The Relationship between Glucocorticoid Receptor Binding to Hsp90 and Receptor Function

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Abstract

In this minireview we summarize evidence that the association of the glucocorticoid receptor (GR) with hsp90 may determine three functional states of the receptor. First, there is a direct correlation between hsp90 binding to the receptor and repression of DNA binding activity. Temperature-dependent dissociation of hsp90 from the cytosolic GR-hsp90 complex is promoted by hormone with simultaneous conversion of the receptor to the DNA binding state. GR that is translated in rabbit reticulocyte lysate binds to hsp90 at or near the termination of receptor translation and is in the non-DNA-binding form. Second, there is a direct correlation between binding of the immunopurified GR to hsp90 and the presence of a high affinity steroid binding conformation of the receptor. GR translated in reticulocyte lysate binds steroid with high affinity, but GR translated in wheat germ extract is not bound to hsp90, does not bind steroid with high affinity, and is in the DNA-binding form. When immunopurified, hsp90-free GR is incubated with rabbit reticulocyte lysate, hsp90 associates with the receptor and high affinity steroid binding capacity is completely reactivated. Third, there is a correlation between binding of hsp90 to steroid receptors and their retention in an inactive “docking” state until the binding of hormone in the intact cell triggers a progression to high affinity nuclear binding sites where the primary events involved in transcriptional activation occur. In contrast to the receptors that are retained in the docking state, the unliganded thyroid hormone receptor proceeds directly to high affinity nuclear binding sites. Consistent with this difference in behavior, the thyroid hormone receptor is translated in reticulocyte lysate in its DNA binding form and is not associated with hsp90.
Introduction

Protein-protein interactions are important for signal transduction in most receptor systems. For example, in the G protein-linked family of membrane receptors, the high affinity agonist binding conformation of the receptor is stabilized through its interaction with the G protein and G protein activity is repressed by unliganded receptor. Hormone binding initiates a series of events which includes dissociation of the receptor-G protein complex and the subsequent interaction of the liberated G protein with the appropriate effector mechanism (for review see Gilman, 1987). Protein-protein interactions are also important in the mechanism of steroid hormone receptors and it is likely that the steroid binding, nuclear localization, DNA binding and transcriptional enhancement functions of these receptors depend on their association with other proteins. One protein that is essential for the proper function of the glucocorticoid receptor (GR) is the 90 kilodalton heat shock protein, hsp90, and the structural and functional aspects of the interaction between the GR and hsp90 will be examined in this review.

The 90kDa heat shock protein is a conserved, ubiquitous, abundant, essential, and predominantly cytoplasmic protein (for review see Lindquist and Craig, 1988). Members of the hsp90 gene family have been cloned from bacteria (Bardwell and Craig, 1987), yeast (Farrelly and Finkelstein, 1984), trypanosomes (Dragon et al., 1987), Drosophila (Hackett and Lis, 1988), and mammals (Moore et al., 1987), the hsp90 proteins display amino acid sequence identity in excess of 40%, and a member of the hsp90 gene family has been found in all species examined (Lindquist and Craig, 1988). In mouse L cells (Lai et al., 1984), as well as in other eukaryotic cell types, hsp90 comprises 1% of the total cytosolic protein and its production is further induced by heat and other stress conditions. Although hsp90 does appear to play a role in thermotolerance, its constitutive presence is essential for normal eukaryotic cell function (Borkovich et al., 1989). It has been shown, for example, that mutation of the hsp90 genes in S. cerevisiae is lethal to that organism, even at 25°C (Borkovich et al., 1989).

There is evidence that hsp90 may play a major role in regulating the function of other regulatory proteins. For example, the transforming protein of the Rous sarcoma virus, pp60<sup>Src</sup>, associates with hsp90 immediately after pp60<sup>Src</sup> translation (Brugge et al., 1981; Operman et al., 1981), and there is evidence that hsp90 represses the tyrosine kinase activity of pp60<sup>Src</sup> prior to its insertion into the plasma membrane (for review see Brugge, 1986). In addition, a pp60<sup>Src</sup> mutant that forms a more stable complex with hsp90 is also transformation defective (Garber et al., 1985). Hsp90 also copurifies with eIF2α kinase and regulates its activity (Rose et al., 1987). In heme supplemented reticulocyte lysates, hsp90 binds to and represses the activity of the eIF2α kinase and dissociation of this heterocomplex correlates with activation of the enzyme (Matts and Hurst, 1989).

