Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmacological and Toxicological Methods 56 (2007) 28 – 33
Journal of Pharmacological and Toxicological Methods
www.elsevier.com/locate/jpharmtox

Original article

A phosphorimager-based filter binding thyroid hormone receptor competition assay for chemical screening

Joseph Chapo, Yanyu Peng, Kelly R. Pitts *

Molecular Pharmacology, Myogen, Inc., Westminster CO, 80021

Received 26 October 2006; accepted 11 December 2006

Abstract

Introduction: A phosphorimager-based filter binding thyroid hormone receptor (THR) competition assay has been developed for use in verifying hits from compound library screens. **Methods:** This method employs in vitro translated ligand binding domains (LBDs) of THRα and THRβ, separation through nitrocellulose via a 96-well vacuum manifold, and analysis of receptor-bound radioactivity by phosphorimaging. **Results:** A standard curve of [I^{125}]T3 showed a linear response over the dynamic range of a competition assay, and a comparison of Sephadex G-25 column separation and gamma counting with en masse filtration and phosphorimaging revealed similar IC_{50} and K_i values when using unlabeled T3 as competitor. In addition, this method produced IC_{50} and K_i values for the known T3 competitors [3,5-Dimethyl-4-(4′-hydoxy-3′-isopropylbenzyl) phenoxy] acetic acid (GC-1) and 3,5-diiodothyropropionic acid (DITPA) similar to those reported elsewhere. **Discussion:** These data suggest that filtration and phosphorimaging adequately and properly reproduces binding values associated with THR competition. Further, this method gave a 3-fold reduction in time and a 40-fold reduction in radioactive waste over the column-based method. These reductions allow for a substantial increase in assay throughput. Taken together, these data suggest that en masse filtration and phosphorimaging is an efficient and tractable method for verifying large groups of putative T3 competitors in vitro.

Keywords: Thyroid hormone receptor; In vitro translation; Radioactive; Competition assay; Filter; Phosphorimager

1. Introduction

The thyroid hormone receptors (THRs) are ligand-dependent transcription factors involved in regulating growth, differentiation, and development. Binding of the natural ligand, thyroid hormone (T3), to the receptors has been shown to modulate expression of various genes including thyrotropin (Shupnik, Greenspan, & Ridgway, 1986), hepatic lipase (Sensel, Legrand-Lorans, Wang, & Bensadoun, 1990), apolipoprotein A1 (Taylor, Wishart, Lawless, Raymond, & Wong, 1996), fatty acid synthase (Moustaid & Sul, 1991), and myosin heavy chains (Dillmann, 1990; Morkin, 1993). Several compounds have been developed and described as competitors of T3 binding to the receptors, and many of these compounds in turn exhibit biological activity (Lim, Nguyen, Yang, Scanlan, & Furlow, 2002; Morkin, Ladenson, Goldman, & Adamson, 2004; Trost et al., 2000). The identification of compounds that act as receptor ligand competitors is facilitated by a robust, tractable competition assay capable of handling large numbers of compounds.

Here we describe a THR competition assay for use in identifying compounds that compete for T3 binding. In contrast to other THR competition assays based on isolated nuclear extracts and gel filtration to obtain receptor-bound radioactivity for gamma counting, this method employs in vitro translated ligand binding domains (LBDs) of THRα and THRβ, separation through nitrocellulose via a 96-well vacuum manifold, and analysis of receptor-bound radioactivity by phosphorimaging. This assay format appears to offer advantages over other reported formats, including a greater capacity for processing large groups of compounds and a substantial reduction in assay processing time and radioactive waste.

* Corresponding author. Gilead Sciences, Inc., 7575 West 103rd Avenue, Westminster, CO, 80021-5426. Tel.: +1 303 544 1747; fax: +1 303 410 6669.
E-mail address: kelly.pitts@gilead.com (K.R. Pitts).

1056-8719/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2006.12.003

2. Materials and methods

2.1. In vitro translation of rhTHR ligand binding domains (LBDs)

In vitro translation was done using a TNT T7 Quick Coupled Transcription and Translation kit (Promega, Madison, WI) with the following modifications. Reactions consisted of 10 μg of human THRLBD cDNA, (α or β), 10 μL 1 mM methionine, 10 μL Superasin RNase inhibitor (1 U/μL; Ambion, Austin, TX), and 20 μL nuclease free water added to 200 μL rabbit reticulocyte lysate. The reaction was placed in 30 °C water bath for 90 min and then placed on ice until use the same day in saturation binding or competition assays.

