IMPLICATIONS FOR PATHOGENIC GENE MUTATIONS*

Structural Evidence for Ligand Specificity in the Binding Domain of the Human Androgen Receptor

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The crystal structures of the human androgen receptor (hAR) and human progesterone receptor ligand-binding domains in complex with the same ligand metribolone (R1881) have been determined. Both three-dimensional structures show the typical nuclear receptor fold. The change of two residues in the ligand-binding pocket between the human progesterone receptor and hAR is most likely the source for the specificity of R1881 to the hAR. The structural implications of the 14 known mutations in the ligand-binding pocket of the hAR ligand-binding domains associated with either prostate cancer or the partial or complete androgen receptor insensitivity syndrome were analyzed. The effects of most of these mutants could be explained on the basis of the crystal structure.

Androgen (AR)1 and progesterone receptors (PR) are members of the superfamily of nuclear receptors that includes the steroid receptors, among others, as well as the vitamin D, thyroid, retinoic acid receptors, and the so-called orphan receptors. In addition, AR and PR are members of a group of four closely related steroid receptors including the mineralocorticoid receptor and the glucocorticoid receptor recognizing the same hormone response element. In general, steroid receptors are comprised of five to six domains and act as ligand-activated transcription factors that control the expression of specific genes. To date, no experimentally determined three-dimensional structure is available for a complete receptor. During the past few years, x-ray structures have been published for two of the domains, the DNA-binding domain as well as for a number of ligand-binding domains (LBD) including LBD-ligand complexes of the estrogen receptor α and β, the PR, the vitamin D receptor, the retinoic acid receptors (X,RXR; acid, RAR), the thyroid hormone receptor, and the peroxisome proliferator-activated receptors (1–13). Despite the low sequence homology of as low as 20% between the LBDs of different nuclear receptor families, all these proteins share a similar fold. They are comprised of up to 12 helices and a small β-sheet arranged in a so-called α-helical sandwich, a kind of fold that up to now has only been observed for the LBDs of nuclear receptors. Depending on the nature of the bound ligand, agonist, or antagonist, the carboxyl-terminal helix H12 is found in either one of two orientations. In the agonist-bound conformation, helix H12 serves as a “lid” to close the ligand-binding pocket (LBP), whereas in the antagonist-bound conformation helix H12 is positioned in a different orientation thus opening the entrance to the LBP.

Androgens and their receptors play an important role in male physiology and pathology. AR binds the male sex steroids, dihydrotestosterone (DHT) and testosterone (14), and regulates genes for male differentiation and development. Therefore, mutations in the androgen receptor gene may lead to several disease states like prostate cancer (PC) or the androgen insensitivity syndrome (AIS). In males, defects in the AR gene result in a spectrum of developmental abnormalities ranging from a phenotypic female to varying degrees of incomplete genital phenotype. These mutations are well documented in the Androgen Receptor Gene Mutations Data Base of the Lady Davis Institute for Medical Research (15).

In this study we present the crystal structure of the human hAR LBD in complex with the ligand metribolone (R1881) in comparison with the crystal structure of the human hPR LBD in complex with the same ligand. AR and PR belong to the same steroid receptor subfamily and share a 54% LBD sequence identity (Fig. 1). A number of different ligands bind with similar binding affinities to both receptors (14). The x-ray structure analysis of both receptors in complex with the same ligand (R1881, Fig. 2) should increase our understanding of ligand specificities. Furthermore, the analysis of published mutant data on the basis of the hAR LBD crystal structure might give us a deeper insight into AR-related diseases.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—The cDNAs coding for hAR and hPR were obtained from the groups of A. Cato (Forschungszentrum Karlsruhe, Germany) and P. Chambon (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France), respectively. The LBDs of...
the hAR (amino acid residues 663–919) and the hPR (amino acid residues 677–933) were amplified by the polymerase chain reaction technology using the appropriate primers and cloned into a pGEX-KG vector (16). The resulting fusion proteins consisted of a glutathione S-transferase, containing a carboxyl-terminal threonin cleavage site, optimized by a glycine-rich “kinker” region followed by the corresponding LBD. The constructs were then transformed into the Escherichia coli strain BL21 (DE3).

