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Hypothesis

A metal-linked gapped zipper model is proposed for the hsp90–glucocorticoid receptor interaction

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In the presence of certain metals, regions of the hormone binding domain of the glucocorticoid receptor (GR) are capable of binding the 90 kDa heat shock protein (hsp90). Using secondary structure prediction methods in correlation with the experimental data, we propose a model which predicts the presence of two widely spaced leucine zipper-like heptads on either side of a central subdomain. The heptads could interact hydrophobically with similar regions on the hsp90 homodimer, bringing putative metal binding residues on each protein close enough to establish a shared metal bridge. The central subdomain between heptads is suggested to contain regions involved in metal binding, steroid binding, and conformational mobility. The hypothetical model that we are proposing therefore addresses the nature of the structural link between hsp90 binding, hormone binding, and conformational changes in the receptor.

Glucocorticoid receptor; 90 kDa heat shock protein; Leucine zipper

1. INTRODUCTION

Binding of glucocorticoids to their cognate receptor in the cell initiates a chain of events which ultimately leads to changes in gene expression. As part of this process, the receptor binds to hsp90 at or near the end of translation to form the 8S complex [1,2]. It has been suggested that the interaction with hsp90 assists in the correct folding of the receptor [3,4]. The formation of the 8S complex promotes the high affinity ligand binding conformation of the glucocorticoid receptor [5–7]. Conformational changes induced by hormone binding have been linked to dissociation of hsp90 from the complex [7]. The receptor is then capable of DNA binding, homodimerization, and altering gene expression [8,9].

Evidence indicates that neither the amino terminal nor the DNA binding domains of the glucocorticoid receptor are primarily responsible for binding hsp90, but rather several different sites within the hormone binding domain are involved in the formation of a stable complex [10]. These and other results further indicate that the hormone-binding domain is a complex structure containing several overlapping, multifunctional regions for ligand binding, dimerization, transcription activation and hsp90 binding [4–7,9–18]. However, little is known about either the secondary structure of the receptor or the conformational changes related to hormone binding and the dissociation of hsp90. By correlating the experimental data on receptor function with secondary structure predictions [19,20], we describe a model for the hsp90–GR interaction which links the structure and the functions of the glucocorticoid receptor.

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2. HYPOTHESIS

We propose that two separate regions within the hormone binding domain of the monomeric glucocorticoid receptor form weak leucine-zippers with the hsp90 homodimer. A hypothetical central subdomain between helices contains regions which contribute to steroid binding, conformational mobility, and metal binding. The partial overlap of these regions in the primary structure of the protein (Fig. 1) would produce a ligand-sensitive, conformationally controlled, regulatory site within the receptor. Steroid binding is predicted to induce a transition in the conformationally mobile region from a random to a helical state. This could, in turn, disrupt the metal link and promote hsp90 release. Further conformational changes could then take place in the receptor which block hsp90 reassociation and favor homodimerization.

2.1. *Weak leucine zipper interactions*

Weak leucine zippers are suggested to form between Region C on hsp90 [21] and LZ-1 and LZ-2 on the

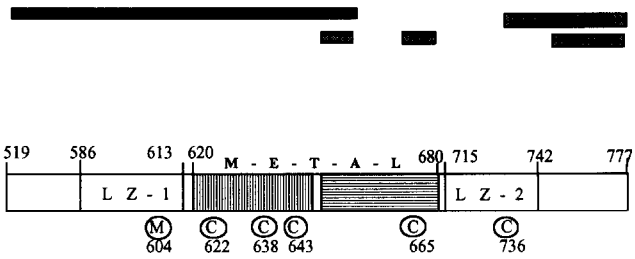

Fig. 1. Predicted subdomain structure for the hGR hormone binding domain. Two separate leucine zipper-like regions (LZ-1 and LZ-2) flank the central subdomain. The central subdomain consists of three partially overlapping regions which function in ligand binding (vertical stripes), conformational mobility (horizontal stripes), and metal binding. The metal binding region may extend into the flanking leucine zippers, as discussed in the text. Residues which can be labelled with ligand [33–35], including cysteines with potential for metal binding, are circled. Shown above are the 16 kDa core hormone binding fragment [40], solid box, and delimited segments required for tight hormone binding [33,38–40], shaded boxes. The numbers shown correspond to amino acid positions in the human glucocorticoid receptor.