2.2. Saturation binding for receptor counting

The number of receptors present after a translation reaction was determined by incubating 1 μL in vitro coupled transcription and translation (IVT) reaction material and 0.1 nM [I^{125}]T3 (Perkin Elmer, Boston, MA) in a total volume of 400 μL of H-400 buffer (20 mM HEPES, 0.5 mM EDTA, 1 mM $MgCl_2$, 400mM KCl, 10% glycerol, pH = 8.0) containing 50 μg/ml calf thymus histones (Calbiochem, San Diego, CA) and 0.1% (w/vol) monothioglycerol (MTG; Sigma, St. Louis, MO) at room temperature. After 1 hr, receptor-bound radiolabel was collected on a 0.45 μm mixed cellulose ester 96-well plate (Millipore, Bedford, MA) by vacuum filtration. Wells were washed once with 100 μL H-400 buffer and individual wells were punched out and counted on a gamma counter (Cobra Autogamma, Packard, Meriden, CT). Counts were used to calculate the femtomoles of receptor present in the IVT material for subsequent use in competition assays. [I^{125}]T3 incubated with blank rabbit reticulocyte lysate routinely showed no significant non-specific binding.

2.3. Gel filtration competition assays

Competition reactions were set up in 12 × 75 mm polypropylene tubes. Each reaction consisted of 100 μL 0.1 nM [I^{125}]T3, 100 μL diluted IVT receptor, and 200 μL cold competitor (T3 or compound) in increasing concentrations. Each reaction contained a total of 25fmol of receptor. All reagent dilutions were made in H-400 buffer containing 50 μg/ml calf thymus histones and 0.1% (w/vol) MTG. Reactions were incubated overnight at 4°C, and then processed on G-25 Sephadex (Sigma, St Louis, MO) columns to separate receptor-bound from free radiolabel. Columns were pre-blocked with H-400 buffer containing 50 μg/ml calf thymus histones and 0.1% (w/vol) MTG as described by Apriletti et al. (Apriletti, vid-Inouye, Eberhardt, & Baxter, 1984). After the reaction volume was added to the column bed, receptor-bound radiolabel was eluted from the column using 1 mL H-400 buffer. The amount of radiolabel in the collected fractions was then determined by gamma counting. K_i and IC_{50} values for each competitor were determined by fitting the dose-response data to a one-site competition model using GraphPad Prism (version 3.0; GraphPad Software, San Diego, CA).

Columns were washed extensively with BSA Wash buffer (15 mM Tris, pH 8.6, 0.2-0.5% BSA) until residual counts reached background (~500 CPM) as assessed by Geiger counting. Typically this was achieved within 5–10 column volumes.

