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Molecular modeling revealed that ligand dissociation from thyroid hormone receptors is affected by receptor heterodimerization

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ABSTRACT

Numerous ligands bind tightly to thyroid hormone receptors (TRs), and exploring the binding and dissociation of these ligands from TRs will increase our understanding of their mechanisms of action. TRs form transcriptionally active heterodimers with retinoid X receptor (RXR); whether this heterodimerization affects ligand dissociation is poorly understood. To investigate the effects of heterodimerization, classical molecular dynamics (MD) simulations and random acceleration molecular dynamics (RAMD) simulations were performed to probe the dissociation of triiodothyronine (T3) from a TRα-RXR ligand binding domain (LBD) heterodimer and the TRα and TRβ LBDs at the atomic level. Seven (I–VII) dissociation pathways were identified for T3. Heterodimerization inhibited pathway I in the TRα-RXR LBD heterodimer, which may block the proper orientation of the helix 12 (H12), therefore affecting the biological functions of TRs. Upon TR heterodimerization, the second most dominant dissociation pathway switched from pathway IV for TRα LBD to pathway II for TRα-RXR LBD. No significant effects of TR heterodimerization were observed on the dominant dissociation pathway III that was located between H3, the H1–H2 loop and the β-sheet. Our study revealed that TR heterodimerization significantly affects T3 dissociation, which provides important information for the study of other TR ligands.

1. Introduction

A member of the nuclear receptor (NR) family, thyroid hormone receptors (TRs) are ligand-regulated transcription factors that are modulated by natural ligands and a broad range of synthesized chemicals. TRs are becoming an important target in drug discovery [1] and environmental toxicity research [2]. Ligands affect thyroid hormone signaling by binding to TRs; therefore, investigating how ligands bind to and dissociate from TRs will increase our understanding of their mechanisms of action.

The TR isoforms, TRα and TRβ, consist of three domains: an N-terminal regulatory domain, a DNA-binding domain (DBD) and a ligand-binding domain (LBD). The LBDs of TRα and TRβ share ~86% similarity at the amino acid level [3]. The monomer form of TR LBD contains a common fold with 12 helices arranged in three layers and several β strands and a nearly identical ligand-binding pocket (one amino acid difference) [4].

Ligands may entry and release from the ligand-binding pocket of TR LBD via multiple pathways. The exploring of dissociation pathways could help us to further understand the mechanism of action of ligands. Martiĭnez et al. revealed that the natural ligand of TR, triiodothyronine (T3) dissociates from the binding pocket of TRα LBD and TRβ LBD monomers [3,5] via three competing pathways. Up to now, there are still no reports on the dissociation of T3 from the dimer or heterodimer of TRs. TRs were recently reported to heterodimerize with retinoid X receptor (RXR), and the TRα-RXR heterodimer is the most transcriptionally active among TR isoforms [6]. The TRα-RXR heterodimer alters the binding and dissociation kinetics of T3 [7]. However, the effects of TR heterodimerization on the T3 dissociation pathways have not been elucidated. Considering that various ligands bind to TR heterodimers, exploring the dissociation of T3 from the TRα-RXR LBD heterodimer will provide the essential framework for studying the dissociation of other relevant ligands.

Here, classical molecular dynamics (MD) and random acceleration molecular dynamics (RAMD) simulations were combined to investigate the effects of TR heterodimerization on T3 dissociation from a TRα-RXR LBD heterodimer (Fig. 1) and from the LBDs of TRα and TRβ. RAMD is an enhanced sampling method that applies a small randomly oriented force to the center of mass of ligands to accelerate ligand dissociation [8]. It is a reliable tool for identifying ligand dissociation pathways from biomacromolecules such as estrogen receptors [9], the retinoic acid receptor (RAR) [10], the vitamin D receptor (VDR) [11], opsin [12], cytochrome P450s [13,14] and dehalogenases [15]. RAMD simulations identified seven (I–VII) T3 dissociation pathways. TR heterodimerization caused the

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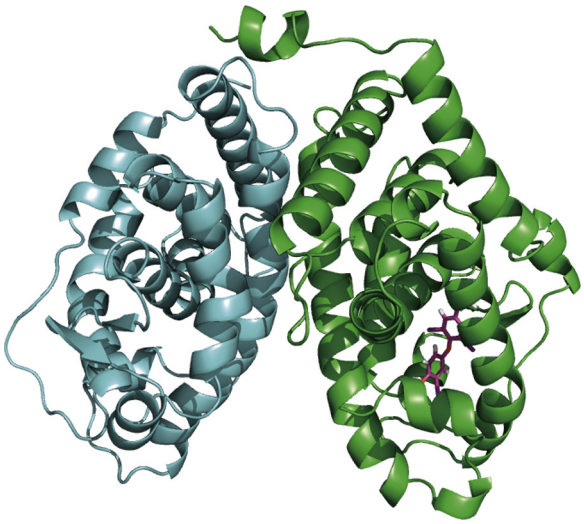

Fig. 1. The crystal structure of TRα-RXR LBD. The RXR LBD (left) and the TRα LBD (right) were represented in cartoon format, and T3 was illustrated in stick format.

second most dominant dissociation pathway to switch from pathway IV for TRα LBD to pathway II for TRα-RXR LBD and resulted in a complete loss of pathway I in TRα-RXR LBD. The results elucidated how TR heterodimerization regulates T3 dissociation at atomic resolution.

