

Gaining ligand selectivity in thyroid hormone receptors via entropy

Leandro Martínez^{a,1}, Alessandro S. Nascimento^{b,1}, Fabio M. Nunes^b, Kevin Phillips^c, Ricardo Aparicio^{b,2}, Sandra Martha G. Dias^{b,3}, Ana Carolina M. Figueira^b, Jean H. Lin^c, Phuong Nguyen^c, James W. Apriletti^c, Francisco A. R. Neves^d, John D. Baxter^{c,4}, Paul Webb^{c,4}, Munir S. Skaf^{a,4}, and Igor Polikarpov^{b,4}

^aInstituto de Química, Universidade Estadual de Campinas, SP 13084-862, Campinas, Brazil; ^bInstituto de Física de São Carlos, Departamento de Física e Informática, Universidade de São Paulo, SP 13566-590, São Carlos, Brazil; ^cMethodist Hospital Research Institute, Houston, TX 77030; and ^dLaboratório de Farmacologia Molecular, Faculdade de Ciências de Saúde, Universidade de Brasília, Campus Universitário Darcy Ribeiro, DF 70910-900, Brasília, Brazil

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Nuclear receptors are important targets for pharmaceuticals, but similarities between family members cause difficulties in obtaining highly selective compounds. Synthetic ligands that are selective for thyroid hormone (TH) receptor β (TR β) vs. TR α reduce cholesterol and fat without effects on heart rate; thus, it is important to understand TR β -selective binding. Binding of 3 selective ligands (GC-1, KB141, and GC-24) is characterized at the atomic level; preferential binding depends on a nonconserved residue (Asn-331 β) in the TR β ligand-binding cavity (LBC), and GC-24 gains extra selectivity from insertion of a bulky side group into an extension of the LBC that only opens up with this ligand. Here we report that the natural TH 3,5,3'-triiodothyroacetic acid (Triaic) exhibits a previously unrecognized mechanism of TR β selectivity. TR x-ray structures reveal better fit of ligand with the TR α LBC. The TR β LBC, however, expands relative to TR α in the presence of Triaic (549 Å³ vs. 461 Å³), and molecular dynamics simulations reveal that water occupies the extra space. Increased solvation compensates for weaker interactions of ligand with TR β and permits greater flexibility of the Triaic carboxylate group in TR β than in TR α . We propose that this effect results in lower entropic restraint and decreases free energy of interactions between Triaic and TR β , explaining subtype-selective binding. Similar effects could potentially be exploited in nuclear receptor drug design.

Triaic | design | mobility

Nuclear receptors (NRs) are conditional transcription factors with important roles in development and disease (1, 2). Although these proteins are targets for existing drugs and pharmaceutical development, structural relationships between subsets of these proteins mean that ligands that target one NR can cross-react with others. This is commonly observed when NRs exist in multiple subtypes, as for thyroid hormone (TH) receptors (TRs), estrogen receptors (ERs), and peroxisome proliferator receptors, but can be a problem with steroid hormone receptors and other closely related receptors.

TH excess leads to cholesterol lowering and fat loss and undesirable effects (altered heart rate, muscle wasting, and bone resorption) (3). There are 2 TRs (TR α and TR β) encoded by different genes. They bind the major active TH (3,5,3'-triiodo-L-thyronine, T₃) with similar affinities but evoke different responses on activation: TR β mediates beneficial effects on cholesterol, whereas TR α mediates harmful effects on heart (3, 4). Synthetic TR β -selective ligands, some of which exhibit liver-selective uptake, reduce serum cholesterol and other atherogenic lipids in preclinical animal models (reviewed in ref. 4). The ligands also reduce body fat without effects on lean muscle in rodents and primates, enhance aspects of reverse cholesterol transport in mice, and improve blood glucose levels in mouse models of type 2 diabetes (4). Two ligands, GC-1 and KB2115, have entered human trials, and the latter has been tested in humans, where it reduces serum LDL cholesterol, lipoprotein(a), and triglycerides (4, 5).