**Protein Expression and Purification**—Fermentation using the corresponding rec E. coli strains expressing hAR LBD was carried out in 2X YT medium in the presence of ampicillin (200 μg/ml) supplemented with 10 μM R1881. Expression was induced with 30 μM isopropyl-β-D-thiogalactoside, and the fermentation (10 liters) was continued at 15 °C for 14–16 h. Cells were harvested by centrifugation and disrupted twice in a continuous high pressure homogenizer (9000PSI) in a buffer containing 50 mM Tris-HCl, pH 8, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 10 μM R1881, 10 mM DTT, and the fusion protein was eluted using the same buffer supplemented with 15 mM reduced glutathione. The eluate was diluted with 100 mM HEPES, pH 7.2, 150 mM NaCl, 0.5 mM EDTA, 10% glycerol, 10 μM R1881, 1 mM DTT, and 0.1% n-octyl-β-glucoside up to a fused protein concentration of 1 mg/ml. A thrombin cleavage (2 NIH units/mg fusion protein) was performed overnight at 4 °C. The protein mixture was further diluted 5-fold with 10 mM HEPES, pH 7.2, 10% glycerol, 10 mM R1881, 10 mM DTT, and 0.1% n-octyl-β-glucoside and loaded onto a Fractogel SO 3 column and eluted with a gradient of 50–500 mM NaCl in a 10 mM HEPES buffer, pH 7.2, 10% glycerol supplemented with 10 mM R1881, 10 mM DTT, and 0.1% n-octyl-β-glucoside. Approximately 2.4 mg of purified hAR LBD were recovered from 1 liter of E. coli cell cultures. Protein concentration was determined with Bio-Rad Protein Assay. Fermentation and purification of the hPR LBD was performed identically, but a HEPES pH 7.2 buffer was used from the beginning.

**Comparative Modeling**—A model of the hAR LBD was built based on the coordinates of the hPR-LBD-progesterone complex (molecule A) (9).

Amino acid substitutions were made based on the sequence alignment of the hAR LBD and hPR LBD using the appropriate primers and cloned into a pGEX-KG vector. Comparative modeling of the hAR-LBD was performed using the coordinates of the hPR-LBD-progesterone complex (molecule A) (9). Mutations presently known for PAIS/MAIS and prostate cancer were introduced in a continuous high pressure homogenizer (9000PSI) in a buffer containing 50 mM Tris-HCl, pH 8, 150 mM NaCl, 10% glycerol, and 10 mM DTT, and the fusion protein was eluted using the same buffer supplemented with 15 mM reduced glutathione. The eluate was diluted with 100 mM HEPES, pH 7.2, 150 mM NaCl, 0.5 mM EDTA, 10% glycerol, 10 μM R1881, 1 mM DTT, and 0.1% n-octyl-β-glucoside up to a fused protein concentration of 1 mg/ml. A thrombin cleavage (2 NIH units/mg fusion protein) was performed overnight at 4 °C. The protein mixture was further diluted 5-fold with 10 mM HEPES, pH 7.2, 10% glycerol, 10 mM R1881, 10 mM DTT, and 0.1% n-octyl-β-glucoside and loaded onto a Fractogel SO 3 column and eluted with a gradient of 50–500 mM NaCl in a 10 mM HEPES buffer, pH 7.2, 10% glycerol supplemented with 10 mM R1881, 10 mM DTT, and 0.1% n-octyl-β-glucoside. Approximately 2.4 mg of purified hAR LBD were recovered from 1 liter of E. coli cell cultures. Protein concentration was determined with Bio-Rad Protein Assay. Fermentation and purification of the hPR LBD was performed identically, but a HEPES pH 7.2 buffer was used from the beginning.