2. Materials and methods

2.1. Preparation of the TR LBD structures

The initial structures for classical MD simulations were obtained from X-ray crystal structures of the TRα-RXR LBD heterodimer (PDB entry: 3UVV, 2.95 Å), the TRα LBD (PDB entry: 2H79, 1.87 Å), and the TRβ LBD (PDB entry: 3GWS, 2.2 Å). T3 and the surrounding crystal water molecules were kept in the structures. The parameters for T3 and the proteins were derived from the general AMBER force field (GAFF) and the standard AMBER 2003 force field [16], respectively, using AmberTools 1.5 [17]. The systems were solvated in the center of a rectangular parallelepiped solvent box surrounded by 9 Å TIP3P explicit waters [18]. The solvated systems of the TRα-RXR LBD heterodimer and the LBDs of TRα and TRβ in complex with T3 were neutralized with 14, 10, and 10 Na^+, respectively, producing final systems with 51,204, 39,658 and 35,613 atoms, respectively.

2.2. MD simulations

Two-stage energy minimization was performed according to a published method [19] to eliminate bad contacts in the three initial TR LBD systems. In the first stage minimization, all hydrogen atoms were minimized keeping heavy atoms fixed and in the second stage, the whole system was minimized. For each stage, 500 steps steepest descent minimization was performed followed by 500 step conjugate gradient minimization. The minimized systems were heated to 300 K in 50 ps. The heated systems containing the TRα-RXR LBD heterodimer, the TRα LBD, and the TRβ LBD were equilibrated and were subjected to productive run in the NPT ensemble at constant pressure (1 atm) for 23, 11 and 36 ns, respectively. The Amber 11 simulation package [17] was utilized for the classical MD simulations using a time step of 2 fs. All the hydrogen atoms were constrained using the SHAKE algorithm [20]. The long-range electrostatic interactions were treated with the particle-mesh-Ewald (PME) method [21] using a non-bonded cutoff of 10 Å.

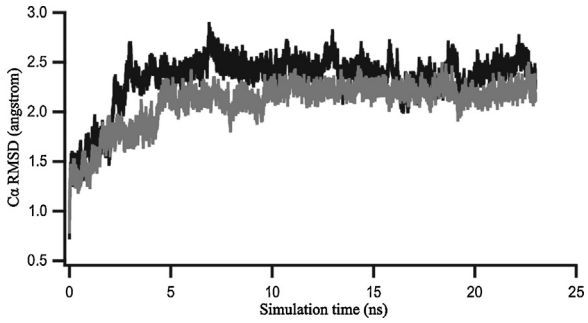

Fig. 2. The Cα RMSD of TRα-RXR LBD as a function of simulation time. The Cα RMSDs of the TRα LBD (top) and the RXR LBD (bottom) were colored in black and gray, respectively.

2.3. RAMD simulations

Multiple snapshots saved from the classical MD simulation trajectories were used as the starting structures for the RAMD simulations. This included 5 snapshots of the TRα-RXR LBD heterodimer in complex with T3 at 3, 8, 13, 18, and 23 ns, 3 snapshots of the TRα LBD in complex with T3 at 1, 6, and 11 ns, and 5 snapshots of the TRβ LBD at 11, 16, 21, 26, and 31 ns. Each snapshot of TRα-RXR LBD, TRα LBD, and TRβ LBD undergoes 30, 30, 20 times RAMD simulations, respectively. The simulations were performed using the NAMD 2.9 program [22] with acceleration parameters of 0.04 and 0.05 kcal/mol Å amu and different random number seeds. The number of steps in one RAMD stint was set to 10 or 20, which corresponds to a minimum distance traveled by the ligand of 0.001 and 0.002 Å, respectively.

3. Results and discussion

3.1. Statistical analysis of MD trajectories

We performed classical MD simulations of the TRα-RXR LBD heterodimer and the TRα and TRβ LBDs in complex with T3 for 23, 11, and 36 ns, respectively. To monitor the conformational changes of the TR LBD upon T3 binding during classical MD simulations, we calculated the root-mean-squared deviation (RMSD) fluctuations of the Cα atom as a function of simulation time (Figs. 2, S1 and S2). Using the minimized complex structures as the reference, the averaged Cα RMSDs of the TRα LBD after heterodimerization and the individual TRα and TRβ LBDs were 2.33, 3.28, and 3.00 Å, respectively. The Cα RMSD of the TRα LBD in the heterodimer was much smaller than that of the monomeric TRα and TRβ LBDs, indicating that heterodimerization induces a more stable conformation of the TRα LBD. The simulations with the TR-RXR heterodimer were performed in the presence of T3 and in the absence of an RXR ligand. As illustrated in Fig. 2, RXR does not undergo a major conformational change (averaged Cα RMSD of 2.09 Å), indicating that the absence of an RXR ligand has no effect on the conformation of RXR.

The binding modes of T3 to TRα-RXR LBD and the TRα and TRβ LBDs were investigated by hydrogen bonding analysis of the recorded conformations. We used the default criteria for a hydrogen bond: the acceptor–donor distance was less than 3.5 Å, and the hydrogen-bond angle was at least 120°. For TRα-RXR LBD in complex with T3, one hydrogen bond formed between the phenol hydroxyl of T3 and His379 of H11, consistent with the X-ray crystal analysis of TRα-RXR LBD [7], which suggested that the correct parameters were used for T3 in the MD simulations. For TRα LBD in complex with T3, T3 formed two hydrogen bonds with His381 of H11 and Ser277 of the β-hairpin. For TRβ LBD in complex with T3, T3 formed two hydrogen bonds with His435 of H11 and Asn331 of the β-hairpin.