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β-apo-13-Carotenone Regulates Retinoid X Receptor Transcriptional Activity Through Tetramerization of the Receptor*

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*Running title: *β-apo-13-Carotenone as an RXR antagonist*

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Background: β-apo-13-Carotenone, a naturally-occurring apocarotenoid, functions as an antagonist of the retinoid X receptor (RXR).
Results: β-apo-13-Carotenone inhibits transactivation of RXRα but does not interfere with coactivator binding to the receptor like the known antagonist UVI3003.
Conclusion: β-apo-13-Carotenone induces the formation of a transcriptionally silent RXR tetramer.
Significance: β-apo-13-Carotenone is a naturally-occurring rexinoid with a novel mechanism of antagonism.

ABSTRACT

Retinoid X receptor (RXRα) is activated by 9-cis-retinoic acid (9cRA) and regulates transcription as a homodimer or as a heterodimer with other nuclear receptors. We have previously demonstrated that β-apo-13-carotenone, an eccentric cleavage product of β-carotene, antagonizes the activation of RXRα by 9cRA in mammalian cells overexpressing this receptor. However, the molecular mechanism of β-apo-13-carotenone's modulation on RXRα transcriptional activity is not understood and is the subject of this report. We performed transactivation assays using full length RXRα and reporter gene constructs (RXRE-Luc) transfected into COS-7 cells and luciferase activity was examined. β-apo-13-Carotenone was compared with the RXRα antagonist UVI 3003. The results showed that both β-apo-13-carotenone and UVI 3003 shifted the dose-dependent RXRα activation by 9cRA. In contrast, results of assays using a hybrid Gal4-DBD:RXRα-LBD receptor reporter cell assay that detects 9cRA-induced coactivator binding to the ligand binding domain demonstrated that UVI3003 significantly inhibited 9cRA-induced coactivator binding to RXRαLBD, but β-apo-13-carotenone did not. However, both β-apo-13-carotenone and UVI 3003 inhibited 9-cRA induction of caspase 9 gene expression in the mammary carcinoma cell line MCF7. In order to resolve this apparent contradiction we investigated the effect of β-apo-13-carotenone on the oligomeric state of purified recombinant RXRα LBD. β-apo-13-carotenone induces tetramerization of the RXRαLBD whereas UVI3003 had no effect on the oligomeric state. These observations suggest that β-apo-13-carotenone regulates RXRα transcriptional activity through inducing the formation of the "transcriptionally silent" RXRα tetramer.

Retinoid X receptors (RXRα, RXRβ and RXRγ) are members of nuclear receptor (NR) family and play a central role in NR-regulated signaling pathways. RXRs are involved in biological processes, including cell growth and differentiation, metabolism, morphogenesis, and embryogenic development (1 – 7) The active form of RXR is a dimer or heterodimer

(8, 9). Besides the RXR homodimer, RXR also forms heterodimers with other NR family members, including retinoic acid receptors (RARs), the vitamin D receptor (VDR), peroxisome proliferator-activated receptors (PPARs), the farnesoid X receptor (FXR) and the liver X receptors (LXRs) (10, 11). RXR naturally forms into tetramers which are transcriptionally inactive (12).

RXRs are primarily made up of two modular domains: a central DNA binding domain (DBD) and a carboxy-terminal ligand binding domain (LBD). In addition to its role in binding of ligands, the LBD contains dimerization motifs and an activation function 2 (AF-2) domain (13, 14). Ligand free RXR represses transcription of target genes through interaction with corepressor proteins. Ligand binding induces a conformational change of the AF-2 helix that releases corepressor protein and allows recruiting of coactivator complexes. Numerous compounds synthesized as antagonists, such as UVI 3003, target the AF-2 helix (13).

Ligand free RXR tends to associate into homotetramers both in solution and when bound to DNA. However, RXR tetramers rapidly dissociate into active dimers upon binding of an agonist such as 9-cis retinoic acid (9cRA). RXR heterodimers bind in regulatory regions of their target genes by associating with response elements (REs). RXR homodimers bind to a retinoid DNA response element (RXRE). Activation of DNA-bound dimers by ligands promotes the recruiting of transcriptional coactivators to the promoters of target genes and enhances transcription rate. In vitro studies have indicated that full-length RXR self-associates into tetramers and the LBD alone is sufficient to mediate tetramer formation with 3-5 nM affinity between the dimers (12, 15). Studies have substantiated the existence in vivo of an RXR tetramer (12, 15). It also has been shown that the RXR tetramer is transcriptionally silent based on the correlation between the transcriptional activity of RXR mutants and their ability to form tetramers (16).

The vitamin A metabolite 9cRA is a ligand of RXR (17). Binding of 9cRA as an agonist induces the dissociation of the tetramer into dimer, which is the first step for RXR activation (18, 19). Carotenoids are polyisoprenoids that are biosynthesized in plants, fungi and bacteria. Approximately 50 – 60 carotenoids that contain at least one unsubstituted β-ionone ring and the correct number and position of methyl groups in the polyene chain exhibit provitamin A activity (20, 21). Dietary provitamin A, β-carotene, can be metabolized in mammals through two pathways (22). β-Carotene oxygenase 1 (BCO1) catalyzes the cleavage of the 15,15′ double bond resulting in two retinaldehyde molecules, and the eccentric cleavage takes place at double bonds other than the central 15,15′ double bond to produce β-apocarotenoids with different chain lengths. β-Apocarotenoids have been detected in foods (23) and the blood of both humans (24) and animals (25). Recently, β-apo-8′-carotenal was detected in plasma after ingestion of β-carotene by a healthy human subject (24). Our previous studies demonstrated that β-apo-13-carotenone functioned as an antagonist in transactivation assay using full length RXRα (26) and the retinoic acid receptors (RAR) α, β, and γ (27). We have reported that β-apo-13-carotenone competes for 9cRA binding to RXRα with an affinity (7–8 nM) identical to 9cRA itself (27). However, the molecular mechanism of β-apo-13-carotenone's modulation of transcriptional activity is not understood yet. This study focused on the mechanism by which β-apo-13-carotenone antagonizes 9cRA induced activation of RXRα. Our results show that β-apo-13-carotenone induces formation of the RXRα transcriptionally-silent tetramer but does not inhibit coactivator recruitment to the isolated LBD.

