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Bioinformatic approaches for study of the thiamine-binding proteins

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Background. The results of numerous current studies indicate that vitamin B_1 (thiamine, Th) is a satellite of acetylcholine (ACh) in nerve cells and neuromuscular junctions. However, the mechanisms underlying the relationship between the functions of these compounds have not been fully elucidated. One of the modern approaches to resolve successfully this issue is the study of target proteins of both ligands using the combination of complex traditional biochemical methods and technologies of structural biology. **Methods.** The proteins isolated from rat brain by affinity chromatography on a Th ligand sorbent were identified using molecular biology, mass spectrometry (MALDI-TOF, Mascot), a variety of bioinformatics tools: AutoDock Vina (version 4.2), PyMOL (version 1.1), SBI resource, PDB, Clustal, AACompIdent. **Results.** The previous studies of thiamine-binding protein (ThBP) have shown its ability to bind Th and hydrolyze Th phosphates, most actively Th triphosphate (ThTP). Now the ThBP was identified as a complex of two proteins: Agrin-LRP4 — components of the nAChR cluster. Identification of ThBP by amino acid (AA) composition gave the same results. Th-binding pockets were predicted in the Agrin-LRP4 complex by docking (confirmed by the corresponding peaks on the mass spectras). The AA residues of Agrin and LRP4 polypeptide chains responsible for Th binding were predicted. The Th, its phosphates, and benfothiamine were superior to adenosine phosphates and ACh in affinity for the Agrin-LRP4 complex, confirming the previous experimental results. Analysis of Th binding sites with the Agrin-LRP4 complex revealed a high similarity with the binding sites of ThDP-dependent proteins. The conformation of the Th when bound to the studied proteins was the same. Docking of the Agrin-LRP4 complex to Th phosphates allowed explain the reasons for their lower affinity compared to Th. The alignments of the LRP4 with a known cytosolic ThTPase was used to test the hypothesis: LRP4 may be a membrane ThTPase. The result showed a pocket in LRP4 similar to the pocket for ThTP in cytosolic ThTPase. We were able to identify the classical ThDP binding site in the AA sequence of LRP4. The ability of the MAGUK p55 to bind Th was also revealed. It may be of interest in the fight against SARS-CoV-2 and other viruses which can lead to neurodegeneration. **Conclusions.** These results broaden the understanding of the molecular mechanisms of vitamin B_1 functioning. It's important for the development of treatment strategies for neurodegenerative and other diseases. Bioinformatics tools made it possible to predict the significance of the interaction of Th with proteins of the Agrin-LRP4 complex (ThBP): 1) stabilization of the Agrin-LRP4 complex; 2) implementation of the functions of the mobile Th pool [1] by the nAChR cluster; 3) hydrolysis of Th phosphates by proteins of the nAChR cluster.

Keywords: vitamin B_1, thiamine-binding proteins, nAChR cluster, neurodegenerative diseases.

REFERENCES

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