Modular Structure of Glucocorticoid Receptor Domains Is Not Equivalent to Functional Independence

STABILITY AND ACTIVITY OF THE STEROID BINDING DOMAIN ARE CONTROLLED BY SEQUENCES IN SEPARATE DOMAINS*

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A long-standing conundrum of glucocorticoid receptors has been why the steroid binding domain is active in hybrid proteins but not in isolation. For this reason, the precise boundaries of the steroid binding domain have not been defined. These questions have now been systematically examined with a variety of receptor deletion constructs. Plasmids encoding amino acids 537–673 of the rat receptor did not yield stable proteins, while the fusion of receptor or non-receptor sequences upstream of 537–673 afforded stable proteins that did not bind steroid. Wild type steroid binding affinity could be obtained, however, when proteins such as β-galactosidase or dihydrofolate reductase were fused upstream of receptor amino acids 537–795. Studies of a series of dfr/receptor constructs with deletions at the amino- and carboxyl-terminal ends of the receptor sequence localized the boundaries of the steroid binding domain to 550–795. The absence of steroid binding upon deletion of sequences in the carboxyl-terminal half of this domain was consistent with improperly folded receptor sequences. This conclusion was supported by analyses of the proteolysis and thermal stability of the mutant receptors. Thus, three independent regions appear to be required for the generation of the steroid binding form of receptors: 1) a protein sequence upstream of the steroid binding domain, which conveys stability to the steroid binding domain, 2) sequences of the carboxyl-terminal amino acids (674–795), which are required for the correct folding of the steroid binding domain, and 3) amino-terminal sequences (550–673), which may be sufficient for steroid binding after the entire steroid binding domain is properly folded. These results establish that the steroid binding domain of glucocorticoid receptors is not independently functional and illustrate the importance of both protein stability and protein folding when constructing mutant proteins.

The presence of separate functional domains in glucocorticoid receptors was first postulated on the basis of protease digestion studies (1). Chymotrypsin digestion removed about half of the receptor to give a 42-kDa (3.6 nm) fragment that still bound steroid and DNA. Trypsin digestion of receptor-steroid complexes liberated an even smaller, about 30-kDa (1.9 nm) steroid-containing fragment that no longer bound DNA. Further support for this domain structure came after the cloning of glucocorticoid receptors, when it was found that the receptor could be divided up into three functionally active domains: an amino-terminal activation domain, a central DNA binding domain, and a carboxyl-terminal steroid binding domain (2, 3).

An initially surprising feature of all the steroid receptors was that functional activity was retained when the domains of different receptors were interchanged for each other or for the segments of other proteins. Particularly notable examples were the swapping of DNA binding domains of two receptors (4, 5) and the fusing of a steroid binding domain either to different positions of the original receptor or to other proteins (6, 7). When the steroid binding domain of the glucocorticoid receptor was fused to other proteins, not only was steroid binding retained, but also the activity of the rest of the fusion protein was usually controlled by steroid binding (6–13). Thus, in most instances, the various domains of the glucocorticoid receptor appeared to function autonomously. In fact, a separate evolution and function of the specific domains has been proposed (14–16). These findings are consistent with reports that transcription factors generally have a modular structure (17) and that individual protein domains, containing 100–200 amino acids (18), can fold independently of the rest of the protein (19–21).

Despite the numerous examples of domain modularity, direct evidence that a given glucocorticoid receptor domain is active in isolation exists only for the DNA binding domain and perhaps the amino-terminal domain. Thus, the fragment corresponding to amino acids 440–525 of the rat receptor, which is only slightly larger than the DNA binding domain (reviewed in Ref. 22), can bind DNA (23, 24). The amino-terminal transactivation domain also possesses transcriptional activity (7, 8), but this activity could be realized only when the domain is part of some larger DNA-binding protein. Thus, deletion of the amino-terminal domain reduced the trans-activation and synergism of intact glucocorticoid receptors (25), while fusion of the amino-terminal domain to the DNA binding domain of GAL4 caused an increased transcriptional activation, and synergism, from tandem arrangements of a palindromic GAL4 binding sequence (26). One example of a biologically relevant activity of the amino-terminal domain in isolation may be squelching (27). However, the observation that three antibodies to amino-terminal sequences do not inhibit squelching but do prevent transactivation (28) suggest that the two activities
Receptor Steroid Binding Activity Requires Multiple Domains

W. H. M. and A. S. M. investigated the effects of different steroid binding domains on receptor function. They found that the steroid binding domain, when isolated from the rest of the receptor, could function independently. This was surprising, as it was thought that the steroid binding domain was only functional in the context of the entire receptor.

