The conformation of the extracellular binding domain of Death Receptor 5 in the presence and absence of the activating ligand TRAIL: A molecular dynamics study

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INTRODUCTION

TRAIL (Tumor necrosis factor Related Apoptosis Inducing Ligand) is an important effector cytokine from the TNF superfamily. It has been shown to selectively induce apoptosis in certain malignant cell lines and HIV infected T-cells, while healthy cells remained unaffected. TRAIL exerts its effect through interaction with receptors belonging to the TNF receptor (TNF-R) superfamily. The interaction sites are formed by two death domain containing receptors, Death Receptor 4 (DR4) and Death Receptor 5 (DR5, also known as Apo2, TRAIL-R2, TRICK-2, and KILLER), which trigger apoptosis through a caspase-dependent pathway. TRAIL mediated apoptosis is regulated by the presence of three antagonistic decoy receptors. The membrane bound decoy receptors DcR1 (TRAIL-R3 or TRID) and DcR2 (TRAIL-R4 or TRUNDD) are present on normal cells while their expression is reduced in certain malignant cell types. The third decoy receptor is the soluble member of the TNF-R superfamily osteoprotegerin (OPG), which is involved in osteoclastogenesis.

The members of the TNF-R superfamily typically are trimeric type I membrane proteins. Their activation was generally believed to be triggered by the attraction and interfacing of three receptor monomers mediated by interaction with the trimeric ligand. This was assumed to lead to the alignment and subsequent interaction of the intracellular domains in turn giving rise to the formation of the intracellular signaling complex by recruitment of downstream effector proteins. In the case of the death receptors the signaling complex is termed the Death Inducing Signaling Complex or DISC, which activates caspases, leading to apoptosis.
Although the mechanism outlined above was widely accepted, it is now clear that several members of the TNF-R superfamily have a propensity to self-associate in the absence of ligand. These receptors may instead reside on the cell surface as preformed complexes, activated by a conformational change or the reorientation of the receptor units rather than merely by crosslinking. They include both TNF receptors (TNF-R1 and TNF-R2), CD40, CD95 (Fas), and Death Receptor 4. In addition, recent results obtained using surface plasmon resonance (SPR) showed that all four receptors for TRAIL can form homodimers and may even form heterodimers, with the exception of the combinations DR5-DcR1 and DR5-DcR2. For TNF-R1, TNF-R2, and CD95, a domain was identified, which mediates the self-association. This domain was termed the preligand association domain and is formed by the membrane distal domain (CRD1). For both TNF receptors this domain consists of two modules, termed A1 and B2, which share a high homology between these receptors. Since CD40 has a homologous domain at the N-terminal, it is expected that this region will also be responsible for self-association of that receptor. The N-terminal cysteine rich domain (CRD) of CD95 only consists of a single B2 module, which apparently is sufficient for self-association. However, in the case of the TRAIL receptors the N-terminal region is formed by a small truncated module, designated N, which is unlikely to mediate self-association.

To gain insight into the self-association of the TRAIL receptors and the mechanism of activation of the Death Receptors, we have examined the case of DR5 binding to TRAIL in detail. TRAIL, together with its receptor DR5, was chosen for these studies, both because of the availability of structures of the complex and because of possible therapeutic interest. The structure of DR5 in complex with TRAIL has been solved by three different methods, with the exception of the combinations DR5-DcR1 and DR5-DcR2. The study provides evidence for the self-association of the unliganded DR5 through the C-terminal cysteine-rich domain of the extracellular region of the receptor, with implications for the mechanism of activation.

**METHODS**

**Starting structures**

Initial structures were based on the available crystal structures (PDB codes 1D4V, 1D0G, and 1DU3). For the simulations of the TRAIL-DR5 complex, the structure given in 1D4V was used as a starting configuration, because this is the only structure with a completely resolved AA-loop in TRAIL. It is worth noting that the residues flanking the AA-loop in structure 1D0G indicate a similar orientation of this loop, whereas in the structure 1DU3 these residues indicate a more extended conformation, lying tighter around TRAIL and having stronger interactions with the receptor units. However,
Self-Association of Death Receptor 5