Some of the best evidence that hsp90 is important in the regulation of protein function is derived from work characterizing its interaction with steroid receptors. Glucocorticoid...
(Housley et al., 1985; Sanchez et al., 1985), mineralocorticoid (Rafestin-Oblin et al., 1989), androgen (Joab et al., 1984), progesterone (Schuh et al., 1985; Catelli et al., 1985), estrogen, and arylhydrocarbon (Perdew, 1988) receptors copurify with the 90 kilodalton heat shock protein. Although there is evidence for all of these receptors that this interaction is of functional importance (for review see Pratt, 1987), the following discussion will focus only on the GR-hsp90 interaction.

**Hsp90 and Repression of DNA Binding Activity of the GR**

It has been known for twenty years that glucocorticoid receptors can exist in two physical states in cytosol preparations. One state is as a large complex of Mr~300,000 and sedimentation value of ~ 9S and the other state is as an Mr~100,000, 4S monomer (Pratt, 1987); When the receptor is in the large complex, its DNA binding activity is repressed, but when it dissociates to the 4S form, it acquires the capacity for high affinity and sequence-specific binding to DNA, a change that is called receptor transformation. During the 1970s, the study of this transformation process with purified systems was hampered because the receptor spontaneously transforms as it is purified. In 1977, it was found that molybdate stabilizes the steroid binding activity of the glucocorticoid receptor (Nielsen et al., 1987) and it soon became clear that molybdate and vanadate stabilize the receptor in its untransformed, non-DNA-binding form (Leach et al., 1979). It was thus possible to purify the non-DNA-binding form of the receptor in the presence of molybdate (Dahmer et al., 1981; Housley and Pratt, 1983), and it was found that in addition to the receptor itself, the molybdate-stabilized complex contained a 90kDa non-steroid-binding protein (Housley et al., 1985), which was identified as hsp90 (Sanchez et al., 1985).

These observations led to the model of receptor transformation depicted in Figure 1. The non-DNA-binding form of the cytosolic GR complex shown in the model consists of a single molecule of the steroid binding protein (Gehring and Arndt, 1985) bound to a dimer of hsp90 (Mendel and Orti, 1988). It should be made clear, however, that recent studies in both glucocorticoid (Bresnick et al., 1990; Sanchez et al., 1990) and progesterone (Kost et al., 1989; Smith et al., 1990) receptor systems provide evidence that the untransformed M,300,000, 9S cytosolic receptor-hsp90 complex is probably a core unit derived from a larger heteromeric structure containing several other proteins in addition to hsp90.

In the intact cell, the hormone somehow drives the conversion of the GR from a transcriptionally inactive to a transcriptionally active state. There is strong evidence that the cytosolic GR-hsp90 complex is derived from the physiologically important transcriptionally inactive “docking” state of the receptor that is turned on by the hormone in the intact cell: (1) Mendel et al. (1986) have shown that hsp90 is associated with GR obtained from hormone-free cells but not from cells that were exposed to hormone at 37°C, a
condition that ensures transformation; (2) Rexin et al. (1988a, b) have cross-linked the GR to hsp90 in intact cells under conditions where the receptor is untransformed; (3) Pratt et al. (1988) have demonstrated a direct correlation between the constitutive versus hormone-inducible properties of GRs produced from modified cDNAs and their recovery from transfected hormone-free cells as the transformed 4S, dissociated receptor protein or as untransformed 9S, hsp90-containing complexes.

Fig 1. Model of glucocorticoid receptor transformation to the DNA binding state. The untransformed, non-DNA-binding form of the GR exists in cytosol as a heterocomplex in association with a dimer of hsp90. The hsp90 binds to the hormone binding domain of the GR and the complex is stabilized by molybdate and some other transition metal oxyanions (indicated by the small globe with the M). Steroid binding promotes the temperature-dependent dissociation of hsp90 with the generation of the DNA binding form of the receptor as indicated by the exposed zinc fingers.

These observations support the proposal that the dissociation of the cytosolic GR-hsp90 complex and the accompanying conversion of the receptor to the DNA binding state that is depicted in Figure 1 constitutes a valid cell-free model of the initial event in steroid hormone action. Exactly how the presence of hsp90 blocks the DNA binding activity is not clear. It could do so either by masking the DNA binding domain directly as the complex is drawn in Figure 1 or it could maintain the receptor in a conformation in which the DNA-binding fingers are not available for interaction. From both the study of mutant GRs produced from transfected cDNAs (Pratt et al., 1988) and direct protein cleavage experiments (Denis et al., 1988a), it is clear that the hormone binding domain of the GR contains the sites of interaction with hsp90. It is also clear that binding of the hormone to
the GR in cytosol promotes both dissociation from hsp90 from the receptor and generation of the DNA binding state (Sanchez et al., 1985; Mendel et al., 1986; Sanchez et al., 1987; Denis et al., 1988b; Meshinchi et al., 1990).