The authors prepared and tested several receptor constructs containing portions of the steroid binding domain. They found that some of these constructs, when expressed in isolation, had wild-type activity. However, other constructs did not function as well.

To understand this, the authors constructed additional receptor constructs using different domains. They found that the steroid binding activity was dependent on multiple domains, rather than just the one present in the isolated domain.

Materials and Methods

The authors used a variety of techniques to study the receptor, including site-directed mutagenesis, PCR, and Western blotting. They also used a variety of reagents, including TAPS, glutathione S-transferase (GST), and dihydrofolate reductase (DHFR).

1 The abbreviations used are: Dex, dexamethasone; DEX-M, [3H]Dex 21-mesydate; PCR, polymerase chain reaction; kb, kilobases; GST, glutathione S-transferase; DHFR (dihydrofolic acid reductase); PBS, phosphate-buffered saline; TAPS, 3-[tris(hydroxymethyl)methyl]-aminopropanesulfonic acid.

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fragments (4.7 kb) was used as the vector for subsequent cloning. The other fragment (1.4 kb) was further digested with SphI to isolate the desired 0.6-kb DHFR sequence. Different lengths of receptor were amplified by polymerase chain reaction from pSVLGR (35). 5’ primers for PCR have an SphI recognition site upstream of the receptor cDNA sequence. 3’ Primers contain receptor cDNA followed by a stop codon plus an EcoRI recognition site. After digestion with SphI and EcoRI, the PCR product was ligated to the above 4.7-kb vector and 0.6-kb DHFR-containing fragments. All the constructs were confirmed by sequencing. Primers used in PCR are as follows. For pdhfr537C: 5’ primer (for this and all constructs starting at 537), 5’-GTATAAGTGACAAATGTTGATCGACGC-3’; 3’ primer (for this and all constructs ending at 795), 5’-CGGAATTCAGTTTAAAAGCGAACC-3’; for pdhfr537–766: 3’ primer, 5’-GGCGAATTCATTAAGCAATCTGTTGACC-3’; for pdhfr537–637: 3’ primer, 5’-GGCGAATTCATTAAGCAATCTGTTGACC-3’; for pdhfr550C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’; for pdhfr555C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’; for pdhfr556C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’; and all constructs starting at 537), 5’-GTATAAGTGACAAATGTTGATCGACGC-3’; for pdhfr537–766: 3’ primer, 5’-GGCGAATTCATTAAGCAATCTGTTGACC-3’; for pdhfr537–637: 3’ primer, 5’-GGCGAATTCATTAAGCAATCTGTTGACC-3’; for pdhfr550C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’; for pdhfr555C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’; for pdhfr556C: 5’ primer, 5’-GTATAAGTGACCAATGTTGATCGACGC-3’. The PCR product was ligated to the above 4.7-kb vector and 0.6-kb DHFR-attaching vector, pSVLGR (35). The PCR product was ligated to the above 4.7-kb vector and 0.6-kb DHFR-attaching vector, pSVLGR (35). The PCR product was ligated to the above 4.7-kb vector and 0.6-kb DHFR-attaching vector, pSVLGR (35).

Growth and Transfection of Cells—Mammary gland cultures of COS-7 cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 5% heat-inactivated fetal bovine serum. Wild type and truncated receptor expression plasmids (10 μg) were introduced into COS-7 cells (6–10/100-mm dish) by standard calcium phosphate transfection methods (36). Briefly, after ~16 h of incubation at 37°C in a 5% CO2 incubator, the excess calcium phosphate and precipitate were removed by washing with phosphate-buffered saline. The supernatant was then purified and ligated to NdeI and BamHI-digested plasmid. The PCR product was digested above PCR-produced dhfr-494C fusion protein sequence. The pSVLTm vector was then purified and ligated to the above PCR-generated dhfr-494C fusion protein sequence.

Western Blotting—Electrophoretic transfer from SDS-polyacrylamide gels to nitrocellulose, conducted in a Trans-Blot (Bio-Rad) apparatus, at 35 mA/gel. Gels were fixed, stained, marked at the positions of the molecular weight markers with Ult-Emit, and fluorographed for 1–2 weeks at 80°C as described (38).

