Heme-based Globin-coupled Oxygen Sensors: Linking Oxygen Binding to Functional Regulation of Diguanylate Cyclase, Histidine Kinase, and Methyl-accepting Chemotaxis

An emerging class of novel heme-based oxygen sensors containing a globin fold and senses environmental O$_2$ via a heme iron complex. Structure-function relationships of oxygen sensors containing a heme-bound globin fold are different from those containing heme-bound PAS and GAF folds. It is thus worth reconsidering from an evolutionary perspective how heme-bound proteins with a globin fold similar to that of hemoglobin and myoglobin could act as O$_2$ sensors. Here, we summarize the molecular mechanisms of heme-based oxygen sensors containing a globin fold in an effort to shed light on the O$_2$-sensing properties and O$_2$-stimulated catalytic enhancement observed for these proteins.

What Is a Heme-based Oxygen Sensor?

Hb, the prototypical O$_2$-binding heme protein, is the initial binding site for external O$_2$ molecules in the body and, as such, is important for numerous important subsequent physiological functions that use oxygen (1, 2). Mb, which has a similar protein structure to Hb, acts as an O$_2$ storage site (1, 2). Hb and Mb additionally perform other functions, such as detoxification of NO via dioxygenation to form nitrate (3, 4). The histidine (E7) imidazole located at the heme distal side in the heme Fe(II)-O$_2$ complex of Hb and Mb plays a significant role in preferentially stabilizing bound O$_2$ via hydrogen bonding and the resistance of globin-O$_2$ complexes to autoxidation (5, 6). An O$_2$-binding site is also present in other heme proteins, including heme-based monoxygenases such as cytochrome P450 and heme oxygenase. As such, many O$_2$-binding heme proteins contain the heme iron complex as their active site/center.

In an extension of this concept, an emerging class of heme-based oxygen sensors uses the heme iron complex as the O$_2$-sensing site for intramolecular signal transduction. In general, the heme-based oxygen sensors are composed of an N-terminal heme-bound oxygen-sensing(binding site, and 2) comparing the heme Fe(II)-O$_2$ complex equilibrium dissociation constant (O$_2$ affinity) and autoxidation rate constant (stability of the heme Fe(II)-O$_2$ complex) of these GCSs with those of other heme-based oxygen sensors containing heme-bound PAS or GAF domains (Table 1).

The Globin-coupled Oxygen Sensor

Globins have been identified in 1185 of 2275 bacterial genomes and 32 of 140 archaeal genomes (16) and further categorized into myoglobin-like globins with a 3/3 $\alpha$-helix fold, sensor globins with a 3/3 $\alpha$-helix fold, and truncated globins with a 2/2 $\alpha$-helix fold. The sensor globins constitute GCSs, protoglobins, and sensor single-domain globins (14–18). It must be noted that not all sensor globins have enzyme function. Moreover, numerous sensor globins involved in signaling have unknown functions that remain to be established, such as GCS from Geobacter sulfurreducens (GsGCS) (Fig. 1) (19). GCSs are defined as chimeric proteins composed of heme-bound globin and functional domains (Fig. 1) (14–18). The globin domain of GCS lacks the entire D-helix and part of the E-helices of Ms and Hbs, which have a common genetic ancestry with sensor oxygenase. As such, many O$_2$-binding heme proteins contain the heme iron complex as their active site/center.

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globs. The resulting shortened globin fold is predicted to have special characteristics that should be beneficial for heme-based oxygen sensors that use the shortened globin fold for oxygen sensing (14–16, 18).

Biochemical characterizations in reconstituted systems using purified enzymes have been reported for only seven of the 400 previously reported putative bacterial GCSs (14–16). Reports on the physicochemical characteristics of these proteins have focused on the structure of the heme environment, with only a few studies investigating biochemical reactions involving specific substrates and products.

