Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Interaction between the androgen receptor and a segment of its corepressor SHP

Natalia Jouravel,[a] Elena Sablin,[a] Leggy A. Arnold,[b] R. Kiplin Guy[b] and Robert J. Fletterick[a]*

[a]Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA, and [b]Department of Chemical Biology and Therapeutics, St Jude Children's Research Hospital, Memphis, TN 38105, USA

Correspondence e-mail: flett@msg.ucsf.edu

Received 14 June 2007
Accepted 17 September 2007

PDB Reference: AR LBD with SHP peptide NR Box 2, 2z4j, r2z4jsf.

The mechanisms of functional repression of the androgen receptor (AR) are crucial for the regulation of genes involved in physiological development as well as for the progression of prostate cancer. To date, only two *in vivo* inhibitors of AR-mediated transcription have been identified: DAX-1 and SHP (small heterodimer partner). SHP is a regulatory nuclear receptor (NR) that lacks DNA-binding and activation domains. Using X-ray crystallography, the interaction between peptide segments of the SHP repressor containing L*xx*LL-like motifs and the ligand-binding domain of AR have been investigated. Under the crystallization conditions used, it was found that of the three NR Boxes present in the SHP protein sequence, only NR Box 2 (LKKIL motif) formed a complex with AR. Determination of the crystal structure revealed that ten amino acids of the SHP peptide (14-mer) are ordered through interactions with AR. Two side chains make unique interactions that were not reported for other AR–peptide complexes. The NR Box 2 of SHP binds to an adaptable hydrophobic groove on the surface of AR in a fashion observed for other NR–L*xx*LL-like complexes. Comparisons of AR structures bound to coactivator peptides and the SHP peptide revealed structural similarity of their binding sites, suggesting that transcriptional AR activity may be inhibited by SHP by competing with AR coactivators.

1. Introduction

The androgen receptor (AR) is a member of the nuclear receptor (NR) family of transcriptional factors that are broadly engaged in normal physiological development and metabolism and play crucial roles in a number of diseases, including cancer. The inhibition of ligand-activated AR is a nonsurgical approach for the treatment of prostate cancer (Kolvenbag *et al.*, 2001; Chen *et al.*, 2004); therefore, the structural principles underlying androgen-receptor inactivation are being intensively studied. Similar to other NR proteins, AR has an N-terminal ligand-independent transcription domain (AF-1), a central DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD), with its ligand-dependent transactivation region being termed AF-2. The AF-2 site is a surface-hydrophobic cavity that is presumed to form owing to agonist androgen-promoted conformational changes of the C-terminal helix 12 (Durand *et al.*, 1994). The agonist-stabilized conformation of AR recruits regulatory proteins necessary to regulate transcriptional machinery and gene expression.

Several families of regulatory coactivator proteins have been identified, including SRC-1, a p160-family member, the CREB-binding protein (CBP)/p300 and the TRAP220–DRIP205–PBP complex (Martin *et al.*, 2005). These coactivators are thought to bind to the AF-2 region of nuclear receptors utilizing an L*xx*LL signature motif (Heery *et al.*, 1997). As for regulatory repressor proteins, only two known direct repressors of AR function have been discovered to date, DAX-1 (NR0B1) and SHP (NR0B2), which are atypical orphan nuclear receptors that lack DBD domains. SHP and DAX-1 display strong sequence similarity in their putative LBD (Seol *et al.*, 1996; Zanaria *et al.*, 1994), but the repression mechanisms of SHP and DAX-1 are thought to differ. DAX-1 has been shown to decrease the nuclear import of cytoplasmic AR or to increase the active export of nuclear AR, thus sequestering it in the cytoplasm (Holter *et al.*, 2002).

The binding motif presented to AR by DAX-1 is structurally and functionally unclear and may lie within the three-dimensional architecture of the receptor (Holter *et al.*, 2002). The suggested mode of AR repression by SHP is that inside the nucleus SHP may compete with coactivators for overlapping but distinct binding surfaces on the receptor (Gobinet *et al.*, 2001). To date, no structural information is available for these proteins or for their interactions with the androgen receptor. The mechanism by which the agonist-bound androgen receptor is repressed is not understood. We have studied the structural aspects of a potential SHP interaction with AR LBD *via* three L*xx*LL-like signature-binding motifs present in SHP.

