Identification of Sphingolipid-binding Motif in G Protein-coupled Receptors

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Abstract

Sphingolipids correspond to a major class of lipids which serve as indispensable structural components of membranes and play an important role in various cellular functions. They constitute ~10–20% of total membrane lipids and are known to form segregated domains in biological membranes. Sphingolipids have been shown to play a vital role in the function of various G protein-coupled receptors (GPCRs). We report here the presence of sphingolipid-binding motif (SBM) in representative GPCRs such as cholecystokinin, oxytocin and secretin receptors, and subtypes of human serotonin receptors. We previously reported the importance of sphingolipids in the function of the serotonin$_{1A}$ receptor, a representative member of the GPCR superfamily, involved in behavioral, cognitive, and developmental functions. In this work, we show that the serotonin$_{1A}$ receptor contains a putative SBM, corresponding to amino acids 205 to 213. In addition, our analysis shows that SBM is an intrinsic characteristic feature of the serotonin$_{1A}$ receptor and is conserved throughout the course of natural evolution. Our results represent the first report on the presence of SBM in serotonin$_{1A}$ receptors and provide novel insight on the molecular mechanism of GPCR-sphingolipid interaction.

Keywords

SBM · Sphingolipids · GPCR · Serotonin$_{1A}$ receptor · CRAC

Abbreviations

CRAC	Cholesterol recognition/interaction amino acid consensus
GPCR	G protein-coupled receptor
SBM	Sphingolipid-binding motif

10.1 Introduction

Sphingolipids are indispensable constituents of cellular membranes of eukaryotes and account for ~10–20% of the entire lipids associated with membranes (Holthuis et al. 2001). They are believed to form laterally segregated domains with cholesterol (also referred as 'lipid rafts') (Brown 1998; Masserini and Ravasi 2001; Jacobson et al. 2007). The concept of these membrane domains assumes relevance as they have been implicated in crucial physiological functions such as cellular sorting, trafficking (Simons and

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van Meer 1988), cellular signaling (Simons and Toomre 2000), and the entry of pathogens into host cells (Riethmüller et al. 2006; Pucadyil and Chattopadhyay 2007; Vieira et al. 2010; Chattopadhyay and Jafurulla 2012; Kumar et al. 2016).

The G protein-coupled receptor (GPCR) superfamily constitutes an important class of proteins employed in signal transduction (Rosenbaum et al. 2009; Chattopadhyay 2014). GPCRs are associated with an array of physiological processes and have therefore emerged as major drug targets (Heilker et al. 2009; Chattopadhyay 2014; Cooke et al. 2015; Jacobson 2015). An estimate of ~50% of present clinically recommended drugs act as either agonists or antagonists to GPCRs (Jacobson 2015). The serotonin$_{1A}$ receptor represents an important G protein-coupled neurotransmitter receptor and plays a vital role in several neurological functions such as behavior, cognition, anxiety, depression, and learning (Pucadyil et al. 2005; Müller et al. 2007; Kalipatnapu and Chattopadhyay 2007; Fiorino et al. 2014). It therefore naturally has emerged as an important target in the development of therapeutics against neurological disorders (Kaufman et al. 2016).

GPCRs are transmembrane proteins, and a large part of the receptor remains embedded in the membrane. Any change in the membrane lipid milieu therefore could influence the structure and function of the receptor. For example, it is estimated from molecular dynamics simulations that the lipid-protein interface accounts for ~38% of the entire surface area of rhodopsin (Huber et al. 2004). Keeping in mind the increasing relevance of the serotonin$_{1A}$ receptor in pharmacology and drug development, interaction of surrounding membrane lipids with the receptor assumes significance. In this context, previous work from our laboratory has demonstrated the requirement of membrane cholesterol and sphingolipids (reviewed in Pucadyil and Chattopadhyay 2006; Paila and Chattopadhyay 2010; Jafurulla and Chattopadhyay 2013, 2015) in the function of the serotonin$_{1A}$ receptor. The influence of sphingolipids on the structure and function of a variety of membrane proteins has been attributed to specific interaction (Snook et al. 2006).