**MCP-GCS: HemAT**

HemAT is likely the MCP-GCS protein that controls the movement direction of archaea and bacteria such as *Halobacterium salinarum* and *Bacillus subtilis* (HemAT-Bs) in an O$_2$ gradient (20, 21). HemAT is the only MCP-GCS in which the molecular characteristics of the heme-bound domain have been well studied. Crystal structures of heme Fe(II) and heme Fe(III)-CN (cyano) complexes of the isolated heme-bound domain of HemAT-Bs have been solved (Fig. 3) (22). The crystal structures revealed that the tyrosine hydroxyl of Tyr-70(B10) in one subunit of the dimer of the heme Fe(II)-bound domain faces the protein surface. In contrast, the tyrosine hydroxyl in the same subunit interacts with CN in the heme Fe(III)-CN complex. Based on the structures of the heme Fe(II) and Fe(III)-CN complexes of HemAT-Bs and amino acid sequence alignment of GCSs, it is possible to speculate about the nature of the O$_2$-binding site (Fig. 3) (14, 15, 23). The O$_2$-binding kinetics of the isolated heme-bound domain of HemAT-Bs are dramatically changed by mutations at Tyr-70(B10) (24). Thus, it appears that the hydroxyl group of Tyr-70(B10) is important for stabilizing bound O$_2$ in the heme Fe(II)-O$_2$ complex via hydrogen bonding and inhibiting autoxidation of the heme Fe(II)-O$_2$ complexes. In contrast, resonance Raman and infrared spectral studies have suggested that Thr-95 should be either at or near the O$_2$-binding site (25–30). However, the crystal structure of the protein suggests that global protein structural changes are needed to explain the involvement of Thr-95 of the E-helix in the O$_2$ interaction (22, 30). Probably, a water molecule(s) would significantly participate in the interaction between O$_2$ and Thr-95. Hydrogen bonds involving Tyr-70 and Thr-95 have been implicated in O$_2$ binding/sensing. It has been suggested that a linker domain present between the N-terminal heme-bound and C-terminal MCP signaling domains plays an important role in intramolecular (and intermolecular) signal transduction (21). Moreover, based on the crystal structure, HemAT is proposed to utilize heterogeneity or negative cooperativity (or conversion from an asymmetrical to a more symmetrical form upon O$_2$ binding) to expand the dynamic rage for detecting O$_2$ and to transfer structural information to downstream HK (22, 24). However, no concrete functional regulation by O$_2$ binding and/or change in redox status has been reported in reconstituted systems *in vitro*, possibly because no specific substrate (and thus, no product) of a HemAT-mediated reac-

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**FIGURE 1. Alignment of heme-bound oxygen-sensing and functional domains of the heme-based oxygen sensors.** Left, the C-terminal HK domain in the two-component system containing N-terminal heme-bound globin, PAS, or GAF domains. Right, the C-terminal functional domains associated with MCP, c-diGMP homeostasis (DGC, synthesis; and PDE, degradation), and unknown functions (transmembrane (TM)) containing the N-terminal heme-bound globin or PAS domain. GCSs are defined as chimeric proteins composed of heme-bound globin and functional domains (14–18). Note that EcDOS and FixL have two tandem PAS domains; the heme iron complex is bound to the first PAS domain in EcDOS and to the second PAS domain in FixL. Similarly, DevS and DosT have two tandem GAF domains; in both cases, the heme iron complex is bound to the first GAF domain. Note that globin folds are not always localized at the N terminus but are inserted into diverse regions, as predicted from the amino acid sequences of sensor globins that are yet to be characterized (14–18). AxPDEA1, A. xylinum PDEA1.