EXPERIMENTAL PROCEDURES

Materials

COS-7 African green monkey kidney cells and MCF7 mammary carcinoma cells from ATCC (Rockville, MD) were cultured in

DMEM supplemented with 10% FBS. Cells were maintained at 37^0C with 10% CO_2. 9-cis Retinoic acid and UVI3003 were purchased from Santa Cruz. β-apo-13-carotenone was synthesized as described previously (27). All other chemicals were from Sigma-Aldrich.

Nuclear Receptor Reporter Cell Assay with Full Length hRXRα

COS-7 cells were cultured in 96-well plates overnight. cDNA of full length human RXRα in pSV sport vector (Addgene) was co-transfected with Renilla (pRL-tk) and Firefly luciferase (RXRE-Luc) reporter constructs into COS-7 cells in serum free DMEM with X-tremeGENE 9 DNA (Roche). Twenty four hours after transfection, COS-7 cells in DMEM with 10% charcoal stripped FBS were then treated with 9cRA in presence or absence of β-apo-13-carotenone or UVI 3003 for an additional 24 hours. Cell lysates were used in the dual luciferase assay (Promega) to determine the activation of hRXRα by 9cRA and the inhibition by β-apo-13-carotenone and UVI 3003. For each experiment, the firefly luciferase (experimental reporter) activity was normalized to Renilla luciferase (control reporter).

Human RXRα-LBD Reporter Cell Assay

Reporter cells expressing human RXRαLBD fused to the GAL-4 DBD (Indigo Biosciences, State College PA) were treated according to the manufacturer's protocol. Reporter cells were incubated with 0, 0.32, 1.6, 8, 40, 200, 1000, and 5000 nM 9cRA for 24 hours at 37 ^{0} C in the presence or absence of fixed concentrations of either β-apo-13-carotenone or UVI 3003. Luminescence was detected with Glomax96 luminometer (Promega).

Quantitative Real-Time PCR

Breast cancer cells MCF7 were cultured in 6-well plates and starved for 24 hours in serum free DMEM. MCF7 cells were then treated with ligands in serum free medium for 4 hours. Total RNA was isolated using NucleoSpin RNA II (Macherey-Nagel). Two micrograms of RNA was reversed-transcribed into cDNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR analysis was performed in quadruplicates with TaqMan Chemistry and probed for caspase 9 (Hs00154260-m1) (Applied Biosystems). Human GAPDH (4333764F) was used as housekeeping gene. The comparative C_t method ($\Delta\Delta C_t$) was used to analyze results.

mRXRαLBD Expression in E. Coli and Protein Purification

N-His-tagged mouse RXRα LBD (pET15b) was transformed into BL21(DE3). The E coli culture was grown at 37^0C to OD600 of 0.6. After induction with 0.5 mM IPTG, cells was incubated for another 2 ~ 4 hours at 25 ^{0} C. Cells were harvested and lysed in lysis buffer (20 mM Tris, 500 mM NaCl, 5 mM imidazole and 3 mM DTT, pH8.0). The supernatant was loaded onto a HisPur Ni-NTA affinity column followed by extensive washing with 20 mM imidazole in lysis buffer. His-mRXRαLBD was eluted with 500 mM imidazole in lysis buffer. The concentrated protein peak fraction was then applied to Hiload Superdex 200 gel filtration column for isolation of mRXRαLBD dimer and tetramer. The gel filtration column was calibrated with protein standards of 13.7, 25, 43, 67, 158, 232, and 440 kDa and blue dextran 2000 in order to confirm the molecular weights of the mRXRαLBD dimer and tetramer. Protein concentration was determined with the Bradford reagent (Bio-Rad) with bovine serum albumin as a standard. The purity of protein was assessed by SDS-PAGE and Coomassie Blue staining.

Gel filtration Chromatography for Detection of mRXRαLBD Dimer and Tetramer

Purified mRXRαLBD dimer (50 μM in monomer concentration) was incubated with β-apo-13-carotenone or UVI 3003 in various concentrations on ice for 3 hours or overnight. Tween 40 was added in order to increase ligand solubility in the aqueous buffer. In other experiments, mRXRαLBD tetramer (50 μM in monomer concentration) was firstly saturated

with 100 μM β-apo-13-carotenone then was incubated with 9cRA. After ligand treatment mRXRαLBD was subjected to gel filtration chromatography on a Superdex 200 HR column controlled by an AKTA FPLC system (GE). The running buffer contained 20mM Tris, 150mM NaCl, pH7.5 at 4^0C. Protein chromatograms were monitored at 280nm. As above, this gel filtration column was also calibrated with proteins of known molecular weight to confirm the retention volumes of the mRXRαLBD dimers and tetramers.

RESULTS

β-apo-13-Carotenone and UVI 3003 Antagonize 9cRA Induced Transactivation of Full Length RXRα

To investigate the effect of β-apo-13-carotenone and UVI 3003 on the functional role of RXRα, an RXRE-luciferase receptor/reporter transactivation assay was performed. Full length hRXRα was transiently co-transfected in COS-7 cells with two reporter plasmids, a firefly luciferase reporter containing RXRE from CRBP-II and Renilla luciferase as an internal control. In transfected cells 9cRA induced luciferase activity in a dose-dependent manner over a concentration range of 5 x 10^{-5} M (50 μM) – 3.2 x 10^{-10} M (0.32 nM), as shown in Figure 1. To determine the antagonist function of β-apo-13-carotenone and UVI 3003, cells were treated with 9cRA in a presence of β-apo-13-carotenone or UVI 3003 at a constant concentration of 200 nM. We observed a shift in the 9cRA dose-response curve induced by both β-apo-13-carotenone and the known antagonist UVI 3003. β-apo-13-Carotenone alone did not induce the activation of RXRα (data not shown). This suggests that β-apo-13-carotenone antagonizes 9cRA activation of full length hRXRα with a similar efficiency as the known antagonist UVI 3003.

