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Mini Review

Binding of YC-1/BAY 41-2272 to soluble guanylate cyclase: A new perspective to the mechanism of activation

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ABSTRACT

Soluble guanylate cyclase (sGC), a heterodimeric heme protein, catalyses the conversion of GTP in to cyclic GMP, which acts as a second messenger in cellular signaling. Nitric oxide activates this enzyme several hundred folds over its basal level. Carbon monoxide, along with some activator molecules like YC-1 and BAY, also synergistically activate sGC. Mechanism of this synergistic activation is a matter of debate. Here we review the existing literature to identify the possible binding site for YC-1 and BAY on bovine lung sGC and its mechanism of activation. These two exogenous compounds bind sGC on α subunit inside a pocket and thus exert allosteric effect via subunit interface, which is relayed to the catalytic site. We used docking studies to further validate this hypothesis. We propose that the binding of YC-1/BAY inside the sensory domain of the α subunit modulates the interactions on the subunit interface resulting in rearrangements in the catalytic site into active conformation and this partly induces the cleavage of Fe–His bond.

1. Introduction

Soluble guanylate cyclase (sGC) serves as a physiological receptor for nitric oxide (NO) [1]. It is a cytosolic heme protein that catalyses the conversion of GTP in to cyclic GMP [1,2]. This enzyme is a heterodimeric protein and contains α and β subunits. Domain structures for sGC have been proposed based on sequence similarity with other proteins, although three dimensional structure for the full protein is not available yet [3,4]. It consists of a sensory domain (termed as H-NOX domain) that binds heme on the N-terminus followed by a PAS domain, a linker helix and finally the catalytic domain at the C-terminus and both the subunits are involved in the domain architecture [3,4].

An iron-protoheme is bound to its β subunit sensory domain through an invariant His residue (His-β105, for bovine sGC) [5]. The resting sGC has a five-coordinate (5c) high-spin Fe^{II}-heme and the His-bound 5c heme is further stabilized through the interaction of its propionate side chains with Tyr135 and Arg139 of the β subunit [6]. Upon NO binding to the heme, the Fe–His (β105) bond is cleaved due to the strong negative *trans*-effect of NO [7,8] resulting in higher enzymatic activity up to 400 fold [8]. However, contradicting models have been proposed to explain the mechanism of this activation [9,10] as the exact mechanism of this activation is not known.

Carbon monoxide (CO) also marginally activates sGC [11]. However, in the presence of certain exogenous compounds such as 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1) or 3-(4-amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-b]pyridine (BAY 41-2272, abbreviated as BAY hereafter) synergistically activate the enzyme to the level of NO-bound sGC [12,13]. Several groups have proposed that the cleavage of the Fe–His bond in sGC and the subsequent formation of CO-bound 5c heme are responsible for the increased activity in the presence of CO and YC-1/BAY) [13–16]. On the other hand, others have argued that the cleavage of the Fe–His (β105) bond is not necessary for the increased activity of sGC–CO [3,17]. However, in a recent study, same group has proposed the presence of 5c sGC–CO as active population [4].

Similarly, the binding site(s) for YC-1 and BAY is also ambiguous. Previously, it was proposed that YC-1 binds in heme pocket of β subunit [18]. However, the same group recently proposed that YC-1 binds next to sensory domain on α subunit [4]. A BAY related compound (BAY 58-2667) was proposed to bind in the heme pocket of β subunit [6]. We also proposed that YC-1 or BAY binds near the heme pocket in the proximity of the vinyl groups for the CO-bound sGC [15,16]. However, others proposed YC-1/BAY binds on α subunit of sGC [19,20].

Crystal structure of sGC is not available, although, in recent times structures of some domains from different organisms including human have been solved [21–25]. However, none of those structures could explain how exogenous compounds modulate

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the activity of CO-bound sGC. In this review we used existing knowledge along with homology modeling and docking studies to propose a model for YC-1/BAY binding to sGC. Present study could explain the rationale for the activation of sGC in the presence of YC-1/BAY.

2. Homology models of sGC sensory domains

Several homology models of sensory domains of both α and β subunits of sGC are available [20,26–28]. However, to correlate with our own experimental observations, models of N-terminal sequences (1–300 amino acid residues) of both α and β subunits of bovine sGC were selected from the data bank and submitted for BLASTP in PDB. β subunit resulted in a hit with 34% identity and 56% similarity with the H-NOX domain from *Nostoc sp.* (PDB ID 2O09) for first 194 amino acids. However, α subunit could not find any similar sequence, even when different lengths of sequences were used. Then, both the sequences were submitted to automated modeling in 3Djigsaw comparative modeling server. PDB IDs 3EEE and 2O09 were automatically picked as templates by the server for α and β subunits, respectively, and were modeled. Models were checked and terminal regions were deleted and the truncated models were used for docking.