**FIGURE 2.** A, c-diGMP is an important second messenger in bacteria. Synthesis (DGC) and linearization (PDE) of c-diGMP, an important second messenger in numerous physiological functions (including biofilm formation), in *E. coli* are conducted by the heme-based oxygen sensor enzymes YddV and EcDOS, respectively. YddV is a GCS, whereas EcDOS is a heme-bound protein with a PAS fold. O$_2$ binding to the heme Fe(II) complexes of these enzymes enhances catalysis to a significant extent. However, because the O$_2$ affinities of the two enzymes are markedly different (5–20-fold) from each other, each enzyme functions to accommodate the local O$_2$ concentration in response to stimuli to maintain c-diGMP homeostasis. This figure is adapted from Ref. 72. B, schematic mechanism of AFGCHK (a GCS) of a two-component system. O$_2$ binding to the heme Fe(II) complex, which is bound to the N-terminal domain via the globin fold, significantly stimulates the C-terminal domain of HK, resulting in autophosphorylation of AFGCHK. Once autophosphorylated, AFGCHK transfers the phosphate group to the cognate response regulator. Although, the enzyme is depicted here as a monomer for the sake of simplicity, all HKs reported to date, including AFGCHK, are dimers (or tetramers). It has been suggested that autophosphorylation is exerted “crosswise” such that the kinase in one subunit phosphorylates the His residue of HK in the other subunit of the dimer (or tetramer). *oa*, amino acids; *Rec*, receiver domain. This figure is adapted from Ref. 44.
tion has been identified. The C-terminal domain interacts with the HK CheA, which is a component of the CheA/CheY two-component signal transduction system in bacterial aerotaxis (20, 21, 31).

**DGC-GCS**

c-diGMP is an important bacterial second messenger involved in mobility, virulence, development, cell-cell communication, and biofilm formation (32–34). In *E. coli*, DGC-mediated synthesis of c-diGMP is carried out by YddV (or *E. coli* DosC (*EcDosC*)), one of the GCSs, whereas degradation of c-diGMP is mediated by the PDE activity of another heme-based oxygen sensor, *E. coli* DOS (*EcDos* (direct oxygen sensor)); or EcDosP (Figs. 1 and 2).

**Azotobacter vinelandii** GReg (*AvGReg*)—Both the full-length and isolated heme-bound domains of GCS from *A. vinelandii* were cloned, overexpressed in *E. coli*, and characterized (35). Spectral and ligand-binding kinetic studies were conducted for recombinant proteins. The heme Fe(III) complex of the full-length enzyme was in the bis-histidine hexacoordinate form. The heme Fe(II) complex of the full-length enzyme was also hexacoordinated with bis-histidine, whereas that of the isolated heme-bound domain was pentacoordinated. O₂ affinities for both proteins were high. The heme Fe(II)-O₂ complex of the isolated heme-bound domain remained stable over several hours. Furthermore, examination of ligand (such as O₂ and NO)-binding kinetic properties suggested that AvGReg plays a role in O₂-mediated NO detoxification via dioxygenation of NO to form nitrate (3, 4, 35). A characterization of the phenotypes of *Salmonella typhimurium* cells overexpressing AvGReg revealed that biofilm formation and swimming motility were influenced by AvGReg (35, 36). Although the DGC activity of AvGReg has not been characterized *in vitro*, these latter results suggest that AvGReg is involved in c-diGMP synthesis *in vivo*.

*Bordetella pertussis* GReg (*BpeGReg*)—BpeGReg was cloned and overexpressed in *E. coli* and was the first GCS that was unequivocally proven to possess DGC activity (36). This study showed that the catalytic activity of BpeGReg is significantly stimulated by O₂ binding to the heme Fe(II) complex. Binding of NO and CO to the heme Fe(II) complex also enhances the catalytic activity of this enzyme. The study provided an interesting homology model based on the crystal structures of HemAT-*Bs* and PleD, a DGC from * Caulobacter crescentus*, and proposed that c-diGMP binds to inhibitory sites, causing feedback inhibition of enzyme activity. More importantly, the middle domain between the heme-bound globin and DGC domains was suggested to be required for proper folding of the DGC domain, but not the heme-bound domain. Relationships between O₂ binding, active dimer formation, and autophosphorylation were also implicated (36). The involvement of BpeGReg in biofilm formation and bacterial motility was demonstrated by protein overexpression in *S. typhimurium* or gene knock-out in *B. pertussis*.

**HemDGC**—HemDGC from *Desulfovibrio psychrophila* was identified and characterized (37). It was shown that only the heme Fe(II)-O₂ complex of HemDGC exhibits DGC activity, whereas heme Fe(II), heme Fe(II)-NO, heme Fe(II)-CO, and...
heme Fe(III) complexes do not. A discussion of the protein structure of the heme distal side emphasized the role of Tyr-55(B10) at or near the O2-binding site (Fig. 3 and Table 1).

