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Conformational Flexibility in the Transmembrane Protein TSPO

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Abstract

The translocator protein TSPO is an integral membrane protein that interacts with a large variety of endogenous ligands such as cholesterol and porphyrins, and is also the target for several small molecules with substantial *in vivo* efficacy. In complex with the TSPO-specific radioligand (R)-PK11195, TSPO folds into a rigid five-helix bundle. However, little is known about the structure and dynamics of TSPO in the absence of high-affinity ligands. Using NMR spectroscopy we show that TSPO exchanges between multiple conformations in the absence of (R)-PK11195. Extensive motions on time scales from pico-to-microseconds occur across all of TSPO's primary sequence and lead to a loss of stable tertiary interactions and the local unfolding of helical structure in the vicinity of the ligand-binding site. The flexible nature of TSPO highlights the importance of conformational plasticity in integral membrane proteins.

Keywords

NMR spectroscopy; membrane protein; dynamics; function

Introduction

Proteins in membranes allow signaling between the interior and exterior of organelles and cells[1]. They thus play an essential role for cellular function and are prime drug targets[2]. In order to allow signal transduction and trafficking of compounds, many transmembrane helical proteins are constitutively active such that binding of small molecules can activate distinct molecular pathways[3]. This exquisite versatility suggests that helical membrane

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Author Contributions

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Competing Financial Interests

The authors declare no competing financial interests.

proteins exist in a multitude of distinct conformations and their efficacy is tightly connected to the underlying dynamic properties[4]. Little is known, however, about the extent and nature of the intrinsic conformational plasticity of helical membrane proteins[5].

The translocator protein (TSPO) was first described as a peripheral receptor for benzodiazepines[6]. TSPO is localized primarily in the mitochondrial membrane of steroid-synthesizing cells[7], and plays an important role in cellular respiration[8], stress adaptation[9] and apoptosis[10]. TSPO expression is up-regulated in activated microglia found in areas of brain injury and neuroinflammation[11]. The protein interacts with a large variety of ligands such as cholesterol, porphyrins and endozepines [11a, 12]. In addition, several small molecules have been developed that are selective for TSPO [13], bind to sites of central nervous system pathology and have substantial *in vivo* efficacy in animal models of neurodegeneration [14] and anxiety models in humans [15]. The best known of these is 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide (PK11195), which specifically recognizes TSPO[13b, 16]. The (R)-enantiomer of PK11195, (R)-PK11195, binds with nanomolar affinity to TSPO and is used for positron emission tomography.

Mammalian TSPO contains 169 residues, which are organized into five transmembrane helices[6, 17]. We previously determined the 3D structure of mouse TSPO (mTSPO) in complex with (R)-PK11195 using NMR spectroscopy[18]. In the complex structure, (R)-PK11195 is tightly bound to a hydrophobic pocket within the helical bundle, which is closed by a long loop[6]. The (R)-PK11195 binding site is distinct from the cholesterol recognition amino acid consensus (CRAC) domain[6, 12a, 19], but overlaps at least partially with that of benzodiazepines[11a]. In complex with (R)-PK11195, the structure of mTSPO is very rigid. In contrast, TSPO appears to be more flexible when no high-affinity ligand is bound[18, 20].

Besides the 3D structure of mammalian TSPO, structures of the bacterial tryptophan-rich sensory proteins from *Bacillus cereus* and *Rhodobacter sphaeroides* have become available[21]. These bacterial tryptophan-rich sensory proteins are homologues to mammalian TSPO, but bind to TSPO-specific ligands such as (R)-PK11195 and cholesterol only with low affinity[21b, 22]. When compared to the 3D structure of mTSPO in complex with (R)-PK11195, the bacterial homologs have the same topology but some helices are rotated (Figure 1). For example, the structures of the bacterial tryptophan-rich sensory proteins strongly differ in the orientation of helices 1 (rotated ~180 degrees between two structures) and 2 (Figure 1b). In addition, some specific NOE contacts were observed in the structure of mTSPO and the protons of the homologous residues are spatially close in the structure of the TSPO-homolog from *Rhodobacter sphaeroides*, while they are distant in the protein from *Bacillus cereus* (Figure 1a). Finally, the structure of the A147T polymorph of mTSPO in complex with (R)-PK11195 is nearly unchanged when compared to the wild-type protein[23], consistent with an unchanged affinity for (R)-PK11195 [24], while the homologous mutation perturbed the structure of the TSPO-homolog from *Rhodobacter sphaeroides* [21b].

