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Analysis of a glucocorticoid–estrogen receptor chimera reveals that dimerization energetics are under ionic control

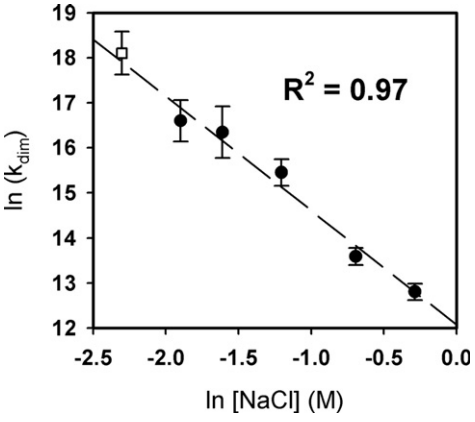
Keith D. Connaghan [a,1], Michael T. Miura [a,1], Nasib K. Maluf [a], James R. Lambert [b], David L. Bain [a,*]

[a] Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, United States
[b] Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, United States

HIGHLIGHTS

- Homologous steroid receptors exhibit large differences in dimerization energetics.
- The molecular origins were probed using a glucocorticoid–estrogen receptor chimera.
- Dimerization energetics of the chimera are coupled to a strong ionic linkage.
- Residues unique to the glucocorticoid receptor constrain ion-regulated assembly.

GRAPHICAL ABSTRACT



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ABSTRACT

Steroid receptors assemble at DNA response elements as dimers, resulting in coactivator recruitment and transcriptional activation. Our work has focused on dissecting the energetics associated with these events and quantitatively correlating the results with function. A recent finding is that different receptors dimerize with large differences in energetics. For example, estrogen receptor-α (ER-α) dimerizes with a $\Delta G = -12.0$ kcal/mol under conditions in which the glucocorticoid receptor (GR) dimerizes with a $\Delta G \leq -5.1$ kcal/mol. To determine the molecular forces responsible for such differences, we created a GR/ER chimera, replacing the hormone-binding domain (HBD) of GR with that of ER-α. Cellular and biophysical analyses demonstrate that the chimera is functionally active. However, GR/ER dimerization energetics are intermediate between the parent proteins and coupled to a strong ionic linkage. Since the ER-α HBD is the primary contributor to dimerization, we suggest that GR residues constrain an ion-regulated HBD assembly reaction.

1. Introduction

Steroid receptors comprise a family of ligand-activated transcription factors [1]. The members include the androgen receptor (AR); the two estrogen receptor isoforms (ER-α and ER-β); the glucocorticoid receptor

* Corresponding author at: Dept of Pharmaceutical Sciences, C-238, University of Colorado Anschutz Medical Campus, 12850 E Montview Blvd, Aurora, CO 80045, United States. Tel.: +1 303 724 6118; fax: +1 303 724 7266.
E-mail address: david.bain@ucdenver.edu (D.L. Bain).
[1] These authors contributed equally to this work.

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(GR); the mineralocorticoid receptor (MR); and the two progesterone receptor isoforms (PR-A and PR-B). As shown in Fig. 1A, all receptors share a centrally located DNA binding domain (DBD), a C-terminal hormone-binding domain (HBD) and a natively disordered N-terminal region. The HBD is thought to be primarily responsible for receptor dimerization in the absence of DNA. Additionally, like the N-terminal region, it contains a transcriptional activation function (AF).

The biochemical model of receptor function posits that upon binding hormone, the receptors translocate to the nucleus, dimerize, and bind to imperfect palindromic response elements typically located upstream of transcriptional start sites. Response element binding is coupled to coactivator recruitment and subsequent transcriptional activation [1]. Although this model has provided a strong qualitative framework for function, it nonetheless remains incomplete. For example, all steroid receptors bind identical or nearly identical response elements *in vitro* yet regulate distinct but overlapping gene networks *in vivo* [2–4]. The quantitative mechanisms by which receptors maintain such functional specificity are largely unknown; our long-term goal is to determine their physico-chemical origins.

As a step toward this goal, we resolved the microstate energetics of steroid receptor–promoter interactions for a majority of the receptors and under identical solution conditions [5–8]. Shown in Fig. 1B are representative assembly states and microscopic interaction parameters for receptor assembly at a promoter containing two hormone response elements (HRE_2). Based on the traditional dimer-binding model, receptors dimerize in the absence of DNA (k_{dim}) and bind as pre-formed dimers to their response elements (k_{int}). Binding to a promoter such as HRE_2 may also be coupled to inter-site cooperativity (k_c). In the context of the traditional dimer-binding model, we find that the receptors analyzed to date share largely identical intrinsic DNA binding energetics (k_{int}). This is not surprising since the receptor DBD is highly conserved both in sequence and in tertiary structure [9–12]. By contrast, dimerization energetics (k_{dim}) vary enormously. For example, our indirect analyses place the ER-α equilibrium dimerization constant at 0.35 nM (−12 kcal/mol), whereas direct determination of PR isoform dimerization reveals constants of 1–2 μM, or ~1000-fold weaker. Surprisingly, GR shows no evidence for dimerization, allowing us to place only a lower limit on k_{dim} at 100 μM (≤−5.1 kcal/mol), or at least 100,000-fold weaker than ER-α. Cooperative binding energetics (k_c) also vary significantly and inversely to dimerization. For example, ER-α exhibits essentially no cooperativity ($k_c = 1.4$) whereas GR maintains strong cooperative stabilization ($k_c = 70$).

