Eur Biophys J (2010) 39:1471–1475
DOI 10.1007/s00249-010-0602-2

ORIGINAL PAPER

A canonical cation–π interaction stabilizes the agonist conformation of estrogen-like nuclear receptors

Núria Queralt-Rosinach · Jordi Mestres

Received: 1 February 2010 / Accepted: 16 March 2010 / Published online: 3 April 2010

Abstract Representative crystal structures of the ligand-binding domain for the majority of nuclear receptors are currently available. A systematic comparative analysis of these structures identified an energetically favorable cation–π interaction that involves an amino acid located at the extreme C-terminal end and appears to form only in the agonist conformation of the estrogen receptor α, glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. It is postulated that this cation–π interaction is used by members of the estrogen-like subfamily to provide additional stabilization to the transcriptional active conformation upon ligand binding.

Keywords Protein stability · Side-directed mutagenesis · Drug design · Agonist binding

Introduction

Nuclear receptors are transcription factors that regulate a variety of biological processes essential to development, reproduction, metabolism, and homeostasis, and thus are major targets for drug discovery in a wide variety of therapeutic areas (Gronemeyer et al. 2004). They distinguish themselves from other transcription factors by the fact that the activity of many of them is controlled through interaction with small lipophilic molecules (such as hormones, fatty acids, bile acids, oxysterols, and xenobiotics) in a hydrophobic core of a highly conserved ligand-binding domain (LBD) (Moras and Gronemeyer 1998). A key structural feature of this LBD is the presence of an amphipathic helix (referred to as the AF2 helix or helix 12), the position of which determines the ability of the nuclear receptor to recruit co-activators or co-repressors (Xu and Lambert 2003). In this respect, small molecules act as regulators of nuclear receptor function by influencing the conformation of helix 12 in the LBD upon binding: in the agonist conformation, helix 12 covers the ligand-binding pocket and creates a new surface for the recruitment of a co-activator that then promotes gene transcription; in the antagonist conformation, helix 12 is displaced and occupies the co-activator binding groove, thus preventing transcriptional activation (Kauppi et al. 2003). However, the intrinsic forces that contribute to the natural stabilization of helix 12 in its transcriptional active conformation remain unclear.

In recent years, cation–π interactions have been recognized to play an important role in a variety of processes such as receptor–ligand interactions, enzyme–substrate binding, antigen–antibody recognition, and protein stability (Dougherty 1996; Prajapati et al. 2006). This type of energetically favorable noncovalent binding force is established when an aromatic amino acid (phenylalanine, tyrosine, or tryptophan) is found within 6 Å of a protonated amino acid (lysine or arginine) (Ma and Dougherty 1997; Gallivan and Dougherty 1999). In this respect, an extensive mutational analysis performed on the C-terminal end of the human androgen receptor (AR), complemented by the construction of a homology model of AR based on the crystal structure of progesterone (PR) in the agonist conformation, identified a putative cation–π interaction between R831 located in helix 9 and F916 from the extreme C-terminal end as being critical for providing the correct ligand-binding conformation of the receptor. In the absence of further structural

N. Queralt-Rosinach · J. Mestres (✉)
Chemogenomics Laboratory, Research Program on Biomedical Informatics (GRIB), Institut Municipal d'Investigació Mèdica and Universitat Pompeu Fabra, Parc de Recerca Biomèdica, Doctor Aiguader 88, 08003 Barcelona, Catalonia, Spain
e-mail: jmestres@imim.es

evidence at the time, it was also indicated that these two residues are conserved throughout the glucocorticoid-like group of nuclear receptors, including glucocorticoid (GR), mineralocorticoid (MR), PR, and AR (Tahiri et al. 2001).

Today, representative crystal structures are available for almost all members of the nuclear receptor family (García-Serna et al. 2006). Accordingly, the aim of this study is to exploit this wealth of structural information to identify and characterize those cation–π interactions that may be critical to stabilize the structure of nuclear receptors in the agonist conformation.