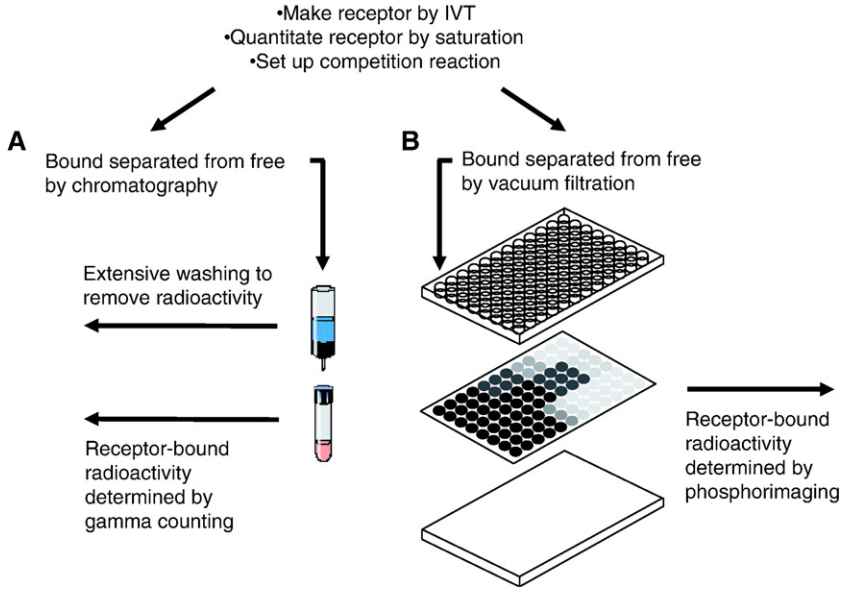

Fig. 1. *Schematic representation of column- vs. filter-based methods.* The initial steps in each method are the same: 1) the THRα and β LBDs are made by in vitro translation, 2) receptor is quantitated in a saturation reaction, and 3) reactions containing putative T3 competitor compounds in dose-response are set up. The methods differ in how the competition reactions are post-processed and analyzed. (A) Bound radioactivity is separated from free by size-exclusion chromatography, with bound being eluted from the column into a tube. Radioactivity is then determined by gamma counting. (B) Bound radioactivity is separated from free by vacuum filtration, with bound being collected on the filter. Radioactivity is then determined by phosphorimaging. IVT, in vitro translation.

2.4. Filter binding competition assays

Competition reactions were set up in polypropylene 96-well deep well plates (1 mL well volume; Matrix Corp., Hudson, NH). Each reaction consisted of 100 μL 0.1 nM [I^{125}]T3, 100 μL (for a final of 25 fmol) diluted IVT receptor, and 200 μL cold competitor (T3 or compound) in increasing concentrations. All reagent dilutions were made in H-400 buffer containing 50 μg/ml calf thymus histones and 0.1% (w/vol) MTG. Reactions were incubated overnight at 4 °C, and then processed over a 0.45 μm Optitran, nitrocellulose membrane sheet (Whatman, Dassel, Germany) secured in a Hybri-dot 96-well blotting vacuum manifold (Whatman-Biometra, Goettingen, Germany). The membrane was pre-blocked by drawing 100 μL of H-400 buffer containing 50 μg/ml calf thymus histones and 0.1% (w/vol) MTG through each blotting well. Reaction volumes were transferred from the deep-well reaction plate into corresponding wells in the vacuum manifold using a 12-channel automated pipette (Biohit, Helsinki, Finland). Receptor-bound radiolabel was then collected on the membrane by vacuum filtration, followed by a wash with 200 μL H-400 buffer. The membrane was removed from the manifold and allowed to air dry for 5 min. Membranes were covered in plastic wrap and exposed to a phosphorscreen for 1 h inside a film cassette. The phosphorscreen was read using a Cyclone phosphorimager (Perkin Elmer, Shelton, CT) and the amount of radiolabel in each reaction spot quantified. K_i and IC_{50} values for each competitor were determined by fitting the dose-response data to a one-site competition model using GraphPad Prism (GraphPad Software).

3. Results and discussion

The aim of this project was to develop a receptor competition assay in a format capable of screening hundreds of compounds for their ability to compete for T3 binding to either the THRα or THRβ. Several criteria needed to be met: 1) abundance of receptor protein, 2) amenable to medium-or high-throughput with the possibility of robotic assistance, 3) capable of multiple replicates at each dose, 4) minimal data collection and analysis time, 5) minimal operator intervention, and 6) minimal radioactive waste. THR binding and competition assays have been described in the literature using a variety of receptor sources, including cell or nuclear lysates (Inoue, Yamakawa, Yukioka, & Morisawa, 1983; Koerner, Schwartz, Surks, & Oppenheimer, 1975; Ladenson, Keiffer, Farwell, & Ridgway, 1986; Schapira et al., 2003; Taylor, Stephan, Steele, & Wong, 1997;Verhoeven et al., 2001), recombinant receptors (Apriletti, Baxter, Lau, & West, 1995; Barkhem et al., 1991; Borngraeber et al., 2003; Nguyen et al., 2002; Pennock, Raya, Bahl, Goldman, & Morkin, 1992), and in vitro translated (IVT) receptors (Borngraeber et al., 2003; Chiellini et al., 1998; Schueler, Schwartz, Strait, Mariash, & Oppenheimer, 1990). We chose to use recombinant human IVT ligand binding domains (LBDs) of THRα and THRβ because of the ease of obtaining fresh receptor protein by this method. In addition, THR assays employ various separation techniques which fall into essentially two categories: size-exclusion chromatography (Apriletti, Eberhardt, Latham, & Baxter, 1981; Barkhem et al., 1991; Borngraeber et al., 2003; Chiellini et al., 1998; Ladenson et al., 1986) and vacuum filter binding (Dow et al., 2003; Inoue et al., 1983; Pennock et al., 1992). Fig. 1A depicts the column chromatography separation method where receptor-bound vs. free radioactivity is separated by size. Pilot studies using this method yielded IC_{50} and K_i values for unlabeled T3 competition (see Fig. 3A) that were similar to those reported elsewhere (Barkhem et al., 1991; Borngraeber et al., 2003; Chiellini et al., 1998; Nguyen et al., 2002). The column-based method, however, failed to meet several of our criteria primarily due to low throughput, need for operator intervention, and quantity of