**Crystallization, Data Collection, and Structure Determination**—Both proteins were dialyzed after purification with buffer containing 50 mM HEPES pH 7.2 for hAR LBD or 10 mM HEPES pH 7.2 for hPR LBD, respectively, 10% glycerol, 10 mM DTT, 0.1% n-octyl-β-glucoside, 10 mM R1881, and 150 mM Li2SO4 and were concentrated up to 3 mg/ml for the hAR LBD-R1881 and up to 4.4 mg/ml for the hAR LBD-R1881, respectively. Vapor diffusion method was used at 20 °C for the hAR LBD complex and at 4 °C for hPR LBD complex. Crystallization experiments had to be set up immediately after concentration. For the hAR LBD-R1881 complex, the reservoir solution contained 0.4 mM Na2HPO4·2H2O, 0.4 mM K2HPO4, 0.1 mM Tris-HCl, pH 8.5, 0.1 mM (NH4)2HPO4, and 5% polyethylene glycol 200. Drops were composed of equal volumes of protein and reservoir solution and were set up using the sitting drop method. Within 2 days crystals appeared and grew to typical dimensions of 50 × 50 × 50 μm3 surrounded by precipitate. Crystals were flash-frozen using a cryoprotecting solution of 60% polyethylene glycol 400 in 0.1 mM Tris-HCl, pH 8.5. Data were collected from one crystal at the European Synchrotron Radiation Facility (Grenoble, France) at beam line ID14-EH4 to a resolution of 2.4 Å. For the hPR LBD-R1881 complex, the reservoir solution contained 10% isopropyl alcohol, and 100 mM sodium citrate in 50 mM HEPES pH 7.5. The hanging drop method was used with a 2:1 ratio of protein and reservoir solution. First crystals appeared after 5 weeks and grew to a size of approximately 160 × 120 × 40 μm3. One crystal was flash frozen using a cryo-protecting solution containing 30% glycerol. Data were collected...
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### Table I

| hAR LBD-R1881 | hPR LBD-R1881 |
|---------------|--------------|
| **Space group** | P2₁,2₁,2₁ | P₂₁ |
| **Unit cell** | a, 54.28; b, 66.14; c, 71.72 Å | a, 58.40; b, 65.01; c, 71.18; Å b, 95.7° |
| **Wavelength (Å)** | 0.9324 | 0.9537 |
| **Resolution range (Å)** | 24.4–2.40 | 12.47–2.80 |
| **N_observations** | 37,443 | 67,655 |
| **N_reflections** | 10,638 | 8,875 |
| **% completeness** | 99.8 (99.9) | 67.0 (68.8) |
| **Redundancy** | 3.5 | 7.6 |
| **Rmerge[a,b]** | 0.078 (0.351) | 0.048 (0.151) |
| **I/σ(I)** | 12.0 | 15.2 |
| **Estimated B_overall** | 49.4 | 45.2 |

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### Table II

**Final refinement statistics for hAR LBD and hPR LBD complexed with R1881**

| hAR LBD-R1881 | hPR LBD-R1881 |
|---------------|--------------|
| **Final R-factor (%)** | 21.0 | 21.7 |
| **Final R-free (%)** | 29.7 | 34.3 |
| **Number of non-hydrogen protein atoms** | 2044 | 4027 |
| **Non-hydrogen protein atoms missing** | 22 | 32 |
| **Non-hydrogen ligand atoms** | 21 | 42 |
| **Solvent molecules** | 26 | 1 |
| **Estimated overall r.m.s. coordinate error (Å)** | 0.47 | 0.53 |
| **Model r.m.s. deviations from ideality** | 0.01/1.7 | 0.0/4.4 |
| **Average B values (Å²)** | 48.3/52.1 | 33.2/28.7 |
| **Ligand/solvent** | 45.2/49.2 | 10.2/3.6 |