To obtain insight the molecular basis for the high-affinity of mammalian TSPO for small molecules from different chemical classes, we here analyze the structural and dynamic

properties of mTSPO. NMR spectroscopy demonstrates that mammalian TSPO exchanges between multiple conformations in the absence of a high-affinity ligand. Extensive motions on time scales from pico-to-microseconds occur across all of mTSPO's primary sequence and lead to a loss of stable tertiary interactions and the local unfolding of helical structure in the vicinity of the ligand-binding site. Based on these data we propose that the conformational plasticity of mammalian TSPO is important for recognition of a wide variety of small molecules.

Results

Mammalian TSPO exchanges between multiple conformations in the absence of a high-affinity ligand

Previously, mTSPO in complex with (R)-PK11195 had been solubilized in foscholine-12[18, 20, 23]. Here we solubilized ligand-free mTSPO in various detergents, from foscholine-10 to foscholine-16 (Figure 2a), which differ in alkyl chain length and aggregation number (Figure 2b). In these detergents, main chain ^1H - ^{15}N resonances as well as tryptophan side-chain signals of mTSPO clustered together in ^1H - ^{15}N correlation spectra. The largest number of cross-peaks was observed for foscholine-12 and foscholine-14, while the spectrum of mTSPO in foscholine-10 had the lowest quality (Figure 2a). However, even for foscholine-12 only about 56% of ^1H - ^{15}N resonances of mTSPO were detected, which are expected on the basis of its primary sequence. Low NMR signal dispersion was previously also observed for mTSPO in n-dodecyl- β -D-maltopyranoside(DDM)[25].

Next, we prepared ^{15}N -labeled material of human TSPO. The primary sequence of human TSPO has an identity of 82% (similarity of 86%) with that of the mouse protein (Figure 3a). Most sequence changes are located in the N-terminal 25 residues. Because the spectra of mTSPO had the best quality in foscholine-12, human TSPO was solubilized in this detergent. Figure 3b shows the ^1H - ^{15}N heteronuclear single quantum coherence (HSQC) spectrum of human TSPO. Both backbone and side-chain ^1H - ^{15}N resonances clustered together and only a fraction of the expected cross-peaks was detected. The low chemical shift dispersion and the absence of many expected cross-peaks indicated that the backbone of mammalian TSPO exchanges between multiple, distinct conformations when no high-affinity ligand is present. Because many ^1H - ^{15}N signals were broadened beyond detection, the exchange between these conformations is rather slow (intermediate on the NMR time scale). Consistent with conformational exchange on multiple time scales, the spectral quality of ^1H - ^{15}N HSQC spectra was dependent on temperature (see Figure 1 in the Supporting Information). The best signal-to-noise and the largest number of cross-peaks was detected at 42 °C, the highest tested temperature, where molecular tumbling is fastest and therefore relaxation losses minimized.

mTSPO lacks distinct tertiary interactions

Figure 4a shows the distribution of ^1H and ^{15}N line-widths against the intensity of the corresponding ^1H - ^{15}N cross-peaks. mTSPO residues differed widely in both their intensities and line-widths. This is a clear sign of distinct degrees of mobility across the mTSPO primary sequence. In contrast, when (R)-PK11195 was bound to mTSPO, NMR signal

intensities and line-widths were more uniform (Figure 4a). In addition, only a single set of NMR resonances was observed for mTSPO in complex with (R)-PK11195, while several residues (see for example the glycine residues in Figure 4b) showed multiple ^1H - ^{15}N resonances in the absence of (R)-PK11195. The presence of multiple NMR resonances for a single residue provided direct evidence for the presence of distinct slowly exchanging conformations in mTSPO (Figures 4b and 4c). Notably, average values for the NMR line-widths across all observed residues were similar in the absence and presence of (R)-PK11195. This suggests that the oligomerization state does not differ. Indeed, we previously showed that mTSPO in complex with (R)-PK11195 is monomeric in foscholine-12,[18] consistent with the binding of a mammalian TSPO monomer to drug ligands[19].