YddV (EcDosC)—In the first report of YddV, a heme-based oxygen sensor DGC from *E. coli* (also designated EcDosC) (38), it was shown that incubation of YddV in a solution containing GTP (the substrate of YddV) and EcDOS (with PDE activity toward c-diGMP) produced linear diGMP (pGpG) as the final product, providing indirect evidence of DGC activity. A separate study from our group (32) monitored the time-dependent decrease in GTP and concomitant increase in c-diGMP in a reconstituted system with the aid of HPLC. These studies unequivocally demonstrated that O2 binding to the heme Fe(II) complex of the N-terminal O2-sensing domain of the YddV molecule markedly stimulates DGC activity (Fig. 2 and Table 1). The heme Fe(III) and heme Fe(II)-CO complexes were also active, whereas the heme Fe(II) and heme Fe(II)-NO complexes were inactive. Analyses of the physicochemical properties of mutant proteins suggested that Tyr-43(B10) is adjacent to the O2-binding site on the heme distal side (Fig. 3) (32, 39). The hydroxyl of Tyr-43(B10) appears to preferentially stabilize O2 bound to the heme Fe(II) complex via hydrogen bond donation, as observed for other GCSs. The electron of O2 bound to the heme Fe(II) atom is partially removed with a negative charge and interacts strongly with proton donors or positive electrostatic fields induced by Tyr-43. However, the residues that are

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**FIGURE 3.** A, heme environmental structures of GCSs and sperm whale Mb. Left, the heme Fe(III)-CN complex of HemAT-Bs (Protein Data Bank code 1OR4) (22). Center, the heme Fe(III) complex of GsGCS (code 2W31) (19). Right, the heme Fe(II)-O2 complex of sperm whale (SW) Mb (code 1A6M) (98). Note that HemAT-Bs and GsGCS are GCSs, whereas sperm whale Mb is a vertebrate globin. The color of the helix corresponds to helix notification in Fig. 38. Distal B- and E- and proximal F-helices are shown in orange, green, and light blue, respectively. B, alignment of amino acid sequences of selected GCSs and sperm whale Mb with helix notification. Helix notification was based on sperm whale Mb and HemAT (14–16, 38, 98). The distal Tyr residue (B10), which binds to O2 in the heme Fe(II)-O2 complex, and the proximal His residue (F8) as a heme-binding site of GCS are shown in blue and red, respectively. The distal His residue (E7) interacting with O2 in the heme Fe(II)-O2 complex of sperm whale Mb is shown in green.
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situated at or near CO- or NO-binding sites in the heme distal side possibly differ from Tyr-43, as suggested from ligand-binding kinetics and resonance Raman spectra of the Tyr-43 mutant proteins. This structural difference in the ligand-bound heme complex in YddV appears to reflect the variations in catalytic regulation by CO and NO. The crystal structure of HemAT suggests that the partial negative charge of Tyr-70(B10) on bound O2 can “pull” hydrogen-bonding donors toward it, whereas CO and NO would not be able to cause such a movement of Tyr-70(B10) (22), similar to the case of the distal Arg side chain for FixL (40, 41). Moreover, it has been speculated that the environmental structure of the heme Fe(II) complex in YddV is significantly different from that of the heme Fe(III) complex, as observed for FixL (40, 41) and EcDOS (42, 43), which are heme-based oxygen sensors with a PAS fold, resulting in heme redox-dependent catalytic regulation. Biofilm formation by E. coli is significantly affected by overexpression of YddV, suggesting again that YddV is involved in biofilm formation in vivo.

**HK-GCS: Anaeromyxobacter sp. Strain Fw109-5**

*AfGcHK*, isolated from the soil bacteria *Anaeromyxobacter* sp. strain Fw109-5, is the first reported GCS with HK activity. The HK activity of *AfGcHK* is markedly stimulated by the binding of an O2 molecule to the heme Fe(II) complex (44). As noted above, evolutionary considerations led to the discovery of GCS enzymes involved in MCP and c-diGMP homeostasis (synthesis), but GCS enzymes with other activities were not known (14–16). On the basis of bacterial genomic sequences, it was predicted that soil bacteria encode a protein containing an amino acid sequence corresponding to the GCS globin fold. In addition, it was thought that the same protein has an HK domain similar to that of other heme-based oxygen sensor HKs involved in two-component systems, such as FixL, DevS, and DosT (Fig. 1, left).