glucocorticoid receptor (Fig. 2). Region C was chosen because it contains a leucine zipper-like M-L-L-L sequence, 1 cysteine, is devoid of proline, and is predicted to have high α-helical content. Furthermore, this region is located within a larger segment which is crucial to the steroid receptor–hsp90 interaction [22]. There are no perfect leucine zippers within the hormone-binding domain of the glucocorticoid receptor. Two helices, however, were selected from several other, conformationally discrete, helical heptad repeats because their involvement in hsp90 binding is consistent with the experimental data, their surfaces show high compatibility for hsp90 binding, and they border the central subdomain and thereby fit a similar model proposed for the estrogen receptor [21].

The model predicts that the association between proteins is relatively weak. First, there are few salt bridges to stabilize the hydrophobic interaction (Figs. 3 and 4). Second, the glucocorticoid receptor heptads are predicted to be short and kinked or broken (Fig. 2). Third, the presence of like charges close to each other would destabilize the interaction between helices (Figs. 3 and 4). These characteristics correlate well with observations that the 8S complex is readily dissociated by increased temperature, dilution, changes in pH, chelation of metal ions, and hormone binding.

2.2. *Metal binding*

The first functionally distinct region in the central subdomain is the hypothetical *metal binding region.* This region contains amino acid residues such as cysteine which are capable of binding metals. Also, this region is predicted to become buried in a hydrophobic interface between the glucocorticoid receptor and a similar potential metal binding region on the hsp90 homodimer. The leucine zippers formed between the glucocorticoid receptor and the hsp90 homodimer are predicted to bring the metal binding residues on each protein close enough to form a shared metal binding site (Fig. 2). A shared metal binding site [23] has also been shown to be involved in dimerization of the MerR metalloregulatory protein [24] and in the binding of growth hormone to the prolactin receptor [25]. Two other proteins the binding of which to hsp90 is stabilized by molybdate [26,27], v-erb A and pp60$^{v\text{-}src}$, have sequences which are similar to the putative metal-binding region of this model.

Divalent zinc was previously suggested to bridge hsp90 and the androgen receptor in the 8S complex [28]. Exogenous transition metal oxyanions, such as molybdate, stabilize the hsp90-bound form of the steroid receptors. Other experiments using glucocorticoid receptor-containing cytosols have demonstrated that an endogenous heat stable, chelatable, anionic, molybdate-like factor stabilizes the hsp90–glucocorticoid receptor complex [29]. A direct physical interaction between hormone-binding domain cysteine sulfhydryl groups on the glucocorticoid receptor and molybdate has been shown [32]. In the present model, two alternative metal binding centers between the glucocorticoid receptor and Region C on the hsp90 dimer have been constructed: (1)