RESULTS

Smallest Segment of Expressed Glucocorticoid Receptor That Retains Steroid Binding Activity—The cDNAs encoding for the intact and truncated receptors of Fig. 1 were transiently transfected into COS-7 cells, which contain very low levels of glucocorticoid receptors (see Fig. 2 below). Cytosolic extracts were then prepared to monitor the presence, and ability to bind steroid, of each receptor construct. The wild type (1–795) and amino-terminal domain truncated (407–795 = 407C) receptors both were stable proteins with very similar steroid binding and Dex-Mes affinity labeling capacity (data not shown). The affinity for dexamethasone (Dex) binding to the 407C receptors was not determined but was expected to be at least that of the smaller 42-kDa chymotryptic fragment of ~413–781 (or -795), which had previously been found to possess 84% of the affinity of the intact receptor (31). By Western blotting, the expression level of wild type and 407C receptors was similar (Fig. 2). The presence of additional, lower molecular weight bands appears due to alternative translational start sites (35, 39).

Neither fragments smaller than 407C, nor larger but containing less of the carboxyl-terminal end of the receptor, displayed significant amounts of steroid binding activity (Fig. 1). Both of the carboxyl truncated receptors 1–766 and 1–673 afforded stable proteins (Fig. 2). The cell-free translated frag-
the synthesis of correctly sized proteins in T7 expression plasmids T7537C and T7537–673, their directed sequences were translationally viable since, in the form of the tracts of COS-7 cells (data not shown). The receptor cDNA cause authentic 16-kDa fragments were stable in cell-free expressed protein was seen in the cytosolic extracts of transiently from COS-7 cell-specific proteases because no appropriately presence of Dex, Dex-Mes, or arsenite (40, 41) to stabilize the 537–673 fragment was also not detected when expressed in the transiently transfected COS-7 cells (Fig. 2). Authentic 16-kDa fragment, corresponding to 537–673 and prepared by trypsin digestion of the receptor fragments. The combination of these results with the nearly wild type level of protein and steroid binding activity seen with the 407C rat glucocorticoid receptor (Figs. 1–3) suggests that the steroid binding domain, which is thought to be encoded by sequences between 547 and 795 (22), is required for the steroid binding activity while upstream sequences, such as 407–546, are required for the steroid binding domain to be stable as a protein. One cause of protein instability is improper folding because incorrectly folded proteins are thought to be rapidly degraded (18, 42–44). We therefore asked whether the receptor sequence of 407–536 was unique in stabilizing the carboxyl-terminal region of the receptor.

Protein Stability of Carboxyl-terminal Constructs Can Be Conferred by Non-receptor Sequences—Constructs encoding the three hybrid receptors of Fig. 4A were prepared by fusing the intact β-galactosidase gene upstream of various carboxyl-terminal portions of the receptor. Earlier studies on the steroid-induced nuclear localization of Z540C in intact cells (13, 33) showed that this fusion protein was stable and bound steroid. We found that both Z540C and Z537C had the same, or slightly higher, affinity for Dex as did the wild type receptor (Figs. 4A versus 1). In both cases, the amount of steroid binding was about 4 times that seen for the mock transfection controls. In contrast, Z537–673 did not give any Dex binding (0.79 ± 0.17 (S.D., n = 5)-fold above mock transfections).

Replacement of the β-galactosidase gene in Z537C by the glutathione S-transferase (GST) gene sequence afforded another stable fusion protein with good steroid binding activity. Transient transfection of GST537C into COS cells gave rise to a 56 ± 22-fold (± S.D., n = 5) increase in the specific binding of [3H]Dex (data not shown).