It remains important to develop compounds with larger therapeutic windows between good and bad effects. Binding modes of 3 selective ligands (Fig. 1) have been characterized at the atomic level. The TR ligand-binding cavity (LBC), like that of other NRs, lies in the core of the C-terminal ligand-binding domain (LBD) (6). TR β -selective binding of GC-1 (\approx 5-fold) and KB141 (\approx 10-fold) is dependent on a single TR subtype-specific residue in the LBC, TR β Asn-331 and TR α Ser-277 (7, 8). These lie in a hydrophilic region of the LBC that contacts charged groups of the T₃ amino propionate group, and pocket swap mutations that reverse the identity of these residues also reverse TR preference for GC-1 and KB141. X-ray structural analysis indicates that TR β Asn-331 repositions Arg-282 relative to its TR α equivalent (Arg-228), and this facilitates hydrogen bond formation between the Arg-282 side chain and the GC-1 or KB141 carboxylate group (7–9). TR β -selective binding of GC-24 (\approx 40-fold) is partly dependent on Asn-331 β but involves another mechanism (10); ligand occupies the LBC and conserved hydrophobic regions of helices (H) 3 and 11 open to form an extension to the LBC that accommodates a bulky GC-24 phenyl group. The TR β H3–H11 region is more flexible than that of TR α in crystal structures, likely explaining subtype selectivity.

Triaic is a TR agonist that binds 2–3-fold selectively to TR β in vitro (11), only moderately less than GC-1. The mechanism of selective Triaic binding has not been explored. In fact, though not previously emphasized, initial comparisons of moderate resolution x-ray structures of rat (r) TR α and human (h) TR β LBDs with Triaic suggested that Triaic fits the TR α LBC better than TR β (6).

In this study, we explored this apparent paradox using new high-resolution structures of hTR-Triaic complexes and molecular dynamics (MD) simulations. MD simulations have accurately described TR LBD dynamics (12–15), and predicted interaction energies of TR/GC-1 and TR/KB141 complexes support the concept that TR β makes stronger contacts with GC-1 and KB141 carboxylate groups than TR α , and that Arg-282 β position is important for this effect (9). Here, simulations

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Conflict of interest: J.D.B. has proprietary interests in Karo Bio AB, which has commercial interests in this area of research.

Data deposition: Coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org [PDB ID codes 3JZB (TR α -Triaic) and 3JZC (TR β -Triaic)].

¹L.M. and A.S.N. contributed equally to this work.

²Present address: Instituto de Química, Universidade Estadual de Campinas, Cx. P. 6154, Campinas, SP 13084-862, Brazil.

³Present address: C3-137, Molecular Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

⁴To whom correspondence may be addressed. E-mail: ipolikarpov@ifsc.usp.br, skaf@iqm.unicamp.br, pwebb@tmhs.org, or jbxaxter918@aol.com.

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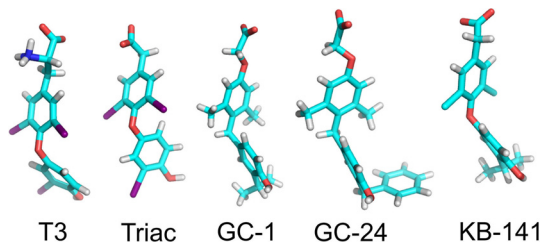


Fig. 1. T₃, Triac, and selective thymimetics GC-1, GC-24, and KB141.

confirm that the TR α LBC makes stronger direct contacts with Triac than TR β . However, we also find that the Triac-TR β complex LBC is larger than the Triac-TR α complex and that this permits greater ligand solvation and increased flexibility of the Triac carboxylate group in TR β . We propose that entropic contributions of these effects account for selective binding and that similar effects could be exploited in design of NR ligands.

Results

Triac Selectively Binds TR β . We examined Triac interactions with hTRs (Fig. 2). We used equilibrium ligand binding assays to measure competition with [¹²⁵I]T₃ for hTR binding. Fig. 2A shows that the competition curve is leftward shifted with Triac relative to T₃ with TR β but not TR α , reflective of higher Triac

affinity for TR β . The ratio of T₃ to Triac IC₅₀ values was 0.97 ± 0.09 with TR α and 2.94 ± 0.52 with TR β (Fig. 2A, Inset), consistent with previous estimates of TR β -selective binding (6, 11). As seen for GC-1 and KB141 (7–9), mutations that reverse the subtype-specific TR LBC residue (hTR β -Asn331Ser and hTR α -Ser277Asn) reverse subtype-specific binding of Triac (Fig. 2B). Analysis of thermal denaturation curves of TR-ligand complexes also revealed that Triac stabilizes TR β more efficiently than TR α (Fig. S1, Table S1) and that Triac protects the TR β LBD against denaturation more effectively than other ligands, suggesting that the Triac-TR β complex is particularly stable (see Discussion).