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### RESULTS AND DISCUSSION

**Protein Expression and Purification**—Glutathione S-transferase fusion proteins can be expressed to very high levels in the E. coli strain BL21 (DE3) (16). This system was used successfully for the production of the hPR LBD (9) and hAR LBD. The expression of soluble fusion proteins strongly depends on the presence of ligand in the cells during fermentation (data not shown). During cell disruption, purification, and concentration, any protein oxidation was avoided by purging all buffers with nitrogen and by using DTT as an antioxidant. Fusion proteins were purified by the use of glutathione-Sepharose and subsequently cleaved with thrombin. Cation exchange chromatography yielded purified LBDs. Concentration was performed with a nitrogen pressure diafiltration system.

**Comparison of Model and Crystal hAR LBD Structures**—The model and the crystal structures of the hAR LBD are very similar with respect to their overall structure, the LBD and the ligand orientation. The root mean square (r.m.s.) deviation between 149 equivalent Cα atoms in helices between the model and crystal structure of the hAR LBD is 1.09 Å. It is comparable to the r.m.s. deviation of 0.84 Å between the crystal structures of the hAR LBD and the hPR LBD-progesterone complex. The most striking difference between the model and the crystal structure was found in helix H6, where an α-helix was identified in the crystal structure in this region, whereas in the hPR LBD-progesterone complex (molecule A) no α-helix is observed.

The ligand orientation in both the hAR LBD-R1881 model and crystal structure is very similar, and the same hydrogen bonds are found.
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Structure Analysis and Comparison of the hAR LBD-R1881 and the hPR LBD-R1881 Complexes—Both crystal structures were analyzed with PROCHECK (35), and their stereochemical quality parameters were within their respective confidence intervals. In the Ramachandran (36) ϕ,ψ plot, 87.7% for the hAR LBD-R1881 and 85% for the hPR LBD-R1881 structures, respectively, lie within the most favored regions. For the hAR LBD-R1881 complex no residue is outside the normally allowed regions, whereas in the hPR LBD-R1881 complex two residues are located in disallowed regions (Asn717 and Ser793 in molecule A), and three residues (Thr736 in molecule A and Asn705 and Ser793 in molecule B) are located in generously allowed regions. These residues are not involved in ligand binding and are located in loop regions that are most probably not involved in ligand recognition. In the hAR LBD-R1881 structure there is only one close contact (2.6 Å) between Met895 and Ala896 carbonyl oxygens. In the hPR LBD-R1881 structure some close contacts were observed, but due to the resolution and completeness of the data this is not surprising. The overall fold of the hAR and hPR LBD-R1881 structures is very similar and also with that of hPR LBD complexed with progesterone (9). On the basis of the secondary structure calculated with PROCHECK (35) according to Kabsch and Sander (37), the hAR LBD-R1881 structure contains 9 α-helices, two 3₁₀ helices, and four short β-strands associated in two anti-parallel β-sheets. The helices are arranged in the typical “helical sandwich” pattern as in hPR LBD-progesterone complex (9), and helices H4, H5, H10, and H11 are contiguous. In hAR LBD-R1881 helix H12 seems to be split into two shorter helical segments, with nine and five residues each, respectively. This observation was not seen in the hPR LBD-R1881 structure, although a bending of helix H12 is also seen here. Fig. 1 shows a comparison between the amino acid sequences of hAR LBD and hPR LBD. A ribbon diagram of the hAR LBD-R1881 structure is shown in Fig. 3 along with a superimposed Cα trace of the hAR LBD-R1881 and hPR LBD-R1881 molecules.

