第278回　化学コースコロキウムのご案内

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16:00-16:55

「Deciphering G protein-coupled receptor signaling at atomic resolution by NMR spectroscopy 」

G protein-coupled receptors (GPCRs) are the largest family of membrane signaling proteins and the target of >40% of all marketed drugs. Nonetheless, molecular details of signal transduction remain obscure despite an explosion of new structures. Crystallization has been impossible without additional modifications that force receptors into a subset of nearly indistinguishable conformations – regardless of the presumed activation state. Yet, in contrast to other proteins, GPCRs are inherently flexible and possess multiple states, which suggests that activation relies on traversing a complex conformational landscape. Nuclear magnetic resonance (NMR) spectroscopy remains the only technique capable of quantifying protein dynamics at atomic resolution, but requires expression in E. coli for isotope labelling (e.g. ^{2}H, ^{13}C, ^{15}N). Overexpression of eukaryotic membrane proteins in prokaryotic systems results in insoluble aggregates that are hopeless to refold under aqueous conditions. However, directed evolution of the neurotensin receptor 1 (NTR1) has recently made possible large-scale prokaryotic expression. Our current efforts focus on the methyl groups of 134 isoleucine, leucine, valine and alanine residues. We are beginning to decipher the structural and kinetic changes of NTR1 upon ligand recognition and heterotrimeric G protein recruitment. At least two different receptor conformations, low and high population states, are visible in the absence of ligands. NT1 agonist stabilizes the low population state – suggesting a conformational selection binding mode. The ligand-binding pocket and G protein interface are allosterically linked as evidenced by chemical shift perturbations following titrations. We have measured methyl dynamics on the picosecond-nanosecond and millisecond-microsecond timescales. NTR1 exhibits ligand-specific motions and the data are currently being globally fitted to elucidate concerted structural dynamics. Combining these kinetic parameters with crystal structures and molecular dynamics simulations will unveil the conformational landscape of ligand binding and GPCR signal transduction.

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