The notion that hsp90 represses the function of the GR and that hormone binding relieves this repression has gained added significance from the observations provided by the molecular biologists. The hormone binding domain of the GR lies in the C-terminal one-third of the protein, and in addition to containing the steroid binding site, deletion experiments have shown that the steroid binding domain contains the features that determine both repression of DNA binding activity and steroid-mediated derepression of DNA binding activity (Danielsen et al., 1987; Hollenberg et al., 1987; Godwski et al., 1987). Several observations have led to the general concept that the C-terminal steroid binding domain acts as a movable hormone regulatory function. For example, Picard et al. (1988) have shown that steroid-dependent regulation of the DNA binding and transcriptional regulatory functions of the GR are retained when the steroid binding domain is repositioned from the C-terminus to the N-terminus of the receptor. It seems clear from the analysis of the transcriptional enhancement activity of chimeric receptors, such as that containing the steroid binding domain of the GR and the DNA binding domain of the estrogen receptor (Kumar et al., 1987), and fusion proteins, such as the adenovirus E1A gene product or the myc oncogene product linked to the steroid binding domain of the GR (Picard et al., 1988; Ellers et al., 1989), that the steroid binding domain can confer both repression of function and steroid-mediated derepression of function to other proteins. At the molecular level, these observations could be explained by the model of Figure 1 where the functional repression of the hormone binding domain is a consequence of its binding to hsp90.

Elimination and Reconstitution of the Requirement for Hormone in Promoting Cytosolic Transformation of Receptors to the DNA-binding State

The mechanism by which the binding of hormone promotes dissociation of hsp90 from the GR in cytosols is unknown. Some insight into this problem may be derived from the study of transition metal oxyanion effects on the receptor. It is known that the hormone binding domain is sufficient for molybdate stabilization of the GR-hsp90 complex (Pratt et al., 1988; Denis et al., 1988a; Bresnick et al., 1989), and it seems quite clear that the metal oxyanion must stabilize the complex through a direct physical interaction with one component or both.

During the 1970s, it was noted that a very small, anionic, heat-stable activity present in cytosol stabilizes a variety of steroid receptors in their non-DNA-binding form (Goidl et al., 1977; Bailly et al., 1977; Sato et al., 1980). This stabilizing factor is ubiquitous, it has the physical properties of a metal anion, and it produces all of the effects on cytosolic glucocorticoid receptors that are produced by the group VI-B transition metal oxyanions
molybdate, vanadate, and tungstate (Leach et al., 1982; Meshinchi et al., 1988). We have proposed that molybdate and the other transition metal oxyanions exert their effects on the receptor by interacting with the binding site for this endogenous metal anion (Meshinchi et al., 1988).

If a metal normally stabilizes the GR-hsp90 complex in cytosol, then the receptor could be expected to behave differently in metal-free cytosol than in normal cytosol containing all of the endogenous metals. The endogenous factor activity binds tightly to the metal chelating resin Chelex-100, and we passed L cell cytosol through Chelex-100 resin to remove endogenous metals in order to study the stability and transformation of the metal-free receptor (Meshinchi and Pratt, 1989; Meshinchi et al., 1990). As expected from previous work, we found that in normal cytosol, both temperature-mediated dissociation of hsp90 and temperature-mediated receptor transformation to the DNA-binding state are hormone-dependent events. In Chelex-treated, metal-free cytosol, however, temperature-mediated dissociation of hsp90 and receptor transformation occur very rapidly in a manner that is no longer hormone-dependent. The two systems are outlined in the schemes shown in Figure 2.

**Fig 2.** Schematic models of transformation in normal cytosol and metal-free cytosol. In normal cytosol containing the endogenous metals, hsp90 dissociation is temperature-dependent and steroid-dependent, but in metal-free cytosol, hsp90 dissociation is temperature-dependent but no longer steroid-dependent.
If boiled cytosol containing all of the metals of the L cell is added to the metal-depleted receptor system, the hormone dependence of both temperature-mediated dissociation of receptor from hsp90 and receptor transformation to the DNA-binding state is reconstituted. These results support the proposal that an endogenous metal anion interacts with the GR to stabilize it in the heteromeric, inactive, non-DNA-binding state in cytosol and that binding of hormone promotes conversion of the receptor to the DNA-binding state through an effect on this metal anion center. To our knowledge, this is the first time that the requirement for hormone has been eliminated and reconstituted in a cell-free system in which receptors are converted from an inactive to an active conformation.

