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Structural basis for the inhibition of AKR1B10 by the C3 brominated TTNPB derivative UVI2008

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ABSTRACT

UVI2008, a retinoic acid receptor (RAR) β/γ agonist originated from C3 bromine addition to the parent RAR pan-agonist 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), is also a selective inhibitor of aldo-keto reductase family member 1B10 (AKR1B10). Thus, it might become a lead drug for the design of compounds targeting both activities, as an AKR1B10 inhibitor and RAR agonist, which could constitute a novel therapeutic approach against cancer and skin-related diseases. Herein, the X-ray structure of the methylated Lys125Arg/Val301Leu AKR1B10 (i.e. AKME2MU) holoenzyme in complex with UVI2008 was determined at 1.5 Å resolution, providing an explanation for UVI2008 selectivity against AKR1B10 (IC_{50} = 6.1 μM) over the closely related aldose reductase (AR, IC_{50} = 70 μM). The carboxylic acid group of UVI2008 is located in the anion-binding pocket, at hydrogen-bond distance of catalytically important residues Tyr49 and His111. The inhibitor bromine atom can only fit in the wider active site of AKR1B10, mainly because of the native Trp112 side-chain orientation, not possible in AR. In AKR1B10, Trp112 native conformation, and thus UVI2008 binding, is facilitated through interaction with Gln114. IC_{50} analysis of the corresponding Thr113Gln mutant in AR confirmed this hypothesis. The elucidation of the binding mode of UVI2008 to AKR1B10, along with the previous studies on the retinoid specificity of AKR1B10 and on the stilbene retinoid scaffold conforming UVI2008, could indeed be used to foster the drug design of bifunctional anti-proliferative compounds.

1. Introduction

The NADPH-dependent aldo-keto reductase (AKR) superfamily consists of approximately 150 phase I drug-metabolizing enzymes that share high sequence similarities. In humans, the subfamily 1B includes the closely related enzymes aldose reductase (AR or AKR1B1), AKR1B10 and AKR1B15, the first two being of biomedical interest. AR has been deeply studied regarding its implication in the polyol pathway and recently also in relation to inflammatory pathologies, including colon cancer [1]. In contrast to the ubiquitous AR, AKR1B10 has a restricted expression in healthy tissues (mostly in colon and small intestine), and is a promising antineoplastic target, as it is overexpressed in several cancer types, including different hepatic, lung, breast and pancreatic tumors [2–6].

Abbreviations: AR, Aldose Reductase; AKR, Aldo-Keto Reductase; AKR1B10, AKR family member 1B10; AKR1B15, AKR family member 1B15; AKR1A1, AKR family member 1A1 or aldehyde reductase; ARIs, AR inhibitors; CAPE, Caffeic Acid Phenethyl Ester; HCC, Hepatocellular Carcinoma; AKME2MU, methylated Lys125Arg/Val301Leu AKR1B10; RA, Retinoic Acid; RAR, RA Receptor; RARE, RAR Element; RXR, Retinoid X Receptor; TTNPB, 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl] benzoic acid.

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AKR1B10 might impact the carcinogenesis process via the reduction of a wide range of substrates, involved in different pathways: Retinoic acid (RA) signaling control [6], detoxification of reactive lipid aldehydes [5], anticancer drugs [6], lipid synthesis promotion [5] and regulation of protein isoprenylation [7]. Many AR inhibitors (ARIs) have been developed, but most of them have been withdrawn in the clinical stage because of their side effects and toxicity. Those adverse effects might have been caused in several cases by cross-inhibition with close enzymes, mostly AKR1B10 and aldehyde reductase (AKR1A1) [8,9]. In the recent years, the focus has been switched from the latter enzyme to AKR1B10, given its higher sequence identity with AR [8,9]. On the other hand, the emergency of AKR1B10 as a drug target has fostered the research for selective inhibitors not cross-reacting with AR. In this regard, about a dozen of AKR1B10 structures have been obtained in the last couple of years [8–11]. UVI2008 is a RAR β/γ agonist originated from a collection of arotinoids with a stilbenoid structure based on the *pan*-agonist TTNPB, only differing in a C3 bromine atom addition [12,13]. Moreover, we previously showed that UVI2008 had the highest potency and selectivity for AKR1B10 among a series of RAR/RXR synthetic retinoids. However, at that time, we could only provide a docking model to support the inhibitory properties of UVI2008, using the V301L AKR1B10 complex with the inhibitor fidarestat as a receptor molecule (PDB ID 4GAB) [14].

Here, we report the X-ray structure of an AKR1B10-$NADP^+$-UVI2008 ternary complex at 1.5 Å resolution, by means of the methylated Lys125Arg/Val301Leu AKR1B10 mutant (i.e. AKME2MU) system previously described [9], showing unequivocally how this synthetic retinoid interacts with AKR1B10. We also show that the parent compound TTNPB is not inhibiting AKR1B10 and that the addition of the C3 halogen atom (either Br or Cl) provides an AKR1B10 specific interaction. Interestingly, this same feature has been recognized as a structural selectivity determinant contributing to RARβ-selectivity [15]. Overall, this three-dimensional structure serves as a framework for the development of more potent compounds bearing two antiproliferative activities, RAR agonism and AKR1B10 inhibition.