Selective activation of TR β by Triac was detectable in cell culture (Fig. 2C). There was a leftward shift in Triac dose-response with TR β relative to TR α at a thyroid response element (TRE)-driven reporter. Average Triac EC₅₀ values were 1.5 nM for TR α and 0.5 nM for TR β , yielding 3-fold TR β selectivity. T₃ was 2.3-fold selective for TR α in this system (EC₅₀ values were 1.5 and 4.2 nM for TR α and TR β , respectively). Thus, Triac was approximately 6-fold more potent than T₃ with TR β .

Triac Makes More Contacts with TR α . We obtained new high-resolution x-ray structures of human TR LBDs with Triac. Previous x-ray structures of TR LBDs with this ligand (7) were of different species (rTR α and hTR β) and obtained at moderate resolution (2.5 Å). Our hTR α -Triac structure was obtained at higher resolution (2.0 Å; Table S2). We also obtained a new hTR β structure at similar resolution to the previous one (2.5 Å) but with increased data redundancy to facilitate the assignments of side chain positions (Table S2).

Relationships between Triac and the hTR α LBC are shown in Fig. 3A and Table 1. Interactions with Triac thyronine rings with the LBC are mostly hydrophobic, except for contacts between His-381 α and the Triac 4' hydroxyl group. The Triac carboxylate group interacts with the hydrophilic region of the LBC, including one interaction not seen in the rTR α structure: the Ser-277 α side chain hydroxyl forms a water-mediated hydrogen bond with an amine group of the Arg-228 α side chain which, in turn, forms a water-mediated hydrogen bond with the Triac carboxylate. The structure also confirms previous reports that the Ser-277 α main chain amine and both Arg-266 α side chain amines form direct hydrogen bonds with the Triac carboxylate.

As in previous hTR β -Triac structures, Asn-331 β hydrogen bonds with Arg-282 β , repositioning the Arg-282 β side chain away from ligand. This differs from Arg-228 α , which forms the water-mediated hydrogen bond with Triac, mentioned above (Fig. 3A). The new structure also reveals a unique aspect of TR β organization: Arg-320 β adopts 2 conformations. The first (Arg320A, productive; Fig. 3B) resembles the previous hTR β structure (7); one amine group of the Arg-320 side chain forms a hydrogen bond with the Triac carboxylate group that seems weak because of poor geometry. The second (Arg320B, nonproductive; Fig. 3B) was not previously detected; here, the Arg-320 side chain rotates away from ligand. Both conformations differ from hTR α , where Arg-266 α side chain amines form well-positioned hydrogen bonds with Triac. B-factors of both conformations of the Arg-320 β side chain (48.5 Å² and 50.6 Å² for A and B conformations) are comparable to each other and neighboring LBC side chains, such as Arg-282 (52.3 Å²) and Arg-316 (40.9 Å²), and lower than Triac (74.4 Å²), suggesting that assignment of double conformation to this residue is realistic.

Together, our x-ray structures suggest that fit of Triac into the TR α LBC is better than the hTR β LBC (Table 1), contrary to predictions about the nature of TR β -selective ligand binding (7). The Triac carboxylate forms 4 hydrogen bonds with the hydrophilic portion of the hTR α LBC but only makes 2, at best, with the equivalent region of hTR β . Of these, the Arg-320 β interaction with the Triac carboxylate is weak because of poor alignment in the A conformation and is broken in the B conformation.