Simulations

Simulations were performed using the Gromacs package for molecular simulations in conjunction with the GROMOS96 43a2 united atom force field. Water was treated explicitly using the Simple Point Charge (SPC) model. The protonation state of ionizable groups was chosen to correspond to pH 7.0. Counterions were added to compensate the net charge of the system. Non-bonded interactions were evaluated using a twin range cut-off of 0.9 and 1.4 nm. Interactions within the shorter range cut-off were evaluated at every step whereas interactions beyond the longer range cut-off were evaluated every 10 steps. To correct for the neglect of electrostatic interactions beyond the longer range cut-off, a Reaction Field (RF) correction was used with $\epsilon_{RF} = 78.0$. In all simulations the system was kept at a constant temperature of 300 K by applying a Berendsen thermostat. Protein and solvent were independently coupled to the heat bath with a coupling time of 0.1 ps. For the simulations of the receptor trimer the pressure was weakly coupled to a reference pressure of 1 bar using a Berendsen barostat, with a coupling time of 1.0 ps and a compressibility of $4.6 \times 10^{-5} \text{ bar}^{-1}$. Simulations of the receptor monomers and of the TRAIL-DR5 complex were performed at constant volume. The time step used for integration of the equations of motion was 0.002 ps. The bond lengths and angle of the water molecules were constrained using the SETTLE algorithm. Bond lengths within the protein were constrained using the LINCS algorithm except for the simulation of the receptor ligand complex, where SHAKE was used together with an algorithm to remove overall rotational and translational motions.

Analysis

Solvent accessible surface

The solvent accessible surface area (SASA) was determined according to the method of Eisenhaber et al. using a solvent probe radius of 0.14 nm. The classification of hydrophobic atoms was based on an absolute charge less than or equal to $-0.2$ e. The interfacial surface area (ISA), that is the area buried by the interaction of two components A and B in the complex, was determined from the difference between the sum of the
surface area of the separate components and the surface area of the complex:

\[ \text{ISA(AB)} = \text{SASA(A)} + \text{SASA(B)} - \text{SASA(AB)} \]

**Decomposition of the mean square displacement**

The mean square displacement (MSD) for each frame with respect to the starting structure was determined, after performing a least-squares fit of all atoms to the starting structure, according to

\[ \text{MSD}_{\text{Total}} = \frac{1}{M} \sum_{i=1}^{N} m_i (x_i - x_{\text{ref}})^T (x_i - x_{\text{ref}}) \]

where \( m_i \) denotes the mass and \( x_i \) the coordinates of atom \( i \). \( N \) is the number of atoms and \( M \) is the total mass. To determine the extent to which the dynamics of the systems could be expressed in terms of rigid body motions, the total MSD was decomposed into two components: a rigid body term and a residual term. The non-rigid body term or the residual component was defined as the MSD obtained after separately fitting each predefined domain to the corresponding domain in the reference structure, again using an all-atom least-squares fit:

\[ \text{MSD}_{\text{Residual}} = \frac{1}{M} \sum_{i=1}^{N} m_i (y_i - x_{\text{ref}})^T (y_i - x_{\text{ref}}) \]

Here \( y \) denotes the coordinates of atom \( i \) after the fitting of the domains. The contribution of rigid body motions to the total MSD was taken to be the difference between \( \text{MSD}_{\text{Total}} \) and \( \text{MSD}_{\text{Residual}} \).

**Principal components analysis (PCA)**

PCA was used to analyze collective motions in the system. In PCA the fluctuations of the individual particles are described in terms of a set of mutually linearly independent collective fluctuations. To perform the PCA, a positional covariance matrix was constructed,

\[ S = \frac{1}{N} \sum_{k=1}^{N} (x_k - \mu)(x_k - \mu)^T = \frac{1}{N} \sum_{k=1}^{N} x_kx_k^T - \mu\mu^T \]

where \( x_k \) is the positional vector representing the \( k \)th frame from the trajectory and \( N \) is the total number of frames. This matrix was subsequently decomposed into a diagonal matrix \( D \) of eigenvalues and a matrix \( P \) of associated eigenvectors:

\[ S = PD P^T \]

Typically, rotational and translational degrees of freedom are removed from the trajectory before construction of the covariance matrix by performing a least squares fit onto a reference structure. In addition to this standard approach, in this study PCA was also performed in which the least squares fit was performed using one specific domain. This exaggerates the fluctuations of the domains relative to the reference domain and was used to highlight hinge-bending motions between the domains of the extracellular part of the DR5.

