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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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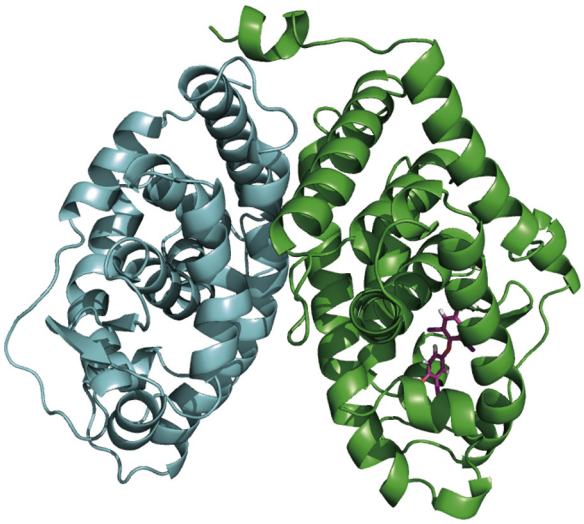

Fig. 1. The crystal structure of TRα-RXR LBD. The RXR LBD (left) and the TRα LBD (right) were represented in cartoon format, and T3 was illustrated in stick format.

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.

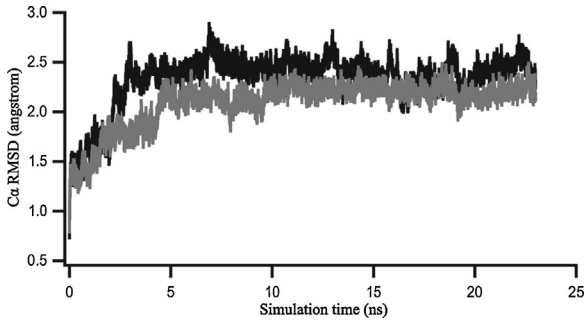

Fig. 2. The Cα RMSD of TRα-RXR LBD as a function of simulation time. The Cα RMSDs of the TRα LBD (top) and the RXR LBD (bottom) were colored in black and gray, respectively.

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.

The conserved His379 in H11 of TRα-RXR LBD, His381 in H11 of TRα LBD and His435 in H11 of TRβ LBD play a key role in anchoring T3 to the binding site and can be resistant to the initial dissociation of T3 from the binding site. The T3 dissociation begins with the breaking of these hydrogen bonds. Two mutations of His435 in TRβ have been identified in patients with abnormal thyroid function [23]. In the ERα LBD, His524 is critical for maintaining the tertiary structure [24].

3.2. T3 egress pathways

Starting from randomly selected MD equilibrated structures, two sets of RAMD simulations were performed for ten or fifteen times with different simulation parameters. A low acceleration parameter of 0.04 kcal/mol Å amu was used in the first 75 RAMD simulations with TRα-RXR LBD, the first 45 RAMD simulations with the TRα LBD complex and the first 50 simulations with the TRβ LBD complex. A second set of RAMD simulations were performed with an acceleration of 0.05 kcal/mol Å amu. Very small acceleration parameters have been used to identify ligand exit pathways that closely resemble the actual dissociation pathways [12,25].

Many RAMD studies of complex biomolecular systems utilize 50–100 trajectories to identify all the dissociation pathways [11]. Our RAMD simulations generated 150, 90 and 100 statistically relevant T3 egress trajectories in TRα-RXR LBD, TRα LBD and TRβ LBD, respectively. The T3 dissociation pathways from the TR LBDs were statistically analyzed (Tables 1–3 and S1).

Our RAMD simulations revealed that T3 dissociated from the three forms of TR LBDs via seven dissociation pathways (I, II, III, IV, V, VI and VII). Three pathways (I, II and III) in TRα LBD and TRβ LBD monomers were also observed by reported locally enhanced sampling (LES) MD simulations and steered molecular dynamics (SMD) simulations [3,5], which validated the accuracy of our RAMD simulations. Figs. 3 and 4 illustrated the position of T3 before egress and the intermediate position of T3 that exits via different pathways. Four additional dissociation pathways, IV, V, VI, and VII were newly discovered.

