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Structural insights into the binding mechanism of IDO1 with hydroxylamidine based inhibitor INCB14943

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ABSTRACT

IDO1 (indoleamine 2, 3-dioxygenase 1), a well characterized immunosuppressive enzyme, has attracted growing attention as a potential target for cancer immunotherapy. Hydroxylamidine compounds INCB024360 and INCB14943 (INCB024360 analogue) are highly effective IDO1 inhibitors. INCB024360 is undergoing clinical trials for treatment of various types of human cancer. Here, we determined the co-crystal structure of IDO1 and INCB14943, and elucidate the detailed binding mode. INCB14943 binds to heme iron in IDO1 protein through the oxime nitrogen. Further analysis also reveals that a halogen bonding interaction between the chlorine atom (3-Cl) of INCB14943 and the sulphur atom of C129 significantly improves the inhibition activity against IDO1. Comparing with the other reported inhibitors, the oxime nitrogen and halogen bond interaction are identified as the unique features of INCB14943 among the IDO1 inhibitors. Thus, our study provides novel insights into the interaction between a small molecule inhibitor INCB14943 and IDO1 protein. The structural information will facilitate future IDO1 inhibitor design.

1. Introduction

IDO1 is a heme-containing non-secretory blood enzyme, which was originally identified and isolated from rabbit in 1967 [1]. IDO1 as the first and rate-limiting enzyme of kynurenine pathway catalyzes the oxidative cleavage of L-tryptophan (Trp) indole ring to generate kynurenine. Except kynurenine, there are several biological active metabolites produced in kynurenine pathway, including kynurenic acid and quinolinic acid (Quin) [2]. The accumulation of toxicity metabolites can inhibit the immune response of T cell and induce T cell inactivation, apoptosis and immunosuppression [3], resulting to disorders in nerve system, including Huntington's disease, Alzheimer's disease and Parkinson's disease [4,5]. IDO1 was well documented to inhibit the proliferation of T lymphocytes, NK cells and plasma cells through regulating Trp metabolism and promote malignant tumor immune escape by inhibiting effector function [6–8]. In addition, IDO1 over-expression has been frequently detected in various cancer cells [7], including breast [9], brain [10], lung [11] and ovarian cancer cells [12], as well as in tumor-draining lymph nodes [13]. Moreover, IDO up-regulation is correlated with poor prognosis in several human cancers [10]. Thus, IDO1 has become an attractive target for cancer immunotherapy. Preclinical investigations have demonstrated that blockage of IDO1 synergized the effect of chemical or radiation therapy [14,15].

Several classes of inhibitors have been reported to date. Examples include 1-methyltryptophan (1-MT) [16], 4-phenyl imidazole (4-PI) analogues [17,18], thiazolotriazole analogue [19], imidazothiazole derivatives [20], hydroxylamidine compounds (e.g., INCB024360 and INCB14943) [21,22], and imidazoleisoindole derivative [23]. Among these reported inhibitors, hydroxylamidine compounds and imidazoleisoindole derivatives exhibit the best inhibitory potency against IDO1 with IC_{50} or K_i values in nanomolar range. For instance, hydroxylamidine compound INCB024360 and its analogue INCB14943 displayed an IC_{50} of 7.4 nM [22] and 19 nM in Hela cells [21], respectively, whereas an imidazoleisoindole derivative NLG919 exhibited a K_i value of 7 nM in vitro or cell based

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assay [24]. Both INCB024360 and NLG919 have been advanced into clinical investigation for the treatment of various human cancers. Most recently, Peng et al. reported several NLG919 analogues co-crystallized with IDO1 [23]. The complex structures revealed that NLG919 analogues spanned and were located deeply in two binding sites (i.e., pocket A and pocket B). Consistent with the other reported complex structures, the NLG919 analogues coordinated with heme iron in IDO1 through a nitrogen atom in the imidazole ring. Further Structure-Activity Relationship (SAR) analysis also demonstrated that the intramolecular hydrogen bond between hydroxyl group and isoindole is critical for NLG919 derivatives to adopt an appropriate conformation to bind with IDO1.

Comparing to NLG919 analogues, INCB024360 and INCB14943 possess entirely different chemical scaffold. The binding details of IDO1 with INCB024360 or INCB14943 remain elusive. Herein, we report the crystal structure of IDO1 in complex with INCB14943. The knowledge of the structural feature of the detailed interaction between IDO1 and INCB14943 will greatly facilitate the structure-based design of IDO1 inhibitors with improved potency.

2. Materials and methods

2.1. Expression and purification

The cDNA fragments of hIDO1 were inserted into pET-28a and verified by sequencing. Proteins were expressed in Rosetta 2 (DE3) cells grown in TB medium. hIDO1 was expressed at 16 °C for 24 h after induction with 0.5 mM isopropyl β-D-1-thiogalactopyranoside at A_{600} = 0.6–0.8. Bacteria were collected by centrifugation, then washed, and resuspended in lysis buffer (20 mM Tris-HCl pH 8.0, 300 mM NaCl, 20 mM Imidazole and 7 mM β-mercaptoethanol) at 4 °C. Cells were lysed by three cycles under high pressure. hIDO1 was purified using a Ni affinity column (GE Healthcare) following standard protocols. Eluted protein was further purified by gel filtration using a Superdex 75 column (GE Healthcare) equilibrated against buffer containing 20 mM MES (pH 6.5), 25 mM NaCl.

