Chapter 19
Sphingolipid-Binding Domain in the Serotonin$_{1A}$ Receptor

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Abbreviations

5-HT$_{1A}$ receptor	5-Hydroxytryptamine-1A receptor
CRAC	Cholesterol recognition/interaction amino acid consensus
DMPC	Dimyristoyl-*sn*-glycero-3-phosphocholine
FB$_1$	Fumonisin B$_1$
GPCR	G-protein coupled receptor
LED	Light-emitting diode
LUV	Large unilamellar vesicle
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
SBD	Sphingolipid-binding domain
Serotonin	5-Hydroxytryptamine

Introduction

Sphingolipids are essential components of eukaryotic cell membranes and constitute 10–20% of the total membrane lipids (Holthuis et al. 2001). They are implicated in crucial cellular functions such as regulation of cell signaling, growth, differentiation, and neoplastic transformation. The distribution of sphingolipids in

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the cellular plasma membrane appears heterogeneous, and it has been postulated that sphingolipids and cholesterol occur in laterally segregated lipid domains (sometimes termed as "lipid rafts") (Brown 1998; Masserini and Ravasi 2001). Many of these domains are believed to be important for the maintenance of membrane structure and function, although analyzing the spatiotemporal resolution of these domains is proving to be challenging (Jacobson et al. 2007). The idea of such membrane domains gains significance since physiologically important functions, such as cellular membrane sorting, trafficking (Simons and van Meer 1988), signal transduction (Simons and Toomre 2000), and the entry of pathogens into cells (Riethmüller et al. 2006; Pucadyil and Chattopadhyay 2007), have been attributed to these domains.

The G-protein coupled receptor (GPCR) superfamily represents the largest class of molecules involved in signal transduction across the plasma membrane (Pierce et al. 2002; Rosenbaum et al. 2009). GPCRs regulate physiological responses to a diverse array of stimuli and mediate multiple physiological processes. As a consequence of this, GPCRs have emerged as major targets for the development of novel drug candidates in all clinical areas (Heilker et al. 2009). The serotonin$_{1A}$ (5-HT$_{1A}$) receptor (see Fig. 19.1) is an important G-protein coupled neurotransmitter receptor and is crucial in a multitude of physiological processes (Pucadyil et al. 2005; Kalipatnapu and Chattopadhyay 2007). It serves as an important target in the development of therapeutic agents for neuropsychiatric disorders. Interestingly, mutant (knockout) mice lacking the serotonin$_{1A}$ receptor exhibit enhanced anxiety-related behavior and represent an important animal model for genetic vulnerability to complex traits such as anxiety disorders and aggression in higher animals (Gardier 2009).

GPCRs are integral membrane proteins with a considerable portion of the protein embedded in the membrane. This raises the obvious possibility that the membrane lipid environment could be an important modulator of receptor structure and function. In case of rhodopsin, for example, it is estimated from molecular dynamics simulation that the lipid–protein interface corresponds to ~38% of the total surface area of the receptor (Huber et al. 2004). Specifically, in the context of increasing pharmacological relevance of the serotonin$_{1A}$ receptor, its interaction with surrounding membrane lipids assumes significance in modulating the function of the receptor in healthy and diseased states (Paila et al. 2008). Work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the function of the serotonin$_{1A}$ receptor [recently reviewed in Paila and Chattopadhyay (2010)]. Interestingly, we previously reported that sphingolipids are necessary for ligand binding and cellular signaling of the human serotonin$_{1A}$ receptor (Jafurulla et al. 2008; Paila et al. 2010). For example, we recently showed that the function of the serotonin$_{1A}$ receptor is impaired upon metabolic depletion of sphingolipids using fumonisin B$_1$ (FB$_1$), a specific inhibitor of ceramide synthase (Paila et al. 2010). In addition, it has been reported earlier that sphingolipids could be necessary for ligand binding of serotonin$_{7a}$ receptors (Sjögren and Svenningsson 2007). The effect of sphingolipids on the conformation and function of membrane proteins could be due to specific interaction. For example, the nerve growth factor receptor tyrosine kinase has been shown to interact directly with gangliosides (Mutoh et al. 1995). It is therefore possible that the serotonin$_{1A}$ receptor enjoys specific interaction with membrane sphingolipids.