β-apo-13-Carotenone and UVI 3003 Inhibit 9cRA Induction of an Endogenous RXRα Responsive Gene

It was previously reported that caspase 9 and Btg2 are direct target genes for RAR-RXR heterodimers (28 – 30). Thus, we asked whether β-apo-13-carotenone and UVI 3003 would inhibit the 9cRA-induced transcription of the endogenous gene caspase 9. For these experiments MCF-7 cells were serum-starved for 24 hours and followed by incubation with 9cRA, β-apo-13-carotenone or UVI 3003 at concentrations of 200 nM for 4 hours. As shown in Figure 2, 9cRA upregulated the expression of mRNA for caspase 9 3-4 fold. Both β-apo-13-carotenone or UVI 3003 inhibited 9cRA-induced gene expression. These data support the results shown above that β-apo-13-carotenone and UVI 3003 antagonize 9cRA induced transactivation of RXRα in transactivation assays.

UVI 3003 Inhibits 9cRA-induced Coactivator Binding to the RXRα Ligand Binding Domain but β-apo-13-Carotenone Does Not

To further characterize the mechanisms of β-apo-13-carotenone and UVI 3003 as antagonists for RXRα, we used cells that stably express a fusion protein containing the Gal4-DBD linked to the ligand binding domain (LBD) of RXRα. The luciferase reporter gene utilized in these assays contains the Gal4 upstream activation sequence (UAS) linked to the luciferase reporter gene. 9cRA activated the transcription, however β-apo-13-carotenone alone did not activate the RXRα reporter assay (Figure 3A). Although we tested co-treatment of β-apo-13-carotenone at several different concentrations (5, 10, 100, 200, 500, 1000 nM) with 9cRA, no marked shift of the 9cRA dose-response curve was observed (Figure 3B). In contrast, 200 nM or 500 nM UVI 3003 prominently shifted the 9cRA dose-response curve, as shown in Figure 3 C. In this assay the activation of RXRα does not require the formation of RXRαLBD dimer. 9cRA binding to the ligand binding domain of RXRα provokes a conformational change of the AF-2 motif that produces a suitable binding surface for recruitment of coactivators. Previous structural studies have shown that the binding of antagonist UVI 3003 to LBD of RXRα disturbs the conformation of helix 12 (H12)

and leads to inhibition of coactivator recruitment (31). Strikingly, in the experiments reported here using the hybrid receptor, β-apo-13-carotenone had no effect on coactivator binding to the RXRαLBD.

β-apo-13-Carotenone Regulates RXRα Through Tetramerization of the Receptor

To further elucidate the mechanism underlying regulation of the RXRα transcriptional activity by β-apo-13-carotenone, we investigated the dimer-tetramer equilibrium of RXRαLBD after exposure to ligand. Mouse RXRαLBD was expressed in E. coli and purified to homogeneity as shown in Figure 4. Gel filtration chromatography on calibrated columns of Superdex 200 was used to isolate the mRXRαLBD dimer and tetramer used in the following experiments. Recombinant mouse RXRαLBD dimer 50 μM (calculated as monomer concentration) was incubated with increasing concentrations (100, 250, 500 μM) of β-apo-13-carotenone on ice for 3.5 hours or overnight. Gel filtration chromatography demonstrated β-apo-13-carotenone induced formation of RXRαLBD tetramer (Figure 5). Treatment with 500 μM of β-apo-13-carotenone for 3.5 hours, in the molar ratio to monomer receptor of 5:1, led to 33% tetramer formation of the RXRαLBD, whereas if treatment was extended overnight 50% tetramer RXRαLBD formed. In contrast, the antagonist UVI 3003 did not induce tetramer formation at any of the tested concentrations (Figure 6) even if incubations were extended overnight (data not shown). Finally, β-apo-13-carotenone saturated RXRαLBD tetramer, in molar ratio 2:1 (β-apo-13-carotenone: monomer RXRαLBD), was incubated with the agonist 9cRA. RXRαLBD tetramer dissociated to dimer with the addition of agonist 9cRA. At 50 μM, in an equal molar concentration to monomer RXRαLBD, 9cRA induced approximately 55% of dimer, whereas higher concentrations of 9cRA almost completely converted tetramer to dimer (Figure 7). The gel filtration chromatography results showed that β-apo-13-carotenone induced the tetramerization of RXRαLBD, which was reversed with addition of 9cRA. In contrast, the antagonist UVI 3003 did not influence the tetramer-dimer equilibrium of RXRαLBD.

DISCUSSION

In this study we characterize the activity of β-apo-13-carotenone as an antagonist to RXRα and reveal the mechanism of RXRα antagonism by β-apo-13-carotenone-induced tetramerization of the receptor. The comparison of experimental data of β-apo-13-carotenone and UVI 3003 indicated that these RXRα antagonists use two distinct mechanisms. β-apo-13-Carotenone, a naturally-occurring β-apocarotenoid which can be obtained from either the diet directly or from eccentric cleavage of β-carotene functioned as an antagonist of RXRα. UVI 3003 is a selective antagonist of RXRα whose inhibitory effect results from an interference of its long side chains with L451 of H12 (31). Both β-apo-13-carotenone and UVI 3003 inhibited 9cRA induced transactivation of full length RXRα in a dual luciferase assay, whereas higher concentrations of 9cRA overcame the inhibition by the two antagonists. Both antagonists inhibited 9cRA induction of the expression of the caspase 9 gene in MCF7 cells.

The tetramerization of RXRα induced by β-apo-13-carotenone is supported by reporter cell based assays. The 'Gal4-DBD:RXRα-LBD' receptor expressed in the reporter cells will bind ligand, translocate to the nucleus, bind to the Gal4 UAS sequence on the reporter gene, recruit co-activator proteins, and lead to the transcription of luciferase. It is important to note that the whole process of transcription in these reporter cells does not require dimerization of RXRα. The conformational change of RXRαLBD due to ligand binding is sufficient to activate coactivator recruitment and subsequent luciferase transcription. β-apo-13-carotenone is inactive in this assay. In contrast, full length RXRα expressed in Cos-7 cells undergoes nuclear translocation, dimer formation upon agonist binding, binding to the RXRE, coactivator recruitment and luciferase

transcription. The distinctive difference in mechanism between the reporter cell assay with Gal4-DBD:RXRα-LBD and the transactivation assay with full length RXRα is that dimer formation is obligatory for the latter; and β-apo-carotenone is only effective as an antagonist in this assay. These observations suggest that β-apo-13-carotenone inhibits 9cRA induced RXRα transcription through the formation of the RXRα tetramer.

