ELSEVIER

Biophysical Chemistry 128 (2007) 156 – 164

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Agonist versus antagonist induce distinct thermodynamic modes of co-factor binding to the glucocorticoid receptor

Rachel R. Kroe [a], Martha A. Baker [a], Martha P. Brown [a], Neil A. Farrow [b], Elda Gautschi [a], Jerry L. Hopkins [a], Roger R. LaFrance [a], Anthony Kronkaitis [a], Dorothy Freeman [a], David Thomson [c], Gerald Nabozny [d], Christine A. Grygon [a], Mark E. Labadia [d,*]

[a] *Department of Biologics and Biomolecular Sciences, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Rd., Ridgefield CT 06877, United States*

[b] *Department of Structural Research, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Rd., Ridgefield CT 06877, United States*

[c] *Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Rd., Ridgefield CT 06877, United States*

[d] *Department of Immunology, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Rd., Ridgefield CT 06877, United States*

Received 30 December 2006; received in revised form 21 March 2007; accepted 24 March 2007
Available online 5 April 2007

Abstract

The glucocorticoid receptor (GR) is involved in the transcriptional regulation of genes associated with inflammation, glucose homeostasis, and bone turnover through the association with ligands, such as corticosteroids. GR-mediated gene transcription is regulated or fine-tuned via the recruitment of co-factors including coactivators and corepressors. Current therapeutic approaches to targeting GR aim to retain the beneficial anti-inflammatory activity of the corticosteroids while eliminating negative side effects. Towards achieving this goal the experiments discussed here reveal a mechanism of co-factor binding in the presence of either bound agonist or antagonist. The GR ligand binding domain (GR-LBD(F602S)), in the presence of agonist or antagonist, utilizes different modes of binding for coactivator versus corepressor. Coactivator binding to the co-effector binding pocket of GR-LBD(F602S) is driven both by favorable enthalpic and entropic interactions whereas corepressor binding to the same pocket is entropically driven. These data support the hypothesis that ligand-induced conformational changes dictate co-factor binding and subsequent *trans*-activation or *trans*-repression.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Glucocorticoid receptor; Dexamethasone; RU-486; Isothermal titration calorimetry; Surface Plasmon Resonance; Analytical ultracentrifugation

1. Introduction

The glucocorticoid receptor (GR) is a ligand-dependent transcription factor that is a member of the nuclear receptor super-family, which includes receptors for mineralocorticoids, estrogens, progestins, androgens, thyroid hormones, and vitamin D. GR is involved in the regulation of genes associated with various functions including inflammation, glucose homeostasis, and bone turnover. GR ligands, including the corticosteroids Dexamethasone and Prednisolone, are commonly used therapeutically for the treatment of diseases such as asthma, allergic rhinitis and rheumatoid arthritis.

In its inactivated state, GR resides in the cytoplasm as a heterocomplex with several chaperone proteins including HSP90. When ligand diffuses across the cell membrane and binds to GR, HSP90 and the other chaperone proteins dissociate. Translocation

Abbreviations: AP-1, activating protein 1; CD, circular dichroism; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DEX, Dexamethasone; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FP, fluorescence polarization; GPCR, G-protein coupled receptor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HEPES, 4-(2-hydroxyethyl)-1-piperasineethanesulfonic acid; HSP90, heat shock protein 90; ITC, isothermal titration calorimetry; LBD, ligand binding domain; LGICR, ligand-gated ion channel receptors; NCoR, nuclear receptor corepressor; NF-κB, nuclear factor kappa B; PMSF, phenylmethanesulfonyl fluoride; SEC-MS, size-exclusion chromatography mass spectrometry; SPR, Surface Plasmon Resonance; TAMRA, carboxytetramethylrhodamine; TCEP, tris(2-carboxyethyl) phosphine hydrochloride; TIF2, transcriptional intermediary factor 2.

* Corresponding author. Tel.: +1 203 798 5129; fax: +1 203 791 6196.
E-mail address: mlabadia@rdg.boehringer-ingelheim.com (M.E. Labadia).

0301-4622/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2007.03.013

of GR into the nucleus follows this dissociation. Once inside the nucleus, GR regulates transcription either through direct interactions with the promoter region of target genes or through interactions with transcriptional factors such as NF-κB and AP-1.

GR can bind to either positive or negative glucocorticoid response elements (GREs) in the nucleus. *Cis*-activation through positive GREs occurs when either GR homo or heterodimers bind to the palindromic consensus sequence GGTACAnnnTGTTCT [1]. *Cis*-repression by GR occurs through an interaction with a negative GRE half site. *Trans*-repression can occur through the direct interaction between GR and transcription factors. Current models of inflammation suggest that *trans*-repression of transcription factors is the primary mechanism by which GR ligands mediate their anti-inflammatory activity. In contrast it is thought that the side effects of GR ligands are primarily mediated through either *cis*-activation or *cis*-repression of gene expression. Current theories postulate that differential recruitment of co-factors to targeted DNA promoter regions by GR may be the underlying mechanism determining selectivity between anti-inflammatory and side effect pathways [2].

GR consists of three main functional domains. The N-terminal region contains a non-ligand-dependent activation domain which is associated with turning on gene expression once GR is bound to DNA. The central region of the GR molecule contains the DNA binding domain which consists of two zinc fingers. The C-terminal region of GR contains the ligand binding domain (LBD). A second activation domain lies within the LBD and is thought to be associated with ligand-dependent regulation of gene expression [3].