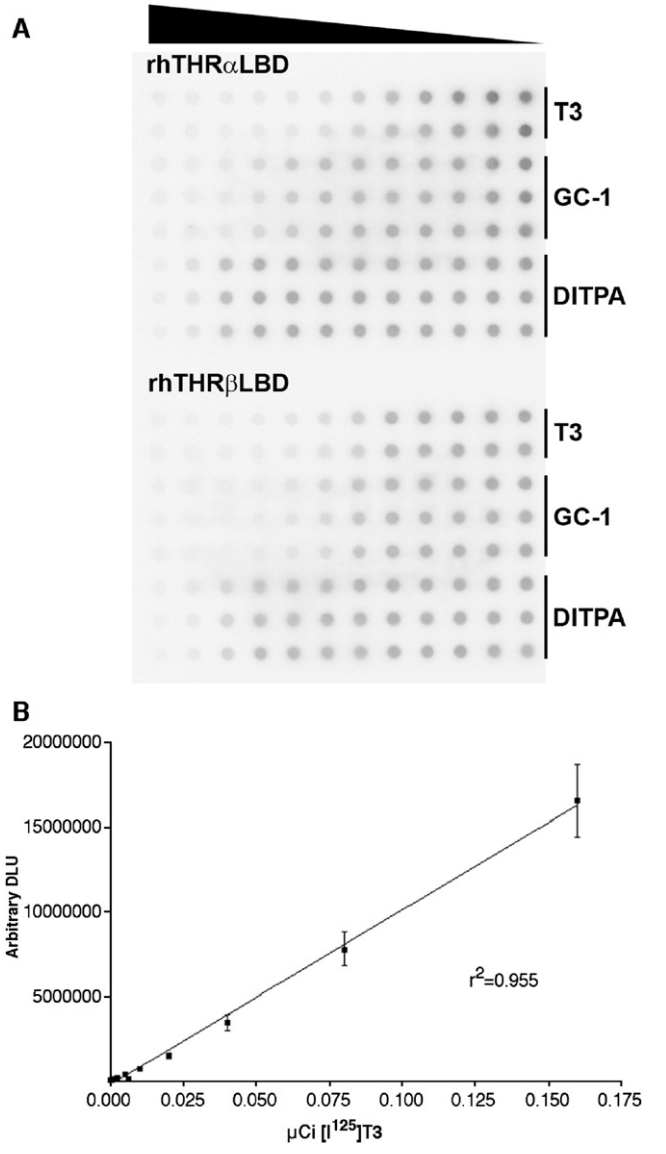

Fig. 2. *Representative phosphorimager data.* (A) Phosphorimages of nitrocellulose membranes containing receptor-bound radioactivity are shown. T3, GC-1, and DITPA competitor concentrations decrease from left to right. (B) The linearity of the phosphorimager is shown using a standard curve of [I^{125}]T3 dilutions. rhTHR, recombinant human thyroid hormone receptor; LBD, ligand binding domain.

radioactive waste. Similar issues exist with reported filter-based assays (Inoue et al., 1983; Pennock et al., 1992), including operator intervention and large wash volumes that contribute significantly to radioactive waste.

