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RESEARCH HIGHLIGHT

The Couple G Protein-Coupled Receptor-G Protein: Preassembly *versus* Agonist-Induced Interaction

Mohammed Akli Ayoub[1,2]

[1]*Biologie et Bioinformatique des Systèmes de Signalisation (BIOS) group, INRA, UMR85, Unité Physiologie de la Reproduction et des Comportements; CNRS, UMR7247, F-37380 Nouzilly, France*

[2] *LE STUDIUM® Loire Valley Institute for Advanced Studies, F-45000 Orleans, France*

Correspondence: Mohammed Akli Ayoub
E-mail: Mohammed.Ayoub@tours.inra.fr
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The investigation of the physical and functional interactions of the G protein-coupled receptors (GPCRs) with their cognate heterotrimeric G proteins constitutes one of the most evolved aspects in this family of cell surface receptors. The development of the energy transfer-based approaches, bioluminescence or fluorescence resonance energy transfer (BRET or FRET, respectively), has strongly contributed to such advance. Using protease-activated receptors 1 and 2 (PAR1 and 2) as model for their multiple G protein coupling profiles, we revealed a preassembly between the two receptors and Gαi1 and Gαo proteins whereas their association with Gα12 occurred only upon receptor activation. Kinetic analysis in real-time and live cells showed important differences in the activation between these two modes of coupling which may be consistent with their implication in the physiological responses of PARs. Together, our finding indicate that preassembly or agonist-promoted association depend on receptor-G protein pair representing the key mechanisms of temporal and spatial regulation and integration of the multiple G protein coupling and signaling of GPCRs.

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The major biological responses such as neurotransmission, secretion, cell growth, cell migration and differentiation are under the control of a large number of stimuli including light, odorants, amino acids, nucleotides, peptides, hormones and neurotransmitters [1-3]. At the molecular level in the target tissues these responses are mediated by a highly specific conserved receptor family named G protein-coupled receptors (GPCRs). In fact, GPCRs constitute one of the largest coding genes with more than 1% of the total genome of the vertebrates [1] and the importance of this family of receptors is illustrated by their implication in many pathologies such as neurological disorders, cardiovascular diseases, cancer, allergies, infectious diseases, diabetes [4, 5]. Therefore, GPCRs constitute the target of about 50% of the drugs used nowadays and continue to be the center of intense investigation and research in both academic and pharmaceutical laboratories [6-8]. Since their discovery our view on GPCRs and their function has considerably evolved with the achievement of important discoveries and the emergence of many new concepts. These advances illustrate the diversity and the complexity of GPCR

function and their implication in physiology and pathophysiology [7, 9-12].

Among the different aspects related to GPCR biology, the physical and functional interactions between the receptors and the different heterotrimeric G proteins and how the multiplicity of GPCR signaling can be regulated have been extensively studied using various assays [13-15]. Recently, BRET and FRET approaches led to shed more light on the mechanisms involved in GPCR-G protein interaction and activation at the molecular level, and more importantly in real-time and live cells [14, 16-18]. These advances are characterized by the emergence of the concept of GPCR-G protein preassembly [19-24] which somehow came to challenge the "free-collision coupling" model postulating that receptor-G protein activation is exclusively agonist-dependent process. Our recent studies on thrombin (PAR1) and trypsin (PAR2) receptors, using BRET and time-resolved FRET (TR-FRET) strongly support the reality of the preassembled concept [19-21]. Interestingly, using three different heterotrimeric G proteins, Gαi1, Gαo, and Gα12, we clearly demonstrated that such preassembly cannot be considered as general feature for all G proteins since it has been observed with some G proteins (Gαi1 and Gαo) but not others (Gα12) [19-21]. In fact, we demonstrated that PAR1 and PAR2 transiently expressed in COS-7 cells form preassembled complexes with Gαi1 and Gαo proteins even in the absence of any activation with their specific agonists (proteases or peptides) [19-21]. Also, the preassembled complexes were insensitive to Pertussis toxin (PTX) indicating that the preassembly cannot be linked anyhow to receptor-G protein precoupling or constitutive activity of the receptor-Gαi1/o complexes [19, 20]. Moreover, real-time assessment of the agonist-promoted activation revealed rapid and transient conformational changes within the preassembled complexes that are totally blocked by PTX and PAR1 antagonist [19, 20] as well as a non-selective G protein inhibitor, BIM-46187 [25]. These observations suggest that preassembly is used for temporal and spatial regulation of the intracellular Gαi1/o-cAMP signaling where PARs-Gαi/o preassembly leads to a faster modulation of cytosolic cAMP levels as well as the localization of the related response in specific domains within the cells. The rapid modulation of cAMP would be more consistent with the rapid and transient agonist-promoted conformational changes within the preassembled complexes rather than agonist-dependent receptor/G protein association. Also, the preassembly may be one the mechanisms involved in the selectivity and efficiency of the signaling pathways by limiting the availability of a given preassembled receptor to interact and activate other G proteins as shown with PAR1 and Gαi1 [20]. In contrast, the mode of interaction with G12 seems to reconcile with the classical GPCR-G protein coupling sine its association with PARs was observed only upon receptor activation [20, 21]. However, the long and stable association kinetics makes atypical this mode of interaction compared to the classical kinetics of G protein activation. It is then unclear how such slow and sustained recruitment of Gα12 can be explained considering the rapid activation of PAR1 and PAR2. The stability of PAR1/2-Gα12 complexes after their formation may be explained by either a non-dissociation of the complexes or many rapid association-dissociation cycles which cannot be detected by BRET or TR-FRET and as a result a continuous signals. However, the recruitment of Gα12 upon PARs activation should be associated with its activation to promote specific and delayed intracellular responses. This is consistent with the strong and sustained effects of thrombin and PAR1 on cell morphology and proliferation where stabilizing PAR1-Gα12 interaction and maintaining their long term activation may be crucial for the control of cell proliferation, differentiation, migration, and oncogenesis [26, 27].

Together, our studies on the interaction between PARs and G proteins using BRET and TR-FRET approaches led to shed more light on the nature of GPCR-G protein coupling at the molecular level. In the context of the multiple coupling and signaling of GPCRs this will definitely help to better understand how GPCR-G protein coupling can be regulated and integrated inside the cells [28]. Indeed, our studies demonstrate different processes involved in the regulation of GPCR-G protein coupling depending on the receptor-G protein couple examined. Therefore, we conclude that the nature of the molecular association between GPCRs and G proteins is characterized by either the preassembly or the agonist-dependent recruitment which depends on the receptor-G protein pair. Moreover, the differential coupling of PARs with Gαi/o versus Gα12 nicely illustrates the complexity of GPCR-G protein coupling and this differential association may constitute a mechanism of control of the multiple coupling and signaling of GPCRs. Of course, the integration of these two concepts with others such as biased signaling and oligomerization of GPCRs would be of great importance. Finally, the next step would be the investigation of the importance of GPCR-G protein preassembly and their agonist-dependent association in physiology or physiopathology.

Conflicting interests

The authors have declared that no competing interests exist.

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