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Human thrombospondin's (TSP-1) C-terminal domain opens to interact with the CD-47 receptor: A molecular modeling study

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ABSTRACT

Thrombospondin-1 (TSP-1) interaction with the membranous receptor CD-47 involves the peptide RFYVVMWK (4N-1) located in its C-terminal domain. However, the available X-ray structure of TSP-1 describes this peptide as completely buried into a hydrophobic pocket, preventing any interaction. Where classical standard methods failed, an appropriate approach combining normal mode analysis and an adapted protocol of energy minimization identified the large amplitude motions responsible of the partial solvent exposure of 4N-1. In agreement, the obtained model of the open TSP-1 was further used for protein–protein docking experiments against a homology model generated for CD-47. Considering the multiple applications of the CD-47 receptor as a target, our results open new pharmacological perspectives for the design of TSP-1:CD-47 inhibitors and CD-47 antagonists. We also suggest a common opening mechanism for proteins sharing the same fold as TSP-1. This work also suggests the usefulness of our approach in other topics in which predictions of protein–protein interactions are of importance.

Thrombospondin-1 (TSP-1)[1], a multifunctional protein that belongs to the extra-cellular matrix compartment, has been largely involved the last years in the modulation of cell-matrix and cell–cell interactions. By this mean, TSP-1 appears to influence a range of cellular functions such as platelet activation, angiogenesis, wound healing, programmed cell death or tumor progression [1,2]. These biological functions are shared by its multifunctional modular domains capable of binding to multiple cell surface receptors. CD-47, also known as integrin-associated protein (IAP), is a transmembrane receptor whose extra-cellular domain belongs to the immunoglobulin like superfamily and has been identified as a central molecular relay of the TSP-1-mediated response in human cells and tissues. TSP-1/CD-47 interactions exhibited a main role in the regulation of the death pathway [3,4] and have also been reported to interfere with the inflammatory process [5,6] and to inhibit nitric oxide-stimulated vascular smooth muscle cell responses [7]. Interestingly, the biological events mediated by the CD-47 receptor involve the TSP-1 bound peptide: RFYVVMWK (4N-1). Recent studies demonstrated that the 4N-1 peptide is also able to wholly reproduce the TSP-1-mediated biological effects, especially in the control of platelet activation and apoptosis [3,8]. The 4N-1 peptide is located at the C-terminal domain of the whole modular protein TSP-1 [9,10]. As a starting point of this article, we observed that the 4N-1 sequence was totally buried in the available X-ray crystallographic structure of the C-terminal domain of TSP-1 (PDB: 1UX6 [11]), a feature not compatible with its direct role in the TSP-1/CD-47 interaction that requires, at least, its partial exposure to the solvent. Together with molecular dynamics (MD) simulations, normal modes analysis (NMA) belongs to the available techniques able to describe protein dynamics at the atomic level. NMA consists in diagonalizing the Hessian matrix whose elements are the second derivatives of the potential energy function with respect to the mass weighted Cartesian coordinates for a minimum energy structure [12]. Although NMA can unfortunately be used only *in vacuo* or with implicit solvent models, many recent examples show that it is particularly adapted to the identification of functional motions of proteins which would not have been sampled easily by classical MD. For instance, NMA has been recently applied to the homopentameric α7 nicotinic receptor (nAChR) for which the first lowest frequency normal mode was sufficient to gain insight into the first step of the opening mechanism [13]. Otherwise, NMA methods have been recently recognized of interest in refining

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[1] *Abbreviations used:* TSP-1, Thrombospondin-1; IAP, integrin-associated protein; MD, molecular dynamics; NMA, normal modes analysis; nAChR, α7 nicotinic receptor; PME, Particle Mesh Ewald; SD, Steepest Descent; Adopted Basis Newton Raphson; ENM, Elastic Network Model.

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macromolecular structures [14], or ligand–protein [15] and protein–protein [16] complexes. Accordingly, NMA was particularly adapted to the study of the TSP-1 C-terminal domain. NMA is otherwise limited by a quadratic approximation of the potential energy function that preliminarily requires to consider a local energy minimum structure; as a consequence of this approximation, even a small displacement along the computed normal coordinates performed with standard techniques produces a large energy variation and unrealistic motions of the protein. We recently described and validated new approaches which combine NMA and energy minimizations under normal coordinates restraints that allow to generate large sets of conformations of the same protein without these drastic changes in energy [17]. In this study, such a protocol was used to explore the possible conformational changes of the C-terminal domain of TSP-1 and highlight how 4N-1 may be involved in the interaction with the CD-47 receptor.

Material and methods

Simulations

Molecular dynamics simulations and normal modes analysis of the TSP-1 C-terminal domain were performed using the PDB: 1UX6 crystallographic structure [11] as a starting point (1.9 Å resolution). A constant temperature and pressure (300 K; 1 atm.) MD simulation of that protein immersed in a water box was performed with the NAMD software [18] using the CHARMM force field [19,20]. An integration time-step of 1 fs was used, the bonds containing a hydrogen atom being held constant with the SHAKE algorithm. Electrostatics interactions were treated with Particle Mesh Ewald (PME) method [21]. A switching function between 8 and 10 Å was used for the Van der Waals interactions.

