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Functional dynamics of cell surface membrane proteins

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ABSTRACT

Cell surface receptors are integral membrane proteins that receive external stimuli, and transmit signals across plasma membranes. In the conventional view of receptor activation, ligand binding to the extracellular side of the receptor induces conformational changes, which convert the structure of the receptor into an active conformation. However, recent NMR studies of cell surface membrane proteins have revealed that their structures are more dynamic than previously envisioned, and they fluctuate between multiple conformations in an equilibrium on various timescales. In addition, NMR analyses, along with biochemical and cell biological experiments indicated that such dynamical properties are critical for the proper functions of the receptors. In this review, we will describe several NMR studies that revealed direct linkage between the structural dynamics and the functions of the cell surface membrane proteins, such as G-protein coupled receptors (GPCRs), ion channels, membrane transporters, and cell adhesion molecules.

1. Introduction

Cell surface receptors are integral membrane proteins that receive specific stimuli from the outside of cells, and transmit signals toward the inside of cells in well-controlled manners. The functions and structures of cell surface receptors are diverse. The major classes of the cell surface receptors are classified into 3 types: (1) seven membrane-spanning G-protein coupled receptors (GPCRs), (2) ion channel-linked receptors, and (3) catalytic receptors that are coupled with an intracellular enzymatic domain (e.g. kinase or phosphatase) [1]. Membrane transporters form another class of integral membrane proteins on the cell surface that actively or passively transport specific substances (e.g. ions, small molecules, and macromolecules) to maintain the homeostasis of the intracellular environments. Since many therapeutic reagents target cell surface receptors/membrane transporters, it is increasingly important to elucidate the structure–function relationships of these receptors at atomic resolution.

Over the past decade, structural understanding of cell surface membrane proteins has dramatically progressed. Growing numbers of structures of integral membrane proteins, including GPCRs, have been solved by X-ray crystallography or NMR, thereby strongly promoting our understanding of how these integral membrane receptors transmit the signals into the cells [2]. However, the crystal structures represent static snapshot of the target protein in the crystal lattice, and the observed conformation may not be the major state in the lipid bilayer environment. Recently, accumulating evidences have suggested that cell surface membrane proteins are structurally more dynamic, and interconvert between multiple conformations in equilibrium, such as between active and inactive conformations [3,4].

In this respect, NMR methods are very useful for obtaining information about the dynamics of the target protein on a wide range of timescales (e.g. from ns to even days) under physiological conditions [5]. Recent advances in the NMR techniques describing conformational dynamics, such as relaxation dispersion, have revealed that the global conformational fluctuations of proteins are tightly related to their functions, such as enzymatic activities [6,7] or molecular recognition [8]. Solid-state NMR can also provide atomic-level structural and dynamical information for heterogeneous biological systems, even in cellular membranes or whole cells. The structures and conformational dynamics of membrane proteins studied by the solid state NMR techniques have been extensively reviewed elsewhere [9].

Here, we define "functional dynamics" as conformational dynamics that are directly related to their function. In order to discriminate the functionally relevant dynamics from random fluctuations of protein conformations, the timescale of the protein dynamics should be correlated with that of their biological functions. In this review, we will cover the four demonstrations of functional dynamics of functionally independent membrane proteins

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by solution NMR. First, we will discuss how the conformational equilibrium of a GPCR determines the signal transduction across membranes. Secondly, we will describe detailed conformational dynamics of the K^+ channel, and directly demonstrate the conformational equilibrium underlying the channel activity in detergent micelles and lipid bilayer mimicking conditions. Thirdly, we will discuss the conformational exchange of the multi-drug resistant transporter, which is crucial for transporting its ligand across the membrane. Finally, we will describe the cell adhesion molecule that mediates the transient adhesion of leukocytes under the influence of hydrodynamic forces, and how the conformational dynamics at the molecular level is related to the phenomena at the cellular level.

2. Conformational equilibrium determines transmembrane signal transduction by GPCR

GPCRs are one of the largest membrane protein families in eukaryotes, and more than 25% of modern drugs target GPCRs. These drugs bind to GPCRs, leading to the induction or inhibition of signal transduction mediated by G-proteins, β-arrestins, and various other effectors *via* GPCRs. Each chemical ligand for a GPCR has a different level of ability to activate or inhibit its target, which is commonly referred to as efficacy, and the ligands are classified according to their efficacies, such as full agonists, partial agonists, neutral antagonists, and inverse agonists. These differences in the efficacies significantly affect the therapeutic properties of the GPCR ligands. In the case of drugs that target β_2-adrenergic receptor (β_2AR), a full agonist offers a clinical advantage over a partial agonist in acute severe asthma, although full agonists are capable of causing more adverse effects. However, the mechanism underlying the differences in the efficacy was not clear, although several structures of GPCRs complexed with ligands have been determined by X-ray crystallography [10,11]. Recently, several NMR studies have revealed that the conformational dynamics of β_2AR provide an explanation for its signal transduction mechanism. Liu et al. incorporated CF_3 probes, which can be observed with high sensitivity and selectivity, into β_2AR. They demonstrated that the ^{19}F-NMR signals from the CF_3 probe at C265 exhibited two components, which are referred to as components I and A, and the population of component A correlated with the efficacy of the ligand [12]. They also characterized the structure of components I and A by paramagnetic relaxation enhancement (PRE) experiments, which suggested that C327 is more exposed to the solvent in I than in A, in an agonist-bound state. Nygaard et al. demonstrated that β_2AR exhibits dynamical feature in the presence of an agonist, which are reduced upon binding with a G-protein-mimetic nanobody [13]. In addition, as described below, Kofuku et al. utilized NMR to clarify the conformational diversity of the transmembrane (TM) region of β_2AR in the inverse agonist-bound, neutral antagonist-bound, weak partial agonist-bound, partial agonist-bound, and full agonist-bound states [14].

