Journal of Inorganic Biochemistry 167 (2017) 12–20

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Focused review

Drug discovery targeting heme-based sensors and their coupled activities

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ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form 8 November 2016
Accepted 16 November 2016
Available online 20 November 2016

Keywords:
Drug discovery
Heme-based sensor
Nucleotide cyclase
Phosphodiesterase
Histidine protein kinase
Nuclear receptor

ABSTRACT

Heme-based sensors have emerged during the last 20 years as being a large family of proteins that occur in all kingdoms of life. A myriad of biological adaptations are associated with these sensors, which include vasodilation, bacterial virulence, dormancy, chemotaxis, biofilm formation, among others. Due to the key activities regulated by these proteins along with many other systems that use similar output domains, there is a growing interest in developing small molecules as their regulators. Here, we review the development of potential activators and inhibitors for many of these systems, including human soluble guanylate cyclase, c-di-GMP-related enzymes, *Mycobacterium tuberculosis* DevR/DevS/DosT (**d**ifferentially **e**xpressed in **v**irulent strain **r**esponse regulator/**s**ensor/***do**rmancy **s**urvival sensor* **T**), the Rev-erb-α and β nuclear receptor, among others. The possible roles of these molecules as biochemical tools, therapeutic agents, and novel antibiotics are critically examined.

1. Introduction

A myriad of heme-based sensor proteins have emerged during the last two decades [2,3]. These biological sensors are modular: they contain at least one input domain that harbors a heme cofactor, and output domains that are involved in the biological response. So far, all domains that are capable of stably binding heme have been demonstrated to be capable of sensing, with examples known for PAS (**P**eriod-**S**im-**A**RNT) [4], GAF (c**G**MP-binding PDE, **a**denylyl cyclase, **F**hlA) [5], globin [6], HNOB (**h**eme **NO** **b**inding) [7], CooA (CO oxidation Activator) [8], LBD (**l**igand **b**inding **d**omain of nuclear receptor) [9], and SCHIC (**s**ensor **c**ontaining **h**eme **i**nstead of **c**obalamin) [10] domains. These input domains' detection of heme ligands can be coupled to a wide range of output domains and signal transduction modes, such as histidine protein kinase, mono- and di-nucleotide cyclases, phosphodiesterases, DNA-binding and protein-binding domains. It is therefore unsurprising that the physiological adaptations controlled by these sensors are very broad. These adaptations include the regulation of blood pressure, circadian clock, CO metabolism, chemotaxis, dormancy/pathogenicity, metamorphosis, symbiosis, biofilm formation or dispersal, among others (Fig. 1).

The modularity of heme-based sensors provides an opportunity to target synthetic small-molecule activators (agonists) and inhibitors (antagonists) to distinct sites within them. These target sites include the heme domain, the output domain, and additional putative regulatory domains (Fig. 2). The latter occur in many sensors, where they are likely to be involved in protein oligomerization and mediation of signal transduction, though their specific regulatory roles are unclear [11–13].

The heme domain is one potential target site for exogenous regulation of heme-based sensors. There are already interesting examples of this kind of regulation for the soluble guanylate cyclase (sGC), which will be further reviewed. One caveat, however, is that any species that acts directly on the heme cofactor of a sensor could potentially act on the heme in hemoglobin and myoglobin, found in high concentration in humans. However, the variety of folds harboring heme allow for the design of agents more selective for these heme domains (e.g. HNOB, PAS, GAF), able to minimize cross-reactions with globin-based proteins, e.g. myoglobin, hemoglobin. This approach has made the heme domain of these systems a valid and suitable target.

Targeting the output domains, rather than the input domains, has been a more common strategy for development of small molecule modulators (Fig. 2A). As we will discuss farther, several interesting molecules are being developed for the regulation of output domains of heme-based sensors. Selective inhibitors have begun to emerge for diguanylate cyclase and histidine kinase: two enzymatic activities that have been shown to have important roles in bacteria [14,15]. There is a clear opportunity to develop novel small molecules for these enzymatic activities, with a much wider application than just heme-based sensors.

One additional and no less interesting opportunity might come from targeting the "extra" putative regulatory domains found in many heme-based sensors that we also call "non-functional" domains (see Fig. 2).

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http://dx.doi.org/10.1016/j.jinorgbio.2016.11.022
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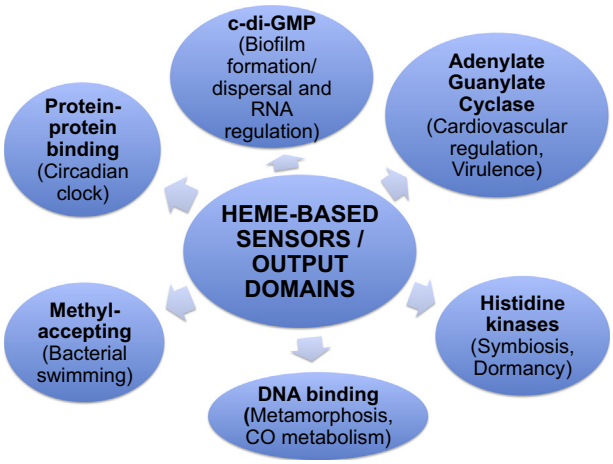

Fig. 1. Heme-based sensors and their associated output domains are involved in key biological activities and are potential targets for the development of small molecule regulators.