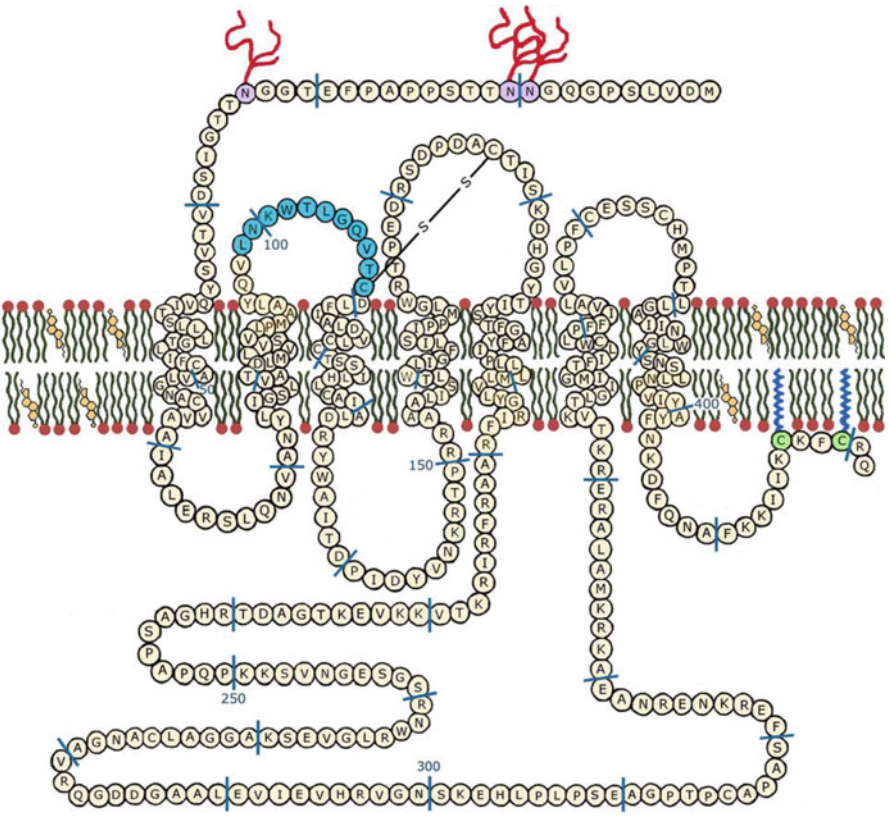

Fig. 19.1 A schematic representation of the membrane embedded human serotonin$_{1A}$ receptor. The membrane is shown as a bilayer of phospholipids and cholesterol, representative of typical eukaryotic membranes. The transmembrane helices of the receptor were predicted using the program TMHMM2. The putative SBD motifs (see text) are highlighted (in *cyan*). The amino acids in the receptor sequence are shown as *circles*. Adapted and modified from Paila et al. (2011)

Previous work by one of us has shown that, in a number of cases, proteins that interact with (glyco)sphingolipids appear to have a characteristic amino acid sequence, termed the "sphingolipid-binding domain" (SBD) (Mahfoud et al. 2002; Fantini 2003; Fantini and Barrantes 2009; Chakrabandhu et al. 2008; Fantini and Yahi 2011; Fantini et al. 2006). In order to explore whether the reported sphingolipid sensitivity of the serotonin$_{1A}$ receptor function (Jafurulla et al. 2008; Paila et al. 2010) could be induced by the SBDs, we examined the presence of SBD motif in the serotonin$_{1A}$ receptor. We report here, using an algorithm (Chakrabandhu et al. 2008; Fantini et al. 2006) based on the systematic presence of key amino acids belonging to hairpin structures, that the human serotonin$_{1A}$ receptor contains a putative SBD. Interestingly, our analysis shows that the SBD motif appears to be an inherent feature of the serotonin$_{1A}$ receptor and is conserved over natural evolution across various phyla. These results constitute the first report of the presence of SBD motif in the serotonin receptor family.

Experimental Section

Materials. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and ovine ganglioside (GM_1) were from Avanti Polar Lipids (Alabaster, AL). The purity of POPC was checked by thin layer chromatography on silica gel precoated plates (Merck, Darmstadt, Germany) in chloroform/methanol/water (65:35:5, v/v/v) and were found to give only one spot in all cases with a phosphate-sensitive spray and on subsequent charring (Dittmer and Lester 1964). The concentration of POPC was determined by phosphate assay subsequent to total digestion by perchloric acid (McClare 1971). DMPC was used as an internal standard to assess lipid digestion. The concentration of SBD peptide in aqueous solution was calculated from molar extinction coefficient (ε) of 5,570 M^{-1} cm^{-1} at 280 nm (Nick Pace et al. 1995). All other chemicals used were of the highest purity available. Solvents used were of spectroscopic grade. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout. The SBD peptide corresponding to the $serotonin_{1A}$ receptor (with a characteristic sequence LNKWTLGQVTC, see Figs. 19.1 and 19.2) was custom synthesized by Schafer-N (Copenhagen, Denmark).