In an X-ray crystal study, atRA has been shown to bind to the transcriptionally silent tetrameric RXRα in a unique conformation (32). Previously, we showed using molecular modeling that when this bound atRA is computationally removed from the tetrameric RXR protein and redocked it assumes the identical position as in the crystal structure (26). In addition, when β-apo-13-carotenone is built in a similar conformation to this atRA and docked into this RXRα tetramer it occupies the same position and has the same conformation as the bound atRA. Alternatively, when we built β-apo-13-carotenone in a conformation similar to RXRα bound 9cRA (33), and attempted to dock this molecule into the dimeric RXRα it assumes a very different position than the agonist ligand. Thus we suggested that β-apo-13-carotenone should be capable of acting as an antagonist of RXRα by stabilizing the transcriptionally silent tetramer, but we had no direct biochemical evidence for that suggestion at that time.

The crystal structure study of the ligand-binding domain of the RXRα suggested that a cavity corresponded to the RXR ligand-binding site and that 9cRA binding triggered a conformational modification of H11 which lead to ligand-dependent transactivation by AF-2 (33). It has also been reported that the tetramerization domain is located in helix 11 at the RXRαLBD and tetramerization does not interfere with the function of helix12 (34). Thus β-apo-13-carotenone could cause tetramerization of RXRα by interacting with helix 11, and not affecting helix 12 (or coactivator binding). In contrast, inhibition of 9cRA induced RXRα transcription by UVI 3003 is due to the blockage of helix 12 as pointed out above (31).

RXRα tetramer formation induced by β-apo-13-carotenone was confirmed biochemically in the present studies by the observations of gel filtration chromatography with purified recombinant mouse RXRα LBD. Comparison between the β-apo-13-carotenone and UVI 3003 treated dimeric RXRα LBD indicates that β-apo-13-carotenone regulates RXRα transcription through tetramerization, whereas inhibition by the antagonist UVI 3003 is due to interference with helix 12. The complete dissociation of the tetramer RXRα LBD saturated with β-apo-13-carotenone to dimer by 9cRA shows that tetramerization is reversible when the agonist is in sufficient concentration. Thus, the equilibrium of RXRα dimer and tetramer could be controlled by the availability of ligands. A model of these various effects of ligands on RXRα is shown in Fig. 8.

In summary, the present study revealed the mechanism of ligand-dependent regulation of RXRα transcriptional activity by the antagonist, β-apo-13-carotenone. The findings imply that tetramerization of RXR and factors that modulate the oligomer state may contribute to regulation of cellular signaling. β-apo-13-Carotenone induced tetramerization could conserve RXRα as an inactive nuclear receptor pool which can rapidly supply dimeric or monomeric RXRα upon 9cRA generation. This may also suggest a ligand-dependent modulation controlling the availability of RXRα for the heterodimerization with other nuclear receptor partners engaged in multiple signaling pathways.

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FOOTNOTES
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The abbreviations used are: RXR-retinoid x receptor, 9cRA-9-cis-retinoic acid, LBD-ligand binding domain, DBD-DNA binding domain, RXRE –retinoid x response element, MCF-7-Michigan Cancer Foundation-7 (a human mammary cancer cell line),

Figure Legends

Figure 1. The antagonist effects of β-apo-13-carotenone and UVI 3003 on transactivation of full length RXRα . COS-7 cells were co-transfected with full length hRXRα, pRL-tk-luc(Renilla) and RXRE-luc(firefly). Twenty-four hours after transfection, cells were treated with 9cRA at concentrations of 0, 0.064, 0.32, 1.6, 8, 40, 200, 1000, 5000,25000, 50000 nM in presence or absence of 200 nM β-apo-13-carotenone or UVI 3003 for another 24 hours in 37 ^{0}C incubator. 9cRA alone (Blue), 9cRA plus β-apo-13-carotenone at 200 nM (Red), 9cRA plus UVI 3003 a 200 nM (Green). Luciferase activity is shown on the y-axis.

Figure 2. β-apo-13-carotenone and UVI 3003 inhibit 9cRA-induced expression of mRNA for caspase 9. MCF7 cells were cultured in 6-well plate and serum-starved for 24 hours before ligand treatment. Cells were incubated with 200 nM ligands (9cRA, β-apo-13-carotenone or UVI 3003) in serum-free medium for 4 hour. The comparative Ct method (ΔΔCt) was used to analyze results. Data are presented as means ± SE, n=5. Antagonist treatment (c) significantly inhibits 9cRA induced Casp9 gene expression (b) at $P < 0.006$).

Figure 3. β-apo-13-carotenone does not antagonize 9cRA induced transactivation in cells expressing Gal4-DBD:RXRα-LBD and a UAS-driven luciferase reporter. Cells were incubated with 9cRA or β-apo-13-carotenone at concentrations of 0, 0.32, 1.6, 8, 40, 200, 1000, and 5000 nM for 24 hours at 37 ^{0} C (A); cells were incubated with these same concentrations of 9cRA in presence or absence of fixed concentrations of β-apo-13-carotenone (B) or UVI 3003(C).

Figure 4. Purification of recombinant mRXRαLBD. Histidine tagged mRXRαLBD (theoretical molecular mass for monomer = 28,821 Da) was expressed in *E. coli* strain BL21(DE3) and purified firstly with a Ni-NTA affinity column. The elution fraction 1 of the affinity column was then applied onto a gel filtration column of Hiload Superdex200 16x60 column to separate the mRXRαLBD tetramer and dimer. A, SDS-PAGE of the fractions from Ni-NTA affinity column. Lane 9 was the mRXRαLBD elution fraction collected for second step purification with gel filtration. B, Separation of mRXRαLBD tetramer and dimer with gel filtration column. mRXRαLBD tetramer was collected between the retention volume of 61.4 ml to 68.4 ml. mRXRαLBD dimer was collected between retention volume 72.4 ml to 84.4 ml. The gel filtration column was calibrated with standard proteins of known molecular weight as described in the text.

