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Characterization of crystal water molecules in a high-affinity inhibitor and hematopoietic prostaglandin D synthase complex by interaction energy studies

Daisuke Takaya[a,1], Koji Inaka[b,1], Akifumi Omura[c,1], Kenji Takenuki[c], Masashi Kawanishi[c], Yukako Yabuki[a], Yukari Nakagawa[a], Keiko Tsuganezawa[a], Naoko Ogawa[a], Chiduru Watanabe[a], Teruki Honma[a], Kosuke Aritake[d,e], Yoshihiro Urade[d], Mikako Shirouzu[a], Akiko Tanaka[a,*]

[a] RIKEN Center for Biosystems Dynamics Research, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan
[b] MARUWA Foods and Biosciences, Inc. 170-1 Tsutsui-cho, Yamatokoriyama, Nara 639-1123, Japan
[c] Asahi Kasei Pharma Corporation, 632-1 Mifuku, Izunokuni, Shizuoka 410-2321, Japan,
[d] International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, Tsukuba, Ibaraki 305-8575, Japan
[e] Daiichi University of Pharmacy, 22-1 Tamagawa, Minami, Fukuoka 815-8511, Japan

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ABSTRACT

Hematopoietic prostaglandin D synthase (H-PGDS) is one of the two enzymes that catalyze prostaglandin D_2 synthesis and a potential therapeutic target of allergic and inflammatory responses. To reveal key molecular interactions between a high-affinity ligand and H-PGDS, we designed and synthesized a potent new inhibitor (K_D: 0.14 nM), determined the crystal structure in complex with human H-PGDS, and quantitatively analyzed the ligand–protein interactions by the fragment molecular orbital calculation method. In the cavity, 10 water molecules were identified, and the interaction energy calculation indicated their stable binding to the surface amino acids in the cavity. Among them, 6 water molecules locating from the deep inner cavity to the peripheral part of the cavity contributed directly to the ligand binding by forming hydrogen bonding interactions. Arg12, Gly13, Gln36, Asp96, Trp104, Lys112 and an essential co-factor glutathione also had strong interactions with the ligand. A strong repulsive interaction between Leu199 and the ligand was canceled out by forming a hydrogen bonding network with the adjacent conserved water molecule. Our quantitative studies including crystal water molecules explained that compounds with an elongated backbone structure to fit from the deep inner cavity to the peripheral part of the cavity would have strong affinity to human H-PGDS.

1. Introduction

Prostaglandin (PG) D_2, produced from arachidonic acid, is important in the pathogenesis of inflammatory diseases[1,2] and regulation of physiological sleep.[3,4] Hematopoietic prostaglandin D synthase (H-PGDS) is one of the two enzymes that catalyze the isomerization of PGH_2 to PGD_2. Inhibition and knockout studies of H-PGDS have indicated its involvement in allergic and inflammatory responses, and thus human H-PGDS is a potential therapeutic target.[1,2,5]

In 2003, Inoue et al. reported the crystal structures of human H-PGDS containing Mg^{2+} and glutathione (GSH), an essential co-factor for the enzyme.[6] The asymmetric unit of the crystal lattice contained two homodimers of H-PGDS. The monomer of H-PGDS contains a large catalytic cavity sandwiched between the *N*-terminal (residues 2-71) and C-terminal (residues 82–199) domains.[6] The catalytic cavity of H-PGDS is subdivided into three regions: the inner cavity surrounded by Arg14, Met99, Tyr152, and Asp96, the central cavity surrounded by Trp104, and the thioate anion of GSH, and the peripheral solvent-exposed part of the pocket.[7,8] In 2004, the first crystal structure of human H-PGDS in complex with an inhibitor was reported.[9] Since then, many inhibitors and candidate drugs against H-PGDS have been developed and reported by pharmaceutical companies.[1,8,10]

In high-affinity inhibitor design, the decision of whether to engage or displace conserved water molecules is an important subject. Thus, the conserved water molecule near Leu199 in the catalytic cavity of human H-PGDS has been drawing attention.[8,10–12] Thorarensen's group

* Corresponding author at: RIKEN Center for Biosystems Dynamics Research, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan.
E-mail address: aktanaka@riken.jp (A. Tanaka).
[1] These authors contributed equally to this work.

