



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.

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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- β 105, 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 (β 105) 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)-1H-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 (β 105) 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* (TtH-NOX) [24] whereas sGC- β -1 resembles H-NOX heme domain of *Nostoc punctiforme* (NpH-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 TtH-NOX. Model of sGC- β -1 is very similar to NpH-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

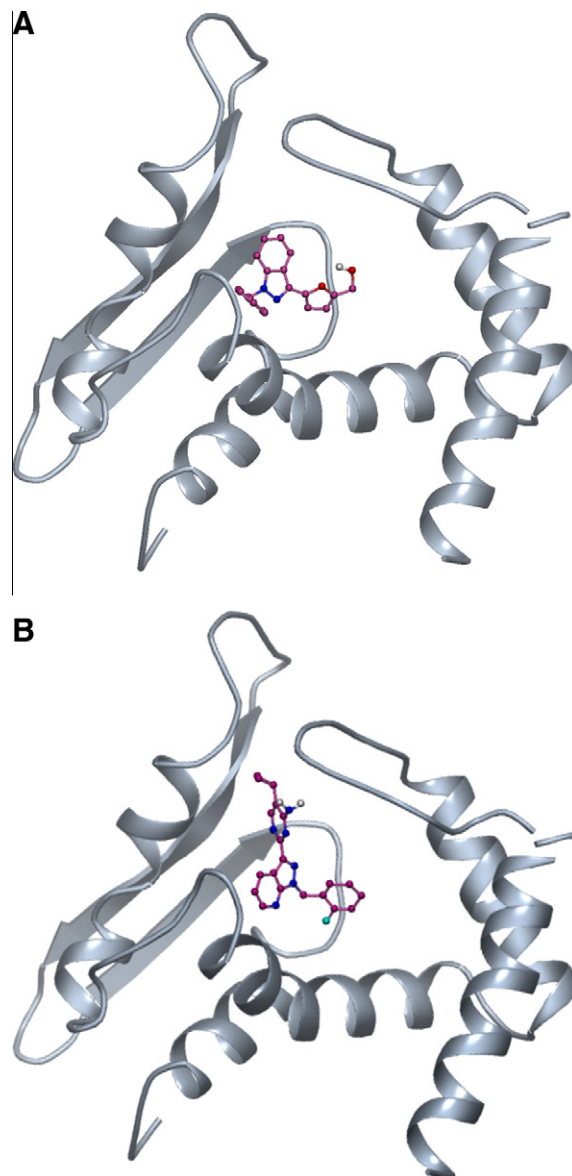


Fig. 1. Docking of (A) YC-1 and (B) BAY inside the sensory domain of α subunit of bovine sGC. YC-1 and BAY are represented as ball and stick model whereas the protein is represented as a cartoon.

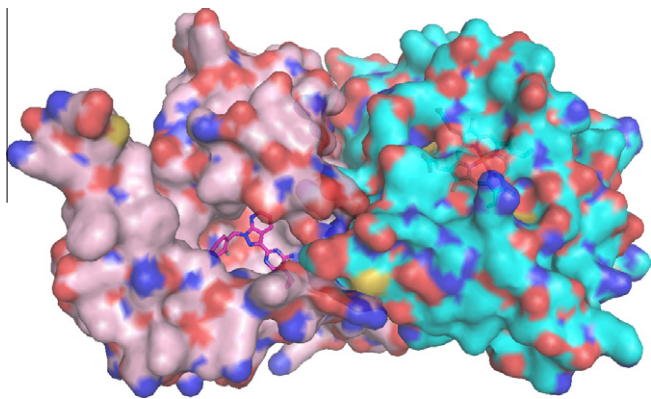


Fig. 2. Subunit interface of sensory domains of α and β subunits of bovine sGC. Purple represents α subunit and cyan represents β subunit, respectively. BAY and heme are seen inside the cavity. YC-1 also occupies the same pocket as that of BAY.

two classical quaternary structures. One is T (tense) state for the low-affinity deoxy-Hb and the other one is the high-affinity R (relaxed) state for liganded Hb [35].