Fig. 1B depicts our filter-based method where receptor-bound radioactivity is collected on a nitrocellulose membrane using a 96-well filter block. This method met most of our requirements, including a format amenable to high throughput, capability for multiple replicates, low operator intervention, and a significant reduction in radioactive waste. A major difference between this method and the column-based method is, rather than determining receptor-bound radioactivity in an eluted, aqueous medium with a gamma counter, the receptor-bound radioactivity collected on the membrane is exposed to a phosphorscreen and determined by phosphorimaging. Fig. 2A shows representative exposures of membranes harboring recombinant human THRαLBD-or THRβLBD-bound radioactivity where cold T3, [3,5-Dimethyl-4-(4′-hydoxy-3′-isopropylbenzyl) phenoxy] acetic acid (GC-1), and 3,5-diiodothyropropionic acid (DITPA) were used as competitors. As competitor concentration decreases (from left to right) the amount of receptor-bound radioactivity increases and the spotting pattern becomes darker. Although it is difficult to discern differences in the intensities of neighboring spots by eye, the dynamic range of the phosphorimager allows these differences to become apparent, with most dose-response curves ranging from ~100,000 to 10,000,000 digital light units (DLU).

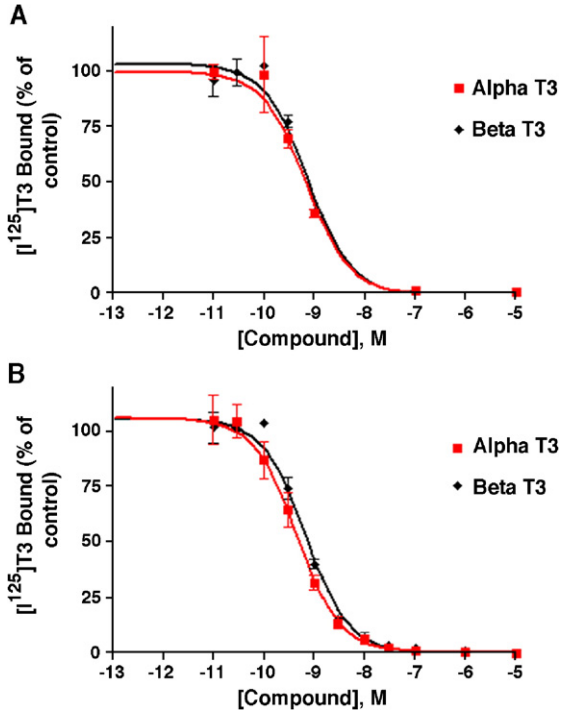

Fig. 3. *Representative T3 competition data obtained by either the column-or filter-based method.* Cold T3 was used in competition assays processed by (A) column- or (B) filter-based methods. Data generated from either method show well-behaved competition curves. Individual data points are mean±SD. Mean K_i values from each method are comparable (Column: 0.158±0.021 nM for rhTHRαLBD; 0.150±0.021 nM for rhTHRβLBD; Filter: 0.140±0.016 nM for rhTHRαLBD; 0.195±0.032 nM for rhTHRβLBD).

Table 1
IC_{50} and K_i values for T3, GC-1, and DITPA using the filter-based method

Competitor	rhTHRαLBD		rhTHRβLBD	
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)
T3	0.374±0.043	0.140±0.016	0.519±0.086	0.195±0.032
GC-1	1.867±0.437	0.700±0.164	0.367±0.077	0.138±0.029
DITPA	83.72±23.60	29.66±8.853	39.22±14.16	14.71±5.310

All values are reported as mean±SEM nM, and are the composites of three separate experiments.

A standard curve using [I^{125}]T3 spotted onto filter paper and exposed to the phosphor screen showed a linear response from the phosphorimager out to 18,000,000 DLU (see Fig. 2B), suggesting that THR dose-response data generated in this manner falls within the linear dynamic range of the imager.