Analysis of putative SBD in $serotonin_{1A}$ receptors. Amino acid sequences of the $serotonin_{1A}$ receptor were obtained from NCBI and Expasy databases. Partial, duplicate, and other nonspecific sequences were removed from the set of sequences obtained. Initial alignment was carried out using ClustalW (Larkin et al. 2007). The sequences used for the analysis belong to diverse taxa that include insects, fish and other marine species, amphibians, and extending up to mammals. After eliminating the relatively divergent parts of the receptor, the sequence was realigned using the same program. The quality of alignment shown in Fig. 19.3b was computed in Jalview, the software used to view the alignment.

Sample preparation. In this study, we have monitored the interaction of a potential SBD corresponding to the first extracellular loop of the $serotonin_{1A}$ receptor with membranes. The sequence of the SBD peptide is: LNKWTLGQVTC (see Figs. 19.1 and 19.2). Fluorescence measurements were performed using large unilamellar vesicles (LUVs) of 100 nm diameter containing (1) POPC, or (2) POPC/GM_1 (90/10 mol/mol), or (3) POPC/cholesterol (60/40 mol/mol), or (4) POPC/cholesterol/GM_1 (50/40/10, mol/mol/mol). All samples contained 2 mol% SBD peptide. In general, 320 nmol of total lipid was dried under a stream of nitrogen while being warmed gently (~35°C). After further drying under a high vacuum for at least 3 h, the lipid film was hydrated (swelled) by adding 1.5 ml of 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4 buffer, and each sample was vortexed for 3 min to uniformly disperse the lipid and form homogeneous multilamellar vesicles. The buffer was always maintained at ~40°C as the vesicles were made. LUVs of 100 nm diameter were prepared by the extrusion technique using an Avestin Liposofast Extruder (Ottawa, Ontario, Canada) as previously described (MacDonald et al. 1991). Briefly, the multilamellar vesicles were freeze–thawed five times using liquid nitrogen to ensure solute equilibration between trapped and bulk solutions, and then

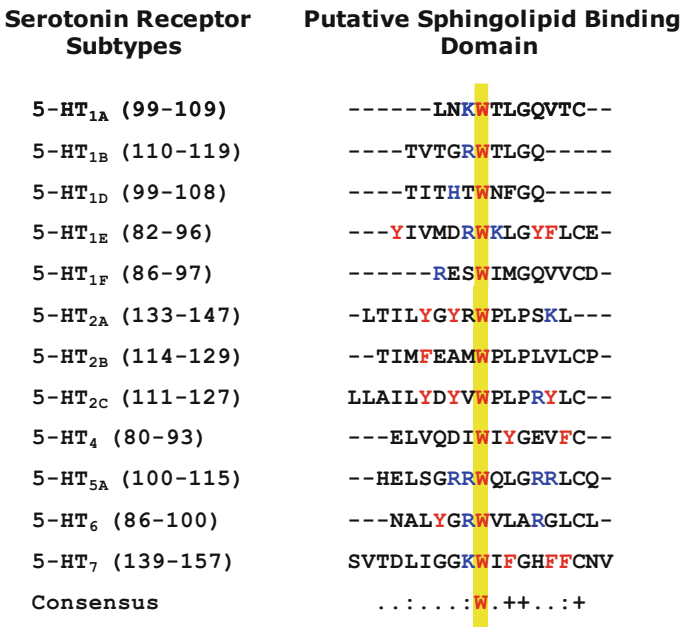

Fig. 19.2 Sequence alignments of the predicted SBD in human serotonin receptor subtypes. Multiple alignments were performed with ClustalW using the serotonin$_{1A}$ (5-HT$_{1A}$) receptor sequence as reference. Aromatic and basic amino acids are color coded *red* and *blue*, respectively. The fully conserved tryptophan residue is highlighted in *yellow*. The positions of amino acid residues are marked in parentheses for various serotonin receptor subtypes. The characters in the consensus sequence correspond to the frequency of the amino acid at the indicated position: "." > 20%, ":" > 40%, "+" > 60%, and the amino acid letter if 100%