2. Material and methods

2.1. Chemicals

UVI2007 and UVI2008 were synthesized as previously described [13]. TTNPB and other chemicals were obtained from Sigma–Aldrich.

2.2. Site-directed mutagenesis

All cDNAs (wild-type and mutants) coding for the proteins used in this work were generated as reported in Refs. [9,16,17], except for the AR Thr113Gln mutant. The latter was obtained using the wild-type AR cDNA cloned into pET-16b as a template and following the manufacturer's instructions for the Quickchange™ Site-Directed Mutagenesis Kit method (Stratagene. Two mutagenic primers were used: 5′- CTACCTTATTCACTGGCCGCAGGGCTTTAAGCCTGGGAAGG-3′, and 5′- CCTTCCCAGGCTTAAAGCCCTGCGGCCAGTGAATAAGGTAG-3′, amino acid positions 106–120 in both cases. Mutated nucleotides are underlined. DNA sequence was verified to ensure that unwanted mutations were absent.

2.3. Enzyme expression and purification

AR (wild-type or Thr113Gln mutant) and AKR1B10 (wild-type or AKME2MU) were expressed and purified as reported [18]. Briefly, *E. coli* BL21(DE3) pLysS cells were grown in 2 × YT medium at 37 °C until $O.D._{600} = 0.6$ was reached. Protein expression was induced for 4 h at 22 °C by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG, GE Healthcare Life Sciences). Proteins were purified using a nickel-charged His-Trap™ HP chelating column (GE Healthcare Life Sciences). The enzymes were eluted by a 0.06–1.0 M imidazole gradient in 50 mM Tris–HCl, pH 8.0, 100 mM NaCl. Fractions containing AKR were pooled and the buffer was exchanged to 10 mM sodium phosphate, 1 mM EDTA, pH 7.4 buffer, prior to flash freezing in liquid nitrogen. AKR1B15 expression and purification is detailed in Ref. [17].

2.4. Enzymatic assays and determination of the IC_{50} values

Activity was measured at 25 °C by monitoring the rate of disappearance of NADPH in 0.6 mL of 0.1 M sodium phosphate buffer (pH 7.4) containing 100 nM enzyme, 0.2 mM NADPH, 6 or 60 mM D,L-glyceraldehyde for AR or AKR1B10, respectively, 1% (v/v) DMSO and various concentrations of inhibitor. The IC_{50} value was determined as the compound concentration that inhibits the enzymatic activity by 50%. IC_{50} was calculated using the Grafit program (version 5.0; Erithacus Software), and the values were determined as the mean of two experiments±the standard error. AKR1B15 IC_{50} determination is detailed in Ref. [17].

2.5. Crystallization, data collection and structure determination

After purification, AKME2MU was subjected to lysine reductive methylation, using a reported methodology [9]. Co-crystallization was obtained by the hanging-drop vapor-diffusion method at 24 °C, using 1 mM protein, 4 mM $NADP^+$ and 40 mM UVI2008. The holoenzyme-ligand solution was mixed with an equal volume of precipitating solution consisting of 100 mM sodium cacodylate, pH 9.0, 30% polyethylene glycol 6000. X-ray data were collected in the X06DA beamline at the Swiss Light Source synchrotron, and processed with the program HKL2000 [19]. The structures were solved by molecular replacement with Phaser [20] (the model used was PDB ID 1ZUA). Finalized sets of atomic coordinates were obtained after iterative rounds of model modification with Coot [21] and refinement with REFMAC5 [22] and PHENIX [23]. Generation of the ligand coordinates and restraints were created using eLBOW [24]. The coordinate data for the solved structure were deposited in the Protein Data Bank with the PDB ID 5M2F. Data collection and refinement statistics are displayed in Table S1 of the Supporting Information (SI). Related figures were prepared with PyMOL (v.1.3; Schrödinger).

2.6. Docking simulations

Docking was performed with AutoDock 4.0 on a Linux workstation, as described before [14]. Briefly, coordinates for all retinoid ligands were generated with eLBOW [24] (for UVI2007 and UVI2110), the Grade Web Server (http://grade.globalphasing.org, for UVI2107), and the PDB for all-*trans*-retinaldehyde (ligand ID RET). Arg125 and Leu301 residues of the holoenzyme-UVI2008 PDB were mutated *in silico* with Coot [21] back to the AKR1B10 wild-type corresponding residues (Lys125 and Val301), and ligands and water molecules were removed before docking. The protein molecule was kept rigid. The dimensions of the grid were 50 × 50 × 50 Å, with a spacing of 0.375 Å between the grid points. In all the cases, the grid was centered at the starting ligand, manually superimposed to crystallographic UVI2008 in Coot. The suitability of the coordinates of the grid center was evaluated by redocking UVI2008. The ligand docking was accomplished by performing 150 runs of a Lamarckian genetic algorithm. Related figures were prepared with PyMOL (v.1.3; Schrödinger).

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