Fig. 3B shows the competition curves generated from data collected using the filter-based method with T3 as the competitor. The K_i values derived from these curves are similar (see Table 1), suggesting no appreciable selectivity of T3 for either receptor subtype. In addition, these values are in good agreement with those generated using the column-based method

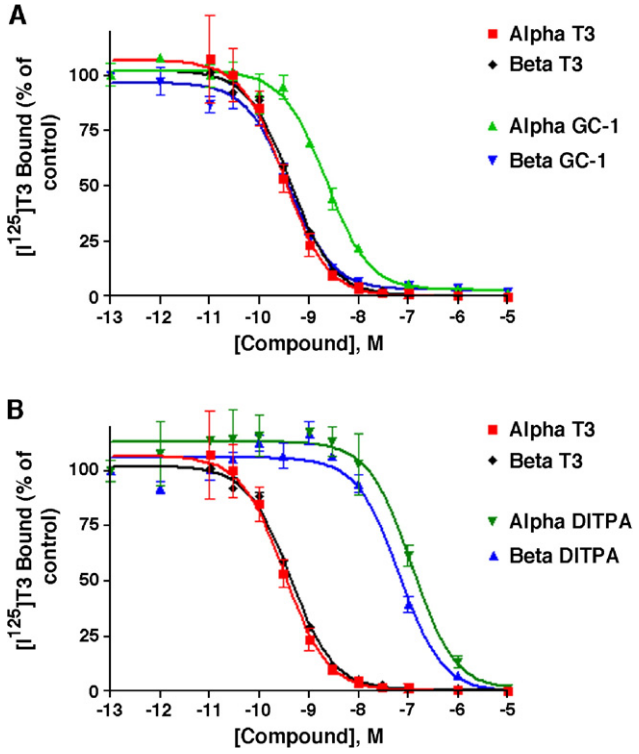

Fig. 4. *Representative GC-1 and DITPA competition data obtained using the filter-based method.* The known thyromimetics GC-1 and DITPA were used in competition assays processed using the filter-binding method. (A) GC-1 exhibits a dose-dependent competition for [I^{125}]T3, with an apparent affinity similar to cold T3 for rhTHRβLBD (compare black T3 curve to blue GC-1 curve) and a ~6-fold reduction in affinity for rhTHRαLBD (compare red T3 curve to green GC-1 curve). (B) DITPA also exhibits a dose-dependent competition for [I^{125}]T3, with similar apparent affinities on both rhTHRαLBD (green curve) and rhTHRβLBD (blue curve), but with a >100-fold reduction in affinity compared to cold T3 controls (black and red curves).

Table 2
Resource comparison of filter- and column-based methods

	Filter-based method	Column-based method
Processing time	2 h	6 h
Radioactive waste	100 mL	7500 mL
Materials cost	$150.00	$150.00
Possible assay points/day	768	192

All values are based on 2×96 assay points processed.

(see curves in Fig. 3A; K_i=0.158±0.021 nM for rhTHRαLBD and 0.150±0.021 nM for rhTHRβLBD) and elsewhere (Barkhem et al., 1991; Borngraeber et al., 2003; Chiellini et al., 1998; Nguyen et al., 2002). A distinct advantage with the filter-based method is the ability to run 12-point dose-response experiments allowing for greater resolution when defining inflection and shoulder regions of a competition curve (compare Fig. 3B and A point placement).

To further benchmark the filter-based method we generated data using two well-characterized thyromimetics as competitors. GC-1 is a high-affinity thyromimetic, exhibiting a THRβ K_i similar to that of T3 and a THRα K_i with ~6-fold less affinity (Chiellini et al., 1998). DITPA, a carboxylic acid analog of T3 (Stasilli, Kroc, & Meltzer, 1959), has been shown to be >100-fold less potent for the THRs compared to T3 with no apparent subtype selectivity (Pennock et al., 1992). Representative data collected using the filter-based method with GC-1 as competitor (Fig. 4A) show a rhTHRβLBD curve (blue) superimposed over the T3 control curves (black and red), whereas the rhTHRαLBD curve (green) is rightward-shifted, suggesting that GC-1 exhibits a similar subtype selectivity as previously reported. The calculated K_i values (see Table 1) indicate a 5.1-fold selectivity was maintained over three separate experiments. Statistical analysis revealed that the differences in GC-1 fold selectivity versus that of T3, as well as the GC-1 $K_{i\alpha}$ versus $K_{i\beta}$ were statistically significant ($p<0.05$). Representative data with DITPA as competitor (Fig. 4B) show competition curves for both rhTHRαLBD (green) and rhTHRβLBD (blue) that are several orders of magnitude rightward-shifted from T3 control curves (black and red). The calculated K_i values (see Table 1) indicate there is no appreciable subtype selectivity and that DITPA is >100-fold less potent than T3 across three separate experiments. Taken together these data suggest that the filter-based method yields data consistent with known thyromimetics as measured in conventional assay systems and can be used to rank-order compounds that may have subtype selectivity.

