Universität Basel

Research Project

Elucidating allosteric signal transmission in the beta1-adrenergic receptor

Third-party funded project

Project title Elucidating allosteric signal transmission in the beta1-adrenergic receptor
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G protein coupled receptors (GPCRs) are an important class of trans-membrane proteins that recognize a multitude of extracellular molecules and transmit their signal to the intracellular side. Despite recent achievements in X-ray crystallography of GPCRs, the high-resolution structures obtained do not capture their intrinsic dynamic properties, which are tightly associated with their function. NMR spectroscopy promises to provide such missing dynamic information. However so far, despite being valuable, only limited information has been obtained by NMR due to the difficult spectroscopic properties of this protein class. At this point, the potential of solution NMR analysis of GPCRs has not been fully realized.

Recently, I have overcome many of the obstacles that hinder the application of solution NMR to study signal transduction in GPCRs. Using a stabilized form of the β1 -adrenergic receptor and a selective isotope labeling method in the baculovirus-insect cell expression system, I was able to acquire well-resolved backbone amide proton-nitrogen correlation spectra. These spectra revealed numerous mechanisms within the receptor that are new, or had been postulated but never observed directly. Thus I have established a system that can now be used to study many more functional mechanisms of GPCRs at atomic resolution. In addition, we have developed an economic method to produce uniformly isotope-labeled (including deuteration) GPCRs in the insect cell systems. This will allow more advanced applications of NMR spectroscopy to GPCRs such as the study of their dynamics by relaxation measurements.

In the present proposal I want to use this system to obtain detailed insights into the receptor's signal transmission mechanism with the aim to understand how the receptor recognizes ligands and passes this information to the G protein in order to modulate its activity. Using state-of-the-art methods of isothermal titration calorimetry, protein rigidity theory, coevolutionary sequence alignment, in vitro

real-time observation of GDP/GTP exchange by fluorescence as well as solution NMR, I want to elucidate the underlying molecular mechanism of the thermodynamic behavior of ligand-receptor interactions, determine a high-resolution allosteric network model of signal transmission, and provide mechanical insights into how different agonists elicit varying levels of G protein activation.
If successful, the results will have implications for the general understanding of GPCR function and the developed methods should be applicable to other GPCRs.

Keywords G protein coupled receptor, protein allostery, nuclear magnetic resonance, thermodynamics, in vitro fluorescent kinetic analysis
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