extruded through polycarbonate filters (pore diameter of 100 nm) mounted in the extruder fitted with Hamilton syringes (Hamilton Company, Reno, NV). The samples were subjected to 11 passes through polycarbonate filters to give the final LUV suspension. In order to incorporate the SBD peptide into membranes, a small aliquot containing 6.4 nmol of the peptide from a stock solution in water was added to the preformed vesicles and mixed well to give membranes containing 2 mol% peptide. Background samples were prepared the same way except that peptides were not added to them. The optical density of the samples measured at 280 nm was ~0.15 in all cases which rules out any possibility of scattering artifacts in the anisotropy measurements. Samples were incubated in dark for 12 h at room temperature (~23°C) for equilibration before measuring fluorescence. All experiments were carried out with multiple sets of samples at room temperature (~23°C).

Steady-state fluorescence measurements. Steady-state fluorescence measurements were performed with a Hitachi F-4010 spectrofluorometer using 1-cm pathlength quartz cuvettes. Excitation and emission slits with a nominal bandpass of 5 nm were used for all measurements. Spectra were recorded in the correct spectrum mode. Background intensities of samples were subtracted from each sample spectrum to cancel out any contribution due to the solvent Raman peak and other scattering

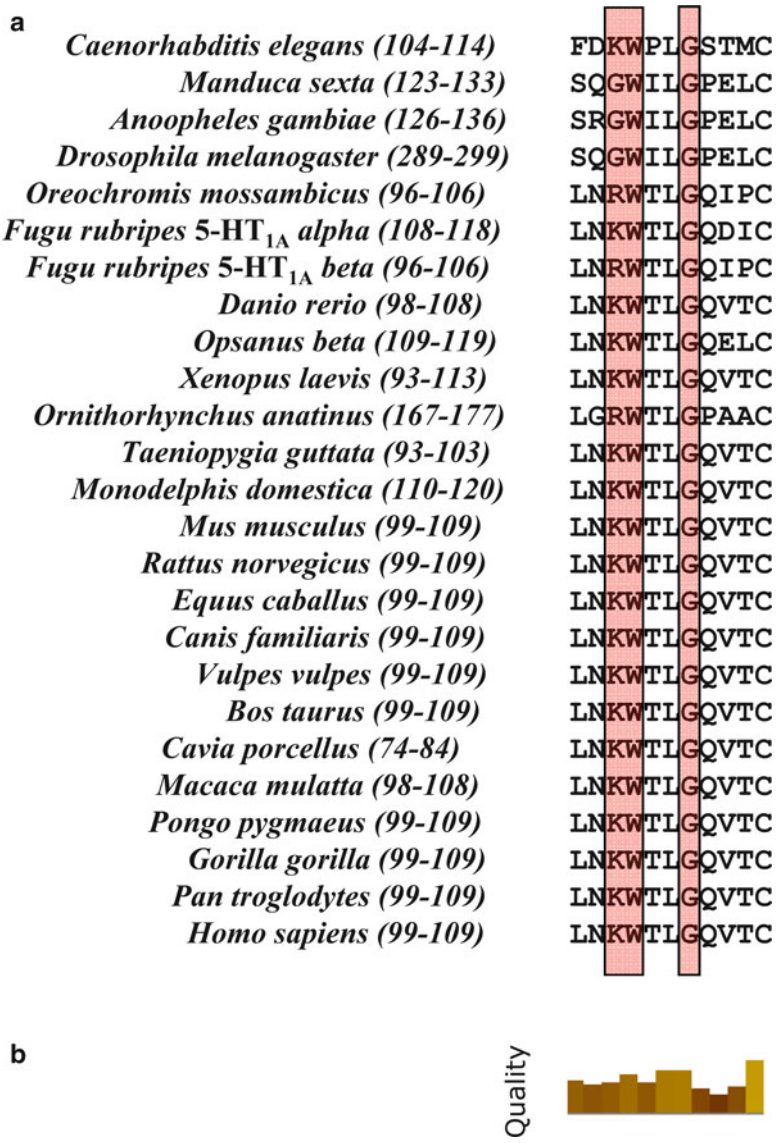

Fig. 19.3 Conservation of the putative sphingolipid-binding domain (SBD) in serotonin$_{1A}$ receptors over natural evolution. *Panel* (**a**) shows the multiple alignment of the serotonin$_{1A}$ receptor from various phyla around the SBD with the conserved residues highlighted. Sequences of *D. melanogaster* and *O. anatinus* were truncated at the N-terminal end, as they did not align to any other sequence, and the sequence of *C. porcellus* is partial. Important residues characteristic of SBD [i.e., K (basic), W (aromatic), and G (turn inducing)] are *highlighted* and appear to be conserved in most species. The positions of amino acid residues are marked in *parentheses* for various species. *Panel* (**b**) is a graphical representation displaying the quality of alignment, with lighter shades representing higher quality. See text for details

artifacts. Fluorescence anisotropy measurements were performed at room temperature (~23°C) using a Hitachi polarization accessory. Anisotropy values were calculated from the equation (Lakowicz 2006):