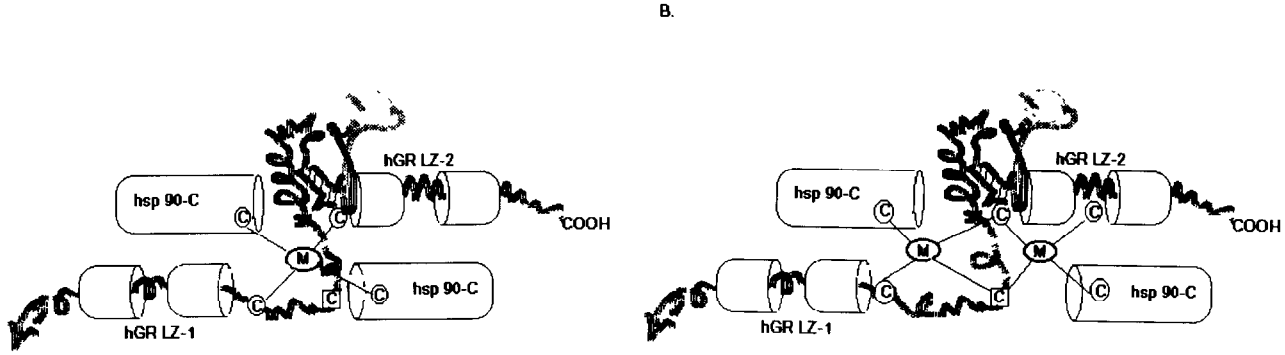

Fig. 2. Schematic representation of the gapped zipper arrangement between LZ-1 and LZ-2 on the hGR monomer and Region C on the hsp90 dimer. The central subdomain, or gap between helices, on the hGR, encompasses regions which contribute to steroid binding, N-terminal to the boxed cysteine; conformational mobility, C-terminal to the boxed cysteine; and metal binding,. The metal binding region is depicted as a single (A) or double (B) metal ion coordination site.

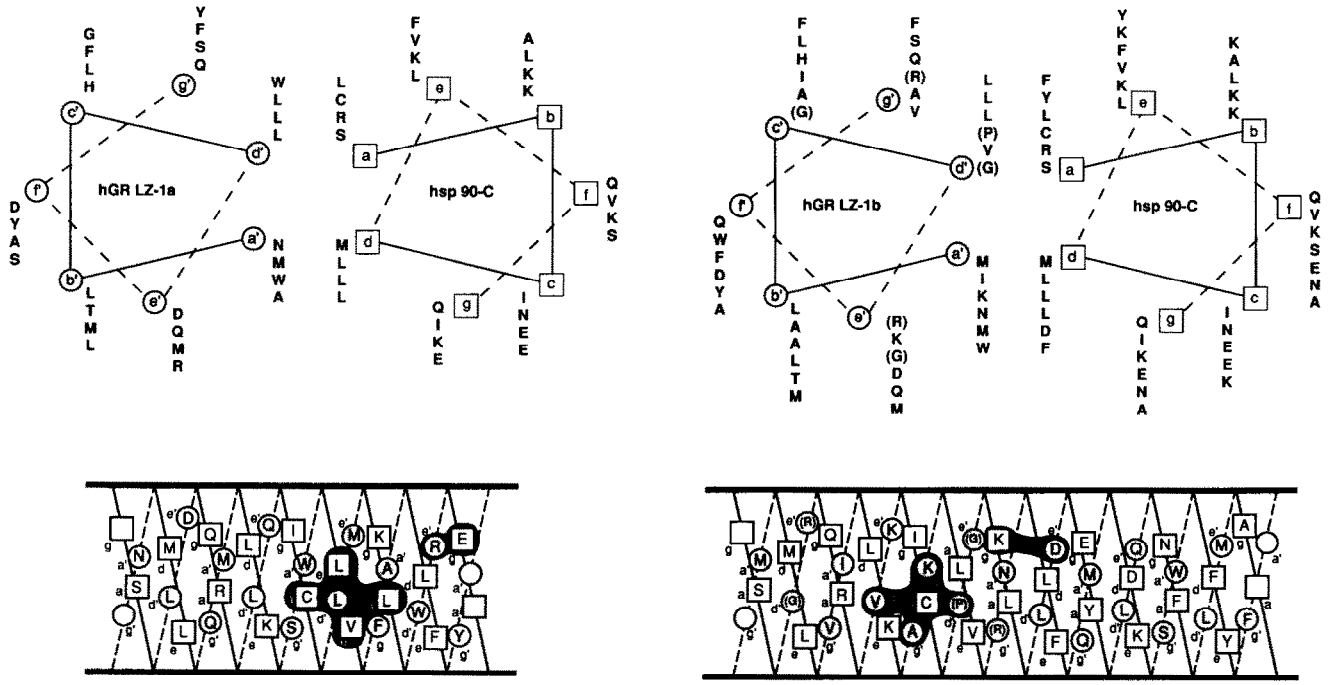

Fig. 3. Hydrophobic interactions between weak leucine zippers. Helical wheel (top) and helical net (bottom) diagrams show the proposed interactive surfaces between hsp90 Region C and one of two possible arrangements for LZ-1, denoted as a or b. Predicted hydrophobic pockets and favorable electrostatic interactions are shaded. Parentheses indicate potential breaks in the helical structure.