2. Methods

2.1. Protein preparation

cDNA encoding AR LBD (residues 662–919) with an N-terminal His_6 tag was cloned into pACYCDuet-1 vector (Novagen) and expressed in *Escherichia coli* BL21(DE3) cells. Protein expression was induced with 1 m*M* IPTG and maintained for 16 h at 288 K in the presence of 50 µ*M* DHT. The pellet derived from 1 l bacterial culture typically yielded 3 mg pure protein after a two-step purification procedure consisting of affinity purification on Ni–NTA agarose (Qiagen) followed by size-exclusion chromatography on Superdex HiLoad 16/60 75 (Amersham Biosciences). The final protein at a concentration of 2–3 mg ml^{-1} was kept in a storage buffer composed of 10 m*M* Tris pH 8, 10% glycerol, 150 m*M* NaCl and 10 m*M* TCEP.

Custom-made peptides (Biosynthesis Inc) encompassing the SHP NR Box 1 (^{21}LYALL), Box 2 (^{118}LKKIL) and Box 3 (^{214}LTRVL) motifs were mixed at a fivefold molar excess with the protein and incubated for 2 h at 277 K; the solution was centrifuged at 20 000*g* prior to crystallization.

2.2. Crystallization, data collection and structure determination

Cocrystals of human SHP Box 2 (^{114}VPSILKKILLEEPS) with AR LBD were grown at 293 K in sitting drops using the vapour-diffusion method with a precipitant solution containing 0.1 *M* bis-Tris propane pH 7.0 and 1.2 *M* potassium sodium tartrate tetrahydrate. Crystals grew in 10 d and were harvested and flash-frozen in liquid nitrogen. Diffraction data were collected to a resolution of 2.6 Å on beamline 8.3.1 at the Advanced Light Source (Berkeley, CA, USA). X-ray diffraction data were integrated using the program *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997). The structure was determined by molecular replacement using the *CNS* program package with the structure of AR LBD (PDB code 1t63) as a search model. The peptide structure was built manually using *QUANTA* (Accelrys, Inc.). Using difference Fourier maps, the structure of the AR–peptide complex was completed with iterations of manual rebuilding in *QUANTA* and refinement in *CNS*. Table 1 summarizes the data-collection and structure-refinement statistics. The final model was refined to 2.6 Å resolution with R and R_{free} values of 20.8 and 24.1, respectively, and includes one molecule of AR LBD (residues 671–918), SHP Box 2 peptide (residues 115–124), DHT and eight water molecules.

Table 1
Summary of data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Molecules per ASU	1
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 55.979, b = 66.542, c = 71.842, $\alpha = \beta = \gamma$ = 90
Resolution range (Å)	25–2.6
Total reflections	8674
Data redundancy	4.4
Completeness (%)	90.5
R_{sym} (%)	9.0
$\langle I/\sigma(I)\rangle$	3.7
Reflections used in refinement	7836
R_{work} (%)/R_{free} (%)†	20.8/24.1
R.m.s. deviation from ideality	
Bond lengths (Å)	0.011
Bond anglse (°)	1.5
Average B factor (Å^2)	44.2

† $R_{work}/R_{free} = \sum||F_o| - |F_c||/\sum|F_o|$, where F_o and F_c are the observed and calculated structure factors, respectively. R_{free} is calculated for 5% of reflections randomly excluded from the refinement.