Figure 5. Gel filtration analysis for tetramerization of RXRα LBD induced by β-apo-13-carotenone. Purified mRXRαLBD dimer (50 μM in monomer concentration) was incubated with β-apo-13-carotenone in concentrations of 0, 100, 250, 500 μM on ice for 3 hours or overnight. Tween 40 of 0.75% (w/v) was added in order to increase ligand solubility in the aqueous buffer. Superdex 200 HR column and elution buffer were well equilibrated at 4 °C with elution buffer consisted of 20 mM Tris, 150 mM NaCl, and 3 mM DTT, pH 7.4. Protein chromatograms were monitored at 280nm.

Figure 6. UVI 3003 does not induce tetramerization of RXRα LBD. Purified mRXRαLBD dimer (50 μM in monomer concentration) was incubated with UVI 3003 in concentrations of 0, 250, 500 μM on ice for 3 hours. Superdex 200 HR column and elution buffer were well equilibrated at 4 °C with elution buffer consisted of 20 mM Tris, 150 mM NaCl, and 3 mM DTT, pH 7.4. Protein chromatograms were monitored at 280nm. Extending the incubation time with 500 μM UVI 3003 overnight did not lead to tetramer formation.

Figure 7. 9cRA dissociates the tetramer of RXRα LBD induced by β-apo-13-carotenone. mRXRαLBD tetramer (50 μM in monomer concentration) was firstly saturated with 100 μM β-apo-13-carotenone then was incubated with 9cRA in concentrations of 0, 50, 100, 250 μM on ice for 3 hours. Protein chromatograms were monitored at 280nm.

Figure 8. Model depicting the effects of ligands on the oligomeric state and transcriptional activity of RXRα. See text for details.

Figure 1

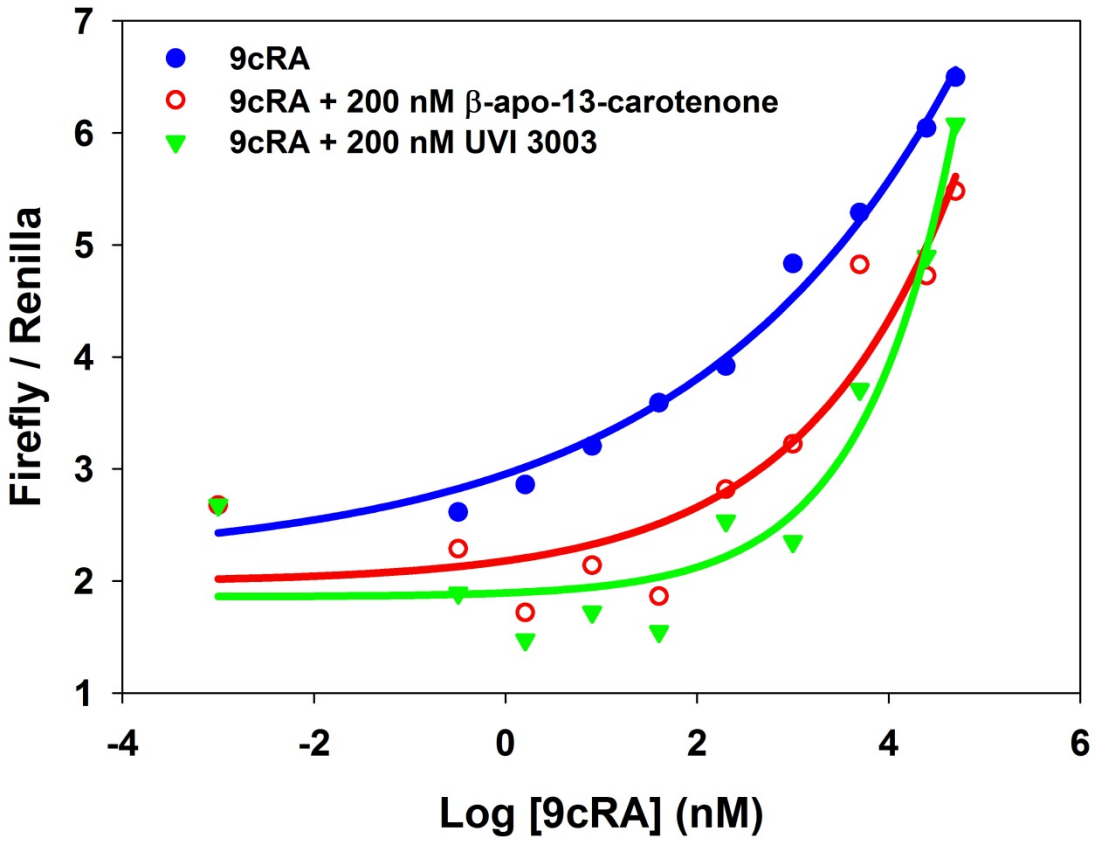
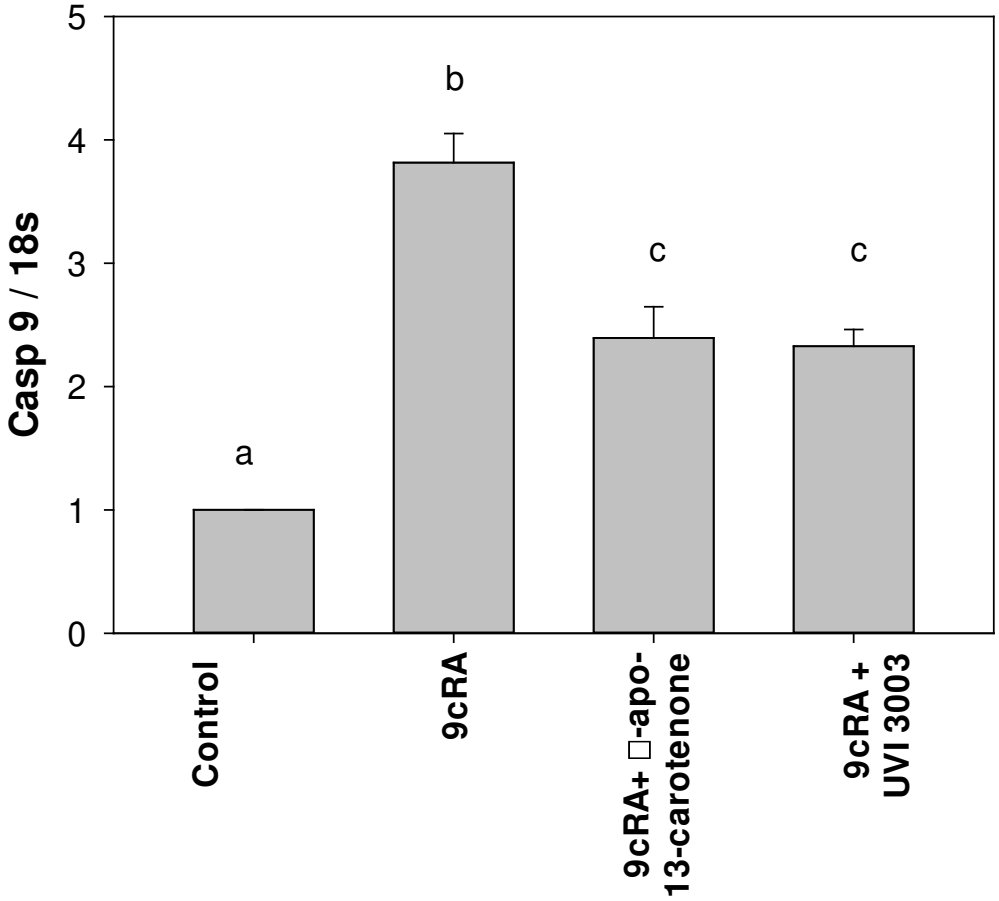