NMR resonances of methyl protons are highly sensitive reporters for folding of proteins as well as protein-protein interactions. Figure 4d compares the ^1H - ^{13}C HSQC region of the methyl protons of mTSPO in the absence and presence of (R)-PK11195. In the presence of (R)-PK11195 several resonances, such as those of L37, I52 and L56, were shifted upfield, demonstrating that these side-chain protons are involved in specific interactions. In contrast, methyl protons of mTSPO in the absence of (R)-PK11195 clustered together. The data show that mTSPO in the absence of a high-affinity ligand lacks many of the specific tertiary interactions, which are characteristic for the rigid mTSPO/(R)-PK11195 complex.

Secondary structure of mammalian TSPO in the absence of (R)-PK11195

Can we better understand what the secondary structure of mTSPO in the absence of a high-affinity ligand is and which parts are most strongly affected by conformational changes? To answer these questions, we aimed to determine the sequence-specific assignment of mTSPO. Because of the extensive conformational heterogeneity this is a difficult task. To overcome this challenge, we recorded a variety of multidimensional NMR experiments (Figure 5a). In addition, we made use of the sequence-specific assignment of mTSPO in complex with (R)-PK11195 [18], and the fact that $\text{C}\alpha/\text{C}\beta$ chemical shifts mostly depend on secondary structure and are less influenced by changes in tertiary structure. In this way, we sequence-specifically assigned 87 mTSPO residues, for which at least one cross-peak was observed in the ^1H - ^{15}N HSQC spectrum (Figure 4). The assigned residues were not distributed uniformly along the mTSPO primary sequence. In the transmembrane helix (TM) 1 and the long TM1-TM2 loop all residues were detected. In addition, several residues in the center of TM2 were present in ^1H - ^{15}N HSQC spectra, as well as residues from the cytosolic side of TM2, the TM2-TM3 loop, the cytosolic half of TM3 and the cytosolic end of TM5 (Figures 5b and 5c). In contrast, conformational exchange broadened most residues of the transmembrane regions TM4 and TM5 beyond detection in ^1H - ^{15}N HSQC spectra.

To quantify the amount of molecular motions in TSPO, we performed NMR relaxation measurements[26]. Heteronuclear NOEs in the transmembrane regions of mTSPO in complex with (R)-PK11195 reached 0.65-0.82, in agreement with its rigid nature (Figure 6a). In contrast, the average value in the five transmembrane regions was only 0.52 when no ligand was present. The low values of the heteronuclear NOE across the primary sequence of mTSPO indicates that all regions of mTSPO, including the transmembrane helices, are affected by extensive pico-to-nanosecond motions. In addition, residues for which multiple

cross-peaks were observed in triple-resonance experiments (G10, G19, G28 (all TM1), G73 (TM2-TM3 loop) and G87 (TM3); Figures 5b and 5c) exchanged on even slower time scales. Notably, some of these minor conformations were more helical by ~3-7% according to the magnitude of their chemical shifts and thus closer to the ligand-bound state.

Circular dichroism previously showed that mTSPO reconstituted in foscholine-12 has a helical content of ~45% when no ligand is bound[20]. In agreement with a high helical content, the chemical shifts of the residues, which were detected in the transmembrane domain, predicted a predominantly helical structure (Figure 5c). In addition, the TM1-TM2 loop, which closes the (R)-PK11195 binding pocket, retained its helix conformation despite the missing ligand. At the same time, however, there were clear differences in the secondary structure of mTSPO in the absence and presence of (R)-PK11195 (Figure 5c). On average, the helical content was decreased by about 10-20% in TM3 and for individual residues it was as low as ~50%. In addition, residues ⁹⁸IFF¹⁰⁰ at the cytosolic end of TM3 were disordered, while they fold into a helical turn when (R)-PK11195 is bound. Besides for TM3, helical structure was not rigidly formed in two other regions of mTSPO. One is TM1, where the transmembrane helix was shortened at the N-terminus and where the linker between TM1 and the short helix, which is located in the TM1-TM2 loop and is indirect contact with (R)-PK11195 in the complex[18], was more flexible. The second one was the C-terminal end of TM5. In the absence of (R)-PK11195 the TM5 helix was shortened by about three residues (N158-G160), as evidenced by both carbon chemical shifts (Figure 5c) and the loss of helix-specific ¹H_N-¹H_N contacts (Figures 6b and 6c). Because many residues in TM4 and TM5 were broadened beyond detection in the ¹H-¹⁵N HSQC spectra, we could not quantify the helical content in these regions. However, the few residues, which were detected there, were in a helical conformation. Together with the observation that addition of PK11195 increased the helical content by only 5-10% [20], the data indicate that most residues in TM4 and TM5 are part of helical structure in mTSPO in the absence of a ligand.