A characteristic amino acid sequence which could represent the conserved sphingomyelin-binding motif in proteins has been previously proposed (Contreras et al. 2012). These authors identified a specific binding motif for sphingomyelin, termed the sphingolipid-binding motif (SBM), in the transmembrane protein p24 (a COPI machinery protein) and demonstrated the headgroup and acyl chain specificity of sphingomyelin in its interaction with this motif. Importantly, these authors further showed that only sphingomyelin with an appropriate dynamic volume would fit into the cavity formed by SBM residues in the transmembrane domain of p24. In spite of the importance of sphingolipids in the structure and function of several membrane proteins, including GPCRs and ion channels (Alves et al. 2005; Harikumar et al. 2005; Sjögren and Svenningsson 2007; Fantini and Barrantes 2009; Slotte 2013; Jafurulla and Chattopadhyay 2015; Jafurulla et al. 2017), limited information is available on specific binding motifs involved in GPCR-sphingolipid interaction. Exploring the possibility of specific interaction between GPCRs and sphingolipids therefore assumes relevance. In the present study, we identified the presence of SBM in representative GPCRs such as cholecystokinin, oxytocin and secretin receptors, and subtypes of human serotonin receptors. Earlier results from our laboratory have shown that membrane sphingomyelin is important for regulating the function of the serotonin$_{1A}$ receptor (Jafurulla et al. 2008; Singh and Chattopadhyay 2012). We therefore explored the presence of SBM in the human serotonin$_{1A}$ receptor and its conservation over the course of natural evolution. Our results show that human serotonin$_{1A}$ receptors contain a putative SBM in transmembrane helix V. In addition, sequence analysis of the serotonin$_{1A}$ receptor from various species across diverse phyla shows that SBM is an inherent characteristic attribute of the receptor and is conserved throughout the course of natural evolution.

10.2 Methods

10.2.1 Identification of SBM in Representative Human GPCRs

The conserved signature sequence for SBM is (I/L/T/V)XX(I/L/T/V)(I/L/T/V)XX(I/L/T/V)(F/W/Y), where X represents any of the 20 naturally occurring amino acids (Contreras et al. 2012; Björkholm et al. 2014). The putative SBMs were identified by visual inspection in cholecystokinin, oxytocin, serotonin, and secretin receptors with the help of regular sequence of SBM (see Fig. 10.1a). The amino acid sequences of GPCRs were obtained from NCBI database. The positions of starting amino acid residues in the corresponding sequences are marked in parentheses.

10.2.2 Sequence Alignments of the Predicted SBM in Human Serotonin Receptor Subtypes

Multiple sequence alignment for various serotonin receptor subtypes was performed with ClustalW (Larkin et al. 2007), using the human serotonin$_{1A}$ receptor sequence as a reference. The positions of starting amino acid residues in various serotonin receptor subtypes are marked in parentheses (see Fig. 10.1b).

10.2.3 Sequence Alignment of the Serotonin$_{1A}$ Receptor and Identification of SBM

The transmembrane helices of the serotonin$_{1A}$ receptor were predicted using the program TMHMM2 (Krogh et al. 2001; see Fig. 10.2). The putative SBM in the serotonin$_{1A}$ receptor is identified as described above. The conservation of sphingolipid-binding motif (SBM) in the serotonin$_{1A}$ receptor during evolution was analyzed by examining amino acid sequences of the receptor over various phyla obtained from NCBI and ExPASy databases (see Fig. 10.3). Partial, duplicate, and other non-specific sequences were removed from the set of sequences obtained. Initial alignment of sequences was carried out using ClustalW. After eliminating the relatively divergent parts of the receptor, the sequence was realigned using the same program. The putative SBMs in the serotonin$_{1A}$ receptor were identified by visual inspection. The amino acid sequences used for the analysis belong to diverse taxa that include insects, fish and other marine species, amphibians, and extending up to mammals. The quality of alignment shown in Figs. 10.1b and 10.3 was computed in Jalview, the software used to view the alignment.

10.3 Results and Discussion

Sphingolipids have been shown to modulate the function and organization of important classes of membrane proteins such as GPCRs (Jafurulla and Chattopadhyay 2015). In the overall context of sphingolipid sensitivity of GPCR function (Sjögren and Svenningsson 2007; Fantini and Barrantes 2009; Paila et al. 2010; Singh et al. 2012; Jafurulla and Chattopadhyay 2015; Jafurulla et al. 2017), we examined whether the sequence of some of the representative GPCRs includes any SBM(s). We identified the presence of SBM in representative GPCRs such as cholecystokinin, oxytocin, serotonin$_{1A}$, and secretin receptors (see Fig. 10.1a). Interestingly, the function of some of these receptors (e.g., the cholecystokinin receptor) has been shown to be modulated by sphingolipids (Harikumar et al. 2005). Sequence analysis of these receptors revealed that while the secretin receptor contains two SBMs (residues 31–39 and 182–190), the cholecystokinin, the oxytocin, and the serotonin$_{1A}$ receptor sequences display only one motif. It is noteworthy that while the characteristic SBM identified in oxytocin and one of the