Figure 2



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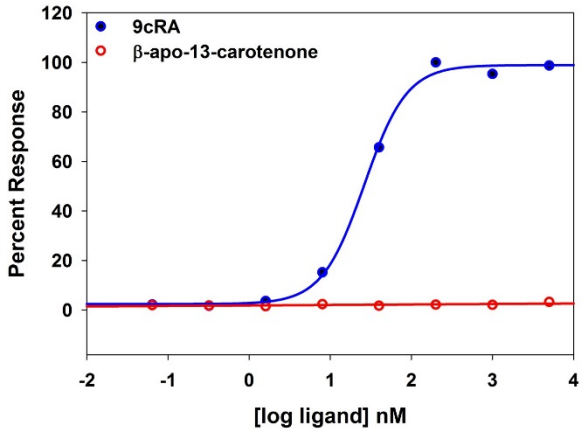

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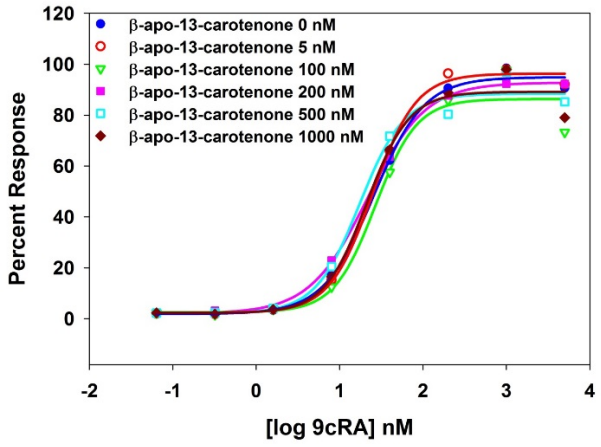

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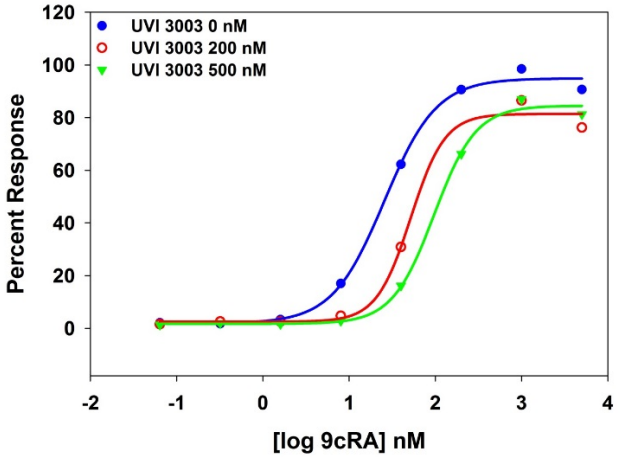
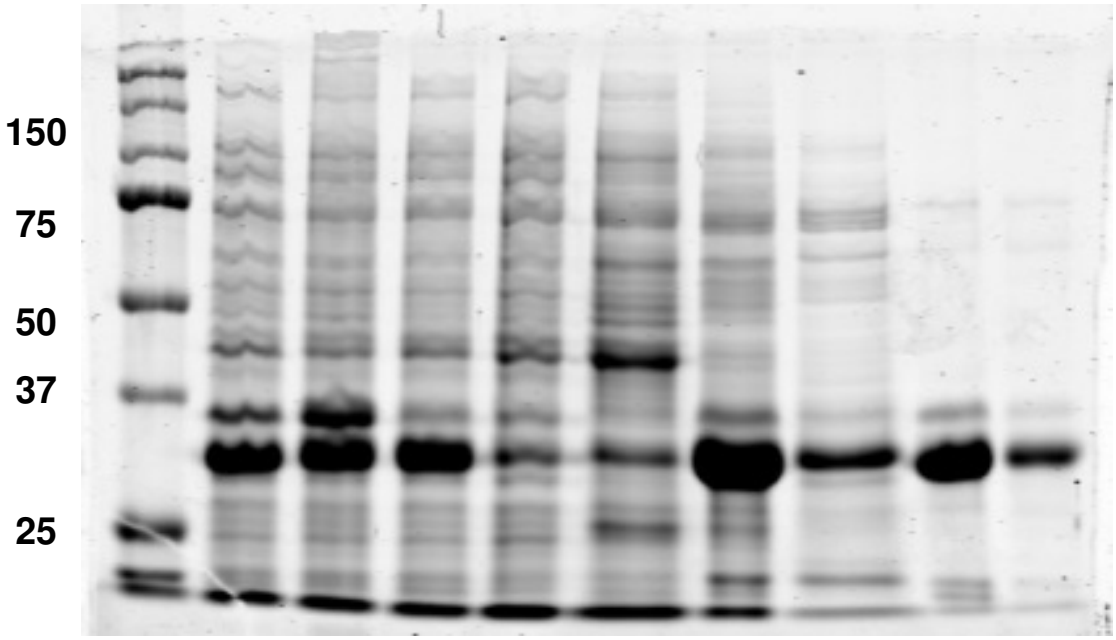