Another series of hybrid receptors was prepared using dihydrofolate reductase (Fig. 4B). The parent receptor construct pMT2/DG (45), here denoted dfrh494C, seemed to have wild type affinity for Dex as witnessed by the reported half-maximal biological response of 10 nM Dex. This was confirmed by the present studies, where Scatchard analysis afforded an affinity comparable to that of wild type receptors (Figs. 4B versus 1). Similarly, removal of receptor sequences up to amino acid 537, to give dfrh537C, had little effect on the affinity of the resulting hybrid receptor (Fig. 4B) or its ability to be covalently labeled by Dex-Mes (data not shown). These data for 537C, Z537C, dfrh537C, and GST537C show that stable molecules with nearly wild type affinity for Dex (and presumably wild type
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Fig. 3. Effect of competing receptor cDNA in the whole cell expression of steroid binding activity of transiently transfected receptors. COS-7 cells were transiently transfected with decreasing amounts of either wild type receptor cDNA (A) or truncated (407C) receptor cDNA (B), plus increasing amounts of competitor DNA (pUC19 or pSVL537–673) and 0.2 μg of β-galactosidase cDNA (pCMVβ) as an internal control, such that the total amount of added DNA was constant at 10 μg. All aliquots of the transfected cell lysates were then analyzed for steroid binding, and the total specific binding per unit of β-galactosidase activity was plotted as described under “Materials and Methods.” C, duplicate aliquots of transfected cell lysates from A and B, with the indicated ratios of plasmids, were separated on an SDS-polyacrylamide (12%) gel. The amount of each receptor protein in various lysates was determined by Western blotting with the anti-receptor antibody aP1 followed by enhanced chemiluminescence using anti-rabbit IgG (location of wild type and 407C receptor is indicated by ▶ and ➡, respectively). For comparison, a sample of authentic 537–673 (≈16-kDa fragment obtained from trypsin digestion of HTC cell receptors) was included.

It should be noted that the same dhfr494C was also transiently expressed as a stable, steroid binding fragment from the same pSVLTm vector that failed in Figs. 1 and 2 to yield stable proteins for 537C and 537–673 (data not shown). Therefore, the inability to obtain the latter receptor fragments did not derive from some defect in the vector.

Minimum Sequence Needed for Steroid Binding Activity in Newly Synthesized Receptors—As with Z537–673, dhfr537–673 did not give any Dex binding (Fig. 4B; 0.83 ± 0.08 (S.D., n = 3) times mock transfection) even though protein of the expected size for dhfr537–673 was evident by Western blotting (data not shown). Two other hybrid receptors with progressively less carboxyl-terminal deletions (dhfr537–710 and dhfr537–766) also afforded no Dex binding (0.9–1.1 times mock transfection (n = 2)) although good amounts of each stable protein were produced (Figs. 4B and 6, bottom panel, and data not shown). The lack of binding with dhfr537–766 was reminiscent of results with the progesterone receptor, where the absence of binding of agonist steroids was associated with the loss of the carboxyl-terminal ~40 amino acids (46). However, the glucocorticoid receptor carboxyl-terminal sequences are not sufficient for binding as constructs containing the complete carboxyl-terminal sequence but lacking 80 or 15 internal amino acids (dhfr537Δ616–695C or dhfr537Δ690–704C, respectively; Fig. 4B) yielded stable proteins that were still unable to bind steroid (0.3–1.1 times mock transfection (n = 3)) or be labeled by Dex-Mes (data not shown). This behavior argues that some property of the sequence 674–795 more fundamental than the presence of the carboxyl-terminal ~40 amino acids, such as proper protein folding, is required for steroid binding activity.

A series of amino-terminal deletions of the receptor sequence in dhfr537C was prepared in order to define this boundary of the steroid binding domain. Removal of 10 or 13 amino acids, to give dhfr547C and dhfr550C, did not affect the ability of the hybrid protein to be affinity-labeled by Dex-Mes and caused less than a 3-fold decrease in the affinity for Dex (Fig. 4B). However, the deletion of 2 more amino acids to give dhfr552C eliminated Dex binding (1.25 ± 0.64 times mock transfection (S.D., n = 5 for two doses)) and dramatically reduced the affinity labeling by Dex-Mes. Further deletions to give dhfr554C and dhfr556C maintained the loss of steroid binding activity (Fig. 4B). These results define the amino-terminal end of the steroid binding domain as either 550 or 551 of the rat receptor. Interestingly, a PCR error generated a point mutation in one clone of dhfr550C that changed Arg-732 to a glutamine. This point mutation eliminated the steroid binding activity of dhfr550C (1.0 ± 0.6-fold above mock transfection (S.D., n = 3)).

Analysis of Steroid Binding Domain Tertiary Structure by Trypsin Digestion—We previously proposed that the 16-kDa tryptic digest fragment of the rat glucocorticoid receptor, corresponding to amino acids 537–673, binds steroids with high
affinity (31) and specificity (32) because the proper tertiary structure had already been achieved and sequences not involved in the actual binding, but which were required for folding, could now be removed. The corollary to this hypothesis is that the 16-kDa fragment should not be formed after trypsin digestion of mutant receptors that do not bind steroid, presumably reflecting an incorrect folding into non-native tertiary structures.

