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The influence of zinc ions on the spatial organization and activation of TIR-domains of the Toll-like receptors

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Motivation and Aim: Proteins of the toll-like receptor (TLR) family are the key components of the human innate immune system [1, 2]. There are 10 different TLRs (TLR1-10) present in the human body. Activation of these receptors initiates the launch of the innate immune response and the inflammatory process. It has been established that TLRs are involved to varying degrees in the development of infectious, autoimmune and neurodegenerative diseases [3].

TLRs have a characteristic type 1 transmembrane protein structure, including large extracellular and intracellular domains, as well as a single transmembrane alpha-helical region. These receptors function in the form of homo- and heterodimers. Despite numerous studies of TLRs, many questions remain unresolved regarding the structural organization of the intracellular domains of TLRs and the mechanism of their activation during signal transduction to activate the immune response.

Methods and Algorithms: To select the conditions for the production of all TLR1-10 proteins, the experimental design method was used, described in detail in the articles [4, 5]. The development of cleaning protocols was based on previously obtained results. For TLR1 TIR (Toll/Il-1 Receptor)-domain, protein production and purification is described in detail in the article [6]. The selected parameters were used for subsequent two-step purification using IMAC and SEC followed by sample concentration. Validation of the secondary structure of proteins was carried out using CD spectrometry and NMR.

Triple resonance spectra were used to determine the main and side chains of TLR1 TIR by NMR. The data obtained were used to calculate the spatial structure automatically with manual correction in areas with increased mobility of the protein chain. Data from

NMR relaxation experiments were used to study the dynamic properties of the protein. More details about the research methodology can be found in the article [7].
Experiments examining the metal binding activity of TLR TIR domains were performed using NMR and analyzed in Mathematica [8]. The research methodology using the example of TLR1 TIR is published in the article [7]. Functional tests of the studied receptors are also described in the article [7].
Results: The conditions for the production and purification of TLR TIR-domains were selected. Based on the data obtained, candidates for structural studies were selected. Structure and dynamics data in solution were obtained for TLR1 TIR. Studies of the metal-binding activity of TLR TIRs have revealed the formation of protein-zinc complexes with nanomolar binding constants. In experiments with point mutants of TIR domains of TLRs, amino acid residues necessary for binding to zinc ions were identified in the sequences of the proteins under study. The results of functional analysis of TLR are demonstrated, according to which the role of zinc ion binding in TLR activation is shown, and the functional significance of previously identified key amino acid residues is presented. The results obtained for TLR1 TIR were published in [7].
Conclusion: Differences have been established between the spatial structures of TLR1 TIR obtained in solution and crystal. The zinc binding activity of intracellular TLR domains was revealed and the functional significance of zinc on the activation of the TLR1/2 receptor was demonstrated. The key role of a number of amino acid residues of TLR TIRs in receptor activation has been revealed. The data obtained significantly add to the knowledge of the functioning of TLR receptors, and in particular, suggest that the zinc-binding ability of the TLR1 TIR-domain is critical for receptor activation.
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