where cysteine ligands within the steroid binding and conformationally mobile regions coordinate a single metal ion (Fig. 2A) or, (2) where additional cysteines in LZ-1 and LZ-2 allow two metal ions to be bound (Fig. 2B). Cysteine ligands were used for modeling because (1) molybdate is known to bind sulfhydryl groups [30]; (2) cysteine binds metals in other proteins [31], and (3) molybdate interacts directly with hormone binding domain cysteines [32]. However, additional experiments are required to identify the actual metal-binding residues.

Interestingly, the secondary structure modeling experiments (Figs. 3 and 4) place a cysteine near to a lysine in both LZ1b and LZ2. The positively charged lysines could therefore lower the pK and stabilize the thiolate anion form of the nearby cysteine residues; this would favor metal binding.

Two studies using deletion mutants [13,18] and synthetic peptides [18] support the proposed model. These experiments identified two critical contact sites for hsp90 binding. One site, corresponding on the human GR (hGR) to residues 626–653, was suggested to contain the metal anion binding site [13]; the other, corresponding on the hGR to residues 568–653, was proposed to provide additional contacts for the hsp90–GR interaction [18]. These segments coincide with the core metal binding region and LZ1 of the model described here (Fig. 2A).

2.3. *Hormone binding*

The hypothetical *hormone binding segment* is the second functionally distinct region in the central subdomain (Fig. 1). This region contains residues that have been implicated in binding hormone, such as methionine-604 and hormone binding domain thiol groups [33–35] (Fig. 1). Most insertions, deletions and point mutations within the hormone binding domain cause a reduction in steroid binding activity [36,37]. However, several delimited segments have also been implicated in tight hormone binding [33,34,38–40] (Fig. 1). Among these is the trypsin-generated, 16 kDa core fragment which binds hsp 90 and hormone but with a 23-fold reduction in affinity for the latter [40]. These data indicate that nearly all portions of the glucocorticoid receptor hormone binding domain are either directly or indirectly involved in the formation of the high affinity steroid binding site. The proposed model, by describing the hormone binding domain as having overlapping segments with specialized, yet interdependent functions, accounts for these observations. Furthermore, the 16 kDa core hormone binding fragment spans LZ1, the ligand binding subdomain, and much of the metal binding region proposed here; most of the other segments required for tight hormone binding [33,34,38,39] coincide with the conformationally mobile or LZ2 regions (Fig. 1). Finally, low levels of arsenite, which may bind vicinal dithiols, were shown to block steroid binding

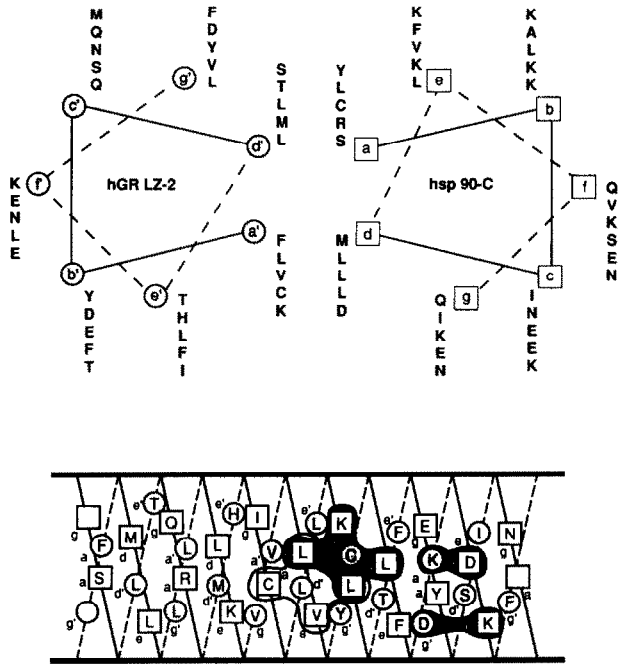

Fig. 4. Hydrophobic interactions between weak leucine zippers. Helical wheel (top) and helical net (bottom) diagrams showing the proposed interactive surfaces between hsp90 Region C and LZ-2. Predicted hydrophobic pockets and favorable electrostatic interactions are shaded.