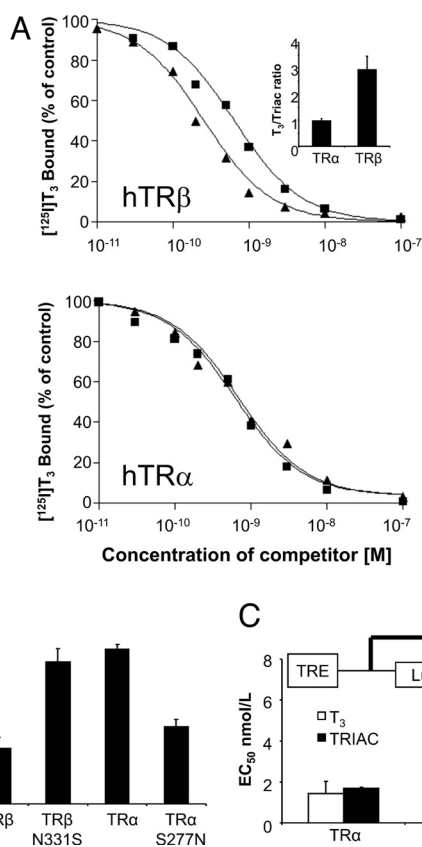


Fig. 2. TR subtype-selective binding and activity of Triac. (A) Competition curves with TR β and TR α obtained with T₃ (squares) and Triac (triangles). Inset: Ratios of T₃/Triac binding for both TRs from several experiments ($n = 3$). (B) Relative K_d values from Triac competition curves ($n = 4$) with TRs and TR mutants were compared with TR β , arbitrarily set to 1. (C) Average EC₅₀ values from luciferase assays using extracts of TR α - and TR β -expressing cells transfected with a TRE-responsive reporter (DR-4) and treated with ligand ($n = 3$).

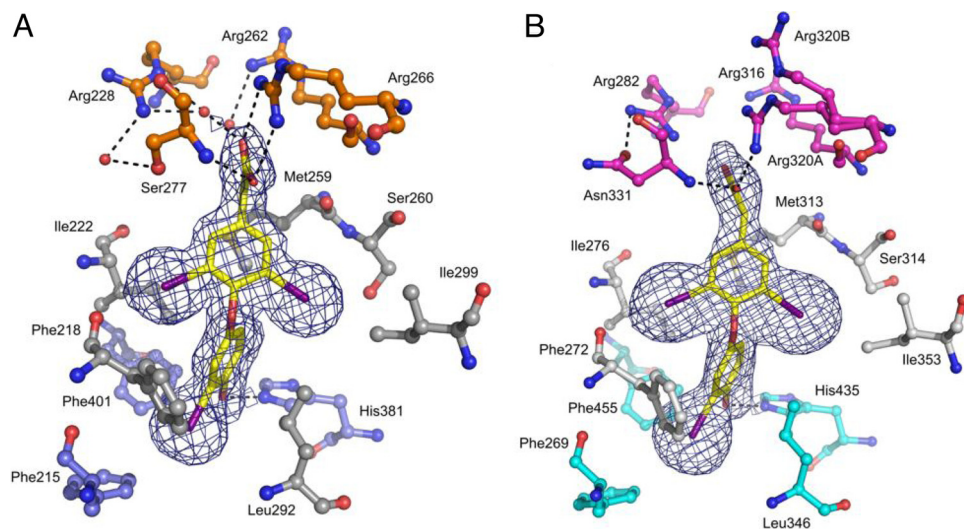


Fig. 3. Better fit of Triac to the TR α LBC. (A and B) Triac interactions with the TR LBCs. Triac is yellow, with electronic density contoured at 1.0 σ . Hydrogen bonds between the Triac carboxylate group and TRs are shown as dotted lines and waters are shown as red balls. (A) Regions of hTR α LBC are in different colors: blue, amphipathic; gray, hydrophobic; and orange, hydrophilic. (B) Equivalent region of hTR β LBC. Cyan, amphipathic; gray, hydrophobic; and purple, hydrophilic. The 2 conformations of Arg-320 are overlaid: A, productive; B, nonproductive.

TR-specific differences in LBC side chain position affect LBC volume around the Triac carboxylate (Fig. 4 A–C). Calculated LBC volume for TR α is 461 \AA^3 , whereas volumes for TR β in the A and B states are 500 \AA^3 and 549 \AA^3 , respectively. Changes in side chain positions in TR β vs. TR α , illustrated by arrows, account for these alterations. Asn-331 β -Arg-282 β interactions that retract the Arg-282 β side chain increase TR β LBC volume in the productive state relative to TR α (Fig. 4 A and B). Repositioning of Arg-320 β in the B conformation accounts for the further increase in TR β LBC volume (Fig. 4 B and C). Similar effects are not seen with TR-T $_3$ structures (Fig. S2); here, Arg-282 β and Arg-228 α side chains interact directly with ligand, resulting in identical LBC configuration and volume.