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}, \tag{19.1}$$

where I_{VV} and I_{VH} are the measured fluorescence intensities (after appropriate background subtraction) with the excitation polarizer oriented vertically and the emission polarizer vertically and horizontally oriented, respectively. G is the grating correction factor that corrects for wavelength-dependent distortion of the polarizers and is the ratio of the efficiencies of the detection system for vertically and horizontally polarized light, and is equal to I_{HV}/I_{HH}. All experiments were done with multiple sets of samples and average values of anisotropy are shown in Fig. 19.5a.

Time-resolved fluorescence measurements. Fluorescence lifetimes were calculated from time-resolved fluorescence intensity decays using IBH 5000F NanoLED equipment (Horiba Jobin Yvon, Edison, NJ) with DataStation software in the time-correlated single photon counting mode. A pulsed light-emitting diode (LED) (NanoLED-15) was used as an excitation source. This LED generates optical pulse at 275 nm of pulse duration 750 ps and is run at 1 MHz repetition rate. The LED profile (instrument response function) was measured at the excitation wavelength using Ludox (colloidal silica) as the scatterer. To optimize the signal-to-noise ratio, 10,000 photon counts were collected in the peak channel. All experiments were performed using emission slits with a bandpass of 6 nm. The sample and the scatterer were alternated after every 5% acquisition to ensure compensation for shape and timing drifts occurring during the period of data collection. This arrangement also prevents any prolonged exposure of the sample to the excitation beam thereby avoiding any possible photodamage of the fluorophore. Data were stored and analyzed using DAS 6.2 software (Horiba Jobin Yvon, Edison, NJ). Fluorescence intensity decay curves so obtained were deconvoluted with the instrument response function and analyzed as a sum of exponential terms:

$$F(t) = \sum_i \alpha_i \exp(-t/\tau_i) \tag{19.2}$$

where $F(t)$ is the fluorescence intensity at time t and α_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i such that $\Sigma_i \alpha_i = 1$. The program also includes statistical and plotting subroutine packages (O'Connor and Philips 1984). The goodness of the fit of a given set of observed data and the chosen function was evaluated by the χ^2 ratio, the weighted residuals (Lampert et al. 1983), and the autocorrelation function of the weighted residuals (Grinvald and Steinberg 1974). A fit was considered acceptable when plots of the weighted residuals and the autocorrelation function showed random deviation about zero with a minimum χ^2 value not more than 1.5.

Intensity-averaged mean lifetimes <τ> for triexponential decays of fluorescence were calculated from the decay times and pre-exponential factors using the following equation (Lakowicz 2006):

$$\langle \tau \rangle = \frac{\alpha_1 \tau_1^{\,2} + \alpha_2 \tau_2^{\,2} + \alpha_3 \tau_3^{\,2}}{\alpha_1 \tau_1 + \alpha_2 \tau_2 + \alpha_3 \tau_3}. \tag{19.3}$$

Results and Discussion

Identification of a Putative SBD in the Serotonin$_{1a}$ Receptor

As mentioned earlier, a common SBD has been identified in a number of proteins (such as HIV-1 gp120, Alzheimer's beta amyloid peptide, and the prion protein) which share little sequence homology (Mahfoud et al. 2002). Although the SBD was originally detected by computer-based structure-similarity searches, it is now possible to predict the presence of such domains on the sole basis of the amino acid sequence of the proteins (Chakrabandhu et al. 2008; Fantini et al. 2006). In spite of high sequence variability of SBDs characterized so far, the systematic presence of key amino acid residues belonging to hairpin structures (loops) makes it possible to generate a robust algorithm for the prediction of SBD from protein sequence databases (Fantini et al. 2006). Application of this algorithm to the extracellular loops of the serotonin$_{1A}$ receptor led to the identification of a potential SBD (LNKWTLGQVTC), corresponding to amino acids 99 to 109 (see Fig. 19.1). Interestingly, this specific sequence contains the characteristic combination of basic (Lys-101), aromatic (Trp-102), and turn-inducing residues (Gly-105), usually found in SBDs (Fantini 2003; Snook et al. 2006). In addition, this motif (SBD) includes Asn and Gln residues, known to be crucial for the binding of cholera toxin B subunit to GM$_1$ (Merritt et al. 1994). These residues are also present in GM$_1$-binding peptides selected by phage library screening (Matsubara et al. 1999). Taken together, these features show that the 99–109 region of the serotonin$_{1A}$ receptor could represent a potential SBD.