Resonance Raman (RR) studies were also applied extensively to identify the nature of strains present in T and R state in hemoglobin. It has been shown that the Fe–His (F8) bond is weak in deoxy-Hb in the T state compared to its isolated chains [36]. This indicates that in the T state tetramer, when protein is strained, the Fe–His bond is stretched slightly and its bond strength becomes weaker. This result demonstrates that in the native Hb $\alpha_2\beta_2$ tetramer in the T state, the inter-subunit interactions actually induce strain in the individual subunits and modify their tertiary structures. When the inter-subunit interactions are relaxed, the strain disappears and the Fe–His bond becomes same as those in the isolated chains [37].

In case of sGC, it has been proposed that the Fe–His bond is very weak. RR studies indicate that Fe–His stretching frequency for sGC is lowest among known heme proteins [8,13–16]. This enzyme is a multi-domain, heterodimeric protein. It is very much likely that the regulation of sGC activation is controlled by its quaternary structure, much like Hb, and heme plays a crucial role in sensing diatomic gas signal, especially NO. CO, on the other hand, is not as potent ligand as NO, at least in the case of sGC. So far, no physiological stimulator for sGC–CO could be identified and only exogenous compounds are known to stimulate sGC–CO. Therefore, the mechanism of synergistic activation of sGC by CO and YC-1/BAY may be different from that of NO.

5. Binding site for YC-1/BAY

YC-1/BAY is large molecule compared to NO or CO and therefore a protein needs a larger pocket to accommodate these compounds. If heme is present in the β subunit, there may not be enough space to further accommodate YC-1/BAY. One possibility is it will ‘stick’ to the surface. Other possibility is to go to another similar pocket. Identification of binding site of YC-1/BAY is a decade old puzzle. It was proposed that it binds in heme pocket [18]. This speculation leads to our interpretation of YC-1 binding near the heme pocket [15,16]. Further, it has been shown that NO-independent BAY 58-2667 could be a mimic of heme and thus occupies heme cavity [6]. However, in a recent paper it has been proposed that YC-1/BAY could bind to the α subunit [4]. This seems more plausible and has been postulated by others as well [19,20]. This leads us to re-interpret YC-1/BAY binding to sGC.

Structural model of sensory domain of sGC- α -1 shows a large cavity that could accommodate YC-1 or BAY. This binding probably brings the structural changes in α subunit which is communi-

cated to β subunit via the subunit interface. This structural change possibly results in the cleavage in Fe–His bond in some population of sGC–CO. Binding of GTP to the catalytic site, which is again composed of both α and β subunits, also exerts ligand induced conformational changes further helping in subunit orientation. This should help catalytic site to reorient itself into a conducive conformation for the cyclization reaction of GTP.

6. Mechanism of activation

Now, the obvious question is, what is the role of Fe–His bond and if its role is different for NO and CO-bound sGC? There is unambiguous agreement among the researcher community that Fe–His bond is cleaved in NO-bound sGC because of negative *trans*-effect. However, it has been shown that NO binding and the cleavage of Fe–His bond itself does not activate sGC–NO fully [38] and additional NO or effector is necessary for full activation by NO [39]. This clearly indicates that local structural change in the sensory domain has to be communicated to the catalytic site for activation. Interactions at the subunit interface possibly play an important role in this allosteric communication. However, in the case of sGC–CO, in the presence of YC-1/BAY, there is no clear agreement among researcher. More studies add more ambiguity to it. For example, over a decade there was a speculation that Fe–His bonds cleavage was not required for maximal activation of sGC [17]. On the other hand, different groups believed that Fe–His bond in sGC–CO was cleaved in the presence of YC-1/BAY [13–16], which was seriously challenged [19]. This resulted in further investigations to show that both 5c and 6c sGC have activity [13,16]. In fact, it was further extrapolated that 6c sGC–CO in the presence of YC-1/BAY has both active and non-active population [4].

Recently, the NMR structure of CO bound H-NOX domain of *Shewanella oneidensis* has been solved for wild type and cavity mutant (H103G) [40]. Analysis of these two structures shows interesting differences. DSSP secondary structure analysis shows wild type is 51% helical (9 helices; 93 residues) and 14% β -sheet (4 strands; 27 residues) whereas the cavity mutant is 51% helical (7 helices; 94 residues) 13% β -sheet (4 strands; 25 residues). There are several structural differences between these two structures. However, most interesting one is the conversion of helix I to loop structure. Superimposition of these structures on β subunit of bovine sGC sensory domain model shows that this region interacts with loop regions of α subunit of sGC. If similar structural changes occur upon Fe–His bond cleavage in sGC, then this type of structural change may alter the interactions on the subunit interface (Fig. 3).

