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Solution NMR studies reveal the location of the second transmembrane domain of the human sigma-1 receptor

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ABSTRACT

The sigma-1 receptor (S1R) is a ligand-regulated membrane chaperone protein associated with endoplasmic reticulum stress response, and modulation of ion channel activities at the plasma membrane. We report here a solution NMR study of a S1R construct (S1R(Δ35)) in which only the first transmembrane domain and the eight-residue N-terminus have been removed. The second transmembrane helix is found to be composed of residues 91–107, which corresponds to the first steroid binding domain-like region. The cytosolic domain is found to contain three helices, and the secondary structure and backbone dynamics of the chaperone domain are consistent with that determined previously for the chaperone domain alone. The position of TM2 provides a framework for ongoing studies of S1R ligand binding and oligomerisation.

1. Introduction

The sigma-1 receptor (S1R) is a membrane chaperone protein present varyingly in both the endoplasmic reticulum (ER) and plasma membranes, where it functions as an accessory protein to a number of ion channels and receptors [1–3]. S1R has been observed to modulate the activity of several ion channels including IP3 receptors [2,4] and voltage-gated channels selective for potassium [5,6], sodium [7], and calcium [8]. S1R has also been shown to interact with acid-sensing ion channels [9], glutamate receptors [10], and dopamine receptors [11]. S1R is highly expressed in the central nervous system (CNS), primarily in the cerebral cortex, hippocampus and cerebellar Purkinje cells [12,13], and binds a large number of small molecules (opiates, antipsychotics, antidepressants, antihistamines, phencyclidine-like compounds, β-adrenergic receptor ligands, cocaine, dimethyltryptamine, progesterone, and sphingosine), many of which have been shown to modulate the effect of S1R on receptors and ion channels (reviewed in [14,15]). Thus, S1R is a potential therapeutic target in the treatment of a range of diseases of the CNS, including schizophrenia, Alzheimer's and Parkinson's diseases, amnesia, depression, amyotrophic lateral sclerosis and addiction.

S1R contains two transmembrane domains connected by a cytosolic domain, and an ER-accessible C-terminal chaperone domain [1,6]. Based on homology to the steroid binding regions of fungal sterol C7–C8 isomerases, the regions from residues 91–109 and 176–194 have been termed Steroid Binding Domain Like I and II (SBDLI and SBDLII, respectively), and shown by mutagenesis and chemical derivatisation studies to be located within the S1R ligand binding site [16,17].

In addition, several residues immediately N-terminal to SBDL1 have been implicated in S1R oligomerisation, including a five amino acid sequence (GGWMG; residues 87–91) proposed to contain a glycophorin A-like GxxxG intramembrane dimerisation motif [18,19]. S1R oligomerisation is ligand dependent and may provide a mechanism by which its activity is regulated [18,20]. An oligomerisation interface at or near SBDLI would provide an obvious structural link to drug binding.

Whereas SBDLII is centered on helix 3 of the membrane associated chaperone domain [17,21], the structure of the region encompassing SBDLI and its relationship to the membrane is not known. Sequence-based predictions of transmembrane helices indicate that SBDLI will have at least some overlap with the second transmembrane helix (TM2). However, these predictions do not converge on a single position for TM2 (Table 1). Of the algorithms tested, some indicate the presence of a transmembrane helix between residues ~80–100, whereas others locate it between residues ~90–110, and at least one algorithm fails to confidently identify any transmembrane helix in this region.

Because of the importance of SBDLI and the adjacent region in ligand binding and receptor oligomerisation, defining the residues that constitute TM2 can shed light on the structural link between

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these two features of S1R function. We previously showed that the S1R chaperone domain could be reconstituted into DPC detergent micelles as a monomeric species capable of binding the ER chaperone BiP [21]. The S1R chaperone domain was found to contain five helices (H1–H5) and a flexible internal region of ~30 amino acids containing at least two regions with propensity to adopt an extended conformation. The fourth helix in the chaperone domain is amphipathic and likely drives its association with membranes. Here we report solution NMR studies of a novel S1R construct (S1R(Δ35)) in which the first transmembrane domain has been removed, enabling a description of the residues within the TM2 helix and the secondary structure of the cytosolic domain. We show that the topology and backbone dynamics of natively purified S1R(Δ35) is consistent with that determined previously for the chaperone domain [21], that TM2 consists of residues 91–107, and that the cytosolic region contains three helices (cH1–cH3). Identification of the TM2 residues provides a framework for further studies of S1R ligand binding and oligomerisation.