Water Compensates for Weaker TR β -Triac Interactions. To determine why Triac is TR β selective, we conducted MD simulations to estimate interaction energies of ligand with TRs and observe dynamics. The simulations used new hTR α and hTR β -Triac structures and included a shell of water and ions to reproduce solution conditions. Computation of interaction energies of both TRs and Triac confirms the impressions obtained from x-ray structures: TR α makes stronger direct contacts with ligand (Fig. S3, Table 2). Interaction energies of Triac with TR α were approximately 20 kcal mol $^{-1}$ stronger than with TR β . However, calculation of Triac interaction energies with the complete system (protein + water + ions) revealed no difference (Fig. S3, Table 2); interaction energies of Triac with water in the TR β LBC are stronger than with TR α (Table 2) and compensate for weaker direct interactions of TR β with Triac.

Fig. 5A shows all 5 water molecules that engage in strong favorable interactions (pair energy ≈ -10 kcal mol $^{-1}$) with Triac

during the TR α simulation; each colored trace corresponds to a different water molecule. At the beginning, only 1 molecule (red) interacts strongly with Triac (favorable interactions). This molecule (red) is replaced by another (blue, first arrow), returns (second arrow), and subsequently is replaced by others (green, third arrow; tan, fourth arrow; black). Thus, only 1 water molecule interacts with Triac at each instant of the simulation, and there is constant exchange of the individual molecules in this position. Analysis of individual simulation timeframes reveals that the water molecule that interacts strongly with Triac cor-

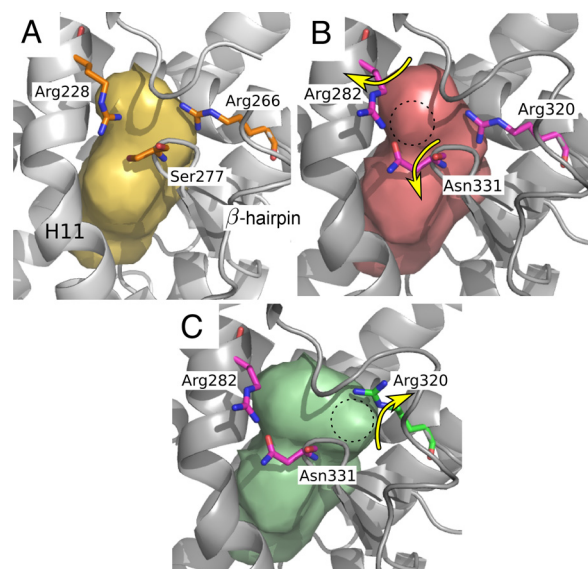


Fig. 4. TR β LBC is larger than the TR α LBC with Triac. (A) Ribbon diagram of TR α showing the LBC in gold space-filling form. Side chains of key Arg residues are shown. (B) Similar view of TR β LBC (pink) with Arg-320 in the productive (A) conformation and movements of TR β N331 and TR β R282 side chains that lead to altered LBC volume relative to TR α (yellow arrows). Note increased volume at the top of the LBC relative to TR α in A (dotted circle). (C) TR β LBC (green), with Arg-320 in nonproductive B conformation and movement of the Arg-320 side chain relative to productive A conformation. Note increased volume on the top right of the LBC relative to TR β in B (dotted circle).

Table 1. Interaction distances of hydrogen bonds between Triac and TR LBCs

HTR α	Distance (\AA)	hTR β	Distance (\AA)
His381	2.62	His435	2.77
Arg266-NH1	3.16	Arg320A-NH1	3.06
Arg266-NH2	3.16	—	—
Ser277	2.83	Asn331	2.63
HOH	2.43	—	—

Table 2. Interaction energies of Triac within MD simulations

Interaction with:	TR α /kcal mol $^{-1}$	TR β /kcal mol $^{-1}$
LBD residues	-66.63	-46.96
Whole environment	-192.74	-192.52
Waters	-18.37	-37.56

responds to the molecule that forms the water-mediated hydrogen bond contact between Arg-228 α and Triac, seen in our high-resolution hTR α x-ray structure (Fig. 5C).