β_2AR possesses nine methionine residues in extracellular loop 1 (ECL1), TM1, TM2, TM4, TM5, and TM6, and M82, M215, and M279 assume distinctly different conformations between the inverse agonist-bound and full agonist/G-protein-bound crystal structures (Fig. 1A) [10,11]. Therefore, these methionine methyl groups can be utilized to investigate the conformations of the TM region of β_2AR in various ligand-bound states by NMR.

In the spectra of the inverse agonist- and full agonist-bound forms, signals that apparently corresponded to the nine methionine residues were detected, suggesting that most of the methionine residues were observed, including those in the TM region (Fig. 1B and C). The spectra were significantly simplified by introducing mutations into the four solvent- or lipid-exposed methionine residues of β_2AR (Fig. 1D and E). In the spectrum of the M82V mutant, two signals were absent in the inverse agonist-bound state, revealing that both of these resonances are from M82 (Fig. 1F). The downfield and upfield resonances from M82 are referred to as $M82^D$ and $M82^U$, respectively. In the full agonist-bound state, one signal was absent in the spectrum of the M82V mutant, revealing that this resonance is from M82 (Fig. 1G). The chemical shifts of the resonance from M82 in the full agonist-bound state were different from $M82^D$ and $M82^U$ in the inverse agonist-bound state. This resonance from M82 in the full agonist-bound state is referred to as $M82^A$.

The ^{13}C and ^{1}H chemical shifts of the methionine methyl signals are reportedly affected by its side-chain conformation and the local environments, including the ring current effects from the neighboring aromatic rings, respectively [15,16]. The side-chain conformation and the local environment that correspond to the chemical shifts of $M82^U$ and $M82^D$ are in good agreement with the crystal structures of β_2AR in complex with inverse agonists, and those of $M82^A$ are in good agreement with the crystal structure with both a full agonist and a G-protein (Fig. 1H). Therefore, we proposed that the $M82^U$ and $M82^D$ signals correspond to the inactive states that cannot directly activate G-proteins, and the $M82^A$ signal corresponds to the active state that can interact with G-proteins. These distinct conformations corresponding to the $M82^U$, $M82^D$ and $M82^A$ signals are referred to as the $M82^U$, $M82^D$, and $M82^A$ conformations, respectively.

To examine the correlation between the ligand efficacy and the β_2AR conformations, the spectra were acquired in the presence of compounds with different efficacies for β_2AR (Fig. 2A and B). In the antagonist-bound state, major and minor resonances that slightly shifted from $M82^U$ and $M82^D$, respectively, were observed. In both the weak partial agonist- and partial agonist-bound states, a signal was observed at a chemical shift between $M82^U$ and $M82^A$, and the chemical shifts in the weak partial agonist-bound state were closer to those of $M82^U$. To examine whether the resonances from M82 in the ligand-bound states undergo conformational exchange, the spectra were recorded at a lower temperature, 283 K (Fig. 2C). As a result, the resonances from M82 in the weak partial agonist- and partial agonist-bound states significantly shifted away from $M82^U$, and the $M82^A$ resonance in the full agonist-bound state slightly shifted away from $M82^U$ at 283 K. These temperature-dependent shifts, together with the chemical shift changes in an efficacy-dependent manner (Fig. 2B) and the good agreement between the observed spectra and simulated data of M82 resonances (Fig. 2D and E), suggested that β_2AR exists in an equilibrium between the $M82^D$, $M82^U$, and $M82^A$ conformations.

Based on the above structural interpretation of the resonances from M82, the following signal regulation mechanism was proposed (Fig. 2F). In the full agonist-bound state, most of the β_2AR molecules assume the active conformation. In the partial agonists-bound states, β_2AR exists in an equilibrium between the inactive and active conformations, and the populations of the two conformations determine the efficacies. In the neutral antagonist alprenolol-bound state, β_2AR exists in an equilibrium between two major inactive conformations, which differ only in the region close to the ligand binding site, and one minor active conformation. The weak basal activity is due to the existence of the minor $M82^A$ conformation. In the inverse agonist-bound state, β_2AR exists in an equilibrium between the two locally different inactive conformations.

The resonances from M215 and M279, which are located on the cytoplasmic side of the TM region, were not observed in the full agonist-bound state (Fig. 1C and E). Nygaard et al. demonstrated that the intensity of these resonances is increased upon binding with a G-protein mimicking nanobody [13]. These results suggested that these signals were broadened due to the

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