As predicted, the autophosphorylation of *AfGcHK* at His-183 and the *AfGcHK*-mediated phosphorylation of Asp-52 and Asp-169 in the response regulator (via a phosphotransfer reaction) were significantly stimulated by the binding of O2 to the Fe(II) heme complex in the N-terminal sensor domain, whereas the heme Fe(II) complex alone showed no catalytic activity. CO binding to the Fe(II) heme complex also enhanced catalysis. These gas-induced catalytic enhancements and the redox-dependent catalytic regulation of *AfGcHK* are similar to those observed for YddV (32).

Tyr-45(B10) appears to be at or near the O2-binding site in *AfGcHK* based on an amino acid sequence alignment of GCSs, the crystal structure of the heme Fe(III)-CN complex of HemAT (Fig. 3), and physicochemical properties of mutant proteins. Again, the hydroxyl of Tyr-45(B10) interacts with O2 in the heme Fe(II)-O2 complex and stabilizes the complex via hydrogen bond donation, as observed for YddV (32).

**GCSs with Unknown Function**

The x-ray crystal structures and physicochemical properties of the isolated heme-bound domain of GsGCS have been reported (Fig. 3) (19). The heme Fe(III) complex of this protein displays bis histidyl hexacoordination, whereas the heme Fe(II) complex is an admixture of penta- and hexacoordinated complexes. Interestingly, distal heme coordination of the heme Fe(III) complex in GsGCS is provided by a His residue unexpectedly located at the E11 topological side, distinct from that at the E7 site of Hb, Mb, and other GCSs. Resonance Raman spectral and ligand (O2, CO, and NO)-binding kinetic studies were additionally conducted. Although no functional properties were examined, GsGCS is the only GCS reported to date that has a C-terminal transmembrane signal transduction domain.

**Specific Characteristics of GCSs**

Globin sensor domains that lack heme binding are known, although their functions have not been determined (45, 46). However, given the accumulating information about the O2-mediated catalytic regulation and physicochemical properties of GCSs, it is worth summarizing specific characteristics of GCSs in comparison with those of non-GCS heme-based oxygen sensors. Note that the characteristics of non-GCS enzymes are not described here in detail; instead, see Table 1 and references therein.

O2 Binding to the Heme Fe(II) Complex Markedly Stimulates Catalytic Activity—For all GCS catalytic reactions reported to date, catalysis is markedly stimulated by O2 binding to the heme Fe(II) complex in the GCS molecule (Fig. 2 and Table 1). This O2-dependent catalytic enhancement appears to be specific for GCSs because other heme-based oxygen sensors (with the exception of EcDOS) behave differently in that their heme Fe(II)-O2 complex is the inactive form, and dissociation of O2 activates catalysis (Table 1). For FixL and *Acetobacter xylinum* PDEA1 (AxPDEA1), both of which have a heme-bound PAS domain, the heme Fe(II)-O2 complex is the inactive form, and the heme Fe(II) complex (O2-free form) is the active form. Similarly, for the heme-based oxygen sensor two-component HKS DevS and DosT, which contain the heme-bound GAF domain as the O2-sensing site, the heme Fe(II)-O2 complex is the inactive form, whereas the heme Fe(II) complex is the active form. Although like FixL and AxPDEA1, EcDOS has a heme-bound PAS domain, its PDE activity toward c-diGMP is markedly enhanced by O2, NO, and CO. However, because the published literature on characterization of GCSs is limited, it is possible that O2-induced catalytic enhancement is not the general case for all GCSs, which may come to light in future investigations.