By contrast, Triac interacts strongly with several waters in the TR β LBC (Fig. 5B). At the beginning, 2 waters (green, blue) interact with Triac. As the simulation evolves, another (red) joins the first 2, and at subsequent times either 3 or 4 water molecules are found in the LBC (favorable interactions). As seen with TR α , individual waters fluctuate between low- and high-energy states (events marked by arrows), implying continuous exchange between positions near Triac in the LBC and the external hydration shell. Visualization of relevant interacting water molecules confirms that they lie close to the Triac carboxylate. A representative frame from the simulation (Fig. 5D) reveals 3 water molecules in contact with Triac, with 2 nearby, all of these molecules displaying high mobility throughout the simulation. We conclude that several waters occupy the space in the TR β LBC near the Triac carboxylate group and bridge charged groups of ligand and TR β protein, explaining how water compensates for weaker direct interactions between TR β and Triac (Table 2; see *Discussion*).

We also performed simulations with TR α and TR β structures in which side chains of subtype-specific LBC residues S277 and N331 were exchanged to create computational builds of TR LBC mutants. These simulations lend further support to the notion that TR β N331 regulates LBC volume and water content in the presence of Triac (Fig. S4). TR α -S277N and TR β -N331S mutant

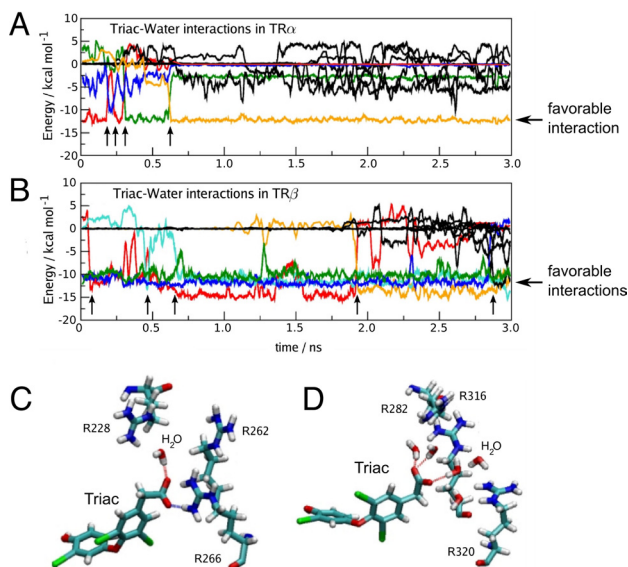


Fig. 5. Triac makes more water contacts in TR β . Interaction energy profiles of most favorable interacting water molecules obtained from MD simulation (A) hTR α and (B) hTR β . Each colored trace represents interaction energies of different water molecules with Triac as a function of time. Arrows indicate water exchange events. Representative views of key amino acids in the hTR α (C) and hTR β (D) LBCs and nearby space in the LBC from the simulation, showing higher hydration level in TR β and that highly favorable interacting waters lie close to the Triac carboxylate. Waters are represented by white/red stick figures, and hydrogen bond interactions with Triac are represented by dotted lines. Note that there are more waters close to the Triac molecule in TR β than in TR α .

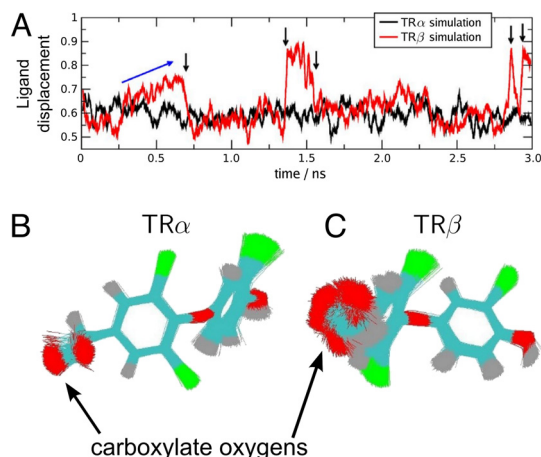


Fig. 6. Triac is more mobile in the TR β LBC. (A) Ligand displacement of Triac over time. RMSD per atom for Triac bound to hTR α (black) and hTR β (red) as a function of time over the simulation. Arrows show Triac conformational transitions in TR β : blue arrow, a smooth transition; black arrows, sharp transitions. (B) hTR α LBC and (C) hTR β LBC indicating increased mobility of the carboxylate in TR β over time. The figures are composed of superimposed Triac conformations from all frames of the simulations. Red, oxygen; green, iodine. The wide distribution of atoms corresponding to the Triac carboxylate oxygens are indicated by the larger space (red).

