Multi-scale molecular dynamics study of the "still" enigmatic protein TSPO

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Translocator protein (TSPO), a mitochondrial membrane protein, has been extensively studied and its functional role is still debated and continues to be enigmatic. While its primarily role could be the cholesterol transport in the mitochondria outer membrane, it is also supposed to be involved in other biological processes, such as steroids biosynthesis or porphyrin transport. In this respect, the protein is now considered as an interesting therapeutic target for Alzheimer's disease, since the cholesterol is a neurosteroids precursor. From a structural perspective, several structures have been already solved. Moreover, the protein is highly dynamic in nature and has been proved to exist in several oligomeric states. Two proteins from bacteria (R. sphaeroides and B. cereus) have been solved by means of X-ray crystallography. The mouse structure, the only solved mammalian form, has been resolved by NMR methodology. However, this last structure was a matter of discussion due to the detergents used. In global, structural information on its implications, mechanism and dynamics along the cholesterol translocation remain scarce and elusive.

In order to gain more insights into this protein, we employed a multi-scale approach based on molecular dynamics (MD) simulations. First, we addressed the oligomerization question by conducting several coarse-grained MD simulations. We considered two different initial configurations: one with pair of TSPO monomers distantly placed in a model of bilayer composed of DMPC/cholesterol mixture (same conditions as in the NMR experiment) and the other with preformed dimer models with different interfaces. Among the entire set of simulations, we observed that not all the dimers were stable, indicating some specificity in dimerization. Furthermore, we identified stable TSPO dimers with high propensity specific interfaces, some of which were consistent with experimental observations. The existence of asymmetric interfaces suggests the potential formation of higher-order oligomers. Refinement of the stable interfaces through all-atoms resolution MD simulations revealed that the dimers formed were highly stable. Importantly, we obtained a detailed view of the molecular mechanism of cholesterol translocation, which involves specific interactions with the protein.

Secondly, because TSPO is known to accommodate a large set of ligands, we explored the molecular mechanism of ligand binding and the impact of ligand binding onto the dynamics of the protein using MD simulations at atomistic resolution. We considered the experimental NMR structure but also, a homology model (HM) of the mouse TSPO, based on the x-ray solved structure of the bacterial TSPOs, in order to assess the impact of the structure quality on the results. Despite having a very similar 3D fold, the calculations revealed that subtle 3D differences affect the protein dynamics: the NMR structure is much more flexible compared to the HM one. Surprisingly, the NMR structure remains dynamic in the presence of the ligand, which is not the case for the HM one. It is so rigid that the ligand becomes unstable and almost leaves the cavity. This sheds light on the versatile dynamics of the protein itself and raises questions about the 3D structure in its proper environment.

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