Sequence Alignment of SBD Among Serotonin Receptor Subtypes

Since the various subtypes of G-protein coupled serotonin receptors share considerable sequence homology (Hoyer et al. 2002), we explored the presence of similar SBD motifs in serotonin receptor subtypes (see Fig. 19.2). The sequence alignments of various subtypes of human serotonin receptors are shown in Fig. 19.2. Multiple alignments were performed with ClustalW using the serotonin$_{1A}$ (5-HT$_{1A}$) receptor sequence as reference. We observed significant variations in the size (from 10 to 19 amino acid residues) and amino acid sequence in the SBD motif in these subtypes. The only fully conserved residue appears to be tryptophan (highlighted in yellow in Fig. 19.2).

SBD Is Conserved Among Serotonin$_{1a}$ Receptors over Natural Evolution

The serotonin$_{1A}$ receptor is an important member of the GPCR superfamily and is estimated to have differentiated ~650 million years ago from the serotonin$_{1A}$ receptor subfamily in the time period during which vertebrates diverged from invertebrates (Peroutka and Howell 1994). In the context of the presence of SBD in the human serotonin$_{1A}$ receptor, we further analyzed whether SBD is conserved during the natural evolution of the receptor. In order to examine the evolution of SBD of the serotonin$_{1A}$ receptor over various phyla, we analyzed amino acid sequences of the serotonin$_{1A}$ receptor from available databases (see Fig. 19.3; the positions of amino acid residues are marked in parentheses for various species). Partial, duplicate, and other nonspecific sequences were removed from the set of sequences obtained. 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. Initial alignment was carried out using ClustalW. Figure 19.3a shows multiple alignments of the serotonin$_{1A}$ receptor from various phyla around the putative SBD with the conserved residues highlighted. It is apparent from this alignment that SBD includes Lys-101, Trp-102, and Gly-105 in the serotonin$_{1A}$ receptor (see Fig. 19.3), and is conserved in most species. The sequences of drosophila (*D. melanogaster*) and platypus (*O. anatinus*) were much longer than the others, prompting us to remove the N-terminus overhang in these sequences. Sequences of *D. melanogaster* and *O. anatinus* were truncated at the N-terminal end, as they did not align to any other sequence, and the sequence of *C. porcellus* is partial. Realignment with ClustalW (after eliminating the relatively divergent parts of the receptor) resulted in conservation of the SBD upto fish and in *C. elegans*. The basic residue of the motif is replaced with glycine in insects, although in *Anopheles*, the basic residue is adjacent to glycine. It therefore appears that putative SBD in serotonin$_{1A}$ receptors is conserved during the course of evolution.

Interaction of the SBD Peptide with Model Membranes

In order to examine whether the peptide corresponding to the putative SBD motif of the serotonin$_{1A}$ receptor could bind membrane glycosphingolipids such as GM$_1$, we carried out experiments with model membranes of POPC and GM$_1$ using the 11-mer SBD peptide. In view of the reported cholesterol-dependent sphingolipid membrane microdomains (Hebbar et al. 2008), we also included cholesterol in samples. The SBD peptide has a single fluorescent residue, Trp-102, and has no other aromatic amino acid residue. This makes the sole tryptophan residue a useful probe to study the interaction of the peptide with membranes. Intrinsic tryptophan fluorescence of peptides and proteins offers a convenient handle to monitor such interactions (Chattopadhyay and Raghuraman 2004). The maximum of fluorescence emission of