**Hsp90 is Required for the High Affinity Steroid Binding Conformation of the GR**

In addition to repressing the DNA-binding activity of the GR, hsp90 must be bound to the receptor for it to have a high affinity steroid binding conformation. We noted many years ago that conditions which transform the steroid-bound GR to the DNA binding state (e.g. heating of cytosol, dilution, salt or increasing pH) inactivates the steroid binding capacity of the unliganded receptor (Leach et al., 1979; Dahmer et al., 1981). These are all conditions that promote dissociation of the GR-hsp90 complex, and reagents, such as molybdate, aluminum fluoride, or hydrogen peroxide which stabilize the complex, prevent both loss of steroid binding capacity and receptor transformation (Bresnick et al., 1988; Housley, 1990). These observations and the observation that the 4S form of the receptor does not bind steroid led to the proposal that hsp90 is required for the GR to have a high affinity steroid binding conformation (Bresnick et al., 1988a, b).

This proposal has been tested in three ways. First, we showed that the steroid binding capacity of immunopurified GR is directly related to the presence of receptor-associated hsp90, and under no conditions does hsp90-free receptor show high affinity steroid binding activity (Bresnick et al., 1989). The receptor bound to hsp90 can be cleaved in the presence of molybdate to yield the hormone binding domain with retention of both bound hsp90 and steroid binding activity. Thus, it seems that the hormone binding domain is not itself sufficient for a competent hormone binding site, but it is sufficient when it is associated with hsp90.

As indicated by the unidirectional arrows in Figure 1, hsp90 is not in free equilibrium with the GR in cytosol. Thus, it is not yet possible to simply add purified hsp90 to purified GR and generate a steroid binding conformation of the receptor. It is known that GR translated in rabbit reticulocyte lysate binds hormone with high affinity, and in a second approach to studying the relationship between hsp90 and steroid binding activity, we have examined the properties of GR produced in cell-free translation systems. We have shown that the GR translated in rabbit reticulocyte lysate is immunoadsorbed by the 8D3 monoclonal antibody directed against hsp90 (Dalman et al., 1989). Wheat germ extract also
supports translation of a full length receptor, but in contrast to the reticulocyte lysate, the receptor produced in wheat germ extract is not immunoadsorbed by the 8D3 antibody and we cannot demonstrate high affinity steroid binding. As might be predicted, GR that is translated in reticulocyte lysate is in a non-DNA-binding form that requires transformation (Dalman et al., 1989), whereas GR translated in wheat germ extract is in the DNA-binding form and does not require transformation (Dalman et al., 1990).

The remarkable stability of the GR-hsp90 complex that is formed on GR translation in reticulocyte lysate has prompted Toft and his coworkers to consider that the lysate may be able to direct reassociation of hsp90 with hsp90-free receptor. Thus, Toft and his coworkers added immunopurified hsp90-free chicken progesterone receptor (PR) to rabbit reticulocyte lysate and were able to form a chicken PR-rabbit hsp90 complex (Toft, personal communication). Accordingly, we have examined reticulocyte lysate-directed reconstitution of the GR-hsp90 complex as a third avenue of testing the hsp90 requirement for high affinity steroid binding activity (Scherrer, Dalman, Meshinchi and Pratt, submitted manuscript). We find that incubation of hsp90-free immunopurified L cell GR with rabbit reticulocyte lysate results in association of the rabbit hsp90 with the mouse GR and this is accompanied by complete reconstitution of high affinity glucocorticoid binding activity.

Evidence that hsp90 is Required for the "Docking" State of the Receptor in Intact Cells

In hormone-free cells, the unliganded forms of most steroid receptors remain in an inactive "docking" complex until the binding of hormone triggers a progression to high affinity nuclear receptor binding sites where the primary events in transcriptional activation occur. In the case of estrogen and progesterone receptors, the unliganded receptor is retained in a docking complex at the nucleus (King and Greene, 1984; Welshons et al., 1984; Perrot-Applanat, 1985), whereas the docking form of the unliganded GR may be cytoplasmic (Antakly and Eisen, 1984; Qi et al., 1989) or nuclear (Sanchez, Hirst, Scherrer, Tang, Welsh, Harmon, Simons, Ringold and Pratt, submitted manuscript), depending on the cell type. Receptors that enter such a docking complex are characterized by being recovered in the cytosol fraction of cells ruptured in hypotonic buffer, they are associated with hsp90, and they are functionally inactive until they are exposed to ligand.

Unlike the steroid receptors that enter a docking complex, the thyroid hormone receptor is tightly associated with the nucleus of the hormone-free cell and high salt is required for its extraction (for review see Samuels et al., 1988). We have found that, in contrast to the GR, the thyroid hormone receptor translated in rabbit reticulocyte lysate is not bound to hsp90 and is translated in its DNA binding form without any requirement for transformation (Dalman et al., 1990). These observations are consistent with the different behavior of this receptor with respect to the classic steroid receptors in the intact cell. Thus, we propose that hsp90 may prevent progression of the steroid receptors to high
affinity nuclear association sites and account for their retention in the inactive docking state in the absence of ligand.

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