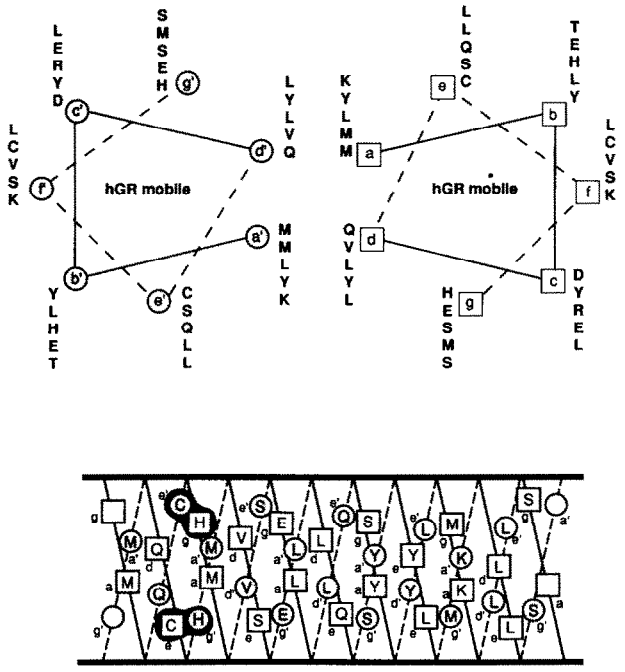

Fig. 5. Potential homodimerization site. Helical wheel (top) and helical net (bottom) representation between conformationally mobile regions when drawn as an α-helix. Potentially reactive residues are shaded. Note the preponderance of hydrophobic residues at predicted interactive surfaces as shown in the helical net diagram.

activity and affinity labeling [41,42]. The blockage of affinity labeling was reversible by DTT [43]. These vicinal dithiols are present in the ligand and metal binding regions proposed here (Fig. 1).

2.4. *Conformational mobility*

The third functionally distinct site in the central subdomain is the hypothetical *conformationally mobile region*, which has equal Chou–Fasman [19] helix- and sheet-forming predictions. For example, ambiguous predictions for the peptide hormone glucagon correlated with structural changes from αv α helix to a β sheet upon binding to the receptor [44]. Similarly, a segment of preproparathyroid hormone which has ambiguous predictions undergoes conformational change in response to changes in solvent hydrophobicity [44]. We propose that steroid binding to the hormone-binding segment of the receptor shifts the conformation of the neighboring mobile region from that of a coil to that of a helix. One possible mechanism is through an increase in the local hydrophobicity brought about by steroid binding. Furthermore, the conformationally mobile region, when modeled as an α helix, forms a potential homodimerization site (Fig. 5). A change in the secondary structure, as suggested here, could therefore favor homodimerization as well as impair hsp90 reassociation.

Because the conformationally mobile region is predicted to contain at least one of the amino acids involved in metal binding, the changes induced by steroid binding are predicted to weaken the metal link between the receptor and hsp90. Once this occurs, the weak leucine zipper would be insufficient to maintain the hsp90–GR interaction and the proteins would dissociate. Additional conformational changes could then take place in the receptor making hsp90 binding sites inaccessible, reassociation difficult, and favoring homodimerization. This reasoning agrees with studies which show that reassociation of hsp90 with the transformed GR has only been achieved in the presence of rabbit reticulocyte lysate [45].

The proposed model is consistent with the available experimental evidence that multiple regions of the GR hormone binding domain are involved in hsp90 binding, and suggests a potential mechanism which links hormone binding with conformational changes in the receptor. We hope that it will assist in the interpretation of current results and serve as a guide for future experiments.

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