The crystal structure coordinates of hAR LBD-R1881 were superimposed with those of hPR LBD-R1881 (molecule A) and hPR LBD-progesterone (molecule A) using LSQKAB (38). For the superposition the main chain atoms except three amino-terminal (Cys669-Pro71) and one carboxyl-terminal (Thr819) residues were used. The r.m.s. coordinate deviations were 1.16 and 1.22 Å, respectively, again an indication of the similarity of the overall fold of these three molecules. In hAR LBD-R1881, Cys669 and Cys844 are very close, and a disulfide bridge between them was modeled, based on the electron density. However there is no supporting biochemical evidence so far, and it should be noted that the temperature factors of both cysteine residues and the adjacent residues are very high. A cis peptide bond is found at position Pro845 in hAR LBD-R1881.

Comparative Modeling—The model of the hAR LBD which is based on the hPR LBD-progesterone complex is very similar to the hAR LBD crystal structure with respect to the overall fold and ligand orientation but shows a stronger bending of helices H10 and H11. Our model structure differs from other published models (39) with respect to the secondary structure alignment. The secondary structure assignment by Yong et al. (39) as compared with the hAR LBD crystal structure is similar between helices H3 and H10 but differs most for helices H11, H12, and the additional helix at the carboxyl-terminal end.

Ligand-binding Pocket Interactions—There are a total of 18 amino acid residues in hAR LBD and hPR LBD that interact with the bound ligand (either R1881 or progesterone). These residues are highlighted in Fig. 1 and included in Fig. 4. Most of these residues are hydrophobic and interact mainly with the steroid scaffold, whereas a few are polar and may form hydrogen bonds to the polar atoms in the ligand.

The hydrogen-bonding scheme to O-3 of R1881 and progesterone is similar but not identical, as shown in Fig. 4. In the hAR LBD-R1881 crystal structure, this oxygen atom forms a hydrogen bond to Arg752 (Arg766 in hPR LBD), but in contrast with the hPR LBD-progesterone complex the distance of 3.9 Å to Gln711 (Gln725 in hPR LBD) does not allow a hydrogen bond. There is a water molecule near O-3 that is hydrogen-bonded to three other residues with a nearly triangular geometry (Arg752 Nε1, Met745 O, and Gln711 Oε1 in hAR LBD; Arg766 Nε1, Met759 O, and Gln725 Oε1 in hPR LBD-progesterone). Two of these residues are acceptors; therefore, a third acceptor atom (O-3 in either progesterone or R1881) in a direction perpendicular to the plane of the triangle is unlikely, also due to unfavorable geometry. The water molecule hydrogen-bonded to Gln711 Nε2 in hAR LBD (Gln725 in hPR LBD) has hydrogen bonds to two other residues (Val688 O and Phe777 O in hAR LBD, Ile699 O and Phe777 O in hPR LBD), and in hAR LBD it is hydrogen-bonded to a further water molecule, the overall hydrogen bond geometry being distorted tetrahedral. In the hPR LBD-R1881 structure, the ligands in molecules A and B possess slightly different hydrogen bond patterns. In molecule A, O-3 of R1881 forms two hydrogen bonds (3.2 Å to Gln725 Nε2 and 2.9 Å to Arg766 Nε2). One water molecule was located in the Fe−Fe electron density.
with the same tetrahedral geometry as observed in the hAR LBD-R1881 structure. In molecule B, the ligand is in a slightly different position, and the hydrogen bond pattern differs from that observed in molecule A. The O-3 of R1881 forms again one hydrogen bond to Arg766 N\textsubscript{H\textsubscript{2}} with a distance of 2.9 Å, whereas the distance to Gln725 N\textsubscript{e} is now 3.7 Å, outside the acceptable range for a hydrogen bond.

