Abstract

G protein-coupled receptors (GPCRs) are the largest family of integral membrane protein receptors present in most eukaryotic cells. They play a key role in signal transduction and understanding their signalling mechanism represents one of the main issues in biology today. In the characterization of the energy landscape of these receptors, at the atomic scale, X-ray crystal atomic structures published during the last decade represent the major breakthrough and contribution in the structural biology of GPCRs. They represent a precious starting point in the understanding of the mechanism of signal transduction by placing structures in the conformational ensemble of these receptors along the activation pathway. To complete these static snapshots that correspond to low energy and highly populated states, a characterization of the whole conformational ensemble and associated kinetic barriers is fundamental to complete the picture.

To this aim we proposed an innovative approach to observe GPCRs dynamic conformational landscape and how it is modulated by ligands and lipids, that are known to play a key role in membrane protein structures and functions (e.g. [1]). One of the most appropriate tool to explore GPCR kinetic barriers is solution state NMR [2]. To do so, we used $^{13}CH_3$ probes immersed in a perdeuterated environment, the most appropriate isotope-labelling scheme to investigate conformational landscapes of large proteins or protein complexes with this spectroscopy [3].

We chose *Escherichia coli* as expression system for its ability to grow in very hostile conditions like 100%-D_2O solutions. In order to overcome the usual expression issues concerning GPCRs, we applied an innovative protocol which targets the expression directly to inclusion bodies [4]. This allows the production of high amounts of proteins (up to 6 mg/litre of culture of pure $^{13}CH_3$-u-^{2}H-GPCRs). Once purified, receptors are folded in amphipols [5] and then transferred to nanometric lipid bilayers or nanodiscs [6]. Importantly quantitative pharmacological measurements indicate that receptors embedded in NLBs following this protocol are stable and fully active in the conditions of the NMR experiments.

NMR investigation of a GPCR in a NLB gave rise to a resolution never achieved in the field thanks to a fine tuned biochemistry and a perdeuteration of the receptor. According to our data, the prototypical receptor, the leukotriene B4 receptor (BLT2), is able to explore multiple different conformations, even in the unliganded state, including the active state. This conformational landscape is further modulated by ligands and lipids. In particular, we observed that an increment in the sterol content of the membrane modifies the distribution of the different conformational states of the receptor in favour of the active one, indicating a positive allosteric regulation of the sterol on the activation of this receptor, as confirmed by GTP-to-G protein binding measurements. This property of the sterol is likely important for the control of the signalling properties of GPCRs [7].

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