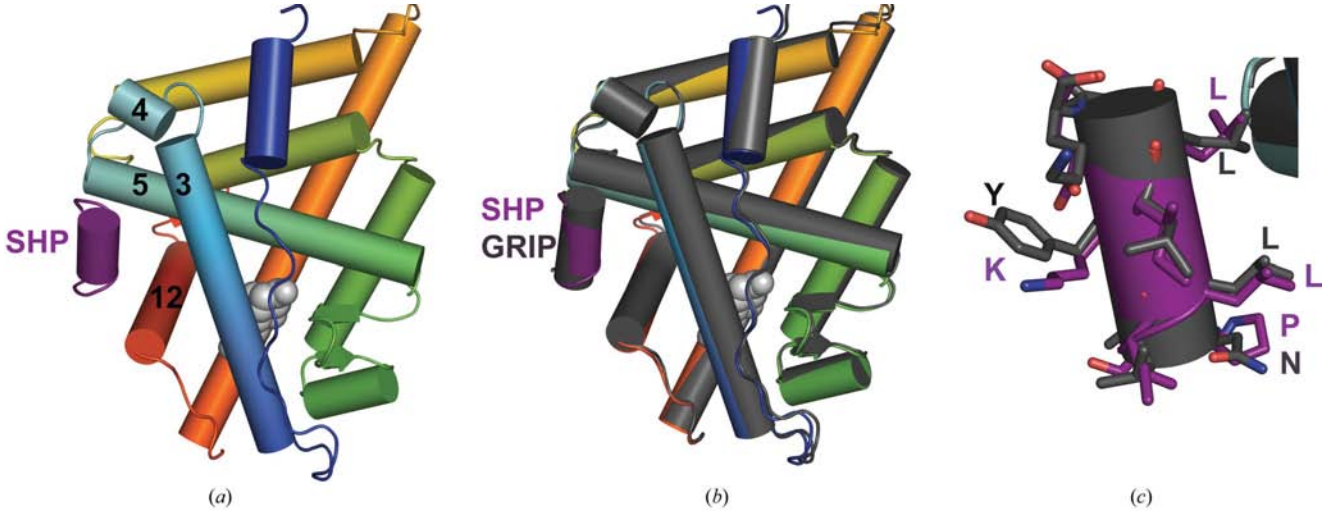

Figure 1
Structure of the SHP LKKIL motif bound to AR–DHT. (*a*) Overall structure of AR LBD in complex with SHP Box 2. The AR helices are shown as rainbow-colored cylinders from the N- to the C-terminus. Helices forming the AF-2 groove are numbered. The SHP L*xx*IL helical motif is colored purple and indicated. The hormone is shown as a space-filling model in gray. (*b*) Superposition of the AR–SHP Box 2 structure (in rainbow colors; the SHP peptide is in purple) with the AR–GRIP Box 3 complex (dark gray). (*c*) A magnified view of the superposed SHP Box 2 (purple) and GRIP Box 3 (dark gray) peptides containing L*xx*LL-like sequence motifs. The helical regions of both peptides are shown as cylinders; side chains are indicated.

Crystallization trials with SHP Box 1 and SHP Box 3 (the other two peptides, each of 14 amino acids) and AR were carried out identically to that described above. The corresponding crystals were grown and X-ray diffraction data were collected to 2.4 and 2.7 Å, respectively, and processed for two representative crystals. Neither SHP Box 1 nor Box 3 peptides were observed in the structures of AR with DHT for these two peptides (data not presented).

Figures were generated with *PyMOL* from DeLano Scientific (http://www.pymol.org).

3. Results and discussion

The three-dimensional structure of SHP is not known, but modeling suggests that SHP is a single intrinsic repression domain, similar to an LBD from the NR superfamily, that spans 257 amino acids. SHP contains three L*xx*LL-like motifs, ^{21}LYALL, ^{118}LKKIL and ^{214}LTRVL, termed Box 1, Box 2 and Box 3, respectively (the accession number for human SHP is NM_021969). These three segments are not readily positioned within SHP using homology modeling because the first is outside the structured region of the SHP LBD and the other two are expected to be in surface loops that connect the 12 helices thought to comprise a typical nuclear receptor LBD.

We have used biochemical assays to determine whether these peptides bind to the AR LBD and visualized their binding modes using X-ray crystallography. Fluorescence polarization assays suggested that only the second motif binds (data not shown). All three L*xx*LL-like motifs were tested in crystallization trials by the mixing the peptide at varying concentrations with freshly purified AR. Well diffracting crystals were readily obtained in all cases under several different crystallization conditions (all favoring hydrophobic interactions) for each peptide and their atomic structures were determined by X-ray crystallography. Two of the peptides did not appear in the crystals, suggesting weak or nonspecific binding. Here, we report the crystal structure at 2.6 Å resolution of the complex of AR LBD with corepressor SHP Box 2 peptide (^{115}PSILKKILLE) containing a helical L*xx*IL motif. The crystal asymmetric unit includes one AR LBD with one bound peptide. The AR LBD adopts a canonical hormone-bound conformation (Fig. 1*a*) with helix 12 of the 12 helices positioned for coactivator binding. Ten of the 14 residues of the bound Box 2 peptide are clearly defined at the AR AF-2 interface, which is formed by helices 3, 4, 5 and 12 of the AR LBD. Significant hydrophobic interactions were found. The AR–SHP Box 2 interaction buries about 930 Å^2 of solvent-accessible surface area at the peptide–protein interface, which is comprised of 24 nonpolar contacts and a hydrogen bond formed by AR residue Lys720 and SHP residue Glu124. The L*xx*LL-like motif ^{118}LKKIL binds as a short α-helix, with the hydrophobic side chains of Leu118 and Leu122 pointing inside the AF-2 groove and making van der Waals contacts with the AR residues Leu712, Val716, Gln738, Met734, Met894 and Ile898, and with Val716, Lys720, Arg726, Val730, Gln733, Met734 and Ile737, respectively. The side chain of SHP Ile121 forms hydrophobic interactions with Val713, Val716 and Met894 of the AR helix 3.