The 17β-hydroxyl group of R1881 forms different hydrogen bonds, when bound to hAR LBD or hPR LBD (Fig. 4). In hAR LBD, the 17β-hydroxy group is hydrogen-bonded to Asn705 O\textsubscript{D\textsubscript{1}} (2.8 Å) and Thr 877 O\textsubscript{G} (2.9 Å). The same pattern is observed in molecule B of the hPR LBD-R1881 complex where the 17β-hydroxyl group of R1881 also forms a strong interaction to Asn719 O\textsubscript{D\textsubscript{1}} (2.8 Å), whereas in molecule A the corresponding distance of 3.5 Å is only in the range of a weak interaction. In contrast to the hAR LBD, in both hPR LBD monomers Cys891 (Thr877 in hAR LBD) shows only a weak interaction with the 17β-hydroxyl group of R1881 (3.7 Å in molecule A and 4.0 Å in molecule B). However, the relative orientation of the Cys891 side chain with regard to the hydroxyl group does suggest that this interaction is relevant to the binding of the ligand.

**Structural Basis for Ligand Specificity in hAR LBD**—The ligand R1881 binds with a relative binding affinity of 290 to the wild-type hAR as compared with a value of 180 for DHT and

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**Fig. 4. Stereo diagrams showing the interactions between the bound ligand and the protein chain in hAR LBD-R1881 (a), hPR LBD-R1881 (molecule A) (b), and hPR LBD-progesterone (c).** Residues included are either hydrogen-bonded or have van der Waals contacts (cutoff distance 4.0 Å) with any of the ligands. Residues Val\textsuperscript{685} and Tyr\textsuperscript{763} in hAR LBD and corresponding residues Ile\textsuperscript{699} and Tyr\textsuperscript{777} in hPR LBD are hydrogen-bonded to other residues or water molecules near the ligand-binding site and are also included. Bound ligand is colored black; conserved residues are colored gray; different residues in hAR LBD and hPR LBD are colored red. Residue labels with an asterisk denote residues that do not have van der Waals contacts within the specified cutoff distance with the ligand. Hydrogen bond distances for the hPR LBD-progesterone complex were calculated from the Protein Data Bank deposited coordinates of molecule A. Figures were produced with MOLSCRIPT (52).
100 for testosterone, respectively (14). As for the wild-type hPR, the relative binding affinity of R1881 is 190 with respect to progesterone (relative binding affinity = 100). Overall, R1881 shows comparable good binding affinities to both receptors, which is also reflected in the orientation of the ligand in the LBPs of the hAR LBD and the hPR LBD (Fig. 4). Thr894 in hAR LBD which is also reflected in the orientation of the ligand in R1881 shows comparable good binding affinities to both receptors. The 17β-acetyl group of progesterone. Not only is there an extra oxygen but the directed decrease in pocket volume caused by the mutation of Thr894 to Leu880 will very likely inhibit the binding of other bulkier ligands such as progesterone. As previously noted (9), there are no strong hydrogen-bonded interactions between the O-20 carbonyl oxygen atom of progesterone and the protein in hPR LBD indicating that the recognition of this group is probably made only through hydrophobic and steric interactions. The hPR LBD can bind R1881 as well as progesterone, and in the crystal structure, the hPR LBD molecule appears to exhibit two different binding modes for R1881. In one similar to that of progesterone (O-3 with two hydrogen bonds to the protein and the 17β function weakly interacting with the protein) and one similar to that of hAR LBD (O-3 with only one hydrogen bond to the protein and the 17β function also hydrogen-bonded to the protein). However, these binding modes do not seem to imply significant changes in ligand position and orientation within the LBP.

