Summary

Nucleobindin-2 (Nucb2) is a multidomain protein, which can be proteolytically cleaved to three peptides: nesfatin-1, -2 and -3. The tertiary structure of the protein includes two EF hand domains, which are responsible for binding Ca^{2+} and Mg^{2+} ions, located in nesfatin-3 sequence. In addition, in nesfatin-1 sequence, we can distinguish a putative Zn^{2+} ion binding motif. Interestingly, only the role of nesfatin- 1 has been identified so far. Nesfatin-1 administered intraventricularly to the rodents inhibits its appetite, suggesting a possible role of the protein in the treatment of obesity. Currently, both the characteristics and the role of the other two nesfatines are unknown. Nucb2 is highly expressed in both the nervous system and peripheral tissues. Interestingly, it has been shown that Nucb2 is involved in many physiological processes, e.g. the regulation of insulin secretion or the control of reproductive process. The structure of Nucb2 is most likely responsible for the multifunctionality of the protein.

The aim of the study was the molecular characterization of Nucb2 and nesfatin-3 from *Gallus gallus*. As the Nucb2 homologues share 85% amino acid sequence similarity, the results of studies conducted on one homologue can be applied to the other homologues. The conducted research was interdisciplinary. The techniques from biochemistry, biophysics and genetic engineering were used during the work. The research was conducted at the Wroclaw University of Science and Technology and Adam Mickiewicz University in Poznań. The research started with the development of an effective method for expressing and purifying both proteins, which allowed for further *in vitro* analyses. *In silico* analyses of Nucb2 sequence showed that Nucb2 has a *mosaic-like* structure consisting of intertwining globular and disordered fragments. The disordered fragments are especially present in nesfatin-3. Circular dichroism (CD), fluorescence and sedimentation velocity analytical ultracentrifugation (SV-AUC) analyses of Nucb2 have clearly shown that Nucb2 and nesfatin-3 belong to the family of partially disordered proteins. In addition, the results of the hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) analysis showed that the Nucb2 protein can be divided into two parts: the amino terminal fragment (nesfatin-1 and -2), with a structure consisting of intertwined ordered and disordered fragments and a completely disordered carboxyl terminal fragment (nesfatin-3).

Metal ions can modulate the structure of proteins, adapting it to perform functions in various physiological processes. Subsequent analyses were aimed at examining the effect of natural protein ligands, Ca^{2+} , Zn^{2+} and Mg^{2+} ions, on both analyzed proteins. Analyzes of CD,

limited proteolysis and SV-AUC showed that both proteins are compacted in the presence of Ca^{2+} ions. HDX-MS results showed that in the presence of Ca^{2+} ions, two loops of EF hand domains undergo compaction. Mg^{2+} ions affect the oligomeric state of both proteins leading to its dimerization. Interestingly, the thermodynamic analysis of the interactions of both proteins with Mg^{2+} ions using isothermal titration calorimetry (ITC) determined that Nucb2 binds one Mg^{2+} ion. On the other hand, isolated nesfatin-3 is characterized by the presence of two identical Mg^{2+} binding sites. The presented results suggest that the presence of nesfatin-1 and -2 may structurally block the binding of the second Mg^{2+} ion to the Nucb2. The greatest difference in the properties of both tested proteins was observed in the presence of Zn^{2+} ions. HDX-MS results showed that the presence of Zn^{2+} ions affects the nesfatin-1 and nesfatin-2, increasing solvent accessibility of peptides located in the Zn^{2+} ion binding motif. In addition, SV-AUC and transmission electron microscopy (TEM) results showed that the presence of an equal and higher concentration of Zn^{2+} ions than 0,3 mM induces high-order oligomers formation and precipitation of Nucb2. On the other hand, nesfatin- 3 is less sensitive to high concentrations of Zn^{2+} ions. Protein precipitation in the presence of higher Zn^{2+} ions concentration was not observed. Moreover, ITC studies have shown that nesfatin-3 binds three Zn^{2+} ions, with the first ion bound with nanomolar dissociation constant. This is a surprising result due to the lack of a known Zn^{2+} ion binding motif in nesfatin-3 sequence. The obtained results suggest the participation of the amino terminal fragment of Nucb2 in the Zn^{2+}-dependent oligomers formation and precipitation of Nucb2. Most likely, both the disordered nature of both proteins and the structural change occurring in the presence of various metal ions determine their participation in various biological processes. Interestingly, isolated nesfatin-3 is characterized by new molecular properties, which has not been shown when the protein was part of Nucb2. The molecular characteristics of both proteins presented in the thesis allow for setting new paths of research focused on understanding the functions of proteins and regulating their activity.