Crystal structures of bacterial homologs provide a glimpse into the conformational ensemble of TSPO

To further understand the structural basis of the conformational flexibility of TSPO, we analyzed the crystal structures of the bacterial homologs of TSPO, the tryptophan-rich sensory proteins from *Rhodobacter sphaeroides* and *Bacillus cereus*[21]. The root-mean-square-deviation (RMSD) between the two bacterial wild-type proteins was 2.65 Å (135 aligned Cα atoms). Comparison of the structures of the bacterial proteins with the structure of the mTSPO/(R)-PK11195 complex, gave RMSD values of 3.30 Å (147 aligned Cα atoms) for *Bacillus cereus* and 3.92 Å (137 aligned Cα atoms) for *Rhodobacter sphaeroides*. It is however important to note that in the crystals formed by the wild-type protein from *Rhodobacter sphaeroides* and its A139T variant, multiple conformations were found (Figure 7a). A wide range of conformations was observed in proximity to the (R)-PK11195-binding pocket (Figure 7b). In particular, the C-terminal end of TM1, the TM1-TM2 loop and the C-terminal ends of TM3 and TM5 varied between different conformers (Figure 7c). Residues ²⁶KPDE²⁹ at the C-terminal end of TM1 were in turn-like structures in the bacterial proteins (Figures 7a, 7b and 7c). In contrast, the homologous residues ²⁶VRGE²⁹ of mTSPO populated a rigid, helical structure in complex with (R)-PK11195 [18], but were

less helical in the absence of (R)-PK11195 (Figure 5c). This suggests that the conformational variability, which was observed in the static structures of the bacterial homologs (Figure 7), is due to the crystallization of distinct conformations of regions, which are dynamic in solution.

Further support for the hypothesis that the different conformations found in the bacterial tryptophan-rich sensory proteins are caused by the crystallization process, is provided by an analysis of the conformations of residues ⁹⁸IFF¹⁰⁰ of mTSPO. These residues are in close proximity to (R)-PK11195 in the complex structure[18], where they fold into a rigid conformation, but are less defined when (R)-PK11195 is absent (Figure 5c). For the homologous region, ⁹⁰VFF⁹², in the tryptophan-rich sensory protein from *Rhodobacter sphaeroides* multiple conformations were observed (Figures 7b and 7c).

The largest conformational excursions in the 3D crystal structures of the bacterial tryptophan-rich sensory proteins were found in TM2 and the TM1-TM2 loop of the wild-type *Rhodobacter sphaeroides* protein (Figures 7c and 7d): TM2 was strongly inclined with respect to the A139T variant, but also with respect to TM2 of the wild-type protein from *Bacillus cereus* (Figure 7d) [21]. In addition, the TM1-TM2 loop could not be detected due to dynamic or static disorder (shown as dashed line in Figure 7d).

Discussion

We showed that mammalian TSPO is flexible in the absence of a high-affinity ligand. Although it retains a high helical content when solubilized in a variety of detergents with distinct properties (Figure 1 and [25]), the tertiary structure is not stable. This suggests that the transmembrane helices of mammalian TSPO move with respect to each other in the absence of high-affinity ligands. In addition, residues that form the ligand binding site are flexible in the absence of (R)-PK11195, but assume a rigid conformation when the ligand is bound (Figure 5). This is most notable for residues ²⁶VRGE²⁹ at the C-terminal end of TM1, which are disordered in the absence of (R)-PK11195, but form a rigid connection to the short cytosolic helix in the TM1-TM2 loop in the mTSPO/(R)-PK11195 complex. The side-chain of V26 of mTSPO is in direct contact with (R)-PK11195 (Figure 1a and [18]), which stabilizes the rigid backbone conformation of ²⁶VRGE²⁹ in the complex. Other residues near the (R)-PK11195 binding site, such as ⁹⁸IFF¹⁰⁰, were also more flexible in the absence of the ligand. In contrast, alanine 8 and isoleucine 66 on the IMS side experienced less perturbation (Figure 4d). The data are therefore best in agreement with a dynamic model of the mTSPO structure, where the structure at the intermembrane side is quite rigid, while that on the cytosolic side is dynamic. Because of this flexibility, a variety of interhelical angles will be populated in the mTSPO ensemble, which leads to strong NMR line-broadening, in particular in transmembrane helices three to five (Figure 5).

