Streszczenie

Analysis of the Relationship Between the Structure and Function of Products of Proteolytic Processing of Nucleobindin-2

Nesfatin-1 (N1), -2 (N2), and -1/2 (N1/2) are products of the proteolytical processing of the N-terminus of Nucleobindin-2 by prohormone convertases (PCs). Nucb2 and/or N1 (Nucb2/N1) are proteins in vivo engaged in the myriad of functions, which include: regulation of energy homeostasis, responses to stress, carcinogenesis, pathogenesis of psycho-neuronal disorders, and blood pressure regulation. On the other hand, there are a few reports about the relationship between the structure and function of Nucb2/N1 as well as about the purpose of the remaining nesfatins. Hence, those studies deserve special attention considering the multi-functional mode of action of Nucb2/nesfatins, their ubiquitous expression throughout the body, and high potential of their medical applications. Additionally, probing the structural differences between the homologs of nesfatins and in their interactions with ligands might shed a new light on the species-specific functions of the peptides. In total, five nesfatin homologs from two species were purified here including human N1, N2, N1/2, and chicken N1, and N1/2. Next, comprehensive structural analysis of nesfatins as well as effects of their interactions with divalent metal cations were undertaken during the project. It is worth noting that so far this is the first and only analysis of this type available in the literature.

The results showed conservation of the highly disordered structure between the human and chicken homologs of apo-N1, both displaying elongated and ellipsoidal shape. In turn, the structure of the homologs of apo-N1/2 was revealed as mosaic with intertwined ordered and disordered regions and extended shape. Structural role of the N2 fragment was suggested based on the obtained data. Moreover, N2 fragment in the context of the N1 fragment (N1/2) seemed to induce different molecular properties of the latter, which in turn where not a simple sum of the effects observed for the isolated fragments. Thus, it is possible that the proteolytical processing of Nucb2/N1/2 can act as an activation mechanism that through the release of the disordered character of N1 enables its interaction with multiple proteins.

Zn(II) had a strong effect both on the observed properties and conformation of nesfatins. Human and chicken homologs of holo-N1 were characterized by a disorder-to-order transition induced by Zn(II), which was associated with a strong decrease in the hydrodynamic volume of the peptides and a strong protection of their backbone against H/D exchange in the M30 region. Moreover, dimerization of the peptides under Zn(II) treatment was also observed for holo-N1 homologs. In contrast to the human homolog, chicken holo-N1 was also more prone to aggregation. Thus, structurization and change in the oligomeric state of holo-N1 might be universal and could facilitate interactions with a different set of the binding partners in vivo. Furthermore, concealment of the anorexigenic core of neuropeptides might affect their function. Interestingly, binding of Zn(II) by human and chicken holo-N1 homologs was also associated with a formation of the amyloid motif, which might indicate their involvement in the neurodegeneration processes. In turn, Zn(II)-binding by hN2 as well as by human and chicken homologs of N1/2 resulted in a strong destabilization of the proteins. Chicken N1/2 was showed to be more susceptible to oligomerization and aggregation under Zn(II) treatment. Additionally, the interaction mode of chicken N1/2 with Zn(II) and its effect seemed to be different than for the human homolog. Interestingly, Zn(II) induced strong exposition of the backbone of human and chicken N1/2 homologs. Particularly, the backbone exposition was the most visible in the M30 and PCs-recognition regions of N1/2 homologs. Thus, it is possible that the proteolytical processing of the N1 precursors, their biological activity, and localization is Zn(II)-dependent.

The in-depth structural analysis of human and chicken homologs of nesfatins described here shed new light on the relationship between their structure and function. Furthermore, the presented data also created a basis for the subsequent studies of this subject.