In pathway I, T3 exited the TRα LBD and TRβ LBD via a hydrophobic cleft between H3, H5 and H12 (Fig. 3B). This cleft is believed to be crucial for coactivator binding [26]. During the dissociation of T3 via pathway I, H12 repositions away from H3 and H3 breaks into two helices (Fig. S3), which was also probed from the reported LES MD simulation [3]. Pathway I was utilized in TRα LBD (7.8%) and TRβ LBD (5.0%). By contrast, pathway I was never identified in the RAMD simulations of the TRα-RXR LBD heterodimer.

Pathway II was located between the beginning of H11, H8 and the Ω-loop between H2 and H3 (Fig. 3C), which is far away from the TR LBD heterodimerization surface. During the ligand dissociation, H11 and H8 move oppositely, which makes T3 easier to exit. Martiĭnez et al. also found that the dissociation of H8 and H11 was accompanied by retraction of the Ω-loop to open the escape cavity [3,5].

In pathway III, T3 exits from a tunnel located at the middle of H3, the H1–H2 loop and the β-sheet (Fig. 3D). This pathway is highly similar to the dissociation pathway C of VDR [11] and pathway A of the RAR γ [10]. Pathway III is the primary pathway in all three TRs: TRα-RXR LBD (37.3%), TRα LBD (41.1%), and TRβ LBD (50%), which shows that the heterodimerization does not change the dominant dissociation pathway of T3. Using SMD simulations, Martiĭnez et al. also revealed that Pathway III is the preferential pathway for T3 to exit from TRα LBD and TRβ LBD, which is in line with our RAMD simulations. The reported random expulsion molecular dynamics (REMD) simulations revealed that the retinoic acid escapes from RARγ LBD mainly via pathway A [10]. This region has great structural flexibility, as illustrated by the classical MD simulation data that indicated expanded passages (Fig. S4) for facile T3 egress. Martiĭnez et al. also explains that the highly mobile region makes T3 exit easier from pathway III [5]. However, the mobility of this region cannot be the only factor. Martiĭnez et al. found that during T3 dissociation through pathway III, the polar groups of the ligand dissociated directly into the aqueous environment and there was a steady increment of the electrostatic attractive interactions, however, the electrostatic interaction was less attractive during dissociation via pathway I [5].