Here we review some of these systems for which regulators have been developed.

1.1. Regulators of soluble guanylate cyclase

Soluble guanylate cyclase is a nitric-oxide (NO) regulated heme-based sensor involved in a variety of important biological processes, including smooth muscle relaxation, platelet aggregation, and neurotransmission. This heterodimeric protein contains a larger α-subunit and a smaller β-subunit. The latter harbors the iron(II)-protoporphyrin IX in a HNOB domain (also known as HNOX), where iron is axially bound to a histidine (H105) and in the unliganded state is pentacoordinated. The human sGC's cyclase activity increases 200 fold upon binding of NO to the heme. The activation of sGC following ligation of NO was once thought to be only due to the rupture of the bond between the heme iron and H105, resulting in pentacoordinate heme iron. However, hemeless sGC was shown to be activated upon binding to organic molecules mimicking heme, whose carboxylate groups are critical for activation, suggesting specific side chains interaction are very important [16]. Additionally, it is now known that pentacoordination is not required for full sGC activation, since the activity of the hexacoordinate carbon monoxide (CO) bound form of sGC in the presence of the synergistic activator YC-1 (5-[1-(phenylmethyl)-1H-indazol-3-yl]-2-furanmethanol) is the same as that of the pentacoordinate NO-bound sGC. Nevertheless, there is evidence, based on resonance Raman spectroscopy, of sGC iron-histidine bond weakening or the formation of a fraction of heme pentacoordinated with CO induced by YC-1 or BAY-412272 [17–19]. Either YC-1 by itself, or CO by itself, enhances cyclase activity (12-fold and 5-fold, respectively), but these effect are much smaller than that of the two effectors combined (about 200-fold) [20]. YC-1 greatly enhances binding of NO due to deceleration of the NO dissociation rate, resulting in sGC that is sensitive to subnanomolar concentrations of NO. Interestingly, the binding affinity and kinetics of CO are unaffected by YC-1, even though it tremendously increases the effect of CO on the cyclase activity of sGC [20,21]. These results have opened new opportunities to develop and apply not only NO donors but also CO donors and small-molecule regulators to modulate sGC.

1.1.1. Nitric oxide donors

NO donors have been investigated as sGC activation agents, showing important pharmacological applications for the control of blood pressure, angina pectoris, ischemia, hypertension, among other disorders

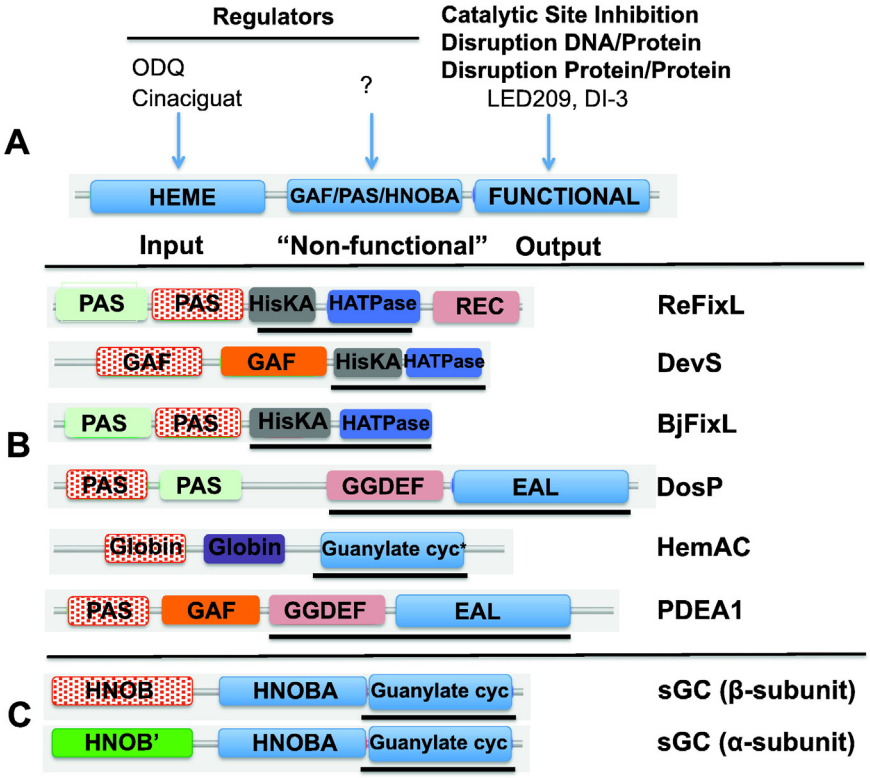

Fig. 2. Potential target sites for the development of small-molecule regulators (A); examples of some domain organizations of heme-based sensors (B); domain organization of sGC (C). The sensory domain where the heme group is bound is in red and stippled; the other sensory domains are putative and are of unknown function. The output domains are underlined in black. Output domains include GGDEF-EAL, which is involved in cyclase and phosphodiesterase activity for c-di-GMP; HisKA-HATPase_c, which has histidine kinase activity; guanylate cyc usually has a GTP cyclase activity that generates cGMP, although in the HemAC protein it has an ATP cyclase activity that generates cAMP; ReFixL also possesses a receiver domain of a response regulator, called a REC domain, in which the aspartate 573 residue can be phosphorylated.