Table 2 shows a resource comparison of the filter-and column-based methods when processing 192 assay points — 96 points for rhTHRαLBD and 96 points for rhTHRβLBD. This is the equivalent of a 12-point dose-response T3 control curve done in duplicate, and two putative T3 competitors over a 12-point dose-response curve done in triplicate for both receptor subtypes. While the cost of materials is equivalent for either method, the major savings occur in processing time (3-fold reduction) and radioactive waste (40-fold reduction). This correspondingly would allow for a 4-fold increase in the number of assay points that could be run per experimental day.

In summary, the phosphorimager-based filter binding THR competition assay described here offers a robust, tractable, and convenient method for verifying compounds with the potential to compete for T3 binding.

Acknowledgement

We thank James Apriletti, Ph.D. at the University of California San Francisco for his assistance in learning the column-based THR competition assay.

References

Apriletti, J. W., Baxter, J. D., Lau, K. H., & West, B. L. (1995). Expression of the rat alpha 1 thyroid hormone receptor ligand binding domain in *Escherichia coli* and the use of a ligand-induced conformation change as a method for its purification to homogeneity. *Protein Expression and Purification*, *6*, 363–370.

Apriletti, J. W., Eberhardt, N. L., Latham, K. R., & Baxter, J. D. (1981). Affinity chromatography of thyroid hormone receptors. Biospecific elution from support matrices, characterization of the partially purified receptor. *Journal of Biological Chemistry*, *256*, 12094–12101.

Apriletti, J. W., vid-Inouye, Y., Eberhardt, N. L., & Baxter, J. D. (1984). Interactions of the nuclear thyroid hormone receptor with core histones. *Journal of Biological Chemistry*, *259*, 10941–10948.

Barkhem, T., Carlsson, B., Simons, J., Moller, B., Berkenstam, A., Gustafsson, J. A., et al. (1991). High level expression of functional full length human thyroid hormone receptor beta 1 in insect cells using a recombinant baculovirus. *Journal of Steroid Biochemistry and Molecular Biology*, *38*, 667–675.

Borngraeber, S., Budny, M. J., Chiellini, G., Cunha-Lima, S. T., Togashi, M., Webb, P., et al. (2003). Ligand selectivity by seeking hydrophobicity in thyroid hormone receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 15358–15363.

Chiellini, G., Apriletti, J. W., Yoshihara, H. A., Baxter, J. D., Ribeiro, R. C., & Scanlan, T. S. (1998). A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. *Chemistry & Biology*, *5*, 299–306.

Dillmann, W. H. (1990). Biochemical basis of thyroid hormone action in the heart. *American Journal of Medicine*, *88*, 626–630.

Dow, R. L., Schneider, S. R., Paight, E. S., Hank, R. F., Chiang, P., Cornelius, P., et al. (2003). Discovery of a novel series of 6-azauracil-based thyroid hormone receptor ligands: potent, TR beta subtype-selective thyromimetics. *Bioorganic & Medicinal Chemistry Letters*, *13*, 379–382.

Inoue, A., Yamakawa, J., Yukioka, M., & Morisawa, S. (1983). Filter-binding assay procedure for thyroid hormone receptors. *Analytical Biochemistry*, *134*, 176–183.

Koerner, D., Schwartz, H. L., Surks, M. I., & Oppenheimer, J. H. (1975). Binding of selected iodothyronine analogues to receptor sites of isolated rat hepatic nuclei. High correlation between structural requirements for nuclear binding and biological activity. *Journal of Biological Chemistry*, *250*, 6417–6423.