Methods

A total of 278 LBD X-ray structures covering 36 nuclear receptors were identified in the *Functional Coverage of the Proteome* (García-Serna et al. 2006) and extracted from the Protein Data Bank (PDB) (Berman et al. 2000). Of these, 226 structures were visually confirmed to be in the agonist conformation and superimposed using the Gaussian-Based Alignment of Protein Structures (GAPS) program (Mestres 2000). Sequence alignments based on the superimposed structures were obtained using the MAMMOTH-mult program (Lupyan et al. 2005). Identification and geometric characterization of all cation–π interactions present in each of the agonist structures were done using the Protein Interactions Calculator (PIC) server (Tina et al. 2007) and subsequently filtered to retain only those interactions involving one amino acid from the extreme C-terminal end (considering a five-residue window). The energetic characterization of the remaining cation–π interactions was performed using the Cation–π Trends Using Realistic Electrostatics (CaPTURE) program (Gallivan and Dougherty 1999). Finally, the relevance of the amino acids involved in the cation–π interactions identified was assessed by examining two mutational databases, namely the androgen receptor gene mutations database (ARDB) (Gottlieb et al. 2004) and the nuclear receptor mutation database (NRMD) (van Durme et al. 2003). Visual inspection, analysis, and graphics of nuclear receptor structures were done with PyMOL (DeLano 2005).

Results

Following on from the earlier suggestion of a cation–π interaction between an arginine located in helix 9 and a phenylalanine from the extreme C-terminal end of AR (Tahiri et al. 2001), focus was first given to the glucocorticoid-like group of nuclear receptors. The results are compiled in Table 1, including distances (in Å) between the cationic atom of the protonated amino acid and the ring centroid of the aromatic amino acid (Tina et al. 2007), and total binding energies (in kcal/mol), derived from the sum of the electrostatic and van der Waals energy contributions (Gallivan and Dougherty 1999), averaged across the corresponding list of PDB chains. In total, 57 side-chains from 45 PDB entries were analyzed. Indeed, an energetically favorable cation–π interaction between R831 and F916 was identified in all 18 side-chains from human AR. Interestingly, the availability of crystal structures for the agonist

Table 1 Geometric and energetic characterization of the cation–π interactions identified in the five members of the estrogen-like subfamily

Nuclear receptor	Organism	Interaction	Distance ± SD	Energy ± SD	PDB chains
NR3A1: estrogen α	*Homo sapiens*	Y526:R548	4.60 ± 0.41	−5.06 ± 1.61	2b1z_A, 3erd_A, 2fai_A, 2fai_B, 1zky_A, 2g5o_A, 1gwr_B, 2g44_A, 2q6j_A, 2q6j_B, 1x7e_A, 1x7e_B, 1ere_A, 1ere_B, 1ere_C, 1ere_D, 1ere_E, 1ere_F
NR3C1: glucocorticoid	*Homo sapiens*	R690:F774	4.97 ± 0.25	−3.13 ± 0.83	1m2z_A, 1m2z_D, 1p93_A
NR3C2: mineralocorticoid	*Homo sapiens*	R896:F981	4.96 ± 0.12	−3.17 ± 0.25	2aax_A, 2aax_B, 2ab2_A, 2ab2_B, 2aa6_A, 2aa6_B, 2a3i_A, 2aa2_A, 2aa5_A, 2aa5_B, 2aa7_A, 2abi_B, 2abi_C, 1ya3_A, 1ya3_B
NR3C3: progesterone	*Homo sapiens*	R845:F930	4.72 ± 0.26	−3.60 ± 0.66	1sqn_A, 1sqn_B, 1sr7_A, 1sr7_B, 1a28_A, 1a28_B, 1zuc_A, 1zuc_B, 1e3k_A, 1e3k_B
NR3C4: androgen	*Homo sapiens*	R831:F916	5.12 ± 0.08	−2.98 ± 0.29	2ax6_A, 2am9_A, 2ax9_A, 1t65_A, 2ax8_A, 2amb_A, 2oz7_A, 2axa_A, 1z95_A, 1xow_A, 2ao6_A, 2ama_A, 2ax7_A, 1gs4_A, 1t63_A, 1t5z_A, 1e3g_A, 1xj7_a
NR3C4: androgen	*Pan troglodytes*	R823:F908	5.15 ± 0.03	−2.89 ± 0.17	1t7r_A, 1t7f_A, 1t7m_A, 1t7t_A, 1t79_A, 1t74_A, 1t76_A, 1t73_A
NR3C4: androgen	*Rattus norvegicus*	R814:F899	5.06 ± 0.20	−3.23 ± 0.31	1i38_A, 1xnn_A, 2nw4_A