Fig. 10.1 Sequence alignment of the predicted sphingolipid-binding motifs in representative human G protein-coupled receptors and serotonin receptor subtypes. (**a**) SBM(s) in cholecystokinin, oxytocin, serotonin$_{1A,}$ and secretin receptors are shown (highlighted in blue). The numbers corresponding to the starting amino acid position in the respective sequences are mentioned in parentheses. (**b**) Multiple alignments of human serotonin receptor subtypes were performed with ClustalW using human serotonin$_{1A}$ receptor sequence as reference. The putative SBM is shown in blue. The positions of amino acid residues are marked in parentheses for various serotonin receptor subtypes. A graphical representation displaying the quality of alignment for serotonin receptor subtypes, with lighter shades representing higher quality. The amino acid sequences of the receptors were obtained from NCBI database, and the protein accession numbers are indicated in parentheses. See text and Methods for more details

motifs in secretin receptors (residues 31–39) show orientation of amino acids similar to what was previously reported (Contreras et al. 2012; Björkholm et al. 2014), SBM of the cholecystokinin, the serotonin$_{1A,}$ and the second motif (residues 182–190) in the secretin receptor was found to be oriented in opposite direction.

In view of the fact that the various subtypes of serotonin receptors share considerable sequence homology (Hoyer et al. 2002), we examined the occurrence of SBM in subtypes of human serotonin receptors. Figure 10.1b shows the sequence alignment of subtypes of human serotonin receptors. Multiple alignments were carried out with ClustalW using the serotonin$_{1A}$ receptor sequence as reference. Interestingly, SBM was found to be conserved in most of the serotonin receptor subtypes analyzed (highlighted in blue in Fig. 10.1b).

Previous work from our laboratory has demonstrated the requirement of sphingomyelin (Jafurulla et al. 2008; Singh and Chattopadhyay 2012) in regulating the function of the serotonin$_{1A}$ receptor. The putative SBM identified in the human serotonin$_{1A}$ receptor is present in transmembrane helix V and comprises of Tyr-205, Iso-206, Leu-209, Leu-210, and Val-213 (see Figs. 10.1 and 10.2). Within SBM, the β-branched residue Ile is found in position 206 in serotonin$_{1A}$ receptors (see Fig. 10.1). These β-branched residues were shown to contribute to interactions between transmembrane helices (Senes et al. 2000) by providing additional rigidity and thereby enhancing London dispersion forces.

It is interesting to note that SBM in the serotonin$_{1A}$ receptor exhibits partial overlap (specifically, in amino acid residues 209–213) with the cholesterol recognition/interaction amino acid

