Cryo-EM Structure of Pre-liganded NAIP5 reveals activation mechanism of NAIP/NLRC4 Inflammasome

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Inflammasome is a cytosolic multiprotein complex formed in response to abnormal or pathogenic stimuli, and it initiates immune response to establish the innate immunity in mammals. Among the known inflammasomes, NAIP/NLRC4 inflammasome is specifically responsible for conferring immunity against various pathogenic bacteria. NAIP (nucleotide-binding domain, leucine rich repeat domain containing protein family (NLR family) apoptosis inhibitory protein) acts as a pathogen recognition receptor whereas NLRC4 (NLR family CARD containing protein 4) serves as a downstream molecule to undergo oligomerization to propagate and amplify the signal initiated by the activated NAIP. NAIP recognizes various bacterial ligands present in the cytosol of phagocytes, and the ligand bound NAIP further binds to NLRC4 and activates it by relieving its auto-inhibition. The activation of one NLRC4 molecule leads to exposure of its buried nucleation surface, which then serves as a binding site for another inactive NLRC4, to get activated. As a result of this process, a wheel-like NAIP/NLRC4 inflammasome composed of only one ligand bound NAIP and numerous NLRC4 molecules is formed. The CARD domain of NLRC4 in inflammasome interacts with CARD domain of pro-caspase-1 through CARD-CARD interactions and mediates the activation of pro-caspase-1. Caspase-1 activates the pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18, which eventually induce the immune response that leads to pyroptosis (a form of programmed cell death) of the infected cell. Although, the information on overall inflammasome formation and its downstream signaling is known, no knowledge on how NAIP exits in inactive state in the absence of pathogenic ligand is available. The information on whether NAIP adopts the same auto-inhibited state as similar to NLRC4 or not, and how it is activated upon binding to ligand would provide valuable insights into the understanding of the activation of inflammasome formation and that further help design and develop the therapeutics for various inflammasome associated auto-immune diseases. Therefore, in this study, we determined the structure of pre-liganded mouse NAIP5 at the resolution of 3.3 Å by cryo-electron microscopy and found that it adopts an unprecedented wide-open conformation, with the nucleating surface fully exposed and accessible to recruit inactive NLRC4. Upon comparing it with the available liganded NAIP5 structures, we further found that the ligand binding could induces ~20° rotation of the winged helix domain (WHD) of NAIP5 and that triggers the conformational change of NLRC4 to propagate the inflammasome signal. Moreover, in our biochemical assays, we observed that the WHD loop of NAIP5 plays key roles in the inflammasome activation by relieving NLRC4 auto-inhibition, and stabilizing the formation of initial-encounter complex between liganded NAIP and active NLRC4. Overall, these data provide key insights into the understanding of the structural mechanisms of pre-liganded NAIP5, the process of NAIP5 activation, and the NAIP-dependent NLRC4 activation.