LBCs acquire some characteristics of the other subtype: there is reversal of Arg-228 α /Arg-282 β position, subtype-specific changes in pocket volume, and water is expelled from the TR β -N331S LBC (Fig. S4 a-c).

Triac Flexibility in TRs. Water compensation of the interaction energies cannot, alone, explain Triac TR β selectivity. However, Triac exhibits increased mobility in the TR β vs. TR α LBC, as indicated by the overall rmsd of Triac atoms. Triac is relatively restricted in TR α , and the ligand oscillates with rmsd amplitudes between 0.5 and 0.7 Å (Fig. 6A). By contrast, Triac exhibits 1 long smooth transition and 5 sharp variations in rmsd in TR β (Fig. 6A, blue and black arrows).

Analysis of individual Triac atoms indicates that the carboxylate oxygens are more mobile (0.011 Å ps $^{-1}$) in TR β vs. TR α (Fig. S5A), owing to increased carboxylate group rotation. Superimposed frames of the simulation reveal that carboxylate oxygens (red) oscillate around well-defined positions in TR α but are delocalized in TR β (Fig. 6B and C). T $_3$ did not exhibit similar movements with either TR (Fig. S5B). We estimated the conformational entropy gain of Triac in TR β vs. TR α from the simulations using an adapted colony potential energy calculation method (ref. 16; *SI Text*, Figs. S6–S8). Predicted conformational entropy gain of Triac in TR β is 1.5 cal K $^{-1}$ mol $^{-1}$, implying a free energy difference of -0.44 kcal mol $^{-1}$, which accounts for the 2–3-fold TR β selectivity of Triac (implied free energy difference -0.45 to -0.65 kcal mol $^{-1}$).

Discussion

In this study, we used biochemical and x-ray structural analysis and MD simulations to investigate Triac interactions with TRs. Triac is a natural TH that displays selective actions in humans (17); it exhibits cholesterol-lowering activities (typically TR β dependent) that are separable from other TR-regulated parameters including heart rate (TR α dependent). Thus, better understanding of Triac selectivity is relevant for understanding of TR activation and thymimetic action *in vivo*.

We confirm that Triac is approximately 3-fold TR β selective *in vitro* (7, 11), close to estimates for GC-1 (4). In addition, we show moderate TR β selectivity in cell culture and find that

selective binding of Triac to TR β is dependent on the nonconserved TR β -LBC residue (Asn-331), like GC-1 and KB141 (7–9). However, x-ray structural data indicate that Triac is not simply a less-selective version of existing compounds; analysis of x-ray structures of hTR-Triac complexes reveals better fit of ligand to the TR α LBC, and this is confirmed by TR protein-Triac interaction energies in our MD simulations, opposite to the case for GC-1 and KB141 (7–9).

Closer analysis of TR x-ray structures coupled with analysis of water and ligand dynamics in MD simulations provides a likely explanation for this apparent paradox. Differences in TR β and TR α LBC amino acid side chain positions that occur with Triac and not T₃ lead to a TR β /Triac complex-specific expansion of pocket volume. The LBC in the TR α -Triac complex is smaller than TR β and contains space for 1 water molecule near the Triac carboxylate. By contrast, the same region of the TR β LBC accommodates 2 to 5 waters. Our calculations indicate that improved water contacts compensate for weaker direct TR β interactions with ligand; water molecules act as bridges between ligand and protein (Table 2). The waters also allow the Triac carboxylate group more flexibility in the TR β LBC than TR α ; Triac carboxylate oxygens are approximately twice as flexible in TR β (Figs. 5B and S5A). Because interaction energies of Triac with both TR systems (protein + water + ions) are similar, we surmise that increases in free energy of association of Triac with TR β stem from the entropic contribution of increased ligand flexibility. Indeed, calculations of entropic gain of Triac binding to TR β based on observed ligand flexibility in simulations (16) agree with binding preferences of Triac for TR β .