2.2. Co-crystallization, data collection, and structure determination

hIDO1 was incubated with INCB14943 in 1:2 M ratio. The mixture (concentrated to 17 mg/ml) was crystallized at 293 K by the sitting-drop vapor diffusion method using the sparse matrix crystallization kit from Wizard I. Co-crystals of hIDO1-INCB14943 were grown in 10% (w/v) PEG-8000, 0.1 M CHES pH 9.5, 0.2 M NaCl. Prior to data collection, crystal was cryo-cooled using the mother liquor with 30% glycerin added as the cryoprotectant. X-ray diffraction data was collected at 100 K using synchrotron radiation on beamline BL19U1 at the Shanghai Synchrotron Radiation Facility. The data were processed with Mosflm [25], and scaled with Aimless [26]. Five percent of the data were randomly selected for the R-free calculation. The molecular replacement was conducted with Molrep [27] using the co-crystal structure of IDO1 with NLG919 analogue (PDB ID: 5EK3) as the starting model. Coot [28] was used to complete the model building. The model was refined using REFMAC5 [29]. The quality of each model was checked using MolProbity [30]. Data collection and processing statistics are listed in Table 1.

3. Results

3.1. Co-crystal structure of IDO1/INCB14943

The crystal structure of IDO1/INCB14943 complex was determined with resolution at 3.2 Å, in $P2_12_12_1$ space group, with two molecules in the asymmetric unit. The density map for two monomers was clear except for residues 1–9 and 361–381 in monomer A, and residues 1–11 and 362–379 in monomer B. IDO1 consists of a large domain and a small domain, as described previously [18]. The large domain holds an endogenous heme, and H346 is coordinated to the heme iron atom (Fig. 1A). The compound INCB14943 is accommodated in the active site of IDO1.

Table 1
Data collection and refinement statistics.

PDB code	5XE1
Data collection	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	87.05, 97.38, 128.47
Resolution (Å)	97.38–3.20 (3.42–3.20)[a]
R_{merge}[b]	0.137 (0.819)
I/σ*I*	8.7 (2.0)
Completeness (%)	98.4 (97.6)
Redundancy	6.7 (6.0)
Refinement	
Resolution (Å)	25.00–3.20
No. reflections	17190
R_{work}[c]/R_{free}[d] (%)	22.78/27.41
No. atoms	
Protein	5888
Ligand/ion	122
B-factors	
Protein	89.18
Ligand/ion	87.05
R.m.s. deviations[e]	
Bond lengths (Å)	0.0089
Bond angles (°)	1.4105
Ramachandran[f]	
Most favored (%)	93.84
Outlier (%)	0.68

[a] The values in parentheses reer to statistics in the highest resolution bin.
[b] Rmerge = ∑hkl∑i|Ii(hkl)- <I (hkl)>|/∑hkl∑iIi(hkl), where Ii(hkl) is the intensity of an observation and <I (hkl)> is the mean value for its unique reflection; Summations are over all reflections.
[c] R-factor = ∑h|Fo(h) - Fc(h)|/∑hFo(h), where Fo and Fc are the observed and calculated structure-factor amplitudes, respectively.
[d] R-free was calculated with 5% of the data excluded from the refinement.
[e] Root-mean square-deviation from ideal values.
[f] Categories were defined by MolProbity.

3.2. Interaction details of INCB14943 with IDO1

In IDO1/INCB14943 complex structure, the density map clearly shows that INCB14943 is covalently bond with heme iron atom through a nitrogen atom in the oxime moiety of the molecule (Fig. 1B). The phenyl ring is situated deeply in pocket A and forms hydrophobic interactions with V130, F164 and L234, as well as a side-to-face π-π stacking interaction with Y126, and a face-to-face π-π stacking interaction with F163 (Fig. 2). In addition, two halogen atoms (3-Cl and 4-F) in the phenyl ring could form distinct interactions with residues in pocket A: the chlorine group (3-Cl) forms a strong halogen bonding interaction with the sulphur atom of C129, whereas the fluorine group (4-F) forms hydrophobic interactions with V130 and F164. These interactions might give a plausible explanation on the previously observation that deletion of fluorine group (4-F) barely affected the activity of INCB14943 derivatives, while replacement of chlorine group (3-Cl) with a methyl moiety caused a dramatic 6-fold potency loss [21]. A hydrogen bond was also observed between the NH_2 group in the furazan moiety with side chain of S263 in IDO1 (Fig. 2B). It was also noteworthy that the hydroxyl group in the oxime moiety could form strong intramolecular hydrogen bond networks with the NH_2 group in the furazan moiety and the linkage NH moiety of the

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