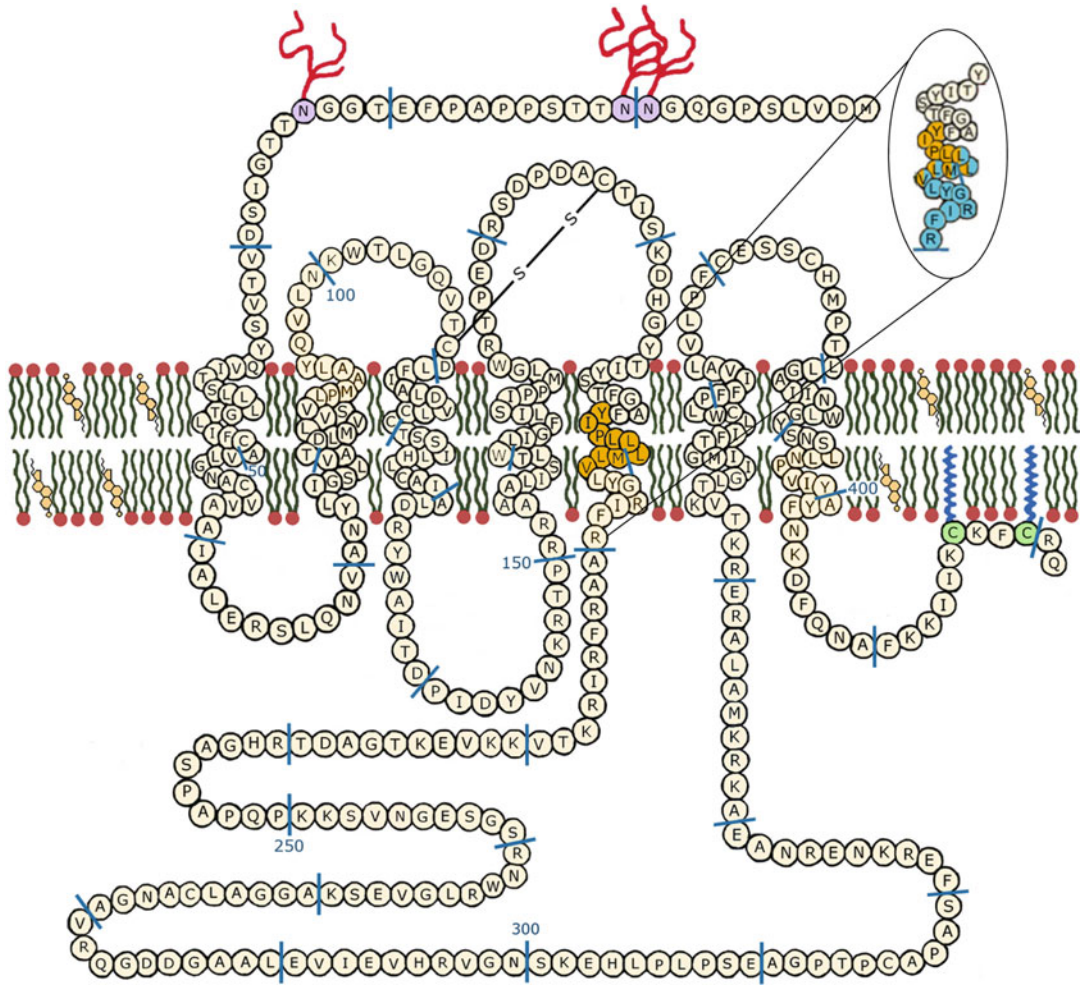

Fig. 10.2 A schematic representation of the membrane-embedded human serotonin$_{1A}$ receptor showing its topological and other structural features. The membrane is shown as a bilayer of phospholipids and cholesterol, representative of typical eukaryotic membranes. The transmembrane helices of the receptor were predicted using the program TMHMM2 (see Methods for details). Seven transmembrane stretches, each composed of ~22 amino acids, are depicted as putative α-helices. The putative SBM in the transmembrane helix V is highlighted (in yellow). The amino acids in the receptor sequence are shown as circles. An enlarged representation of transmembrane helix V of the human serotonin$_{1A}$ receptor showing the overlap of putative SBM highlighted in yellow and CRAC highlighted in cyan in the transmembrane helix V is shown. The common residues corresponding to both SBM and CRAC are shown in a combination of yellow and cyan. (Adapted and modified from Paila et al. 2009)

consensus (CRAC) motif identified earlier by us in transmembrane helix V (Jafurulla et al. 2011; see Fig. 10.2). This observation assumes significance in light of our previous results that showed both cholesterol (Pucadyil and Chattopadhyay 2006; Paila and Chattopadhyay 2010; Jafurulla and Chattopadhyay 2013) and sphingolipids (Jafurulla and Chattopadhyay 2013, 2015) to be essential for the function of the serotonin$_{1A}$ receptor. Such overlapping interaction sites for cholesterol and sphingomyelin in the serotonin$_{1A}$ receptor could help in understanding the mode of regulation of receptor function by these important membrane lipids. Previous results from our laboratory have shown the presence of a sphingolipid-binding domain (SBD) in the