A convenient method for identifying the 16-kDa fragment has been by affinity labeling with Dex-Mes (31). Dex-Mes covalently labels only one amino acid (Cys-656) in the wild type receptor (47). Furthermore, the mutation of Cys-656 to glycine in the full-length receptor not only increases the affinity of [3H]Dex binding (39) but also blocks the covalent labeling of receptors by Dex-Mes (32). As shown in Fig. 5, covalent labeling by [3H]Dex-Mes of both the intact receptor and the 16-kDa tryptic fragment was prevented by the Cys-656 to glycine mutation. The fact that the 16-kDa fragment contains two other cysteines that are in close proximity to Cys-656 (32, 48) but were not labeled by [3H]Dex-Mes in the Cys-656 Gly mutant further argues that the tertiary structure of the 16-kDa fragment is similar to that of the intact receptor.

The tertiary structure of the steroid binding domain in the above mutant receptors was probed by looking at the ability of trypsin digestion to yield the 16-kDa steroid binding fragment. The presence of the 16-kDa fragment was ascertained by a combination of Western blotting with the antibody aP1, [3H]Dex binding, and [3H]Dex-Mes labeling. As expected, mutant receptors that did not bind steroid (pSVLGR1–766, pSVLGR1–673, dhfr537–690–704C, dhfr537–766, and dhfr537–673) did not produce a 16-kDa fragment on Western blots (data not shown). Likewise, no [3H]Dex binding was observed after trypsin digestion of pSVLGR1–766 (data not shown). In contrast, trypsin digestion of the steroid binding chimeras dhfr494C and dhfr537C gave, in each case, the correct 16-kDa fragment with good [3H]Dex binding and the correct sized product on both Western blots (data not shown) and SDS gels of affinity-labeled material (Fig. 6). Thus, the formation of the 16-kDa fragment after trypsin digestion seems to be an accurate probe of receptor tertiary structure.

dhfr537C is missing the lysine at position 536 that would be cleaved by trypsin to generate the 16-kDa fragment (31). However, the dhfr537C contains two lysines in the linker (sequence = KKDAC) between dhfr and the receptor sequences that appear to substitute for Lys-536 in the generation of 16-kDa fragments. Surprisingly, trypsin digestion of two other steroid binding chimeras, dhfr547C and dhfr550C, yielded none of the 16-kDa-like fragment that was expected from trypsin cleavage at the same linker region lysines and eliminated all [3H]Dex binding and [3H]Dex-Mes labeling (Fig. 6 and data not shown). Thus, the ability of trypsin digestion to yield a 16-kDa-like fragment appears to be a more sensitive probe of receptor tertiary structure.
Receptor Steroid Binding Activity Requires Multiple Domains

Previous attempts to express the isolated steroid binding domain of the glucocorticoid receptor in a form that retained appreciable amounts of steroid binding activity have been unsuccessful. We now show that the solution to this problem requires the presence of two specific receptor domains. The first domain is the full steroid binding domain corresponding to residues 550 and/or 551 and marks the set of positions as the "degron" (51), within the sequence of 537–795 (Figs. 1 and 4).

According to this scheme, the blockage of a specific inactivating region would have to involve nonspecific interactions of 407–536 to account for the equivalent stabilizing effects seen upon fusion of the unrelated proteins β-galactosidase, dihydrofolate reductase, and glutathione S-transferase. Alternatively, a non-specific stabilization might reflect a weak hydrophobic interaction with the chaperones, such as hsp90, that are required for steroid binding activity (52, 53). Regions upstream of amino acid 537 are not normally thought to be associated with hsp90 (54, 55), but it should be noted that amino acids 506–514 of the receptor, which are recognized by the antibody AP64 (56), are no longer accessible to AP64 after hsp90 binding to receptors (13).

The second domain required for steroid binding is, obviously, the steroid binding domain. However, the precise limits of the steroid binding domain have never been defined (22). The cell-free translated fragment 547–795 displayed very low affinity (1/350 of full-length receptors) (29), presumably due to protein instability since the affinity of the same fragment present as a free translated fragment 547–795 displayed very low affinity.

Our stabilizing sequence does not correspond to the highly conserved region of amino acids 587–643 (rat numbering) (22) within the steroid binding domain that has been reported to decrease proteolysis of mouse glucocorticoid receptors in intact cells (49). This segment of 587–643 was clearly insufficient to prevent proteolysis of glucocorticoid receptors in intact cells (49). This segment of 587–643 was clearly insufficient to prevent the degradation of newly synthesized hybrid proteins.