conformation of AR from *Pan troglodytes* and *Rattus norvegicus* allowed confirmation that both geometric and energetic parameters of this cation–π interaction are very much preserved across species. Overall, the average distance between the positively charged carbon in the arginine and the centroid of the aromatic ring in the phenylalanine involved in the cation–π interaction in AR (R831:F916) was 5.1 Å, with both residues interacting favorably with an average total binding energy of −3.0 kcal/mol. Similar results were obtained for the cation–π interactions identified in the three side-chains of GR (R690:F774) and the 15 side-chains of MR (R896:F981), with consistent interaction distances of 5.0 Å and binding energies of −3.2 kcal/mol. In contrast, the cation–π interactions found in the ten side-chains of PR (R845:F930) occurred at slightly shorter distances of 4.7 Å and with more favorable binding energies of −3.6 kcal/mol. The level of conservation of this cation–π interaction is illustrated in Fig. 1a, in which representative structures of GR, MR, PR, and AR have been superimposed. As can be observed, in all four nuclear receptors, the two amino acids involved in the cation–π interaction are arranged in the energetically most favorable stacked geometry (Gallivan and Dougherty 1999).

Having confirmed structurally the existence of a cation–π interaction involving one amino acid from the extreme C-terminal end in the four members of the glucocorticoid-like group of nuclear receptors, we moved onto investigate whether a similar type of interaction could also be present in members of other nuclear receptor groups. Accordingly, all remaining LBD structures in the agonist conformation were submitted to the PIC server (Tina et al. 2007) and then filtered to retain only those interactions involving one amino acid located within the five last positions at the extreme C-terminal end. This systematic analysis of all nuclear receptor agonist structures identified a candidate cation–π interaction in the estrogen receptor subtype α (ERα) which, to the best of our knowledge, has been long overlooked. However, most interestingly, both the nature of the amino acids involved in the cation–π interaction and their relative position in the secondary structure elements did not match those previously found in AR and strongly conserved in GR, MR, and PR. In ERα, the cation–π interaction involved a tyrosine residue in helix 11 (Y526) and an arginine from the C-terminal end (R548). Table 1 summarizes the geometric and energetic characterization of the Y526:R548 cation–π interaction found consistently in 18 side-chains from 10 PDB entries of ERα. On average, the distance between the positively charged carbon in the arginine and the centroid of the aromatic ring in the tyrosine involved in the cation–π interaction in ERα was slightly shorter than that found in AR (4.6 versus 5.1 Å) but energetically much more favorable (−5.1 versus −3.0 kcal/mol). Figure 1b illustrates the cation–π interaction in one representative structure of ERα in the agonist conformation. As can be observed, the structure of ERα lacks the long tail present in GR, MR, PR, and AR after helix 12 and thus cannot reach the residues in helix 9 to anchor helix 12. Still, quite remarkably, ERα seems to have evolved to end up using the same mechanism found in glucocorticoid-like receptors to stabilize the transcriptional active conformation but with the residues involved in the cation–π interaction placed in completely different locations.