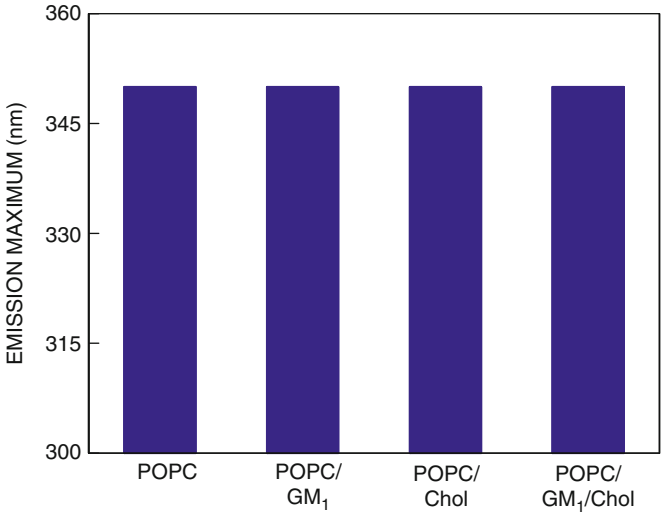

Fig. 19.4 Emission maxima of SBD peptide in membranes (LUVs) of varying lipid composition. The ratio of SBD peptide/POPC was 1:50 (mol/mol) and the concentration of SBD peptide was 4.27 μM. The excitation wavelength was 280 nm in all cases. Data shown are representative of at least three independent measurements. See Experimental Section for other details

the tryptophan residues in SBD peptide in buffer is 350 nm. This emission maximum is consistent with the aqueous exposed environment of the tryptophan residue in the SBD peptide (Lakowicz 2006). The emission maximum (see Fig. 19.4) or fluorescence intensity (not shown) of the SBD peptide did not exhibit any shift in presence of POPC LUVs and remained at 350 nm. In addition, the emission maximum did not exhibit any shift even when the lipid compositions of LUVs were changed to POPC/cholesterol, or POPC/GM_1, or POPC/cholesterol/GM_1. Figure 19.5a shows the steady-state fluorescence anisotropy of the tryptophan residue of the SBD peptide in LUVs of varying lipid composition. As is apparent from the figure, there is no significant change in anisotropy values under these conditions. Fluorescence lifetime serves as a faithful indicator of the local environment in which a given fluorophore is placed (Prendergast 1991). Fluorescence lifetimes of the tryptophan residue of the SBD peptide in LUVs of varying lipid composition are shown in Table 19.1. All fluorescence decays could be fitted with a triexponential function. We chose to use the intensity-averaged mean fluorescence lifetime as an indicative parameter since it is independent of the method of analysis and the number of exponentials used to fit the time-resolved fluorescence decay. The mean fluorescence lifetime was calculated using (19.3) and shown in Fig. 19.5b. The figure shows that the mean fluorescence lifetime of tryptophans in the SBD in POPC LUVs is ~3.27 ns. The mean fluorescence lifetime of SBD does not change significantly in presence of POPC/GM_1 LUVs (~3.22 ns). The mean fluorescence lifetime changes to 2.92 and 2.89 ns, in presence of POPC/cholesterol and POPC/cholesterol/GM_1 LUVs, respectively. It is difficult to pinpoint a specific reason for changes in fluorescence lifetime, since it is sensitive to