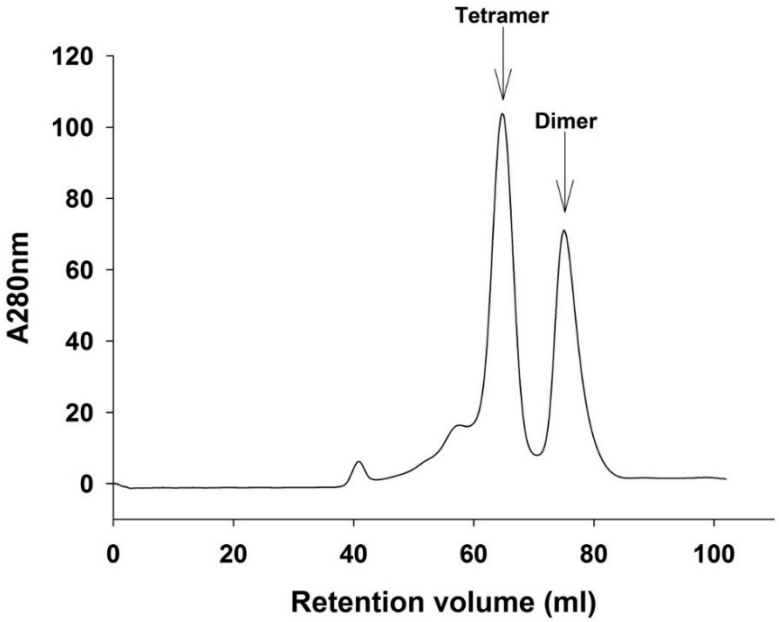
C

Figure 4A



1	2	3	4	5	6	7	8	9	10
Maker	Whole lysate	Pellet	Super-natant	Flow through	Wash 1	Wash 2	Wash 3	Fraction 1	Fraction 2

Figure 4B



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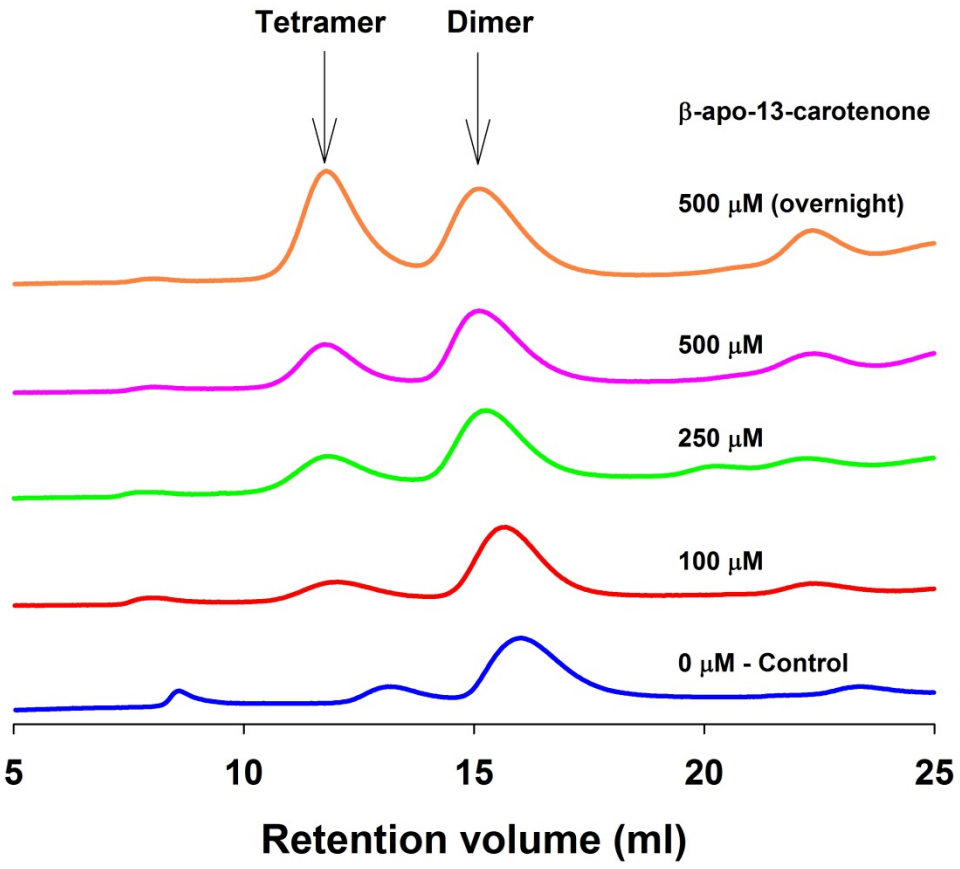