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at Pfizer reported that the complex structures of H-PGDS with nanomolar potency inhibitors all showed the hydrogen bonding between the conserved water molecule and ligands. They tried to displace the water molecule by their new ligands to enhance the binding affinity due to the entropic gain; however, they concluded that the maximal affinity for H-PGDS required ligands to form a hydrogen bonding with the water molecule.[13] Therefore, we aimed to identify all the crystal water molecules binding stably in the H-PGDS catalytic cavity and quantify their contributions to ligand binding by the fragment molecular orbital (FMO) calculation method.[14] This information will be valuable for future drug-design of high-affinity H-PGDS inhibitor molecules.

The interactions important in the ligand-protein binding are hydrophobic and electrostatic interactions, and hydrogen bonding. To understand these interactions the quantum mechanics (QM) calculation method is one of powerful approaches, because the method takes into consideration the effects of donating and withdrawing electrons and π-π interactions. The FMO method has been developed to apply the QM calculations to large biomolecules.[14–20] The method is achieved by dividing a large molecule into small pieces called fragments, such as amino acid residues, water molecules, ions, and ligands. The FMO method evaluates the electronic states of each fragment pair, and indicates their interaction magnitude in individual fragment levels with the Inter-Fragment Interaction Energy (IFIE).[21] Further, the Pair Interaction Energy Decomposition Analysis (PIEDA) indicates the energy components of IFIE: electrostatic interaction (ES), dispersion interaction (DI), charge transfer with higher-order mixed terms energies (CT + mix), and exchange-repulsion (EX).[20,22,23] The ES component mainly reflects the hydrogen bonding and Coulomb interaction energies, and the DI component reflects the CH-π and π-π interaction energies. Thus ES and DI components are important for protein-ligand interactions.

In this paper, we designed and synthesized a high-affinity ligand for human H-PGDS, and then their complex crystal structure was determined. Based on the structure, FMO calculations were performed to quantify the ligand-protein interactions in fragment levels to reveal all the stably bound water molecules in the cavity and their contributions to the ligand binding.

2. Materials and methods

2.1. Synthesis of F092

2.1.1. General

First, 4-(2-oxopyrrolidin-1-yl)benzoic acid (**1**) was converted to the *N*-Boc aniline (**2**) via a Curtis rearrangement reaction under conventional conditions, and then the deprotection reaction of the Boc group was accomplished using HCl in 1,4-dioxane to produce 1-(4-aminophenyl)pyrrolidin-2-one (**3**). Subsequently, the amidation reaction of **3** and 2-(pyridin-2-yl)pyrimidine-5-carboxylic acid was performed by the addition of propylphosphonic anhydride (T_3P®), to provide F092 (**4**) in good yield.

Progress of all reactions was monitored on Merck pre-coated silica gel plates using ethyl acetate (EtOAc)/hexane as a solvent system. Spots were visualized by irradiation with ultraviolet light (254 nm). Column chromatography was performed using Yamazen silica gel 60 (230–400 mesh). Proton (^{1}H) NMR spectra were recorded on a Bruker Avance 400 instrument using tetramethylsilane as an internal standard. Chemical shifts are given in parts per million (ppm) (δ relative to the residual solvent peak for ^{1}H). The following abbreviations are used: singlet (s), doublet (d), triplet (t), and multiplet (m). ESI mass spectrometry was performed by UPLC/MS (Waters).

2.1.2. tert-Butyl (4-(2-oxopyrrolidin-1-yl)phenyl)carbamate (2)

Diphenyl phosphorazidate (DPPA) (0.251 ml, 1.2 eq.) was added dropwise to a solution of 4-(2-oxopyrrolidin-1-yl)benzoic acid (**1**) (201 mg, 1 eq.), tert-BuOH (0.93 ml, 10 eq.) and trimethylamine (Et_3N) (0.272 ml, 2.0 eq.) in toluene (4.8 ml) at room temperature (rt). The resulting pale yellow solution was stirred at 120 °C for 16.5 h under N_2 atmosphere. The resulting brown mixture was cooled to rt and saturated $NaHCO_{3(aq)}$ (5 ml) was added to the mixture. The mixture was extracted twice with EtOAc (10 ml), and the combined organic extracts were washed with brine (5 ml), dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica eluted with hexian-EtOAc (50:50, 30:70 and 20:80) gave **2** (183 mg, 68%) as a white solid; R_F (33:67 hexane-EtOAc) 0.45; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (dd, J = 2.4, 2.0 Hz, 2H, Ph), 7.42 (d, J = 4.4 Hz, 2H, Ph), 3.78 (t, J = 7.2 Hz, 2H, NCH_2), 2.45 (t, J = 7.2 Hz, 2H, CH_2CO), 2.04 (tt, J = 7.2, 7.2 Hz, 2H, CH_2), 1.47 (s, 9H, tert-Bu); ESI-MS m/z calculated for $C_{15}H_{20}N_2O_3^+$ H^+ $[M+H]^+$; 277.2. Found: 277.3.