To visualize the effect of ligand binding on the extent of motion within the system the trajectories were projected onto a selected set of eigenvectors, revealing the sampling along these vectors. The projection of a frame \( x(t) \) from a trajectory on a set of eigenvectors is given by

\[ p(t) = P^T (x(t) - \mu) \]

In addition, the loadings of individual atoms with a selected set of eigenvectors were also determined. The loadings indicate the extent to which the positional fluctuations of a given particle are described by a specific eigenvector. For each atom the loadings of the \( x, y, \) and \( z \) coordinates were calculated and summed to reveal the direction of the fluctuation along that eigenvector in Cartesian space. A higher value of the loading, represented as the length of the arrow, means the motion of the particle is more strongly correlated to the component specified. The matrix of loadings was calculated according to

\[ A = PD^{1/2} \]

The nature of the collective motions was further analyzed by taking the extreme projections of the trajectories along a selected set of eigenvectors and processing these using the program DynDom.53 DynDom determines the relative motions of domains, calculates hinge axes, and determines hinge-bending residues and rotation angles.

Principal components analysis was performed using the available tools within the Gromacs package and using Matlab.54 The visualization of the results from the principal components analysis was performed using Pymol and POV-Ray.56

**RESULTS AND DISCUSSION**

Self association of the unbound receptors

To investigate the fate of the individual receptors in the absence of TRAIL, a series of simulations of the three receptor units within the 1D0G complex from which TRAIL was removed were performed. A summary of the events observed in the simulations is presented in Figure 2(A), which shows a sequence of stereo images covering the first 5 ns of a simulation in which the three receptor units are constrained to the XY plane to mimic the effect of attachment to the membrane. A similar sequence of events was also observed in two replicate simulations performed with different starting velocities. As can be seen in Figure 2(A),
following the removal of TRAIL from the complex, the three receptor monomers rapidly self-associate. To confirm that this association is driven by interactions between the receptors as shown in Figure 2(A) and is not an artefact due to interactions between periodic images, the minimum distance between receptors in different periodic images was determined. The distance was found to increase from 2.4 to 2.9 nm during the simulations showing that, despite the large changes in the relative orientation of the individual receptors evident in Figure 2(A), there are no interactions between periodic images. The complex obtained in the absence of TRAIL is not well ordered. This may reflect the limited time scale of the simulation. While there is a strong tendency for the receptors to aggregate, 20 ns is not sufficient to allow a system of this size to fully equilibrate. The final structures from the three independent simulations show significant differences in terms of the packing between the receptors and while indicative the final structure shown in Figure 2(A) is expected to rearrange further with time. In fact, as discussed below, in each of the simulations it was seen that initially two of the receptor units interacted with each other with the third unit following slightly later. This leads to an asymmetric arrangement of the receptor units in the aggregate. This is reflected in the distances between the Val138 Cα atoms (an indicator of the relative positions of the C-terminal positions) and is illustrated in Figure 2(B) which gives a bottom view of the aggregate formed in one of the simulations of the unliganded DR5 trimer, showing the relative orientations of the membrane proximal (CRD2) domains.

The aggregation of the receptors is associated with the burial of approximately 5.2 nm² of solvent exposed surface area per interaction interface. The surface buried is predominantly hydrophobic with the ratio of hydrophobic to hydrophilic surface area buried being between 1.2 and 1.5 depending on the precise interface formed in a given simulation. It is expected that the burial of this hydrophobic surface area provides the change in free energy that drives the association.57–60 The surface area per residue buried in the interfaces in the simulations of

Figure 2
Self-association and C-terminal positions of Death Receptor 5 and interface surface areas (ISA). (A) Cross-eyed stereo images summarizing events observed in the simulations of the free receptor trimer. The sequence of images shows the evolution of the system over the first five nanoseconds and demonstrates the rapid aggregation of the receptor units. The images provide a top view of the Death Receptor, looking down from the N-termini. (B) Bottom view of the final structure of a simulation of the free receptor trimer. The outer triangle connects the C-terminal positions as present in the crystal structure, whereas the inner triangle connects the C-terminal positions as observed after 20 ns of simulation. Colors are as above. (C) Bottom view, looking up from the C-termini, of the crystal structure of the TRAIL-DR5 complex, with the C-terminal C-alpha atoms shown as corners of a triangle. Colors are as indicated above. (D) Bottom view of the final structure obtained from the TRAIL-DR5 complex simulation. The outer triangle connects the C-terminal positions as present in the crystal structure, whereas the inner triangle connects the C-terminal positions as observed after 35 ns of simulation. The TRAIL subunits are colored in different shades of green, the DR5 units are shown in yellow, orange and red.