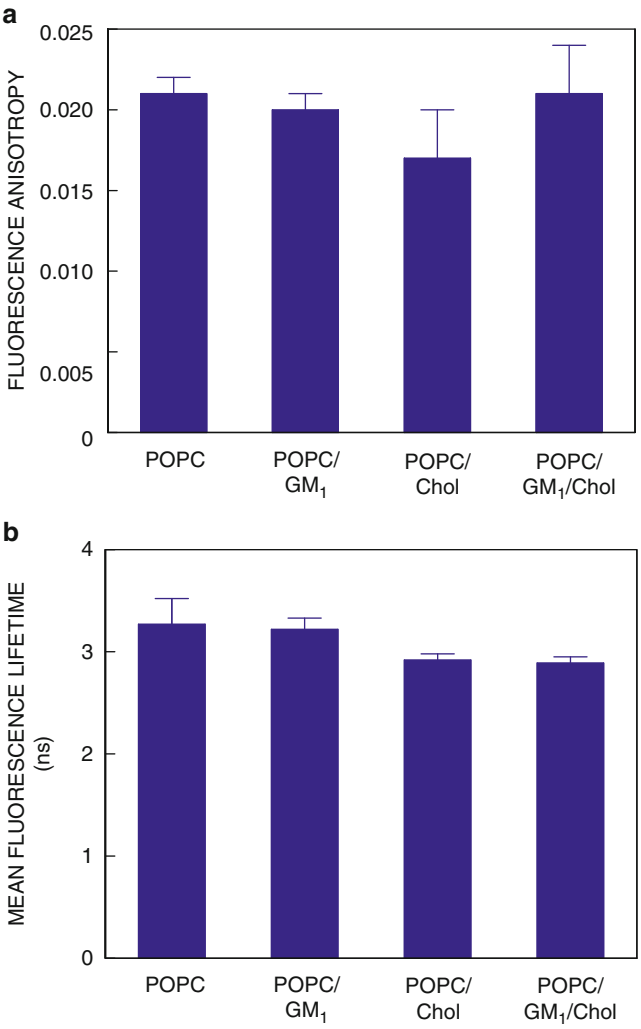

Fig. 19.5 (**a**) Fluorescence anisotropy of SBD peptide in membranes (LUVs) of varying lipid composition. The excitation wavelength was 280 nm, and emission was monitored at 350 nm. Measurements were carried out at room temperature (~23°C). Data shown are means ± SE of at least three independent measurements. (**b**) Mean fluorescence lifetimes of SBD-peptide in membranes (LUVs). Mean fluorescence lifetimes were calculated using (19.3). The excitation wavelength was 275 nm corresponding to pulsed light-emitting diode source, and emission was monitored at 350 nm. Data shown are means ± SE of at least three independent measurements. All other conditions are as in Fig. 19.4. See Experimental Section for other details

a number of factors (Berezin and Achilefu 2010). Taken together, these results indicate lack of appreciable binding of the isolated 11-mer SBD peptide to membranes, monitored utilizing intrinsic tryptophan fluorescence.

Table 19.1 Representative fluorescence lifetimes of SBD-peptide in presence of membranes[a]

Condition	α_1	τ_1	α_2	τ_2	α_3	τ_3
POPC	0.15	0.53	0.77	2.40	0.08	6.40
POPC + GM_1	0.12	0.34	0.70	2.07	0.18	4.86
POPC + Chol	0.15	0.34	0.67	2.08	0.18	4.61
POPC + GM_1 + Chol	0.13	0.33	0.72	2.10	0.15	4.71

[a]Excitation wavelength was 275 nm corresponding to pulsed light-emitting diode source, and emission was monitored at 350 nm. The ratio of SBD peptide/POPC was 1:50 (mol/mol) and the concentration of SBD peptide was 4.27 μM. See Experimental Section for other details

Previous work from our laboratory has shown the crucial requirement of membrane cholesterol in the organization and function of the serotonin$_{1A}$ receptor (Kalipatnapu and Chattopadhyay 2007; Paila et al. 2008; Paila and Chattopadhyay 2010; Pucadyil and Chattopadhyay 2004). In this context, we recently reported the presence of cholesterol recognition/interaction amino acid consensus (CRAC) motifs in the serotonin$_{1A}$ receptor (Jafurulla et al. 2011). The CRAC motif represents a characteristic structural feature of proteins that are believed to result in preferential association with cholesterol (Li and Papadopoulos 1998; Epand 2006). It is defined by the presence of the pattern –L/V–$(X)_{1–5}$–Y–$(X)_{1–5}$–R/K–, in which $(X)_{1–5}$ represents between one and five residues of any amino acid. The serotonin$_{1A}$ receptor sequence contains CRAC motifs in putative transmembrane helices II (residues 90–101), V (residues 208–219), and VII (residues 394–405) (Jafurulla et al. 2011). Interestingly, the SBD motif we propose here has some overlap with the CRAC motif proposed earlier (specifically, in residues 99–101). This is particularly relevant in the context of the reported cholesterol-dependent sphingolipid membrane microdomains (Hebbar et al. 2008). In any event, both cholesterol and sphingolipids are necessary for the function of the serotonin$_{1A}$ receptor and an interplay between these membrane lipids could be significant. In addition, we showed earlier that CRAC motifs are inherent characteristic features of the serotonin$_{1A}$ receptor and are conserved over natural evolution (Jafurulla et al. 2011). Interestingly, our current analysis shows that the SBD motif is conserved over natural evolution across various phyla in serotonin$_{1A}$ receptors. However, experiments with the 11-mer SBD peptide in model membranes utilizing intrinsic tryptophan fluorescence did not show significant binding, probably highlighting the importance of the overall "context" of the receptor architecture in lipid–GPCR interactions. Future studies with synthetic peptides encompassing both the CRAC domain and the putative SBD of the serotonin$_{1A}$ receptor (e.g., the 20-mer LPMAALYQVLNKWTLGQVTC) could provide further insight in this regard. Nonetheless, our results of the presence of SBD in serotonin$_{1A}$ receptors is in overall agreement with previous literature in which copatching of a fraction (~30%) of the serotonin$_{1A}$ receptor with GM_1 was reported (Renner et al. 2007).

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