Figure 6

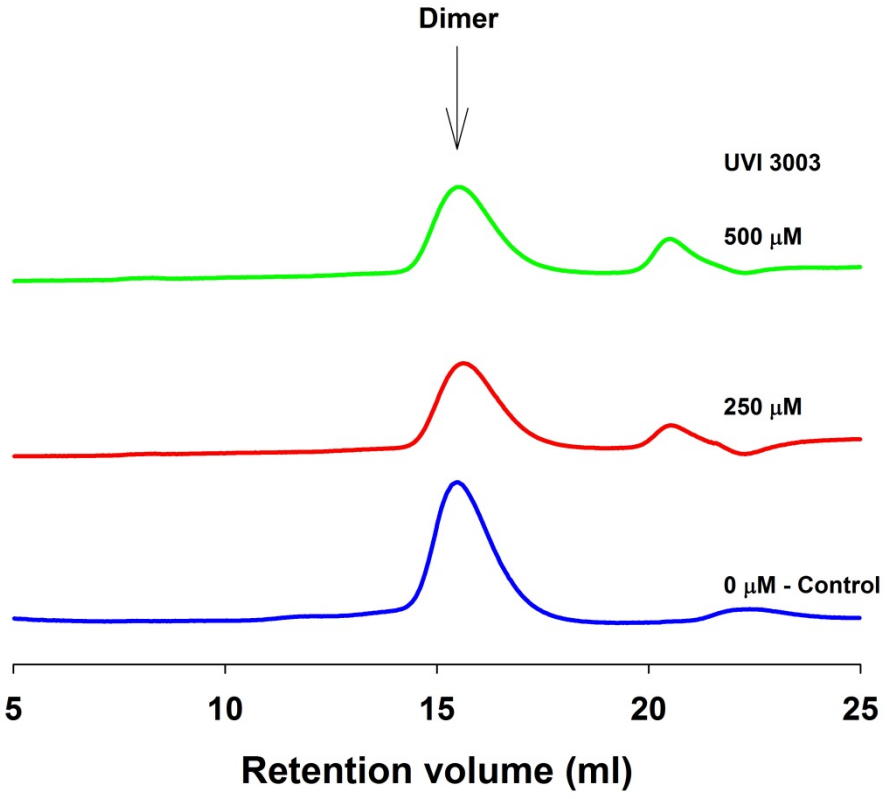


Figure 7

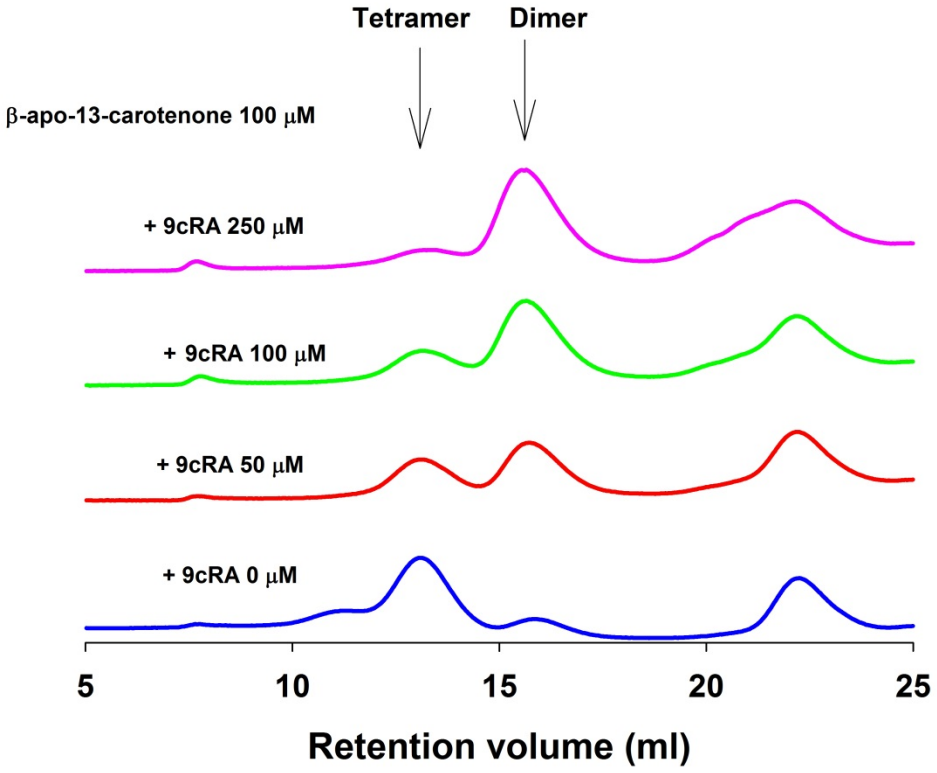
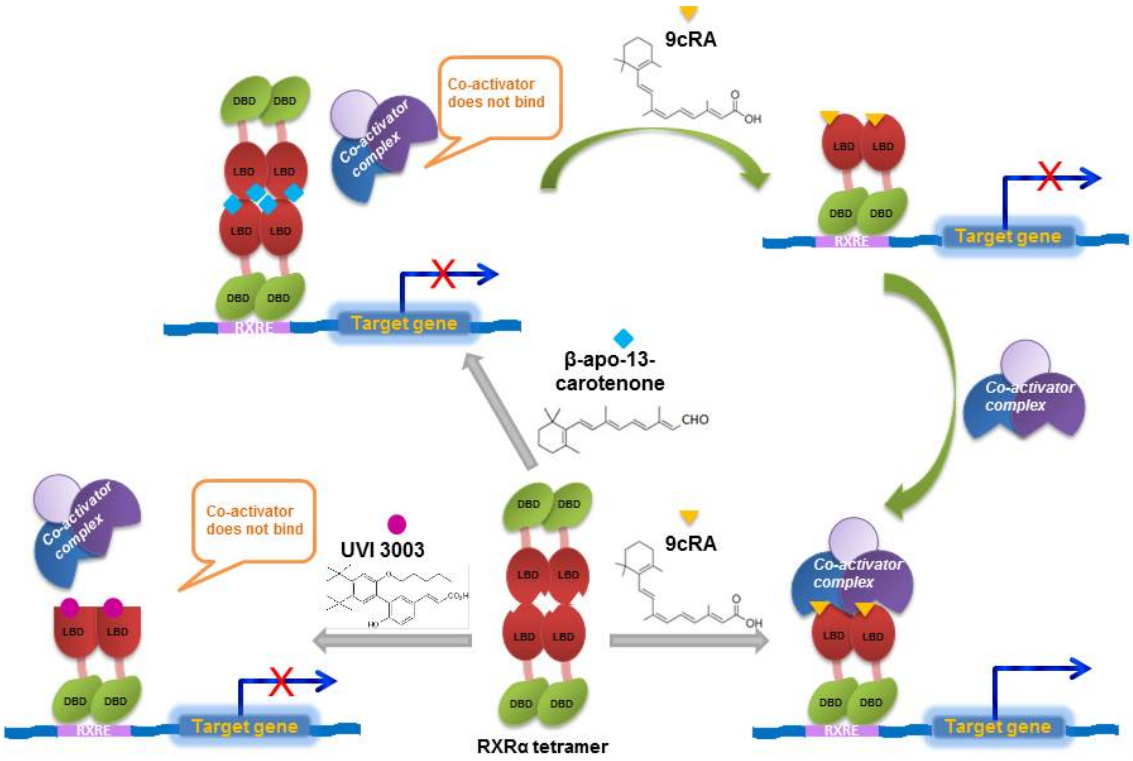


Figure 8



Metabolism:

β-apo-13-Carotenone Regulates Retinoid X Receptor Transcriptional Activity Through Tetramerization of the Receptor

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