Figure 3
(A–C) Residue contributions to interface surface areas involving Death Receptor 5 in three simulations, time averaged over the last nanosecond. (D) DR5 residue contributions to the TRAIL-DR5 interface surface area time averaged over the first nanosecond of the simulation. (E) DR5 residue contributions to the TRAIL-DR5 interface surface area time averaged over the last nanosecond (34–35 ns). (F) Changes in residue contributions to the TRAIL-DR5 interface surface, given as the difference between the time averaged values from 34–35 and 0–1 ns. The different colors indicate the different surfaces in each system.
The TRAIL-DR5 complex

The rapid association of the receptor units observed in the simulations after the removal of TRAIL is in agreement with recent SPR studies by Lee et al., suggesting that the Death Receptor 5 has a propensity to homo-oligomerize.27 Interestingly Lee et al.27 propose that the dominant species is a dimer. The simulations, in contrast, suggest the formation of an asymmetric trimer, with the binding of two of the receptor units being stronger than that of the third. Note it is not possible from the current simulations to determine whether the dimer or the trimer is the most important species physiologically. For one, the effective concentration in the receptor within the simulation cell is much higher than that on the surface of the cell (assuming a homogenous distribution). Second, as the simulation cell contains only three receptor units the formation of a trimer is favored. Finally, the tighter interaction of two out of the three receptors could reflect the fact that the system might not be fully equilibrated. However, if, as suggested in the simulations, the third receptor unit binds much more weakly, this interaction could be easily overlooked in the SPR experiments leading to the conclusion that dimers were the predominant species.

The possibility that the receptors are pre-associated has major implications for the mechanism of activation. If the receptors are pre-associated, then activity must depend on the precise orientation or the relative positions of the extracellular binding domains and not merely on the bringing together of the intracellular death domains. For example, if dimers are the dominant species on the surface of the cell, the activation of DR5 by TRAIL could involve the crosslinking of receptor dimers, according to the expanding network hypothesis.61 or the rearrangement of receptor dimers to form trimers. Alternatively, TRAIL may simply enforce symmetry onto a preformed but asymmetric trimer. In either case, the rearrangement of the extracellular domains leading to the repositioning of the cytosolic domains would be the critical step in the activation of the associated effector proteins (FADD).

Collective motions

Besides affecting structural properties, such as the conformation of the receptor units or their relative orientation, the binding of TRAIL will also affect the dynamic properties of the receptor units. To investigate possible changes in the collective motions within the system associated with the binding of TRAIL to DR5, principal component analysis (PCA) was performed on the receptor in the TRAIL-DR5 complex, the unliganded receptor trimer and the individual receptor monomers. It was found that the CRD1 and CRD2 domains are in practice quite rigid. This is illustrated in Figure 4(D), which shows the contribution of the interdomain motions to the total mean
square displacement. As can be seen, most of the fluctuations involve the relative motion of one domain with respect to the other. For this reason, it was chosen to perform the PCA using only one of the domains to align the molecules in order to highlight the relative motions between the domains. Such a linear transformation of
the coordinates results in a quadratic transformation of the positional covariance matrix changing the quantitative, but not the qualitative information. It is important to note, however, that the order of the principal components obtained after PCA is affected with more weight being given to inter-domain motions. Note also that the data presented in Figure 4 represents the combined results for the three receptor units simulated as free monomers (green), as the unliganded-trimer (blue) and as the complex (orange). Results were combined for each set of simulations separately and projected onto the eigenvectors obtained for that set. There was, however, a close correspondence between the eigenvectors of the different sets (data not shown).

The first 12 principal components and their (relative) cumulative sums are given in Table I. As can be seen, the unliganded trimer has the largest range of conformations, followed by the free monomer. As expected, the binding of TRAIL dramatically decreases the conformational freedom of the receptor. Figure 4 summarizes the results of the PCA with regards to the first two eigenvectors, which describe the hinge-bending motions of CRD1 and CRD2. The flexion point identified by comparing the bound DR5 with TNF-R135 appears to be part of a very flexible hinge region connecting two otherwise freely moving domains. DynDom analysis of the extreme projections of each set of trajectories on these two eigenvectors was used to determine the total angle of rotation around the hinge axes. In the case of the free receptor monomer and the free receptor trimer the rotations were very similar (49° and 39° compared to 55° and 44° respectively). In the presence of TRAIL, these rotations were reduced to 26° and 20°.