In this scenario, in the absence of three dimensional structure of both active and inactive conformation, it is possibly not possible to explain the mechanism of activation precisely. One needs to remember that heme domain serves as a marker for overall structural change, which is still unknown. Comparison with similar model proteins to draw a conclusion could be counterproductive. For example, the crystal structure of H-NOX (or SONO) domain of microbial proteins might not be conclusive either as the allostericity could not be correlated using these models, like in the case of myoglobin and Hb.

There are numerous examples of ligand induced allosteric structural changes in the literature. In a multimeric protein, it becomes more relevant. Active sGC being a heterodimer, binding of YC-1/BAY as well as GTP, its substrate, possibly induces allostericity. Effects of YC-1/BAY are more prominent in the sensory regions showing effects in the heme environment. On the other hand, effect of GTP binding in the catalytic side is less prominent on sensory domain.

The mechanism of NO and CO induced activation may not be exactly the same. In the case of NO, Fe–His bond is broken first and that reorients the catalytic site towards the active

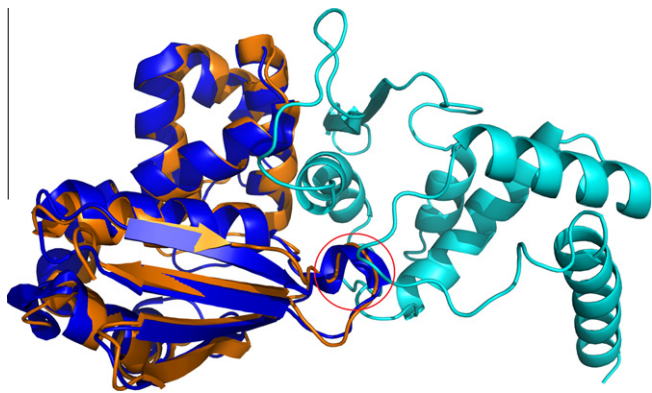


Fig. 3. Superimposition of H-NOX domain of *Shewanella oneidensis* on the bovine sGC model. Coordinates from 2KII and 2KIL are superimposed on the model of β -subunit of sGC. Blue cartoon represents CO bound wild type whereas gold represents CO bound H103G mutant in the presence of imidazole. Heme of H-NOX structure and the model of β -subunit of sGC are not shown in the figure for clarity. Helix to loop conversion region in H-NOX structure is marked with a red circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conformation and interactions on the subunit interface relay this information to the catalytic site. In the case of CO, binding of CO to the heme only marginally reorients the catalytic site without breaking the Fe–His bond. YC-1/BAY binding inside the sensory domain of the α subunit first modulates the interactions on the subunit interface resulting in rearrangements in the catalytic site into active conformation and this leads to the cleavage of Fe–His bond. Bonding of His to the heme iron actually renders some kind of strain to the protein and NO or CO and YC-1/BAY binding removes the strain and thus a quaternary structural change is induced to activate the enzyme. Changes in interaction patterns of vinyl and propionate side chains of heme in active and inactive conformation [4,16] supports this hypothesis.

7. Concluding remarks

CO, produced by the degradation of heme by heme oxygenase, has been confirmed as a signaling molecule. However, produced physiological concentration of CO may not be enough to activate sGC as such and it should be consumed immediately after synthesis as it lacks vesicular storage [41]. Existence of YC-1 or BAY like compounds under physiological conditions could be useful for the synergistic activation of sGC. *In vitro*, these xenobiotic compounds modulate the sGC activity in the presence of CO by reorienting the quaternary structure of sGC.

In summary, we explained the binding of YC-1/BAY to CO-bound sGC and the mechanism of synergistic activation. We propose that YC-1/BAY bind to the α subunit of sGC. This binding alters the interactions on the subunit interface and thus help catalytic site to reorient in an active conformation. As a result of this conformational change, Fe–His bond in sGC is broken in the presence of YC-1/BAY and GTP. However, till the three dimensional structure of full sGC is solved, explanation of mechanism of activation will be speculative.

Statement of conflicts of interest

There is no conflict of interest among the authors in this work.

Note added to the proof

Recently a structure of heme deficient *Nostoc sp.* H-NOX in BAY 58-2667 bound form has been solved and published [42]. The

structure shows that BAY 58-2667 occupies the heme cavity. This work also emphasizes the importance of inter-domain interaction.

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