The identification of a key stabilizing cation–π interaction that appears to be present exclusively in five members of the estrogen-like subfamily among all nuclear receptors motivated a more in-depth analysis of this subfamily. Accordingly, the sequence alignment of all nine members of the estrogen-like subfamily of nuclear receptors is presented in Fig. 2. From the alignment, it becomes apparent that the cation–π interaction observed in ERα is not possible

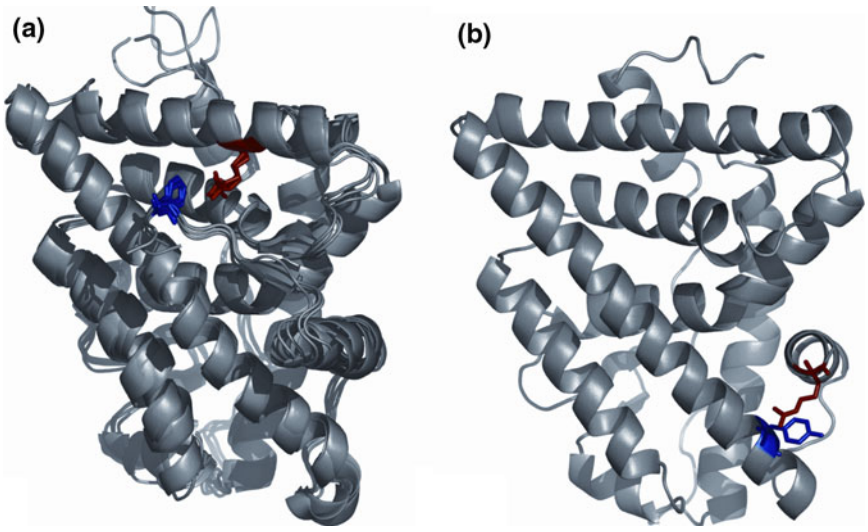

Fig. 1 **a** Structural superposition of the LBD agonist conformation in GR (1m2z_A), MR (2a3i_A), PR (1a28_A), and AR (1e3g_A) and **b** agonist conformation structure in ERα (3erd_A). The amino acids involved in the cation–π interaction are highlighted as sticks (*red*: protonated residue; *blue*: aromatic residue)

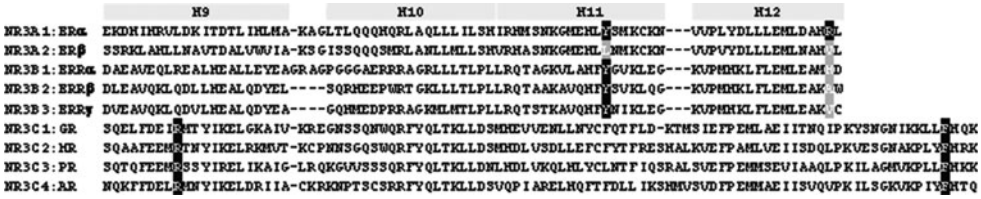

Fig. 2 Sequence alignment between all members of the estrogen-like subfamily of nuclear receptors. Amino acids involved in the cation–π interaction in ERα, GR, MR, PR, and AR are highlighted in *black*. In those alignment positions, conserved and nonconserved amino acids in ERβ, ERRα, ERRβ, and ERRγ are also highlighted, in *black and grey*, respectively

in ERβ, since the Y:R interacting pair of residues in ERα (highlighted in black) has mutated to L:V in ERβ (highlighted in grey). The absence in ERβ of the additional stabilization provided by the cation–π interaction to the agonist conformation of ERα may contribute to the observation that, when slightly increasing the size of substituent groups on an ERβ agonist, the agonism on ERβ disappears and the ligands act as pure ERβ-selective antagonists (Katzenellenbogen et al. 2001). As regards to the three estrogen-related receptors, they all conserve the tyrosine involved in the cation–π interaction of ERα (highlighted in black), but they all lack also the corresponding cationic partner (highlighted in grey). Therefore, a stabilizing cation–π interaction cannot occur in estrogen-related receptors. Again, this may partially explain why, for example, diethylstilbestrol acts as an agonist in ERα but as an antagonist in ERRγ (Gronemeyer et al. 2004). In fact, it has been hypothesized that the steroid-binding capacity in the estrogen-like subfamily was acquired rather late during nuclear receptor evolution (Escriva et al. 1997). Under this assumption, the additional stabilization provided by a cation–π interaction to the agonist conformation of ERα, GR, MR, PR, and AR could well be an evolutionary signature of these nuclear receptors to further stabilize the conformation of these nuclear receptors in the transcriptional active form.