The third principal component describes the degree of deflection and is complementary to the hinge-bending motions described by the first two components. The fourth principal component describes a twisting motion of CRD1 relative to CRD2. Again, DynDom analysis was used to determine the total twist based on the extreme projections of the trajectory. The degree of twist was much larger in the case of the free monomer and trimer (81° and 91° respectively) than for the bound trimer (43°). The higher order principal components capture primarily local motion.

In summary, the motions within the free monomer and the free trimer were found to be very similar. It was observed that in the simulation of the unbound trimer more bent conformations were sampled, giving rise to slightly larger eigenvalues, suggesting a higher degree of motility. The presence of TRAIL strongly inhibits the hinge-bending and twisting motions. This is reflected in the narrow distributions of the projections on the first two eigenvectors seen in Figure 4(B,C). Interestingly, the differences between motions of the receptor units within trimers with and without TRAIL is almost exclusively due to differences in the rigid body motions as measured by the between-domains component of the mean square displacement (MSD) shown in Figure 4(D). Thus, the receptor could be considered to be a mechanical construct in which the interactions are determined by hinge-bending and twisting motions.

### Comparison with experiment

In their SPR experiments Lee et al. found that the on-rate (association) was slower than the off-rate (dissociation)
The activation of DR5 by TRAIL

The simulations suggest that TRAIL affects three factors presumably necessary for alignment of the intracellular domain and the activation of DR5. First, TRAIL enforces threefold-symmetry onto the receptor complex. In the absence of TRAIL, the receptor units associate to form an asymmetric trimer preventing the alignment of the intracellular domains. Second, TRAIL restricts the conformational freedom of the extracellular binding domains. This would increase the probability that the intracellular domains align and form an active complex. Third, the binding of TRAIL is associated with a large change in the degree of twist within the receptor units with respect to the unbound form. This change in twist angle will affect the relative orientation of the membrane-spanning α-helical domains and in turn alter the alignment of the cytosolic domains. We note that a similar twist mechanism has previously been proposed for the dimeric erythropoietin receptor62 and more recently for the growth hormone receptor.63

Note, the simulations can only provide information on the nature of conformational changes associated with the binding of TRAIL. It is known that other factors are also involved in the process of activation. For example, experiments involving the treatment of resistant carcinogenic cells with resinveratrol suggest that there is a requirement for Death Receptors to be localized in lipid rafts.64 Most likely a combination of factors, including those listed above, is needed to explain the almost binary on/off effect that TRAIL exerts when initiating apoptosis.65

The association and structural changes observed in the simulations after the removal of TRAIL from the complex could in principle be verified by experiment. If activation involved the crosslinking of initially independent receptors by a multidentate ligand, then the binding response would be expected to be dependent on the concentration of the receptor. In addition it would be expected to show a distinct maximum as increasing concentration of the ligand will eventually lead to the formation of inactive one-to-one complexes.66 If the receptor is pre-associated, these effects would not be observed. The importance of the precise spacing or orientation of the receptor could be assessed by introducing specific spacers into the transmembrane domains to vary the relative positions of the intra- and extracellular domains as has been done in related systems.62,67

Finally, we note that the work represents one of the few examples in which protein-protein self-association has been seen in simulation. This area has recently attracted attention in regard to the process of dewetting of hydrophobic surfaces.68,69 While in the current case the interactions between the surfaces are not sufficiently regular to investigate whether similar processes to that observed by Liu et al.69 occur the work does highlight the fact that the simulation of the assembly of large scale regulatory complexes in atomic detail is becoming possible.

CONCLUSIONS

Simulations of the Death Receptor 5, both unbound and in a complex with its ligand TRAIL have been performed. It was found that in the absence of TRAIL, the receptor units rapidly self-associate to form an asymmetric trimeric complex stabilized by interactions between the CRD2 domains. This finding is in line with recent experimental studies, suggesting that a number of cytokine receptors, including the four receptors for TRAIL, exist as preformed complexes. The asymmetric nature of the unbound complex with the interaction to the third receptor unit being relatively weak might also explain the observation of dimers as opposed to trimers in SPR studies. The simulations suggest that the unbound DR5 receptors initially reside at the cell surface as asymmetric trimers. We propose that in these complexes the distance and relative orientation between the C-termini of the extracellular domains of the receptor prevent the formation of an active trimeric cytoplasmic death domain. The primary role of TRAIL is to enforce threefold symmetry on the complex, which leads to the repositioning of the receptor units. In addition, TRAIL strongly restricts the conformational freedom of the extracellular domains, which might also contribute to stabilizing the activated state of the death complex.

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REFERENCES