2. Materials and methods

2.1. Protein sample production

An ACA-free gene construct (GeneArt) containing an N-terminal $(His)_6$-tag or a $(His)_9$-tag, a Factor Xa cleavage site, and residues 36–223 of human S1R was subcloned into the pCOLD-I vector (Takara) and confirmed by sequencing. The substitution C94A, shown to have no effect on ligand binding in full-length guinea pig S1R [17], was introduced to prevent intermolecular disulfide bond formation during purification. The N-terminal sequence preceding the S1R residues 36–223 was MNHKVHHHHHHIEGRHM or MNHKVHHHHHHHHHHIEGRHM. The S1R(Δ35) plasmid and a pMazF plasmid containing the gene for the RNA interferase MazF (Takara) were transformed into C43(DE3) cells. Transformed cells were grown to an OD_{600} of 0.8–0.9, cold shocked on ice, and incubated for 45 min at 15 °C. Cells were pelleted and washed with M9 salt solution, pelleted a second time, and then resuspended into a 10-fold lower volume of isotopically labeled media. Cells were incubated for a further 45–60 min at 15 °C before induction with 2.5 mM IPTG. Expression proceeded for 16 h at 15 °C. Membranes were collected by centrifugation at 200000×*g*, and incubated overnight in a solution containing 6 M guanidine, 200 mM NaCl, 1% Triton, and 20 mM Tris at pH 8.0. S1R(Δ35) was separated by Ni affinity chromatography and dialyzed against water to remove guanidine. The precipitated protein was resolubilized in hexafluoro-2-propanol and purified by HPLC on a C3 reverse phase column over a gradient from buffer A (95% water, 5% acetonitrile, 0.1% trifluoroacetic acid) to buffer B (57% 2-propanol, 38% acetonitrile, 5% water, 0.1% trifluoroacetic acid). Fractions containing S1R(Δ35) were pooled and lyophilized. For the native purification, the resuspended membranes were incubated overnight with 1% dodecylphosphocholine (DPC). The solubilized membranes were purified by Ni affinity chromatography in 20 mM DPC and subsequently gel filtrated into a low DPC concentration (3.3 mM) in a Superdex200 column (GE). Both methods yielded approximately 40 mg of pure protein per liter of labeled media. The lipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was added from powder to the sample to a *q* ratio of 0.1, where *q* = [lipid]/[detergent].

Table 1
Sequence-based predictions of S1R transmembrane domains.

Algorithm	TM1 (residues)	TM2 (residues)	Refs.
DAS	12–29	99–107	[34]
HMMTOP	13–37	83–107	[35]
Membrain	8–27	89–108	[36]
MEMSAT-SVM	15–30	91–106	[37]
PHOBIUS	9–30	89–111	[38]
PRED-TMR2	9–30	81–100	[39]
SOSUI	13–34	86–108	[40]
SPOCTOPUS	9–29	88–108	[41]
TMHMM	9–31	89–111[a]	[42]
TMMOD	12–32	Not predicted	[43]
TMpred	9–28	81–101	[44]

[a] TMHMM indicated an increased transmembrane helix probability for these residues, but did not identify it as a transmembrane domain.

2.2. Circular dichroism

Samples for circular dichroism (CD) contained 12.6 or 7.3 μM protein of S1R(cd) or S1R(Δ35), respectively, in DPC:DPPC mixed micelles at a *q* ratio of 0.1, and 20 mM potassium phosphate at pH 6.5. CD spectra were collected at room temperature on a Jasco J-815 circular dichroism spectropolarimeter from 250 to 200 nm with 10 accumulations. Data were smoothed with a Savitsky–Golay filter [22] using a window of 11 points and then corrected for concentration and number of residues.

2.3. NMR spectroscopy and data analysis

NMR experiments for resonance assignments were recorded on ^{2}H, ^{15}N, and ^{13}C labeled samples in DPC:DPPC mixed micelles at a *q* ratio of 0.1, in 20 mM potassium phosphate at pH 6.5. Spectra were recorded at 600 MHz (^{1}H) on a Bruker Avance III HD spectrometer equipped with a TCI CryoProbe at 41 °C. Backbone resonance assignments were obtained for 160 of the 181 non-proline S1R residues using a conventional set of TROSY-based experiments (HNCA, HNCACB, CBCA(CO)NH and HNCO) collected with non-uniform sampling (NUS). For NUS, random sampling schedules were used, with typically 15% of the total number of points collected. Spectra were reconstructed with compressed sensing using qMDD [23], processed using NMRPipe [24], and analyzed using NMRView [25]. The assigned chemical shifts have been desposited to the BMRB with accession code 25410.

The secondary structure of S1R(Δ35) was predicted from backbone $^{1}H_N$, ^{15}N, ^{13}C, $^{13}C\alpha$ and sidechain $^{13}C\beta$ chemical shifts using TALOS-N [26]. ^{15}N R_1, R_2 and ^{1}H–^{15}N heteronuclear NOE values were measured with TROSY-based sequences collected on a 0.4 mM ^{15}N, ^{13}C-labeled S1R(Δ35) sample at 600 MHz using the following relaxation delays: 10, 175, 350 and 525 ms (R_1), and 0, 17, 51 and 85 ms (R_2). The recycle delays for R_1, R_2 and heteronuclear NOE experiments were 1.5 s.

3. Results

The S1R(Δ35) construct contains the cytosolic domain, the region of the second transmembrane domain, and the chaperone domain, but lacks the first transmembrane domain (predicted in residues 9–30) and approximately eight lumenal, N-terminal residues. S1R(Δ35) was reconstituted for NMR studies either from inclusion bodies or from the *Escherichia coli* membranes (Fig. 1A). Well resolved backbone amide NMR spectra of S1R(Δ35) could be obtained in mixed micelles containing the detergent DPC and relatively small amounts of the lipid DPPC. By contrast, a subset of the resonances for S1R(Δ35) in DPC alone were weak or missing, indicating conformational exchange. The assigned backbone amide resonances are shown in Fig. 1B. No significant chemical shift differences were observed between reconstituted S1R(Δ35) purified from membranes or inclusion bodies. The effective size of the protein in DPC micelles was assessed by SEC-MALS and TRACT [27]