Discussion

Although the existence of a cation–π interaction involving one amino acid from the extreme C-terminal end in the agonist conformation of AR had been suggested earlier on the basis of mutational studies (Tahiri et al. 2001), the current availability of multiple crystal structures has allowed for observing its occurrence at the molecular level and providing a geometric and energetic characterization. In addition, a systematic scan across all available nuclear receptor structures in the agonist conformation confirmed the existence of this peculiar cation–π interaction in the remaining members of the glucocorticoid-like group (GR, MR, and PR) and, most remarkably, identified for the first time the use of this type of interaction in ERα.

One means of establishing the relevance of the amino acids involved in this cation–π interaction for the function of nuclear receptors is through mutational analyses. Indeed, inspection of mutational databases (Gottlieb et al. 2004; van Durme et al. 2003) reveals that mutating either R831 (R831L (Chen et al. 2000), R831Q (Chen et al. 2000; Brown et al. 1990; Yaegashi et al. 1999)) or F916 (F916A (Tahiri et al. 2001), F916P (Tahiri et al. 2001), F916L (Radmayr et al. 1997)) in AR causes complete androgen insensitivity (CAIS) that can be explained, in some cases, by the inability to form a functional AR–steroid complex and hence the impossibility of AR to activate transcription of genes essential for male sex differentiation during fetal development (Brown et al. 1990). In contrast, mutating F916 to a tyrosine—F916Y (Tahiri et al. 2001)—and thus retaining the ability to form a cation–π interaction with R831, results in transcriptional activation not significantly lower than that of wild-type AR. All these findings are very much in consonance with the R831:F916 cation–π interaction being a key stabilizing force of the agonist conformation in AR. Unfortunately, mutational analyses of similar scope are not available for GR, MR, PR, and ERα. Only for MR, mutation of F981 to an alanine—F981A (Couette et al. 1998)—was found to reduce the amount of aldosterone bound to the entire receptor by 70% and its affinity by 20-fold. Accordingly, the present findings may guide future mutational analysis on estrogen-like nuclear receptors both to confirm the crucial functional role of the amino acids involved in the cation–π interaction of ERα, GR, MR, PR, and AR, but also potentially to enhance the stability of the agonist conformation of ERβ, ERRα, ERRβ, and ERRγ upon ligand binding.

Acknowledgments Grant sponsor: Spanish Ministerio de Educación y Ciencia and Instituto de Salud Carlos III; Grant number: BIO2008-02329.

References

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. Nucleic Acids Res 28:235–242. http://www.rcsb.org/

Brown TR, Lubahn DB, Wilson EM, French FS, Migeon CJ, Corden JL (1990) Functional characterization of naturally occurring mutant androgen receptors from subjects with complete androgen insensitivity. Mol Endocrinol 4:1759–1772

Chen CP, Chern SR, Chen BF, Wang W, Hwu YM (2000) Hamartoma in a pubertal patient with complete androgen insensitivity syndrome and R(831)X mutation of the androgen receptor gene. Fertil Steril 74:182–183

Couette B, Jalaguier S, Hellal-Levy C, Lupo B, Fagart J, Auzou G, Rafestin-Oblin ME (1998) Folding requirements of the ligand-binding domain of the human mineralocorticoid receptor. Mol Endocrinol 12:855–863

DeLano WL (2005) The case for open-source software in drug discovery. Drug Discov Today 10:213–217. http://www.pymol.org/