Ladenson, P. W., Kieffer, J. D., Farwell, A. P., & Ridgway, E. C. (1986). Modulation of myocardial L-triiodothyronine receptors in normal, hypothyroid, and hyperthyroid rats. *Metabolism*, *35*, 5–12.

Lim, W., Nguyen, N. H., Yang, H. Y., Scanlan, T. S., & Furlow, J. D. (2002). A thyroid hormone antagonist that inhibits thyroid hormone action in vivo. *Journal of Biological Chemistry*, *277*, 35664–35670.

Morkin, E. (1993). Regulation of myosin heavy chain genes in the heart. *Circulation*, *87*, 1451–1460.

Morkin, E., Ladenson, P., Goldman, S., & Adamson, C. (2004). Thyroid hormone analogs for treatment of hypercholesterolemia and heart failure: past, present and future prospects. *Journal of Molecular and Cellular Cardiology*, *37*, 1137–1146.

Moustaid, N., & Sul, H. S. (1991). Regulation of expression of the fatty acid synthase gene in 3T3-L1 cells by differentiation and triiodothyronine. *Journal of Biological Chemistry*, *266*, 18550–18554.

Nguyen, N. H., Apriletti, J. W., Cunha Lima, S. T., Webb, P., Baxter, J. D., & Scanlan, T. S. (2002). Rational design and synthesis of a novel thyroid hormone antagonist that blocks coactivator recruitment. *Journal of Medicinal Chemistry*, *45*, 3310−3320.

Pennock, G. D., Raya, T. E., Bahl, J. J., Goldman, S., & Morkin, E. (1992). Cardiac effects of 3,5-diiodothyropropionic acid, a thyroid hormone analog with inotropic selectivity. *Journal of Pharmacology and Experimental Therapeutics*, *263*, 163−169.

Schapira, M., Raaka, B. M., Das, S., Fan, L., Totrov, M., Zhou, Z., et al. (2003). Discovery of diverse thyroid hormone receptor antagonists by high-throughput docking. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 7354−7359.

Schueler, P. A., Schwartz, H. L., Strait, K. A., Mariash, C. N., & Oppenheimer, J. H. (1990). Binding of 3,5,3′-triiodothyronine (T3) and its analogs to the in vitro translational products of c-erbA protooncogenes: differences in the affinity of the alpha-and beta-forms for the acetic acid analog and failure of the human testis and kidney alpha-2 products to bind T3. *Molecular Endocrinology*, *4*, 227−234.

Sensel, M. G., Legrand-Lorans, A., Wang, M. E., & Bensadoun, A. (1990). Isolation and characterization of clones for the rat hepatic lipase gene upstream regulatory region. *Biochimica et Biophysica Acta*, *1048*, 297−302.

Shupnik, M. A., Greenspan, S. L., & Ridgway, E. C. (1986). Transcriptional regulation of thyrotropin subunit genes by thyrotropin-releasing hormone and dopamine in pituitary cell culture. *Journal of Biological Chemistry*, *261*, 12675−12679.

Stasilli, N. R., Kroc, R. L., & Meltzer, R. I. (1959). Antigoitrogenic and calorigenic activities of thyroxine analogues in rats.. *Endocrinology*, *64*, 62−82.

Taylor, A. H., Stephan, Z. F., Steele, R. E., & Wong, N. C. (1997). Beneficial effects of a novel thyromimetic on lipoprotein metabolism. *Molecular Pharmacology*, *52*, 542−547.

Taylor, A. H., Wishart, P., Lawless, D. E., Raymond, J., & Wong, N. C. (1996). Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein AI promoter. *Biochemistry*, *35*, 8281−8288.

Trost, S. U., Swanson, E., Gloss, B., Wang-Iverson, D. B., Zhang, H., Volodarsky, T., et al. (2000). The thyroid hormone receptor-beta-selective agonist GC-1 differentially affects plasma lipids and cardiac activity. *Endocrinology*, *141*, 3057−3064.

Verhoeven, F. A., Moerings, E. P., Lamers, J. M., Hennemann, G., Visser, T. J., & Everts, M. E. (2001). Inhibitory effects of calcium channel blockers on thyroid hormone uptake in neonatal rat cardiomyocytes. *American Journal of Physiology. Heart and Circulatory Physiology*, *281*, H1985−H1991.