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Molecular determinants of the recognition of ulipristal acetate by oxo-steroid receptors

I. Petit-Topin [a,b], M. Fay [a,b], M. Resche-Rigon [c], A. Ulmann [c], E. Gainer [c], M.-E. Rafestin-Oblin [a,b], J. Fagart [a,b,d,e,*]

[a] Inserm U773, Centre de Recherche Biomédicale Bichat-Beaujon CRB3, Paris, France
[b] Université Denis Diderot, Site Bichat, Paris, France
[c] Laboratoire HRA Pharma, Paris, France
[d] Inserm U693, Le Kremlin – Bicêtre F94276, France
[e] Faculté de Médecine Paris-Sud, Univ Paris-Sud, UMR-S693, Le Kremlin – Bicêtre F94276, France

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ABSTRACT

The human progesterone receptor (PR) plays a key role in reproductive function in women. PR antagonists have numerous applications in female health care including regular and emergency contraception, and treatment of hormone-related pathological conditions such as breast cancer, endometriosis, and leiomyoma. The main factor limiting their long-term administration is the fact that they cross-bind to other oxo-steroid receptors. Ulipristal acetate (UPA), a highly potent PR antagonist, has recently come onto the market and is much more selective for PR than the other oxo-steroid receptors (androgen, AR, glucocorticoid, GR, and mineralocorticoid, MR receptors) and, remarkably, it displays lower GR-inactivating potency than RU486. We adopted a structural approach to characterizing the binding of UPA to the oxo-steroid receptors at the molecular level. We solved the X-ray crystal structure of the ligand-binding domain (LBD) of the human PR complexed with UPA and a peptide from the transcriptional corepressor SMRT. We used the X-ray crystal structure of the GR in its antagonist conformation to dock UPA within its ligand-binding cavity. Finally, we generated three-dimensional models of the LBD of androgen and mineralocorticoid receptors (AR and MR) in an antagonist conformation and docked UPA within them. Comparing the structures revealed that the network of stabilizing contacts between the UPA C11 aryl group and the LBD is responsible for its high PR antagonist potency. It also showed that it is the inability of UPA to contact Gln642 in GR that explains why it has lower potency in inactivating GR than RU486. Finally, we found that the binding pockets of AR and MR are too small to accommodate UPA, and allowed us to propose that the extremely low sensitivity of MR to UPA is due to inappropriate interactions with the C11 substituent. All these findings open new avenues for designing new PR antagonist compounds displaying greater selectivity.

1. Introduction

The critical role of progesterone (P4) in reproduction led to the development of synthetic P4 analogs, which found clinical applications in women's health care, for purposes such as oral contraception and post-menopausal hormone therapy. Most of the biological effects of P4 and progestins result from their binding to the progesterone receptor (PR), a ligand-activated transcription factor [1,2]. This realization has stimulated the development of receptor antagonists for both regular and emergency contraception, and for the treatment of hormone-related disorders, such as breast cancer, endometriosis, and leiomyoma. The first steroidal PR antagonist to be developed was mifepristone (RU486), which is marketed since 1988 in France and since 2000 in USA. Despite its

Abbreviations: AR, androgen receptor; DMEM, Dulbecco's minimal essential medium; FCS, fetal calf serum; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GST, glutathione S-transferase; HEK, human embryonic kidney; IPTG, isopropyl-β-D-thiogalactoside; LBD, ligand binding domain; MR, mineralocorticoid receptor; NR, nuclear receptor; P4, progesterone; PDB, Protein data bank; SMRT, silencing mediator of retinoic and thyroid receptors; SPRMs, selective progesterone receptor modulators; UPA, ulipristal acetate.

* Corresponding author Address: Inserm U693, Faculté de médecine Paris-Sud, 63, rue Gabriel Péri, Le Kremlin – Bicêtre, F94276, France. Tel.: + 33 1 49 59 67 02.
E-mail address: jerome.fagart@inserm.fr (J. Fagart).

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high potency in antagonizing the action of P4 in subnanomolar concentration ranges, RU486 binds to and inactivates the glucocorticoid receptor (GR), to a lesser extent the androgen receptor (AR), and weakly the mineralocorticoid receptor (MR) [3–8]. Other steroidal compounds were then developed by pharmaceutical companies, which were intended to reduce their cross-reactivity with the other oxo-steroid receptors [9]. All these molecules harbor a bulky C11-aryl substituent and are derived from testosterone [Mifepristone (RU486), Asoprisnil (J-867), Lonaprisan, (ZK230211), Onapristone (ZK98299), ORG-31710], or from progesterone [Ulipristal acetate (UPA, CDB-2914, VA2914), Telapristone acetate (CDB-4124)]. UPA, which was first marketed in 2009 in EU and then in the USA in 2010, harbors the same C11 substituent as RU486 but is characterized by having a 17β-methylketone and a 17α-O-acetyl group. Whereas RU486, in association with prostaglandins, is used solely for medical termination of pregnancy, UPA has a broad range of therapeutic applications including emergency contraception and the preoperative treatment of uterine fibroids [10]. It has been proposed that UPA has greater bioavailability than RU486 [11]. Moreover, UPA has been shown to be more efficient than RU486 when administered per os. Importantly, it has been reported that the anti-glucocorticoid activity of UPA is 12 times lower than that of RU486 [7].