Pathogenic Gene Mutations in the hAR LBD Gene—Constitutional mutations in the AR gene can cause the AIS by impairing androgen-dependent male sexual differentiation to various degrees. Complete AIS (CAIS) leads to an unequivocally external female phenotype; PAIS (partial or incomplete AIS) comprises a wide spectrum of clinical phenotypes; and MAIS, a mild form of AIS, is connected to forms of undervirilization (40). About 50% of the mutated residues reported in the hAR LBD are found to be involved in PC and in AIS (15). These mutations are distributed all over the LBD but seem to be accumulated in helices H4 and H5, a region involved in ligand binding. Comparison of the solvent accessibility of these mutated residues revealed that a nearly even distribution is found between buried, medium, or fully accessible residues. Table III lists all those mutations in or near the AR LBP which are known to be involved in AIS and prostate cancer, their location with respect to secondary structural elements, as well as the potential effect of the mutations. The location of these mutations in the three-dimensional structure of the hAR LBD-R1881 is shown in Fig. 5, and it can be seen that the mutations involved in PC cluster mainly near the R1881 17β hydroxyl group, whereas those involved in AIS are arranged mainly around the other parts of the ligand. This is in contrast to mutations involved in PC/MAIS. A structural effect can be associated with the substitution. The location of these mutations in the three-dimensional structure of the hAR LBD-R1881 is shown in Fig. 5, and it can be seen that the mutations involved in PC cluster mainly near the R1881 17β hydroxyl group, whereas those involved in AIS are arranged mainly around the other parts of the ligand. This is in contrast to mutations involved in PC/MAIS. A structural effect can be associated with the substitution.

**Table III**

| Mutation in AR aar in PR Location in LBD Vicinity of ligand Comment |
|-------------------------------------------|---------------------|------------------|-----------------|
| Prostate cancer Leu703–His 715 H3 D | His: too close contacts to Phe576, hydrophobic environment for His; Met745, Phe767, Leu880 Ile: either too close to Arg752 or Phe764 | Leu side chain too close to Leu880 in the 2 most often observed side chain conformations for Leu |
| Met749–Ile 763 H5 A | No H-bond partner for ligand 17β-OH Hydrophobic environment for Gly: Leu703, Met740, Phe767 |
| Thr877–Ala 891 H11 D | 2 energetically favorable conformations for Ser similar to the O" or C' position of Thr | |
| Thr877–Ser 891 H11 D | | |
| Leu880–Gln 894 H11 D | | |
| Phe893–Leu 905 Loop H11/H12 D | | |
| CAIS Asn705–Ser 719 H3 D | Ser: too small for H-bond partner to ligand 17β-OH | |
| Leu707–Arg 721 H3 A | Arg: too elongated for this area | |
| Met742–Val 763 H5 A | Val: branched aar, C" too close to ligand | |
| PAIS/MAIS Gly708–Ala 722 H3 C | No hindrance for Ala | |
| Gly708–Val 722 H3 C | Val: too close to Trp741, Met805, ligand | |
| Met742–Val 756 H5 C/B/C | Val fits into LBP but environment is less tightly packed, the LBP is enlarged | |
| Met742–Ile 756 H5 B/C | Ile: fits into LBP but environment is less tightly packed, the LBP is enlarged | |
| Met745–Thr 759 H5 A | Thr: too close to ligand | Met: too close to Met743, Leu877, ligand |
| Val762–Met 760 H5 B | Gln: too small for H-bond partner to ligand O-3 Ser: no stacking with A-ring of ligand possible | |
| Arg762–Gln 766 H5 A | | |
| Phe764–Ser 778 S1 A | Ser: no stacking with A-ring of ligand possible | |
| Met787–Val 801 H7 B | No hindrance for Val, but fewer contacts to Val746, Leu877, and ligand | |

For convenience, the equivalent positions of the amino acid residues (aar) in the hPR LBD are given. Bold numbers indicate available mutant data in the PR. All mutations are taken from the androgen receptor gene mutations data base (Ref. 15 and references therein).
close (4.3 Å in molecule A and 4.4 Å in molecule B) to O-20 of progesterone. However, bacterial extracts of a mutated hPR LBD (C891S or C891V) showed a large decrease in relative binding affinity for progesterone, and the purified mutated hPR LBD was completely inactive in binding assays (17).

Mutations in the LBP Observed in CAIS—The three mutations in the hAR LBP described for CAIS are substitutions that considerably change the size of the respective amino acid side chains, N705S (40, 42), L707R (43), and M749V (40, 44). This change in size alters the LBP such that the local structure and interactions to the ligand are disturbed.

