Crystal Structure of the Catalytic Domain of Human Phosphodiesterase 5 with Bound Drug Molecules

In 1992, nitric oxide (NO) was recognized by Science magazine as a molecule of the year and ever since NO has become an increasingly popular target of investigation. Why has such a small molecule received so much attention? It is primarily because of the extraordinary biological effects and medically relevant processes mediated by NO. NO exerts part of its effect by binding to the heme of guanyl cyclase which leads to the production of cyclic GMP (cGMP; Fig. 1). In the immune system, macrophage cells produce cGMP and NO as part of the oxidative cytotoxic arsenal resulting from macrophage stimulation (i.e., infection).

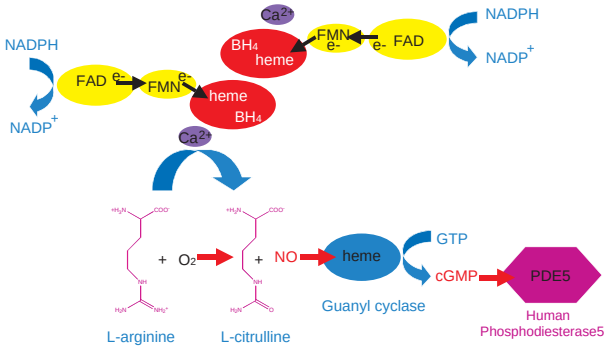

Fig. 1. Schematic representation of how PDE5 functions.

Phosphodiesterases (PDEs) are a superfamily of enzymes which degrade the intracellular second messengers cGMP and cyclic AMP (cAMP). As essential regulators in cyclic nucleotide signaling with diverse physiological functions, PDEs are drug targets for the treatment of various diseases including heart failure, depression, asthma, inflammation, and erectile dysfunction. Of the twelve PDE gene families, the cGMP-specific PDE5 is the principal cGMP-hydrolysing activity in the human corpus cavernosum tissue. It is well known as the target of the drugs sildenafil citrate (Viagra®, Pfizer), tadalafil (Cialis®, Lilly-ICOS), and vardenafil (Levitra®, Bayer & GlaxoSmithKline) for the treatment of erectile dysfunction. Despite the pressing need to develop selective PDE inhibitors as therapeutic drugs, only cAMP-specific PDE4 structures have been available [1]. To discover PDE inhibitors by rational structure-based drug design, we have determined the three-dimensional structures of the catalytic domain (residues 537-860) of human PDE5 complexed with the three drug molecules, sildenafil, tadalafil and vardenafil [2], at SPring-8 beamline **BL44B2** and at PSL (Pohang Light Source).

The catalytic domain of PDE5 has three helical subdomains, an N-terminal cyclin-fold region, a linker region and a C-terminal helical bundle (Fig. 2). The overall topology is similar to that of the homologous PDE4, although PDE5 has only 23% sequence identity with PDE4 in the catalytic region. The active site of PDE5 is located at the center of the C-terminal helical bundle domain. The substrate pocket is approximately 10 Å deep, with a narrow opening and a wide inner space, giving a total volume of about 330 Å^3. It is composed of four subsites, a metal binding site (M site), core pocket (Q pocket), hydrophobic pocket (H pocket), and lid region (L region) (Fig. 3(a)). Overall, the M site and Q pocket are similar to those of PDE4, but the H pocket and the L region show significant structural differences from those of PDE4 (Fig. 4).

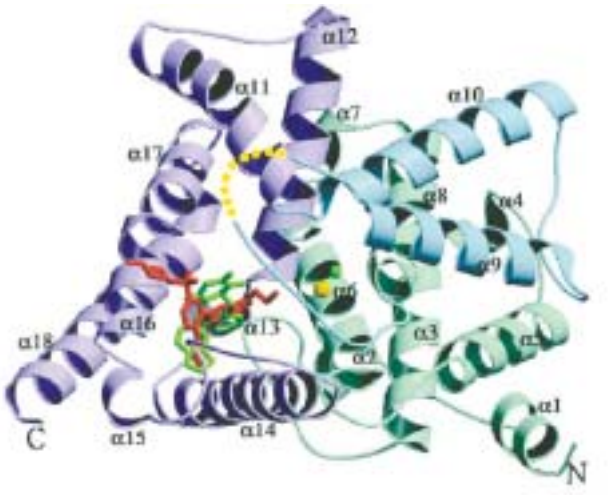

Fig. 2. Overview of PDE5 complex structure. The catalytic domain of the PDE5 molecule can be divided into three subdomains: N-terminal cyclin-fold domain (residues 537-678 in gray), linker helical domain (residues 679-725 in light green) and C-terminal helical bundle domain (residues 726-860 in violet). The bound sildenafil and tadalafil molecules are overlapped and shown by stick models (red and green, respectively) [2].