2.1.3. 1-(4-Aminophenyl)pyrrolidin-2-one (3)

To a solution of **2** (183 mg, 1.0 eq.) in 1,4-dioxane (0.67 ml) was added 4 M HCl in 1,4-dioxane (3.3 ml) at rt. The resulting solution was stirred at rt for 3 h to precipitate. Obtained precipitate was filtered, washed three times with 1,4-dioxane (5 ml), and dried under reduced pressure at 40 °C for 18 h to give **3** (116 mg, 83%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (brs, 2H, NH_2), 7.73 (d, J = 8.8 Hz, 2H, Ph), 7.33 (d, J = 8.8 Hz, 2H, Ph), 3.83 (t, J = 7.2 Hz, 2H, NCH_2), 2.50 (t, J = 7.2 Hz, 2H, CH_2CO), 2.07 (tt, J = 7.2, 7.2 Hz, 2H, CH_2); ESI-MS m/z calculated for $C_{10}H_{12}N_2O^+$ H^+ $[M+H]^+$; 177.1. Found: 177.3.

2.1.4. N-(4-(2-oxopyrrolidin-1-yl)phenyl)-2-(pyridin-2-yl)pyrimidine-5-carboxamide (4)

A solution of propylphosphonic anhydride in EtOAc (50 wt%, 6.45 ml, 2 eq.) was added to a solution of **3** (1.07 g, 1.1 eq.), 2-(pyridin-2-yl)pyrimidine-5-carboxylic acid (920 mg, 1.0 eq.) and Et_3N (5.10 ml, 8.0 eq.) in CH_2Cl_2 (45.7 ml) at rt. The resulting solution was stirred at rt for 1 h to cause precipitation, which was collected by filtration and washed with EtOAc (2 x 10 ml). The obtained solid was dried under reduced pressure at 40 °C for 18 h to give **4** (840 mg, 51%) as a white solid. Saturated $NaHCO_{3(aq)}$ was added to the filtrate, then the mixture was stirred at rt for 30 min to give a precipitate, which was collected by filtration and dried to give **4** (597 mg, 36%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H, NH), 9.43 (s, 2H, NCH), 8.80 (d, J = 3.6 Hz, 1H, NCH), 8.47 (d, J = 8.0 Hz, 1H, NCH), 8.03 (t, J = 8.0 Hz, 1H, CH), 7.78 (d, J = 9.2 Hz, 2H, Ph), 7.69 (d, J = 9.2 Hz, 2H, Ph), 7.59 (dd, J = 3.6, 8.0 Hz, 1H, CH), 3.85 (t, J = 7.2 Hz, 2H, NCH2), 2.07 (tt, J = 7.2, 7.2 Hz, 2H, CH2); ESI-MS m/z calculated for $C_{20}H_{18}N_5O_2^+$ H^+ $[M+H]^+$: 360.1. Found: 360.3.

2.2. Purification of Histag H-PGDS

Human Histag H-PGDS was expressed and purified as described previously.[11] Briefly, the DNA fragment of the full-length H-PGDS gene (NCBI accession no.: NP 055300) fused with a 6x-Histidine tag at the *N*-terminus was cloned into the pET28a vector (Novagen, Madison, WI, USA) and expressed in *E. coli* one shot BL21 (DE3) (Thermo Fisher). The cells were grown in LB medium at 37 °C, induced with 1.0 mM IPTG, and then cultured further for 4 hr at 37 °C. The cells were collected and disrupted by sonication in 50 mM phosphate-buffer (pH 6.6), containing 2 mM $MgCl_2$, 0.4 mg/ml lysozyme, and 6 μg/ml DNase/RNase. After removal of the cell debris by centrifugation, the supernatant was filtered through a 0.45 μm PVDF filter and applied to a GSH-Sepharose 4B column. After washing the column with 50 mM phosphate-buffer (pH 6.6) containing 2 mM $MgCl_2$, the protein was eluted with 50 mM Tris-HCl, pH 9.0 containing 10 mM GSH. The eluted H-PGDS fractions were washed three times by PBS(-) and concentrated using an Amicon Ultra filtration device. Protein concentrations were determined with a Bradford protein assay kit (Bio-Rad).