To analyze the similarities and differences between the AR–SHP structure and those of previously reported NR–L*xx*LL complexes (Darimont *et al.*, 1998; Shiau *et al.*, 1998; Svensson *et al.*, 2003), we superposed the AR–SHP Box 2 structure with the previously determined AR–GRIP Box 3 structure (PDB code 1t63; Figs. 1*b* and 1*c*). The positioning of the L*xx*IL motif of SHP in the AF-2 groove of AR closely mimics the positioning of the L*xx*LL motif of the coactivator GRIP. The root-mean-square deviation between the backbone atoms of the two peptides is 0.89 Å for ten Cα atoms. This deviation is greater than the experimental error and we observe a systematic translation and rotation of the two peptides. The buried surface areas compare favorably, but the buried surface for the SHP peptide is intermediate between the largest and the smallest coactivator–AR interfaces. The mechanism of SHP repression is most certainly direct competition with coactivators. For SHP to win the competition at the AF-2 site there must be either a higher relative concentration of SHP or greater affinity. Two factors might enhance SHP binding: (i) the binding motif may be preordered in SHP before binding to the AF-2 site, in contrast to the known disorder found for motifs in coactivators (Darimont *et al.*, 1998), and (ii) adjacent elements of SHP outside the Box 2 motif may contribute to the affinity. A crystal structure of a larger fragment of SHP with AR remains an important goal to understand this aspect of repression.

In conclusion, our structure shows that the binding modes of corepressor/coactivator-derived L*xx*LL-like peptides to AR LBD are structurally very similar, thus suggesting that the AF-2 region is utilized as a 'communicating' binding site for both repression and activation of AR. Therefore, transcription mediated by the AF-2 region would be regulated by competing coregulators and influenced by their binding affinities and abundance, resulting in dynamic gene regulation.

This project was supported by NIH RO1 DK058080 (RKG, PI; RF, subcontract PI), the American Lebanese and Syrian Associated Charities (ALSAC), St Jude Children's Research Hospital (SJCRH), US Army/Department of Defense IDEA Award W81XWH-05-1-0545 (RF, PI) and SHIFA Biomedical SBIR Subcontract R43 CA 119673, Search for Anti-Androgen Compounds to Overcome Resistance of Current Drugs (SA-M, PI; RF, subcontract PI).

References

Chen, C. D., Welsbie, D. S., Tran, C., Baek, S. H., Chen, R., Vessella, R., Rosenfeld, M. G. & Sawyers, C. L. (2004). *Nature Med.* **10**, 33–39.
Darimont, B., Wagner, R., Apriletti, J., Kushner, P., Stallcup, M., Baxter, J., Fletterick, R. & Yamamoto, K. (1998). *Genes Dev.* **12**, 3343–3356.
Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R. & Chambon, P. (1994). *EMBO J.* **13**, 5370–5382.
Gobinet, J., Auzou, G., Nicolas, J., Sultan, C. & Jalaguier, S. (2001). *Biochemistry*, **40**, 15369–15377.
Heery, D. M., Kalkhoven, E., Hoare, S. & Parker, M. G. (1997). *Nature (London)*, **12**, 733–736.
Holter, E., Kotaja, N., Makela, S., Strauss, L., Kietz, S., Janne, O. A., Gustafsson, J., Palvimo, J. J. & Treuter, E. (2002). *Mol. Endocrinol.* **16**, 515–528.
Kolvenbag, G. J., Iversen, P. & Newling, D. W. (2001). *Urology*, **58**, 16–23.
Martin, P., Lardeux, V. & Lefebvre, P. (2005). *Nucleic Acids Res.* **33**, 4311–4321.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Seol, W., Choi, H. S. & Moore, D. D. (1996). *Science*, **272**, 1336–1339.
Shiau, A., Barstad, D., Loria, P., Cheng, L., Kushner, P., Agard, D. A. & Greene, G. L. (1998). *Cell*, **95**, 927–937.
Svensson, S., Ostberg, T., Jacobsson, M., Norstrom, C., Stefansson, K., Hallen, D., Johansson, I. C., Zachrisson, K., Ogg, D. & Jendeberg, L. (2003). *EMBO J.* **22**, 4625–4633.
Zanaria, E., Muscatelli, F., Bardoni, B., Strom, T. M., Guioli, S., Guo, W., Lalli, E., Moser, C., Walker, A. P., McCabe, E. B., Meitinger, T., Monaco, A. P., Sassone-Corsi, P. & Camerino, G. (1994). *Nature (London)*, **372**, 635–641.