In this study we set out to characterize how UPA binds to PR and to identify the molecular determinant responsible for its high selectivity for PR as compared to the other oxo-steroid receptors, and also the determinants responsible for its lower cross-reactivity with GR than RU486. To do this, we adopted a structural approach. We solved the X-ray crystal structure of the PR ligand-binding domain (LBD) complexed with UPA and a co-repressor peptide. We took advantage of the previously solved X-ray crystal structure of the GR LBD complexed with RU486 [12] to dock UPA within the ligand binding cavity. The crystal structures of the AR and MR LBD complexed with an antagonist ligand were not available and so we followed a homology modeling approach by generating three-dimensional models of the AR and MR LBD in an antagonist conformation and then docking UPA within the ligand-binding cavity. These structures allowed us to characterize how UPA binds to the oxo-steroid receptors and identified the contacts responsible for the high PR-selectivity of UPA.

2. Materials and methods

2.1. Chemical products used

RU486 (mifepristone; 11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-propinyl-4.9-estradiene-3-one) and Ulipristal acetate (UPA) (17α-acetoxy-11β-(4-N,N-dimethylaminophenyl)-19-norpregna-4,9-diene-3,20-dione) were from HRA Pharma (Paris, France). Aldosterone (4-pregnen-11β,21-diol-18-al-3,20-dione), dexamethasone (11β,16α)-9α-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione), progesterone (4-pregnen-3,20-dione), testosterone (17β-hydroxy-4-androsten-3-one), and all other products were purchased from Sigma-Aldrich (St Louis, MO, USA). The SMRT peptide (residues 2346 to 2362, TNMGLEAIIRKALMGKY, human, isoform 1, SMRTα) was synthesized by Eurogentec (Seraing, Belgium).

2.2. Expression vectors

The expression vectors pchGR, pchMR, and pchPR code for human GR, MR, and PRB, respectively [13–15]. The human AR expression vector pcDNA-hAR was kindly provided by G. A. Coetzee. Expression vector pchMR$_{A773G/S810M}$ codes for the mutant MR$_{A773G/S810M}$ [16]. pGexhPRLBD vector codes for the fusion protein between GST and the PR LBD [15]. The plasmid pcβgal, which contains the β-galactosidase sequence, was used to standardize the transfection experiments [17]. The reporter vector GRE2Luc was the kind gift of A. Biola-Vidamment and M. Pallardy.

2.3. Protein expression and purification

Fermentation using the BL21Codon Plus (DE3) RIL strain from Stratagene (Amsterdam, The Netherlands) transformed with the pGexhPRLBD vector was carried out in the presence of 50 μM UPA. Expression was induced by incubating with 200 μM isopropyl-β-D-thiogalactoside (IPTG) for 16 h at 15 °C. After centrifuging and freezing the bacterial pellet, the bacteria were disrupted by sonication in TENG buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 0.1% n-octyl-β-glucoside, pH 7.5 at 4 °C) supplemented with 50 μM UPA. The lysate was clarified and loaded onto a GSTrap column (Amersham, Les Ulis, France). The fusion protein was eluted with 15 mM of reduced glutathione in the TENG buffer. After diluting the eluate to a protein concentration of 1 mg/ml in the presence of the SMRT peptide (2 fold excess mol/mol compared to the GST-LBD fusion protein), the fusion protein was cleaved by exposing it to thrombin protease (50 units/mg of fusion protein) overnight at 4 °C. The protein mixture was diluted a further 10-fold in HENG buffer (10 mM HEPES, 10% glycerol, 0.1% n-octyl-β-glucoside, pH 6.8 at 4 °C) supplemented with 50 μM UPA, loaded onto a cation-exchange column (Hitrap SP XL from Amersham, Les Ulis, France), and eluted with a 0–500 mM NaCl gradient in the HENG buffer supplemented with 50 μM UPA. The fractions containing the LBD were pooled and concentrated to a protein concentration of 3.8 mg/ml.

2.4. Crystallization, structure determination and model refinement

Crystals were grown over two weeks at 20 °C in hanging drops containing 1 μl of protein solution and 1 μl of well buffer (15% PEG 3350, 50 mM Tris, 400 mM NaCl, 10% glycerol, pH 8.5). Before the data were recorded, the crystals were flash-frozen in liquid nitrogen without adding any cryoprotective agent. Diffraction data was collected to a resolution of 1.50 Å on the id23eh2 beam line from the European Synchrotron Radiation Facility (ESRF, Grenoble, France) using a MarCCD detector. The data set was integrated and scaled using XDS [18] to a 2.41 Å resolution. The structure was solved by molecular replacement using Phaser [19], with the coordinates of the PR LBD complexed with asoprisnil (PDB ID: 2OVH [20]) as the search model. Several rounds of manual rebuilding using the Sigma A weighted 2Fo-Fc electron density maps, followed by simulated annealing and individual isotropic B-factor refinements, were performed using CNS [21]. Solvent molecules were located in a Fo-Fc map contoured at 2σ. The final model was validated using PROCHECK [22]. All structural figures were produced using DINO (http://www.dino3d.org).

2.5. Cell culture and transfection

HEK 293 T cells were routinely cultured in a high-glucose DMEM medium (Invitrogene, CergyPontoise, France), 20 mM HEPES, 2 mM glutamine, 1X non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin supplemented with 10% fetal calf serum (FCS) in a humidified atmosphere at 37 °C and with 5% CO_2. One day before transfection, the cells were seeded at 3×10^6 cells/80 mm diameter culture petri dish and cultured overnight in the same medium. Six hours before transfection, the FCS supplemented medium was replaced by the same medium supplemented with 10% dextran-charcoal treated FCS. Transfections were carried out using the calcium phosphate precipitation method. The calcium phosphate