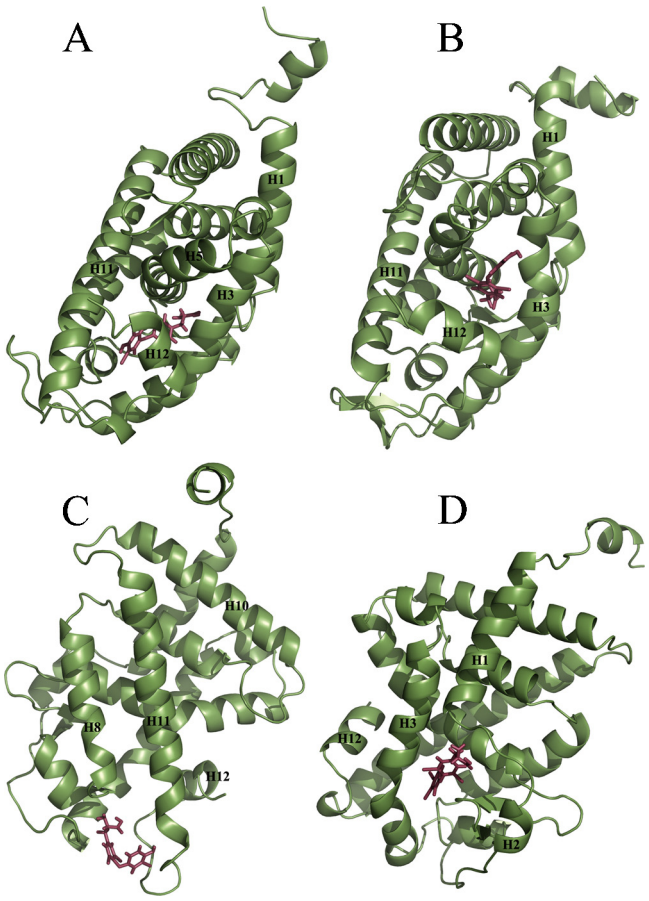

Fig. 3. The initial position of T3 before egress (A) and the intermediate position of T3 in pathway I (B), II (C), and III (D). The TRα LBD is illustrated in cartoon format, and T3 is represented in stick format.

In pathway IV, T3 exits from a tunnel between H1–H2 loop and the β-sheet loop (Fig. 4A). The pathway IV is the secondary pathway in TRα LBD. During the dissociation of T3 through pathway IV, the H1–H2 loop and the β-sheet loop can provide highly flexibility to facilitate T3 egress, which is similar to the dissociation of T3 through pathway III. The pathway IV is highly similar to the dissociation pathway B of 1α,25-dihydroxyvitamin D3 in VDR [11] and to the second pathway B of retinoic acid in RARγ LBD [10].

T3 can also exit along pathway V that is located between H8, H7 and β-sheet of TRα LBD (Fig. 4B). This region is sterically dense and the ligand egress involves in rearrangements of local structures, therefore, this pathway is least frequently utilized (3.3–4%) in TRα-RXR LBD, TRα LBD, and TRβ LBD (Tables 1–3). The pathway V is quite same to the unexpected exit pathway C of retinoic acid in RARγ LBD. The dissociation of retinoic acid through pathway C results in a reduction of interactions of ligand carboxy end with solvents and causes a loss of electrostatic field in this region that can guide the binding of ligands to RARγ LBD [10].

In pathway VI, T3 exits through a narrow cleft between the middle of H11, H8, H6 and the beginning of H3 (Fig. 4C). The pathway VI is the secondary pathway of T3 in TR β LBD, which is quite similar

Table 1
Summary of RAMD simulations of TR α-RXR LBD heterodimer.

Pathway	Acceleration (kcal/Å/g)	r_{min} (Å)	N (step)	Number of egress					
				3 ns	8 ns	13 ns	18 ns	23 ns	Total (ratio)
I	0.04, 0.05	0.001, 0.002	10, 20	0	0	0	0	0	0 (0%)
II	0.04, 0.05	0.001, 0.002	10, 20	6	8	6	5	4	29 (19.3%)
III	0.04, 0.05	0.001, 0.002	10, 20	13	12	11	10	10	56 (37.3%)
IV	0.04, 0.05	0.001, 0.002	10, 20	5	3	4	8	5	25 (16.7%)
V	0.04, 0.05	0.001, 0.002	10, 20	0	2	1	1	1	5 (3.3%)
VI	0.04, 0.05	0.001, 0.002	10, 20	3	2	2	2	4	13 (8.7%)
VII	0.04, 0.05	0.001, 0.002	10, 20	3	3	5	3	5	19 (12.7%)

Table 2
Summary of RAMD simulations of TR α LBD.

Pathway	Acceleration (kcal/Å/g)	r_{min} (Å)	N (step)	Number of egress			
				1 ns	6 ns	11 ns	Total (ratio)
I	0.04, 0.05	0.001, 0.002	10, 20	1	2	4	7 (7.8%)
II	0.04, 0.05	0.001, 0.002	10, 20	3	2	2	7 (7.8%)
III	0.04, 0.05	0.001, 0.002	10, 20	11	14	12	37 (41.1%)
IV	0.04, 0.05	0.001, 0.002	10, 20	5	4	4	13 (14.4%)
V	0.04, 0.05	0.001, 0.002	10, 20	1	2	1	3 (3.3%)
VI	0.04, 0.05	0.001, 0.002	10, 20	4	2	2	8 (8.9%)
VII	0.04, 0.05	0.001, 0.002	10, 20	4	4	4	12 (13.3%)

to the dissociation pathway D of retinoic acid in RARγ LBD. Ligand egress through this pathway also needs the rearrangement of local structures, therefore, it was observed at a low frequency [10].