Currently, two major ligand-binding sites in the structure of mammalian TSPO have been identified. The binding site, which was observed in the 3D structure of the mTSPO/(R)-PK11195 complex[18, 23], is common to several radioligands according to competition experiments[27]. The second binding site is also located near the cytosolic side of the membrane and is formed by residues 147-159 in transmembrane region five. This second

binding site is used by cholesterol[12a, 19] and small molecules, which have been developed for therapeutic applications and are similar to cholesterol[28]. The two binding sites do not directly overlap, but are spatially close potentially allowing allosteric changes. Our structural analysis of mTSPO in the absence of (R)-PK11195 indeed supports such allosteric changes. Chemical shifts (Figure 5c) and NOE contacts (Figure 6) demonstrated that residues N158-S159, which are part of the cholesterol-binding motif, are disordered in the absence of (R)-PK11195, but fold into a stable helical conformation upon binding of (R)-PK11195. The conformational stabilization of the CRAC motif might then influence the interaction of mammalian TSPO with cholesterol. Because a 3D structure of TSPO in complex with cholesterol is so far not available, we currently do not know if cholesterol binding might vice versa modulate binding of ligands to the (R)-PK11195 binding site.

Is the flexibility of mammalian TSPO, when solubilized in detergent, caused by the known shortcomings of detergents to mimic a real membrane? This is unlikely, because of several reasons. First, NMR spectroscopy showed that the mTSPO structure is highly flexible in a variety of detergents, including detergents such as n-dodecyl- β -D-maltopyranoside, which is widely used for crystallization of integral membrane proteins (Figure 2 and [25]). Secondly, both mouse and human TSPO showed strong conformational exchange broadening (Figure 3). Thirdly, addition of the TSPO-specific ligand (R)-PK11195 to mTSPO, which had been solubilized in foscholine-12, stabilizes a unique, rigid conformation of mTSPO in complex with a single (R)-PK11195 molecule[18], in agreement with a known 1:1 stoichiometry of the interaction[19]. Fourth, solid-state NMR spectra of ligand-free mTSPO reconstituted into liposomes had only low quality in agreement with conformational heterogeneity, but improved spectral properties in the presence of PK11195[25]. Fifth, even in the crystal structures of the bacterial homologs of TSPO strong conformational variability was observed: the orientation of the TM2 helix strongly differs in the TSPO-homolog from *Rhodobacter sphaeroides* when compared to that of *Bacillus cereus* (Figure 1b and Figure 7). Thus, although the type of detergent is likely to influence the time scale and degree of motion, as suggested by differences in NMR spectra in different detergents (Figure 2), the combined data demonstrate that crucial tertiary interactions are missing in mammalian TSPO in the absence of a high-affinity ligand, resulting in large scale motions of the transmembrane helices and the loss of local structure around the ligand-binding sites.

mTSPO solubilized in foscholine-12 is monomeric both in the absence of a high-affinity ligand and when bound to (R)-PK11195 (Figure 4a and [18]). Consistent with the functionally important monomeric state of mammalian TSPO, TSPO binds drug ligands as monomer[19]. At the same time, TSPO can also form covalent and non covalent dimers and higher-order oligomers, although the exact role of TSPO oligomerisation in physiological and pathological conditions is still unknown[29]. In contrast to mammalian TSPO, the tryptophan-rich sensory protein from *Rhodobacter sphaeroides* occurs mostly as dimers[22, 30], and was found in different dimeric and trimeric arrangements in the crystal lattice[21b] (see Figure 2 in the Supporting Information). This suggests that the dynamic properties of TSPO might further – beyond binding of (R)-PK11195 – be tuned by variations in lipid environment[31] and interaction with cellular partners[7a].