Model of α subunit contains residues Glu77 to Val232 whereas β subunit contains Met1 to Glu182. Overall structural model of sGC-α-1 resembles H-NOX domain from *Thermoanaerobacter tengcongensis* (*Tt*H-NOX) [24] whereas sGC-β-1 resembles H-NOX heme domain of *Nostoc punctiforme* (*Np*H-NOX) [25]. Both the domains from sGC-α-1 and sGC-β-1 have similar α/β fold. Typical SONO or H-NOX fold has seven helices and one four-stranded antiparallel β-sheet [23,24]. From the N-terminus, there are six helices followed by two strands, followed by a helix and then two strands. In the model of sGC-α-1 two helices and two strands are missing as first helix from the N-terminus and seventh helix along with last two strands could not be modeled. This matches well with the reported model [20]. Also, invariant His105 in β subunit is replaced by Leu171 in α subunit. There is a large cavity in the model of sGC-α-1, where heme is accommodated in the case of *Tt*H-NOX. Model of sGC-β-1 is very similar to *Np*H-NOX and matches well with the models reported [25–28].

3. Automated docking

Docking studies were carried out using web based DockingServer [29]. Coordinates of YC-1 and BAY, prepared using JChem software, were submitted along with the coordinates of the proteins. Energy minimization of ligand molecules were automatically carried out by DockingServer using MMFF94 force field [30]. Then docking calculations were carried out for the protein models. AutoDock tools automatically added essential hydrogen atoms, solvation parameters, etc. [31]. Autogrid program was used to generate affinity (grid) maps of $20 \times 20 \times 20$ Å grid points and 0.375 Å spacing [31]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [32]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied. To find the interface between α and β subunits, PatchDock server was used [33,34].

To check the efficiency of docking, first heme was docked in the β subunit of the model. The orientation of heme matched well with the related structure (data not shown). Finally, YC-1 and BAY were modeled in the α subunit although some of the residues on the loop region were removed by the software. YC-1 and BAY occupied the cavity inside the α subunit (Fig. 1). In fact, this possibility was raised in a similar sGC α-1 subunit from *Manduca sexta* [20]. However, how this will affect the structure/activity relationship is still unknown. To judge this further, α subunit was docked on the β subunit to find the interface. Automated docking studies using PatchDock resulted in an interface between α and β subunits (Fig. 2).

4. Allostery and subunit interface

Allostery plays a major role in protein functions and thus controls many cellular processes and substrate or ligand induced conformational changes in protein structure often control this allosteric regulation. One of the classic examples is oxygen binding in hemoglobin (Hb), which has been studied extensively. X-ray crystallographic studies of Perutz and others showed that Hb has

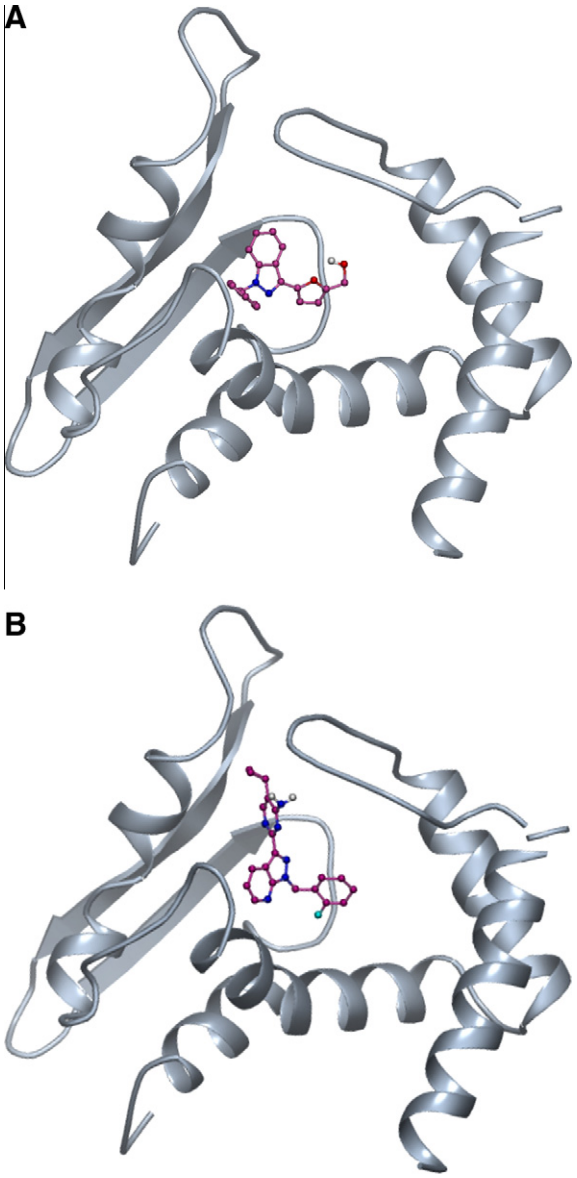

Fig. 1. Docking of (A) YC-1 and (B) BAY inside the sensory domain of α subunit of bovine sGC. YC-1 and BAY are represented as ball and stick model whereas the protein is represented as a cartoon.