Pathway VII is located at the confluence of H12, the H11–H12 loop and the beginning of H3 (Fig. 4D), which is similar to the dissociation pathway A of 1α,25-dihydroxyvitamin D3 in VDR [11]. T3 egress through this pathway involves the relocation of H12. The percentages by which T3 exited via pathway VII from TRα-RXR LBD (12.7%), TRα LBD (13.3%), and TRβ LBD (10.0%) were similar, indicating the negligible effects of TR heterodimerization on this pathway.

3.3. The effects of heterodimerization on the pathways

The recently resolved crystal structure of the TRα-RXR LBD heterodimer revealed a surface region comprised of H11, H10, the loop connecting H10 and H11, the middle of H9 and the H9–H8 loop [7]. Compared with the TRα LBD monomer, the binding of RXR LBD to TRα LBD packs H9, H10, H8, H6, H5, and H3 more tightly, decreasing the average Cα RMSD of the TRα LBD (Figs. 2, S1 and S2). Pathway I is located at a cleft surrounded by H3, H5 and H12 and the binding of RXR LBD causes this cleft sterically denser. Before the egress of T3 through pathway I, it needs the rearrangements of local structures to facilitate T3 to exit. However, H12 of the TRα-RXR LBD heterodimer is tightly packed against H3 and H5 and this region is hardly to undergo major conformations. Consequently, the open passage of pathway I present in TRα LBD is blocked in TRα-RXR LBD, and T3 egress via pathway I does not occur in the heterodimer.

The loss of pathway I cause T3 to egress from other alternate exit route. As summarized in Tables 1–3, the rate of T3 egress via pathway II increased from 7.8% for TRα LBD and 5.0% for TRβ LBD to 19.3% for TRα-RXR LBD, making pathway II the secondary pathway in the TRα-RXR LBD heterodimer. TR heterodimerization causes the switch of the second most dominant dissociation pathway from pathway IV of TRα LBD to pathway II of TRα-RXR LBD. The difference in secondary pathways indicated that the TR structural variations upon the heterodimerization altered the dissociation kinetics of T3 [7].

After receptor heterodimerization, conformational changes in the receptors occur, and the allosteric interactions between the heterodimers may create complexes with unique properties [27]. There is ample scientific evidence that receptor heterodimerization modulates combinatorial gene expression by altering the kinetics of ligand association/dissociation from TRs [7,28,29]. A recent MD study on the dimerization of estrogen receptors demonstrated the suppression of the E2 dissociation pathway and the modification of the raloxifene escape routes [30]. The RAMD simulations reported here revealed substantial heterodimerization-induced alterations in the dissociation pathways, including the suppression of pathway I. We speculate that upon TR-RXR binding, the heterodimerization affects the T3 dissociation pathways and consequently influences thyroid hormone signaling, which alters a diverse range of physiological processes.

The increase in the incidence of thyroid diseases, such as thyroid cancer and thyroid autoimmune disease [31], warrants the development of potent TR agonists and antagonists that modulate TR activity. Because the TRα-RXR heterodimer is transcriptionally

Table 3
Summary of RAMD simulations of TR β LBD.

Pathway	Acceleration (kcal/Å/g)	r_{min} (Å)	N (step)	Number of egress					
				11 ns	16 ns	21 ns	26 ns	31 ns	Total (ratio)
I	0.04, 0.05	0.001, 0.002	10, 20	2	0	1	1	1	5 (5.0%)
II	0.04, 0.05	0.001, 0.002	10, 20	0	3	2	0	0	5 (5.0%)
III	0.04, 0.05	0.001, 0.002	10, 20	8	9	9	12	12	50 (50.0%)
IV	0.04, 0.05	0.001, 0.002	10, 20	1	1	1	1	1	5 (5.0%)
V	0.04, 0.05	0.001, 0.002	10, 20	2	1	0	0	1	4 (4.0%)
VI	0.04, 0.05	0.001, 0.002	10, 20	4	1	4	5	2	16 (16.0%)
VII	0.04, 0.05	0.001, 0.002	10, 20	2	3	2	1	2	10 (10.0%)