The crystal structure of GR-LBD(F602S) in complex with Dexamethasone (DEX) and a peptide derived from coactivating co-factor transcriptional intermediary factor 2 (TIF2) has been solved by Bledsoe et al. [4]. This structure reveals that, when agonist (DEX) is bound to GR-LBD(F602S), two charge clamps mediate the selectivity for TIF2 recognition by GR. The crystal structure of GR-LBD(F602S) in complex with the antagonist RU-486 shows a large conformational change in helix 12 [5] that has been postulated to block the ability to bind coactivator. Co-immunoprecipitation studies with GR and the corepressor NCoR show that when RU-486 is bound, NCoR interacts with both the N and C-terminus of full length GR [6]. Consistent with the structural data, MS-based deuterium exchange measurements suggest that RU-486 binding provokes the unfolding of helix 12. These measurements also imply that the binding sites for TIF2 and NCoR are largely overlapping. Here we further describe how distinct co-factors bind to a similar or overlapping GR-LBD (F602S) pocket via differences in thermodynamic recognition.

2. Experimental procedures

2.1. Peptides

Both TIF2 and NCoR peptides were obtained from AnaSpec Inc. The TIF2 sequence used was KENALLRYLLDKDD while the NCoR-ID2 sequence was ASNLGLEDIIRKALMGSFD [8]. The same TIF2 peptide was obtained from AnaSpec Inc. with a TAMRA fluorophore directly linked to the N-terminus.

2.2. Protein expression and purification

The GR-LBD(F602S) was expressed as a glutathione-*S*-transferase (GST) fusion protein in *Escherichia coli* BL21 (DE3) cells [4]. Cells were grown at 37 °C in LB medium to an OD_{600} of 0.8. The temperature was then reduced to 15 °C. Once the cells attained an OD_{600} of 1.0, protein expression was induced with 30 μM isopropyl-β-D-galactoside and 200 μM Dexamethasone was added to the Media. The cells were harvested 15 h post induction and frozen at −80 °C.

Cells were thawed and mixed with 4 °C lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM KCl, 10% Glycerol, 0.5 mM EDTA, 10 mM DTT, 1 mM PMSF, 4 μg/mL Pepstatin A, 4 μg/mL Leupeptin, 0.04% CHAPS, 2 M Urea and 50 μM Dexamethasone) at a ratio of 5 mL/g of cells. The cells were homogenized on ice using a PolyTron PT 2100 (Kinematica AG, Switzerland) then sonicated with a Branson Sonifier 450 (Converter, USA). The cell lysate was centrifuged at 44,000 rpm for 60 min at 4 °C.

Using an AKTA Prime chromatography system (Amersham, Sweden) the clarified lysate was purified by affinity chromatography on Glutathione Sepharose 4B resin (GS4B) (Amersham, Sweden). While the protein was bound to the resin the urea was removed with a linear gradient of 4 °C wash buffer (50 mM Tris–HCl, pH 8.0, 150 mM KCl, 10% Glycerol, 0.5 mM EDTA, 10 mM DTT, 0.1% CHAPS and 50 μM Dexamethasone). The GR-LBD(F602S) was cleaved from the GST-tag by incubating the resin overnight at 4 °C with thrombin protease (USB, USA) in the presence of 2.5 mM $CaCl_2$. The thrombin flow through was collected and dialyzed into 4 °C IEX buffer A (10 mM Tris–HCl, pH 7.8, 10% Glycerol, 0.5% CHAPS, 1 mM DTT and 50 μM Dexamethasone). The GR-LBD(F602S) was further purified by anion exchange chromatography on Micro Prep 25 Q resin (BioRad, Ca). The 25 Q resin was eluted using a linear gradient with 4 °C IEX buffer B (1 M NaCl and IEX buffer A). The GR-LBD(F602S) was dialyzed at 4 °C into a storage buffer (20 mM HEPES, pH 7.4, 200 mM NaCl, 10% Glycerol, 0.04% CHAPS, 1 mM DTT and 35 μM Dexamethasone). The final product, GR-LBD(F602S) contains 258 residues and has a molecular weight of 29,798 g/mol.

2.3. Ligand exchange

Purified GR-LBD(F602S) was diluted to 10 μM in a pH 7.4 buffer containing 20 mM HEPES, 200 mM NaCl, 1 mM TCEP and either 10 μM Dexamethasone or 10 μM RU-486. The samples were exhaustively dialyzed to ensure complete exchange of ligand. Ligand exchange was confirmed by SEC-MS where the percentages of DEX, RU-486 both bound to GR-LBD(F602S) and free in solution were quantified. After dialysis GR-LBD (F602S) was concentrated in a Vivaspin 10,000 MWCO PES concentrator (Vivascience, Germany). The final protein concentration was determined using a Biorad DC Protein Assay.

2.4. Confirmation of ligand exchange

Ligand exchange was confirmed using size exclusion chromatography–mass spectrometry (SEC-MS). This method was

Download English Version:

https://daneshyari.com/en/article/5372323

Download Persian Version:

https://daneshyari.com/article/5372323

Daneshyari.com