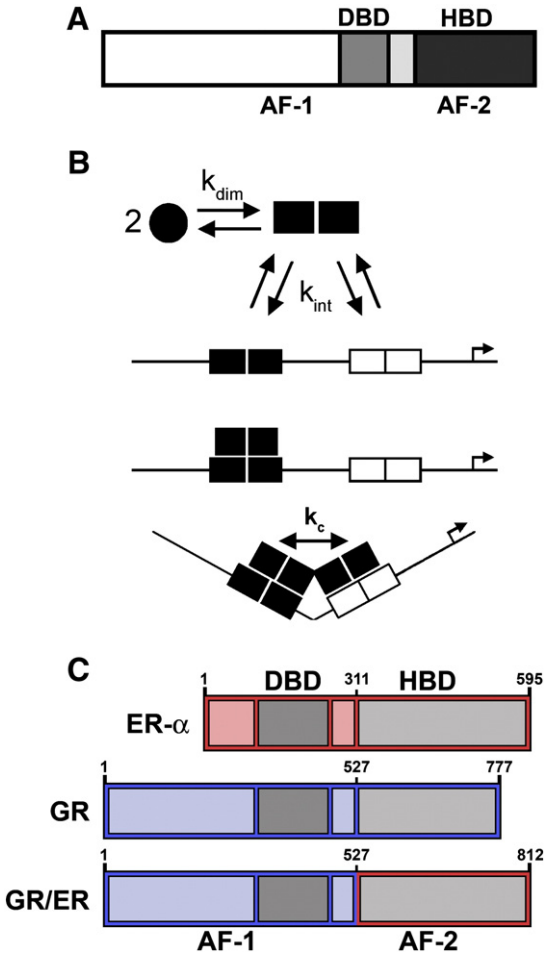

Fig. 1. Schematic representation of human steroid receptors, receptor-promoter binding, and chimeric GR/ER receptor. (A) Generic primary structure schematic. Functional domains are as indicated: DBD, DNA binding domain; HBD, hormone binding domain. An activation function is located within both the N-terminal region and the HBD (AF-1 and AF-2, respectively) (B) HRE_2 promoter assembly model. Macromolecular species and interactions are as indicated: circles, hormone-bound receptor monomers; squares, receptor dimers. Dimerization (k_{dim}) is coupled to response element binding (k_{int}); complete occupancy is coupled to an inter-site cooperative interaction (k_c). Arrow refers to the direction of transcriptional start site. (C) Chimeric GR/ER; N-terminal region and DBD of GR is fused to the HBD of ER-α. Amino acid number is indicated above each receptor. Functional regions are as indicated for Panel A.

We have speculated that the ability of steroid receptors to maintain large differences in promoter binding energetics serves as a framework for generating receptor-specific gene regulation. As described in more detail in our previous work, simulations demonstrate that such differences allow preferential promoter occupancy as a function of promoter architecture—even in the presence of multiple receptor populations competing for identical DNA binding sites. Importantly, these results are consistent with our recent studies demonstrating that the energetics of receptor–DNA interactions *in vitro* are the primary determinant of sequence-specific gene regulation *in vivo* [13]. Thus a critical concern is to identify the molecular forces responsible for receptor-specific differences in energetics, particularly for the (at least) 100,000-fold difference in ER-α and GR dimerization.

Unfortunately, ER-α and GR dimerization energetics are not accessible experimentally. Indeed, ER-α dimerization affinity could only be estimated by indirect methods [7]. By direct analysis we can only place an upper limit for ER-α dimerization and a lower limit for GR. We therefore created a chimeric receptor, replacing the HBD of GR with that of ER-α (GR/ER; see Fig. 1C). We then used analytical ultracentrifugation and quantitative DNase footprint titrations to examine GR/ER self-association and promoter binding energetics; transient transfection assays were used to examine *in vivo* transcriptional activity. We find that the chimera is functionally active in a cellular environment, consistent with previous reports [14]. However, our thermodynamic dissection of GR/ER dimerization reveals energetics intermediate between the parent proteins and a strong linkage to net ion release. Noting that the HBD of ER-α is thought to be the primary contributor to dimerization, we suggest therefore that residues unique to GR structurally constrain an ion-dependent HBD assembly mechanism.

2. Materials and methods

2.1. Construction of GR/ER cDNA

The human GR DNA sequence corresponding to residues 1–527 and the human ER-α DNA sequence corresponding to residues 311–595 were generated by PCR amplification using the oligonucleotides:

GR5′: 5′-CGATGGATCCGAATGGACTCCAAAG-3′
GR 3′: 5′-GATCGCTAGCCTCACCCCTACCCTGGTGTCA-3′
ER-α 5′: 5′-GATCGCTAGCACGGCCGACCAGATGGTCAGT-3′
ER-α 3′: 5′-AGATCTCGAGTCAGACCGTGGCA-3′

Following amplification, the products were digested with *NheI* and ligated overnight. The ligation product was digested with *BamHI* and