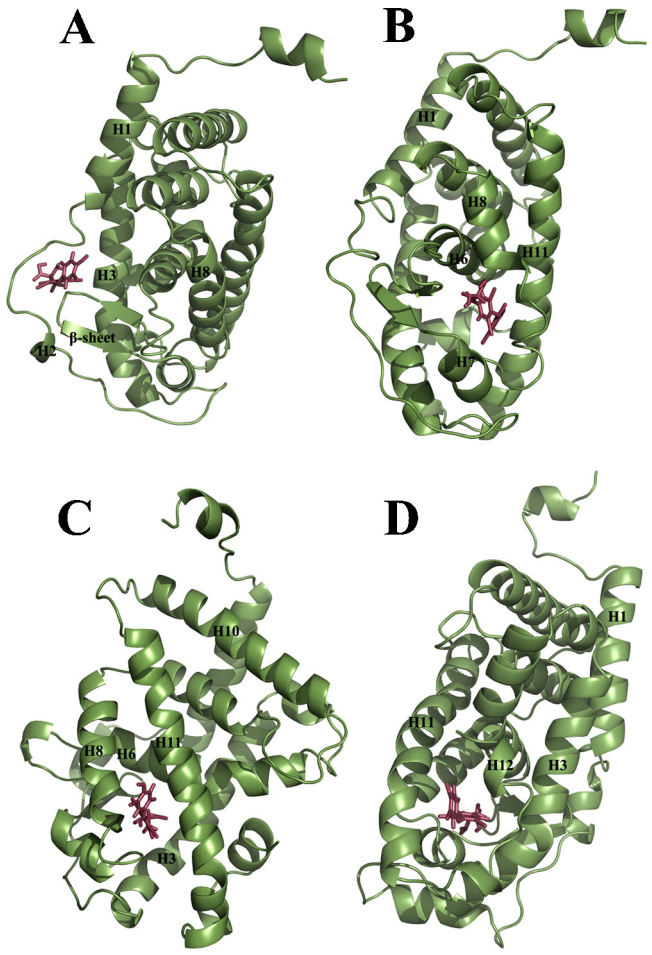

Fig. 4. The intermediate position of T3 in pathway IV (A), V (B), VI (C), and VII (D). The TRα LBD is illustrated in cartoon format, and T3 is represented in stick format.

active and heterodimerization significantly affects ligand dissociation, TR heterodimers and monomers should both be considered potential drug targets for the rational design of selective TR modulators. Many environmental chemical pollutants are structurally similar to T3 and have been demonstrated to bind to TRs with both agonist and antagonist effects on thyroid hormone signaling. Therefore, the ability of these toxic chemicals to bind to TR heterodimers should be evaluated when studying their mechanisms of action.

4. Conclusion

Classical MD and RAMD simulations were utilized to probe T3 dissociation from a TRα-RXR LBD heterodimer, the TRα LBD, and the TRβ LBD. Seven exit pathways were identified. The same exit pathway, pathway III, predominated for all three TR LBDs, but the second most dominant pathway was influenced by TR heterodimerization. The absence of pathway I in TRα-RXR LBD was discovered to be a direct consequence of the structural change that occurred upon binding of TRα LBD to RXR. In summary, traditional MD combined with RAMD led to the identification of novel mechanisms of T3 dissociation at atomic resolution and revealed the significant effects of TR heterodimerization. Detailed ligand dissociation mechanisms from TRs at atomic resolution are not experimentally observable but can be inferred using MD and RAMD, which require resolved crystal structures. Currently, only the tertiary structure of the TR-RXR LBD heterodimer has been resolved. The full assessment of T3 egress from TRs will require crystal structures of other TR heterodimers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jmgm.2013.06.001.

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