To our knowledge, entropic effects have not been explicitly invoked to explain subtype-selective binding of synthetic TR or NR ligands. It is clear that such effects play important roles in several protein–ligand interactions (18, 19); examples include improved inhibitor binding (20, 21) and many other aspects of protein/small molecule interactions, such as ligand dissociation paths (22) and ion permeation (23). It is interesting to evaluate previous NR structures in the light of our results. TR β selectivity of GC-24 stems mostly from the extension of the LBC, but Asn-331 β also contributes to selectivity, and contacts between the GC-24 carboxylate and this part of the LBC seem to be suboptimal (10). Thus, entropic influences could contribute to selective binding of GC-24. Improved affinity of vitamin D receptor ligands has been associated with factors shown to be important here, including water in the LBC (24), increased LBC volume, and multiple ligand conformations (25–27). Two groups, including ours, found that water can substitute for charged regions of NR LBCs at little or no energetic cost in MD simulations, lending support to the idea that water substitutes for loss of direct protein contacts (17, 18, 28). However, perhaps the best example of links between entropic contributions and subtype selectivity may come from binding modes of the ER ligand THC [(R,R)-5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysen-2,9-diol], a selective agonist that binds ER β with 4-fold higher affinity and antagonizes ER α (29). X-ray structures reveal that the ER β LBC, generally smaller than ER α , expands with THC to reduce direct ligand contacts (30). This seems strongly analogous to the TR-Triac case.

It will be important to assess entropic and enthalpic contributions to TR ligand binding affinity *in vitro*. Classically, this is addressed with isothermal titration calorimetry, which measures heat variation (ΔH) from a system at set temperature and free energy of ligand association (ΔG). Standard versions of this approach rely on rapid ligand binding (<1 s), but NR ligand association kinetics are slow (31). It is theoretically possible to

correct for this effect by extending the experiment, but lability of standard TR preparations over long times at higher temperatures and the hydrophobic nature of ligands precludes reliable results. We continue to work on this problem, but one piece of experimental evidence favors an entropic contribution to Triac binding: the TR β -Triac complex is more resistant to thermal denaturation than the equivalent TR β -T₃ complex (Fig. S1). Such effects have previously been linked to entropic contributions in free energies of ligand association (23, 32).

Our observations imply that standard approaches to improve TR and NR ligand binding by increasing complementarity of ligand and LBC may not identify the tightest binding or most selective ligands. Will it be possible to use enthalpy/entropy compensation for design of new TR β -selective thymomimetics? Rational consideration of enthalpy and entropy in ligand binding is a difficult issue (33). However, combinations of biochemical/structural analysis and MD simulations could permit intelligent approaches. Ye et al. (8) found that TR β selectivity of Triac was reduced from 2.9 to 1.3 with a larger 1-substituent in L-3'5'3'-triiodothyropropionic acid. Given that the Triac carboxylate group improves ligand selectivity because it explores more conformations at this position in the larger pocket, it may be possible to exploit entropic influences in NR ligand design by targeted reduction of substituent size near regions of the LBC that can vary in volume.

Materials and Methods

Protein Purification. hTR β LBD (202–461) and hTR α LBD (148–410) were expressed as described previously (34). Ligand was added to 5-fold excess after cell disruption by sonication. Both proteins were concentrated by centrifugation before crystallization.

Ligand Binding. T₃ and Triac binding affinities were determined by saturation binding assays as described previously (35). HeLa cells that express TR α or TR β were transfected with a DR-4 element-driven luciferase reporter (35).

Protein Crystallization and Data Collection. hTR α 1 crystals grew in conditions previously described (34). hTR β 1 crystals grew in conditions established for other hTR β complexes (7). Datasets were collected using a Mar345db image plate mounted at a Rigaku rotating anode X18 source equipped with Osmic Confocal MaxFlux mirrors operating with CuK α radiation. During data collection, crystals were kept on a nitrogen stream at 100 K.

Structure Solution. Datasets were reduced and merged using MOSFLM/SCALA. Structures of hTR α LBD and hTR β LBD with Triac were solved by molecular replacement using TR-T₃ complexes as search models. Molecular replacement used MOLREP, and structures were refined using REFMAC and PHENIX. To gain in precision of measurements of reflection intensities and to obtain a robust data for x-ray structure refinement, we measured redundant datasets for hTR α and hTR β crystals (Table S2).

MD Simulations. New hTR α and hTR β structures with Triac were used. Simulated systems contained LBD, Triac, and a 15-Å-thick solvation layer with 16,500 water molecules and counter ions. Water and ions were added with Packmol (36), and 3-ns-long simulations were performed with NAMD (37) using CHARMM parameters (38). Ligand parameters and the protocols for equilibration and simulations are described in ref. 14. Binding cavities and volumes were computed using VOIDOO (39). Figures of molecular structures were prepared with Pymol (40).

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