In the AR LBD and PR LBD crystal structures, Asn705 or Asn719, respectively, is one of the hydrogen bond partners to the ligand R1881 but not to progesterone. If this residue is substituted to Val in hPR LBD, only a moderate effect was observed on the binding activity of progesterone, considering the Kd and half-life values (17). In the crystal structure of the hPR LBD progesterone complex, Asn719 is involved in the stabilization of the loop between H11 and H12, via hydrogen bond between its N atom and Glu744 O. In the hAR LBD, an identical stabilization is found. An N705S mutation, observed in a patient suffering from CAIS, would have a 2-fold effect, destabilization of the structure and loss of a hydrogen bond partner for the ligand.

In the described hAR mutant L707R, the structure integrity disturbance is also reflected in the binding constants. Considering a van der Waals cutoff distance of 4.0 Å, the side chain of Leu707 makes close contacts with the A-ring of R1881 as well as five residues in the protein chain as follows: Val685, Ala687, Glu712, Phe764, and Leu768. These residues are located in a loop region between H2 and H3, within H-3, and in strands S1 and S2. Clearly, such a variation in the size of the side chain would have a large impact, not only in the LBP but in disrupting the overall protein fold itself. The mutated receptor shows undetectable binding affinity to the ligand R1881 as obtained by Scatchard plot analysis, and no transcriptional activity is found (43).

Mutations in the LBP Observed with PAIS/MAIS—Seven described mutations in the hAR LBP are associated with PAIS/MAIS, and multiple substitutions were observed for amino acids at position 708 (45) and 742 (46). In the hAR LBD crystal structure, a substitution of Gly708 to alanine should be tolerated, whereas a valine at this position would interfere with ligand binding. The closest distance of the Cβ atom of an alanine residue to the ligand would be 3.0 Å; however, the Cγ atoms of a valine would be too close to the ligand atoms (1.5 Å).

In all steroid receptors, the steroid is stabilized by a hydrogen bond between the A-ring of the ligand and an arginine (Arg752 in hAR). A smaller amino acid residue at this position (mutation to glutamine in hAR) should have a dramatic impact on ligand binding as the stabilization of the A-ring would be severely hampered due to the lack of an electrostatic interaction (47, 48). A similar effect has been reported for the hPR receptor where a mutation (R766H) resulted in a low or even non-detectable binding affinity. The side chain of histidine is too small to serve as a hydrogen bond partner to the O-3 atom in progesterone (17).

In the hAR mutation F764S, R1881 shows a similar binding affinity as the wild-type receptor, but a rapid ligand dissociation is observed (49). In the crystal structure, Phe764 is involved in the stabilization of the A-ring position. A smaller amino acid like serine would allow binding of the ligand but very likely would not contribute to the tight binding of R1881.

Mutations M742V or M742I both dramatically reduce the binding affinity of R1881 (46). Although Ile and Val fit into the LBP, the changed environment is less tightly packed, and the LBP is enlarged, thus affecting the binding of the ligand.

However, not all mutations can be related to a disturbance of the structure. In case of the M787V mutation in the hAR LBD, it was found by Scatchard analysis that R1881 and DHT binding were undetectable or strongly reduced (50). The lack of androgen binding was thought to be the cause for AIS. In the crystal structure, a methionine to valine substitution could be tolerated. The lack of binding affinity found for R1881 may account for a destabilization in the LBP as the Met779 side chain is in van der Waals contact with other amino acids like Val760 and Leu887 as well as ligand atoms.

Of the 20 amino acid residues involved in ligand interaction as discussed above, only 6 have no mutations been reported so far. In addition, for many of the published mutated receptors no ligand binding data are available. However, the effects of most of the characterized mutants could be explained on the basis of the crystal structure.

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