The binding mode of tadalafil is different from that of sildenafil (Figs. 3(a) and 3(b)). Tadalafil makes no interaction with the L region of the protein. The Q pocket also makes different interactions between the two ligands. The Oe atom of Gln forms a single, not bidentate, hydrogen bond with NH of tadalafil. The H pocket, occupied with an ethyloxy group in the sildenafil complex, is filled with the methylenedioxyphenyl group of tadalafil. The more extensive interactions with the H pocket may be one of the reasons that tadalafil maintains a high affinity without binding to the L region. A comparison of the structures of the three complexes suggests obvious modifications (for example, changing the ethyloxy group of sildenafil to fit to the H pocket) of the inhibitors in order to improve their binding affinity and selectivity. Vardenafil shows a binding mode very similar to that of sildenafil.

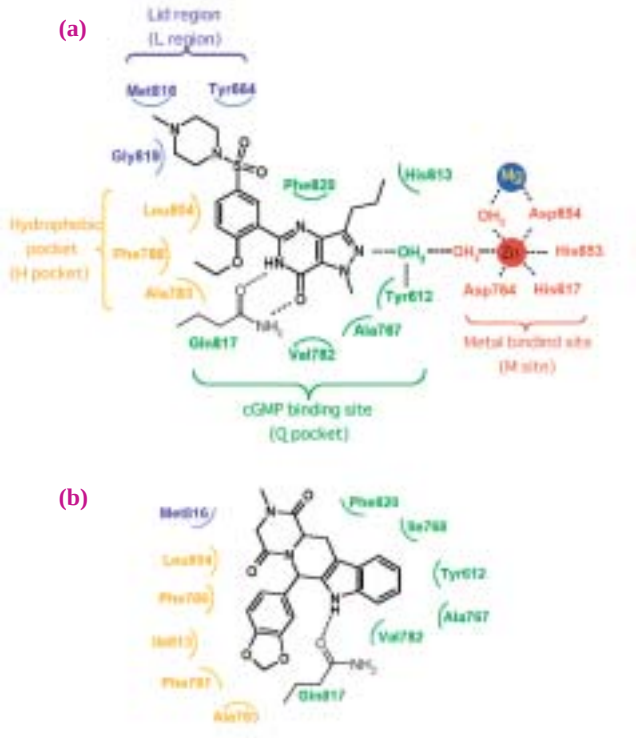

Fig. 3. **(a)** Schematic representation of interactions made by the sildenafil in complex with PDE5. Residues in four subsites are colored differently: M subsite in red, Q subsite in green, H subsite in yellow and L region in blue. The hydrogen-bonding interactions are only shown in dashed lines. **(b)** Schematic representation of interactions made by the tadalafil in complex with PDE5. The colors used are the same as those in **(a)** for the residues in four subsites.

In conclusion, we have presented here the three-dimensional structures of PDE5 complexed with the three inhibitors in clinical use, sildenafil, vardenafil, and tadalafil. Our study provides the first clear picture of the different binding modes of these ligands in the active site of PDE5. We believe that these structural studies will assist the discovery of potent and selective PDE inhibitors that exhibit improved pharmacological profiles.

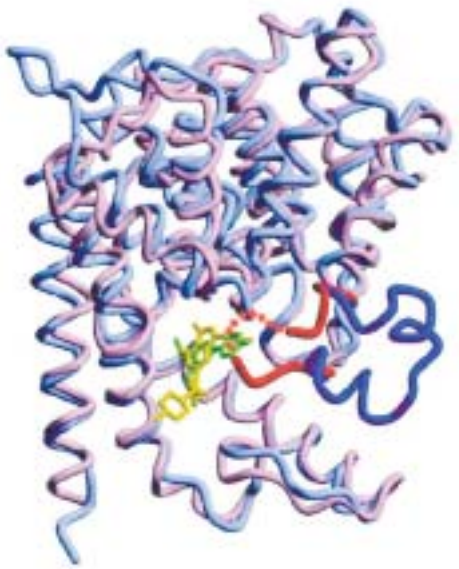

Fig. 4. Comparison of PDE5 and PDE4 active sites. Superimposed structures of PDE5 and PDE4 showing a difference in folding at the active site. The backbone worm presentations of PDE5 and PDE4 are shown in red and blue, respectively. To emphasize the structural difference, only the backbone traces of aa.304-325 of PDE5 and aa.660-680 of PDE4 are saturated with their respective colors. The stick model of sildenafil is shown in yellow, and that of zardaverine in green [2].

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References

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