NMA was achieved with the DIMB method [22] as implemented in the CHARMM package. Up to 100 lowest frequency normal modes were computed with a convergence criterion of 10^{-6} on eigenvectors using the same force field as for MD simulations. Minimizations under normal mode restraints of the intermediate structures along the first ten internal lowest frequency normal modes were carried out as described previously [17]; to obtain energetically relaxed models of the protein along the normal modes, energy minimization of intermediate structures was achieved using a normal mode restraint potential added to the internal standard potential as described recently in other studies [23] and of the form:

$$\frac{k_{\mathrm{d}}}{2}(d-d_{\mathrm{r}})^2+\frac{k_{\mathrm{trans}}}{2}\left|\mathbf{r}_{\mathrm{CM}}-\mathbf{r}_{\mathrm{CM}}^{\mathrm{r}}\right|^2+\frac{k_{\mathrm{rot}}}{2}\left|\sum_{i=1}^{N}m_i\left(\mathbf{r}_i^{\mathrm{r}}\times(\mathbf{r}_i-\mathbf{r}_i^{\mathrm{r}})\right)\right|^2$$

where in the first term d is a given displacement along the normal mode coordinate corresponding to a given mode of interest, d_{r} the desired restraint distance value and k_{d} the force constant. d is defined by projecting the vector of mass weighted coordinate differences with respect to the energy minimized conformation onto the normal mode vector. The two other terms of the equation are added to prevent global translation and rotation displacements, in which k_{trans} and k_{rot} are the corresponding force constants, $\mathbf{r}_{\mathrm{CM}}$ and $\mathbf{r}_{\mathrm{CM}}^{\mathrm{r}}$ are the Cartesian coordinate vectors of the center of mass of the displaced structure and of the energy minimum structure for which the modes where computed, respectively; $\mathbf{r}_{\mathrm{i}}$ and $\mathbf{r}_i^{\mathrm{r}}$ are the Cartesian coordinate vectors of atom i with respect to the center of mass of the displaced structure and of the minimum energy structure, respectively [23]. The force constants used in the calculations were k_{d} = 10.000 kcal mol^{-1} Å^{-2}, k_{trans} = 1.000 kcal mol^{-1} Å^{-2} and k_{rot} = 10^{-6} kcal mol^{-1} Å^{-4}.

Energy minimizations along a given mode were performed for different d_{r} values (expressed in MRMS with respect to the X-ray energy minimized structure) in the range −2.0 to 2.0 Å with steps of 0.1 Å along both directions. A negative value for the MRMS corresponds to a displacement along the reversed direction of the normal mode vector.

The restraint potential allows to obtain a series of low-energy structures along the normal modes directions without being limited by the harmonic approximation inherent to NMA. The minimizations were carried out by successively combining the Steepest Descent (SD) and Adopted Basis Newton Raphson (ABNR) methods until an energy gradient of 10^{-6} kcal mol^{-1} Å^{-2} was reached.

Motions were analyzed using self-developed programs and figures were generated either with VMD [24] and Tachyon version 0.98 or Pymol (http://pymol.sourceforge.net/).

Building of a CD-47 model and protein–protein docking experiments

The entire extra-cellular part of the human CD-47 receptor was first identified as belonging to the V set domains of the immunoglobulin like superfamily in the SCOP database [25,26] (SCOP code d1pkoa) with the FUGUE program [27] aimed to predict the 3D fold of proteins from their sequences. Without any doubt on the identified fold (Z score = 16.87), the structure of the extra-cellular domain of CRIg (PDB: 2ICC) was chosen as a template and a multiple sequence alignment including all the known CD-47 sequences in the swissprot database was produced with ClustalW [28]. 200 models of the whole extra-cellular part of the human CD-47 receptor (Swissprot accession number Q08722) were built with the MODELLER software [29] and ranked according to their score, including a disulfide bridge linking Cys23 and Cys96 residues. The model sharing the best score was then selected to realize protein–protein docking experiments toward the open conformation of TSP-1 (MRMS = 2 Å) with the GRAMM-X protein–protein docking server (http://vakser.bioinformatics.ku.edu/resources/gramm/grammx) without including any constraint.

Multiple structures analysis

The SCOP database [25,26] was screened to identify other proteins sharing the same fold as the TSP-1 C-terminal domain (Class: all β proteins; Fold/superfamily: Concanavalin A like lectins/glucanases). The first member of each subsequent family was then downloaded (PDB: 1A8D, 1D2S, 1GBG, 1GV9, 1IKP, 1J1T, 1JHN, 1KQR, 1KRI, 1NLS, 1OQ1, 1Q2B, 1S2B, 1S2K, 1SAC, 1UYP, 1W6N, 1WD4, 1XNB, 2SLI). The proteins were structurally superimposed with VMD [24] and the STAMP algorithm [30]. A 3D statistical analysis of residues distributions was performed with the Volmap facility as implemented in VMD.

Results

Opening of the 4N-1 cleft

To understand how the 4N-1 peptide may be accessible to the exterior and escape the protein, we initially performed an explicit water molecular dynamics simulation (MD) of the whole C-terminal Ca^{2+} bound domain of TSP-1 lasting 12 ns. No opening motion of the interesting region was characterized. This was not surprising as the residues surrounding the 4N-1 peptide were mainly hydrophobic. Opening of a protein cleft during MD on the nanosecond time-scale is usually driven by water molecules and it is difficult in some cases to sample such a large conformational change with