Fig. 10.3 Multiple alignment of putative SBM in the serotonin$_{1A}$ receptor over various phyla across natural evolution. The conserved signature sequence for SBM (highlighted in blue) is (I/L/T/V)XX(I/L/T/V)(I/L/T/V)XX(I/L/T/V)(F/W/Y) residues, where X represents any of the 20 naturally occurring amino acids. As shown, the putative SBM is conserved from fish to humans. The numbers corresponding to the starting amino acid position in the respective sequences are mentioned in parentheses. Amino acid sequences of serotonin$_{1A}$ receptors are from NCBI and ExPASy databases, and the protein accession numbers are indicated in parentheses. A graphical representation displaying the quality of alignment with lighter shades representing higher quality is shown below. See text and Methods for more details

serotonin$_{1A}$ receptor (Chattopadhyay et al. 2012) that showed an overlap with the CRAC motif in transmembrane helix II. We recently demonstrated using coarse-grain molecular dynamic simulations that GM_1 (monosialotetrahexosylganglioside) predominantly interacts at the extracellular loop 1 specifically at the proposed SBD site of the serotonin$_{1A}$ receptor in a cholesterol-dependent manner (Prasanna et al. 2016). These results provided better understanding of the importance of such overlap of SBD and CRAC motifs. With this background, we plan to explore in our future studies the possible role of SBM (identified in the present study) in the interaction of sphingomyelin with the serotonin$_{1A}$ receptor and dependency of any such interaction on membrane cholesterol.

We further explored the conservation of SBM in the serotonin$_{1A}$ receptor over natural evolution. For this, we examined the amino acid sequences of the serotonin$_{1A}$ receptor from various organisms across diverse phyla (see Fig. 10.3;

the position of the first amino acid in the alignment is denoted in parentheses). We analyzed the amino acid sequences from various species that belong to diverse taxa which include insects, fish, amphibians, and extending up to mammals. Figure 10.3a shows multiple sequence alignment of the serotonin$_{1A}$ receptor from diverse phyla in the region of the putative SBM with the conserved amino acid residues highlighted. It is evident from this sequence alignment that SBM (Tyr-205, Iso-206, Leu-209, Leu-210, and Val-213) identified in the serotonin$_{1A}$ receptor of *Homo sapiens* (see Figs. 10.1 and 10.2) is conserved in most species included in the study. Initial sequence alignment performed using ClustalW demonstrated that SBM is conserved in the majority of species. Realignment with ClustalW (after deleting the comparatively diverse fractions from the sequence of the serotonin$_{1A}$ receptor) displayed conservation of SBM across various phyla studied (see Fig. 10.3). These results therefore show that SBM is conserved over natural evolution and corresponds to an inherent characteristic attribute of the serotonin$_{1A}$ receptor.

Sphingolipids are enriched in neural tissue and play an important role in the metabolism, survival, and regeneration of the nervous system (van Echten-Deckert and Herget 2006; Piccinini et al. 2010). Regulation of neuronal sphingolipid metabolism has been shown to be crucial, with any deregulation resulting in severe neurodegenerative diseases (Zeidan and Hannun 2007; Piccinini et al. 2010; Prinetti et al. 2011). For example, sphingolipids have been shown to be critical players in the pathogenesis of Alzheimer's disease (Ariga et al. 2008; van Echten-Deckert and Walter 2012) and Parkinson's disease (Wu et al. 2012). In particular, deregulation of sphingomyelin content has been reported in neurological disorders such as Alzheimer's disease, schizophrenia, Parkinson's disease, and Niemann-Pick disease (Bienias et al. 2016). On the other hand, it is interesting to note that imbalance in serotonergic signaling is implicated in Alzheimer's disease, schizophrenia, Parkinson's disease, anxiety, and depression (Tan et al. 2011; Wirth et al. 2017). Importantly, signaling mediated by the serotonin$_{1A}$ receptor is shown to be a crucial target in the pharmacotherapy of schizophrenia and Parkinson's disease (Sumiyoshi et al. 2007; Haleem 2015). In view of the reported role of serotonin$_{1A}$ receptors in neurological disorders associated with deregulation of sphingomyelin, along with our previous results on role of sphingomyelin in the function of the receptor (Jafurulla et al. 2008; Singh and Chattopadhyay 2012), SBM identified in serotonin$_{1A}$ receptors assumes greater relevance.

Recent advancements in GPCR-lipid interaction have improved our overall understanding of GPCR function in the context of human physiology. Although pharmacological and signaling aspects of GPCRs have been studied in detail, features highlighting their interaction with membrane lipids such as sphingolipids and cholesterol are recently beginning to be addressed. Our present results, along with previous observations by us and others, could therefore help to understand the molecular basis of the observed role of sphingolipids in the function of GPCRs in general and the serotonin$_{1A}$ receptor in particular. In summary, these results could prove to be useful in understanding malfunctioning of GPCRs in neurodegenerative disorders involving sphingolipids.

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