Conclusions

Ligand-driven modulation of conformational ensembles plays a key role for recognition of soluble proteins[32] and determines functional versatility of membrane proteins[4a, 5, 33]. For example, major structural rearrangements in G-protein coupled receptors were suggested[4a], and intrinsic motions help multi-drug transporters in recognition and transport of small molecules[4c]. While in many transporters transitions between outward-facing and inward-facing conformations occur, the ligand-free TSPO is best characterized by a broad ensemble of conformations that lacks crucial tertiary interactions. Extensive molecular motion on time scales from pico-to-microseconds occur across all of TSPO's sequence and lead to local unfolding of helical structure in the vicinity of the two distinct binding sites for small molecules and cholesterol. The flexible nature of TSPO highlights the importance of conformational plasticity of integral membrane proteins.

Experimental Section

Protein preparation

Human and mouse TSPO (cDNA clone MGC:6086 IMAGE:3493196) were expressed in *E. coli* in M9 minimal medium with $^{13}\text{C}_6\text{-D-glucose}$ as carbon source and $^{15}\text{N-NH}_4\text{Cl}$ as nitrogen source. For deuteration the minimal medium was supplemented with 100 % D_2O . TSPO was purified and reconstituted in 2 % of either fos-choline-10, fos-choline-12, fos-choline-14 or fos-choline-16 as described previously [18–20].

NMR spectroscopy

NMR measurements were carried at 42 °C (unless otherwise stated) for 0.5–0.9 mM protein solution in 10 mM sodium phosphate buffer, pH 6.0. Spectra were processed with the software TopSpin (Bruker) and analyzed using CARA [34]. For sequence specific backbone assignment, TROSY versions of 3D HNCO, HNCA, HNcoCA, HNcoCACB, HNCACB, and HNCB and 3D ^{15}N -edited NOESY-HSQC [35] were recorded on a 800 MHz Bruker Avance III spectrometer of mTSPO solubilized in 2% ^2H -fos-choline-12 with 7% (v/v) D_2O . Helical propensities based on the backbone chemical shifts H^{N} , C, $\text{C}\alpha$ and $\text{C}\beta$ were calculated using TALOS-N [36]. The temperature dependence of conformational exchange was probed using 2D ^1H - ^{15}N HSQC spectra of mTSPO at temperatures ranging from 42 °C to 5 °C. 2D ^1H - ^{13}C and 2D ^1H - ^{15}N HSQC spectra of mTSPO/(*R*)-PK11195 were recorded on a 900 MHz Bruker Avance III spectrometer. $\{^1\text{H}\}$ - ^{15}N NOE spectra of mTSPO were acquired with a recycle delay of 10 s on a 700 MHz Bruker Avance III spectrometer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) Experimental contacts between transmembrane helices and with (R)-PK11195 in the 3D structure of the mTSPO/(R)-PK11195 complex, which were reported in [18], are indicated. The homologues residues are more than 7 Å apart in the structure of the tryptophan-rich sensory protein from *Bacillus cereus* (PDB id: 4RYQ; [21a]). Orange arrows indicate the displacement of homologous residues from the position expected on the basis of the mTSPO structure. A8, L17, G18, V26, Y65, L89 and W93 are identical in mouse and human TSPO. The lower panel shows the structure of the tryptophan-rich sensory protein from

Rhodobacter sphaeroides (PDB id: 4UC3:A). The positions of several homologues residues are similar to those observed in the 3D structure of the mTSPO/(R)-PK11195 complex. Notably, TM1 of the tryptophan-rich sensory protein from *Rhodobacter sphaeroides* is rotated by ~180 degrees when compared to TM1 in the protein from *Bacillus cereus*. b) Comparison of the 3D structures of the tryptophan-rich sensory proteins from *Bacillus cereus* (green; PDB id: 4RYQ) and *Rhodobacter sphaeroides* (pink; PDB id: 4UC3). The TM1-TM2 loop residues, which were not observed in the 3D structure of the protein from *Rhodobacter sphaeroides*, are indicated by dots. The RMSD over 135 aligned C α atoms ($d < 5 \text{ \AA}$) between the structures of the two bacterial homologs is 2.65 $\text{\AA}$.

Figure 2.

a) ^1H - ^{15}N HSQC spectra of mTSPO in different detergents: fos-choline-10 (Fos-10), fos-choline-12 (DPC), fos-choline-14 (Fos-14) and fos-choline-16 (Fos-16). b) Critical micelle concentrations (CMC) and aggregation numbers of the detergents used for mTSPO.

