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Changes in the structure and dynamics of the intracellular domain of Toll-like receptors 1 under the action of Zn^{2+}

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Motivation and Aim: Toll-like receptors (TLRs) are key players in the innate immune response and may serve as targets against inflammatory, neurodegenerative, and autoimmune diseases [1]. The mammalian TLR family includes 13 members, 10 of which are found in humans. These receptors can recognize various molecular patterns associated with bacterial and viral pathogens [2]. The molecular mechanism of receptor activation is considered to be known: ligand binding causes the receptor dimerization, which activates the interaction of intracellular domains (TIR domains) with adapter proteins. In particular, TLR1 forms a complex with TLR2 when bound to lipopeptides or lipoteichoic acid.

Despite the large amount of data on TLR, many questions remain unresolved when considering the activation of the receptor from within the cell. Primarily, it is not known why the TIR domain interacts with adapter proteins exclusively in the dimeric state and how the interaction occurs, why TIR domains do not homodimerize *in vitro* (with the exception of TLR10). In present work the results of a study of the cytoplasmic domain of TLR1 in solution and crystalline form, and the effect of metal ions on the functioning of the receptor.

Methods and Algorithms: Methods for obtaining and purifying the protein are described in detail in the article [3]. Selected parameters were used for optimal protein production and subsequent two-stage purification using MCAC followed by sample concentration. Triple resonance spectra were used to assign the protein backbone and side chains by NMR. The obtained data were used to calculate the spatial structure in automatic mode with manual correction in regions with increased mobility of the protein chain. Metal binding experiments were analyzed by NMR spectroscopy. More detailed methods for studying the structure of TLR and the metal-binding activity of the protein are provided in the article [4].

Results: Data on the structure and dynamics of TIR TLR1 in solution were obtained. Studies of the TIR TLR1 metal-binding capacity revealed the formation of a TIR TLR1/Zn2+ complex with the nanomolar dissociation constant mediated by interactions with residues C667 and C686. Possible structures of the complex were predicted *in silico* (Fig. 1). Functional assays for the heterodimeric TLR1/2 receptor showed an effect of zinc concentration on TLR1 activity. The introduction of the C667A mutation leads to a decrease in receptor activity [4].

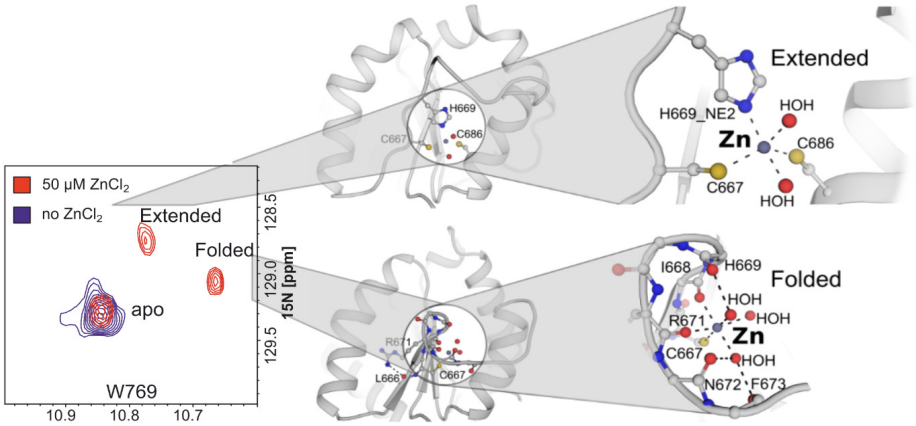

Fig. 1. Overlay of fragments of 1H, 15N-HSQC spectra corresponding to the region with signal W769 indole NH. Each zinc-bound state has its own mode of coordination (obtained *in silico*)

Conclusion: It was found that the spatial structure of TIR TLR1 in solution differs from the crystal structure in the BB-loop region due to non-native disulfide bonds that occur during crystallization. The resulting protein structure suggested the presence of a zinc-binding site, that was experimentally confirmed. The results obtained in this work suggest that the zinc-binding ability of the TLR1-TIR domain is crucial for receptor activation.

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