and copper(II) results into an enhanced affinity of GHK for the metal ion.

All the interpretations of the results are summarized and presented in an organic manner in the discussion. These results are scholarly presented in this section and, if judged from a wider perspective, they demonstrate that there are a lot of still undisclosed features in the copper coordination chemistry of His- containing peptides which await to be clarified. That is of primary importance to better understand the physiological role of many of these ligands. Indeed, the results and their interpretation presented here open a window on the comprehension of these features.

Overall, Mrs Karolina Bossak-Ahmad has presented a doctoral thesis which deserves an undoubtful scientific attention, and that I evaluate as excellent. For all the reasons described in this review I feel this dissertation deserves honorable mention, for the solid contribution provided to

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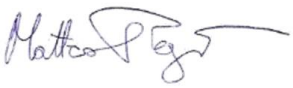
the comprehension of the thermodynamics and the structure in solution of copper(II) complexes with oligopeptides containing His at the N-term, and their models.

Should you need any additional information, please do not hesitate to contact me

Best regards

Prof. Matteo Tegoni



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