**DISCUSSION**

Previous attempts to express the isolated steroid binding domain of the glucocorticoid receptor in a form that retained appreciable amounts of steroid binding activity have been unsuccessful. We now show that the solution to this problem requires the presence of two specific receptor domains. The first domain is the full steroid binding domain corresponding to residues 550–795; the second is a stabilizing sequence that lies outside of the steroid binding domain.

Our stabilizing sequence does not correspond to the highly conserved region of amino acids 587–643 (rat numbering) (22) within the steroid binding domain that has been reported to decrease proteolysis of mouse glucocorticoid receptors in intact cells (49). This segment of 587–643 was clearly insufficient to stabilize the 537–673 and 537C peptides in our system (Figs. 1–3). Instead, the stabilizing sequence of the rat receptor was found to lie between amino acids 407 and 536 (Fig. 1), which is outside our defined steroid binding domain of 550–795. This positioning was unexpected considering the ability of the functional steroid binding domain to be moved between chimeric proteins in a modular fashion (6, 7). It seems unlikely that there is any unique stabilizing sequence because the effect of the receptor amino acids 407–536 could be mimicked by non-receptor proteins, such as β-galactosidase (Fig. 4A), dihydrofolate reductase (Fig. 4B), and glutathione S-transferase that contained no homologous sequence (data not shown).

The mechanism of this stabilizing sequence is not known. It could involve protein-protein contacts to block the action of an inactivating region (44), such as a PEST sequence (50) or a "degron" (51), within the sequence of 537–795 (Figs. 1 and 4). According to this scheme, the blockage of a specific inactivating region would have to involve nonspecific interactions of 407–536 to account for the equivalent stabilizing effects seen upon fusion of the unrelated proteins β-galactosidase, dihydrofolate reductase, and glutathione S-transferase. Alternatively, a non-specific stabilization might reflect a weak hydrophobic interaction with the chaperones, such as hsp90, that are required for steroid binding activity (52, 53). Regions upstream of amino acid 537 are not normally thought to be associated with hsp90 (54, 55), but it should be noted that amino acids 506–514 of the receptor, which are recognized by the antibody AP64 (56), are no longer accessible to AP64 after hsp90 binding to receptors (13).

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766 indicates that it must be somewhere between 767 and 795 (Fig. 4B). A 30-fold decrease in affinity was observed after removing the five carboxyl-terminal residues of 407C to give 407–790 (29) while all binding and biological activity disappeared with the deletion of the terminal 14 amino acids (58). Furthermore, internal deletions (dhfr537Δ690–704C and dhfr537Δ616–695C) eliminated Dex binding (Fig. 4) while the removal of the two amino acids 780 and 781 caused a 4-fold decrease in affinity (59). Thus, deletions throughout the carboxy-terminal half of the receptor can be detrimental to the steroid binding activity without affecting the stability of the final protein. We therefore conclude that the minimum sequence of the steroid binding domain with wild type affinity in a newly synthesized protein can be defined as extending from 550 (or 551) to 795.

The final issue is whether the entire steroid binding domain is needed for steroid binding activity or only for the folding into the tertiary structure. The fact that the affinity of the 16-kDa glucocorticoid receptor fragment of 537–673 (31, 32) appears to be ∼20-fold lower than that of the full-length receptor (31) argues that proteolytically released sequences between 674 and 795 do augment, but are not required for, a significant binding affinity. However, this is in contrast with the absence of binding in proteins that are translated with the absence of the carboxyl-terminal half of the receptor (64). How- ever, deletion of the two amino acids 780 and 781 caused a 4-fold decrease in affinity (59). Thus, deletions throughout the carboxy-terminal half of the receptor can be detrimental to the steroid binding activity without affecting the stability of the final protein. We therefore conclude that the minimum sequence of the steroid binding domain with wild type affinity in a newly synthesized protein can be defined as extending from 550 (or 551) to 795.

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Modular Structure of Glucocorticoid Receptor Domains Is Not Equivalent to Functional Independence: STABILITY AND ACTIVITY OF THE STEROID BINDING DOMAIN ARE CONTROLLED BY SEQUENCES IN SEPARATE DOMAINS

Min Xu, Pradip K. Chakraborti, Michael J. Garabedian, Keith R. Yamamoto and S. Stoney Simons, Jr.


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