Dougherty DA (1996) Cation–pi interactions in chemistry and biology: a new view of benzene, Phe, Tyr, and Trp. Science 271:163–168

Escriva H, Safi R, Hänni C, Langlois M-C, Saumitou-Laprade P, Sthéhelin D, Capron A, Pierce R, Laudet V (1997) Ligand binding was acquired during evolution of nuclear receptors. Proc Natl Acad Sci USA 94:6803–6808

Gallivan JP, Dougherty DA (1999) Cation–π interactions in structural biology. Proc Natl Acad Sci USA 96:9459–9464. http://capture.caltech.edu/

García-Serna R, Opatowski L, Mestres J (2006) FCP: functional coverage of the proteome by structures. Bioinformatics 22:1792–1793. http://cgl.imim.es/fcp/

Gottlieb B, Beitel LK, Wu JH, Trifiro M (2004) The androgen receptor gene mutations database (ARDB): 2004 update. Hum Mutat 23:527–533. http://androgendb.mcgill.ca/

Gronemeyer H, Gustafsson JA, Laudet V (2004) Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discov 3:950–964

Katzenellenbogen BS, Sun J, Harrington WR, Kraichely DM, Ganessunker D, Katzenellenbogen JA (2001) Structure-function relationships in estrogen receptors and the characterization of novel selective estrogen receptor modulators with unique pharmacological profiles. Ann N Y Acad Sci 949:6–15

Kauppi B, Jakob C, Färnegårdh M, Yang J, Ahola H, Alarcon M, Calles K, Engström O, Harlan J, Muchmore S, Ramqvist AK, Thorell S, Ohman L, Greer J, Gustafsson JA, Carlstedt-Duke J, Carlquist M (2003) The three-dimensional structures of antagonistic and agonistic forms of the glucocorticoid receptor ligand-binding domain. J Biol Chem 278:22748–22754

Lupyan D, Leo-Macias A, Ortiz AR (2005) A new progressive-iterative algorithm for multiple structure alignment. Bioinformatics 21:3255–3263. http://ub.cbm.uam.es/mammoth/mult/

Ma JC, Dougherty DA (1997) The cation–pi interaction. Chem Rev 97:1303–1324

Mestres J (2000) Gaussian-based alignment of protein structures: deriving a consensus superposition when alternative solutions exist. J Mol Model 6:539–549

Moras D, Gronemeyer H (1998) The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol 10:384–391

Prajapati RS, Sirajuddin M, Durani V, Sreeramulu S, Varadarajan R (2006) Contribution of cation–pi interactions to protein stability. Biochemistry 45:15000–15010

Radmayr C, Culig Z, Glatzl J, Neuschmid-Kaspar F, Bartsch G, Klocker H (1997) Androgen receptor point mutations as the underlying molecular defect in 2 patients with androgen insensitivity syndrome. J Urol 158:1553–1556

Tahiri B, Auzou G, Nicolas JC, Sultan C, Lupo B (2001) Participation of critical residues from the extreme C-terminal end of the human androgen receptor in the ligand binding function. Biochemistry 40:8431–8437

Tina KG, Bhadra R, Srinivasan N (2007) PIC: protein interactions calculator. Nucleic Acids Res 35:W473–W476. http://crick.mbu.iisc.ernet.in/~PIC/

van Durme JJJ, Bettler E, Folkertsma S, Horn F, Vriend G (2003) NRMD: nuclear receptor mutation database. Nucleic Acids Res 31:331–333. http://www.receptors.org/NR/

Xu EH, Lambert MH (2003) Structural insights into regulation of nuclear receptors by ligands. Nucl Recept Signal 1:e004

Yaegashi N, Uehara S, Senoo M, Sato J, Fujiwara J, Funato T, Sasaki T, Yajima A (1999) Point mutations in the steroid-binding domain of the androgen receptor gene of five Japanese patients with androgen insensitivity syndrome. Tohoku J Exp Med 187:263–272