Figure 3.

a) Alignment of the primary sequences of mouse and human TSPO. b) ^1H - ^{15}N HSQC spectra of human TSPO in fos-choline-12.

Figure 4.

a) Comparison of NMR line-widths and normalized signal intensities in ^1H - ^{15}N correlation experiments of mTSPO in the absence (black) and presence (red) of (R)-PK11195. b) Selected region of ^1H - ^{15}N HSQC spectra of mTSPO in the absence (black) and presence of (R)-PK11195 (red). Without ligand, several residues (marked by *) have multiple signals arising from distinct slowly exchanging conformations. c) Evidence for multiple, slowly exchanging conformations with different helical contents. 3D TROSY HNCA strips of G28 and G83. Minor conformations are marked by *. The upfield shift of the C α resonances of L82 and G83 demonstrates that the minor conformation of transmembrane region 3 is more helical than the major conformation. d) Selected region of ^1H - ^{13}C correlation experiments of mTSPO in the absence (black) and presence (red) of (R)-PK11195. Selected resonance assignments are indicated. The low dispersion of methyl signals of mTSPO in the absence of (R)-PK11195 supports the lack of a stable tertiary structure.

Figure 5.

a) Sequence-specific backbone assignment of mTSPO in the absence of a ligand. 3D TROSY HNCA strips at 315 K of the S16-F25 fragment comprising TM1 and the TM1-TM2 loop. b) Schematic representation of residues detected in ^1H - ^{15}N HSQC spectra of mTSPO. c) Helix content in mTSPO revealing an unstable transmembrane structure when no ligand is present. Helical content was determined from the difference between experimentally observed chemical shifts (black: mTSPO; grey: mTSPO/(R)-PK11195 complex) and random coil values. The schematic representation on top marks the position with stable (mTSPO/(R)-PK11195 complex) or transient (mTSPO) helical structure. Regions of mTSPO represented by white bars were strongly exchange broadened, hashed parts were disordered.

Figure 6.

a) Steady state $\{^1\text{H}\}$ - ^{15}N NOE for backbone amide nitrogens of mTSPO in the absence (black) and presence (grey) of (R)-PK11195. Average errors were determined from the signal-to-noise ratio in the NMR spectra and were 1% and 8% for mTSPO and mTSPO/(R)-PK11195, respectively. b,c) Strips from 3D ^{15}N -edited NOESY-HSQC spectra of (a) 0.9 mM ^2H (~60%), ^{13}C , ^{15}N mTSPO and (b) 0.6 mM ^2H (~60%), ^{13}C , ^{15}N mTSPO/(R)-PK11195, both in ^2H -DPC micelles. Only in complex with the ligand, helix-specific H^{N} - H^{N} NOE contacts were observed for N158-G160. The helical region is marked by a dashed line.

Figure 7.

a) Superposition of the backbone conformers observed in crystals of the tryptophan-rich sensory protein from *Rhodobacter sphaeroides*: chain A of PDB id 4UC3 (wild-type protein; orange), chain A of PDB id 4UC2 (A138T variant in P212121 space group; yellow), chain A (green), B (cyan), C (red) of PDB id 4UC1 (A138T variant in C2 space group). Residues that have the largest pairwise root-mean-square deviation (see panel c) are colored blue. Side-chains of selected residues are shown and labeled. b) View from the cytosol. c) Residue-specific root-mean-square-deviation between different conformers of the tryptophan-rich sensory protein from *Rhodobacter sphaeroides*. The location of helices as found in the bacterial homologs is indicated on top. d) Comparison of the cytosolic side of the 3D structures of mTSPO (black; PDB id: 2MGY), wild-type tryptophan-rich sensory protein from *Rhodobacter sphaeroides* (red; PDB id: 4UC3), A138T variant of the

tryptophan-rich sensory protein from *Rhodobacter sphaeroides* (cyan; PDB id: 4UC1) and wild-type tryptophan-rich sensory protein from *Bacillus cereus* (green; PDB id: 4RYQ). The TM1-TM2 loop was not visible in the crystal of wild-type tryptophan-rich sensory protein from *Rhodobacter sphaeroides* (red; PDB id: 4UC3), but has been indicated by a dotted line for visualization purposes.