**GCS Active Sites Contain Distal Tyr and Thr Residues**—On the basis of amino acid sequence alignments of GCSs and crystal structures of heme Fe(II) and Fe(III)-CN complexes of HemAT-Bs, it was suggested that the side chain phenolate of the Tyr residue (B10) is located in the heme distal side and is at or near the O2-binding site for most GCSs (Fig. 3). Although the crystal structure of the heme Fe(II)-O2 complex of GCSs has not been determined, O2-binding kinetics and resonance Raman studies of heme Fe(II)-O2 complexes of wild-type and mutant HemDGC, YddV, and *AfGcHK* proteins suggest that the Tyr residue could be located at or near the O2-binding site (Table 1). The hydroxyl of the Tyr residue (B10) would stabilize bound O2 in the heme Fe(II)-O2 complex via hydrogen bond donation in GCSs. In all cases, O2 binds to the Fe(II) atom first.
and partially removes an electron to generate a negative charge, which then interacts with proton donors or positive electrostatic fields induced by the Tyr or Thr residue. In contrast, for HemAT-BS, resonance Raman spectral studies suggest an interaction between Thr-95 and the O₂ molecule of the heme Fe(II)-O₂ complex probably via a water molecule(s) and implicate a hydrogen-bonding network composed of Tyr-70 and Thr-95 in O₂ recognition.

The only crystal structure for the heme Fe(II)-O₂ complex of the sensor globin (but not GCS), protoglobin from Methanosarcina acetivorans, has been reported (47). The O₂ molecule in the heme Fe(II)-O₂ complex is not stabilized by hydrogen bonding to the protein. The residue closest to O₂ (Phe-93(E11) at 3 Å) is affected by conformational disorders. However, the amino acid residue interacting directly with O₂ bound to the heme Fe(II) complex could not be unequivocally distinguished in the structure.

DevS and DosT, which contain a heme-bound GAF domain, have a Tyr residue adjacent to the active site in the heme Fe(II)-O₂ complex (48–55), whereas FixL (40, 41, 56–63) and EcDOS (42, 43, 64–72), which contain a heme-bound PAS domain, have an Arg residue at the corresponding position in the sensing site (Table 1). Interestingly, although the protein folding of the GAF domain is similar to that of the PAS domain, the amino acid in the distal pocket adjacent to the ligand-binding site in the heme Fe(II)-O₂ complex is different between the GAF and PAS folds.

Thus, the O₂-binding sites of heme-based oxygen sensors are different from those of Hb and Mb, where O₂ in the heme Fe(II)-O₂ complex interacts with a His residue (E7) in the distal side. For the oxygen sensors, it is probably important for the O₂ association/dissociation process to respond smoothly to O₂ concentration in the environment without being influenced by environmental pH or ionic strength. This is likely why Tyr, Thr, or Arg, but not the His imidazole, is used for the oxygen-binding site of these heme-based oxygen sensors.

Note that the His (E7) side chain preferentially stabilizes O₂ bound to the heme Fe(II) complex in all vertebrate Hbs and Mbs. However, distal Gln (E7) and Tyr (B10) side chains stabilize bound O₂ in many invertebrate Hbs, particularly those with extremely high O₂ affinities (73). In contrast, the hydroxyl group of Tyr (B10) can destabilize bound O₂ by the non-bond electrons or partial negative charge on the oxygen atom of the side chain in Cerebratulus lacteus Hb (99).

High O₂ Affinity—For the GCSs reported to date, the heme Fe(II)-O₂ complex is the active form, whereas the heme Fe(II) complex is the inactive form. The equilibrium constants for the dissociation of O₂ from the heme Fe(II) complexes of GCSs are low (between 0.077 and 14 μM) as shown in Table 1; thus, the O₂-binding affinity for the heme Fe(II) complex is very high. These equilibrium dissociation constant values are in contrast to those of heme-based oxygen sensors containing the heme-bound PAS fold, such as FixL and EcDOS, where the corresponding values are 140–340 μM. However, the value for DevS (0.58 μM) is in the same range as that for GCSs. This suggests that GCSs, as well as DevS/DosT, act under anaerobic or semi-anaerobic (micro-aerobic) conditions and need to sense changes in the concentration of trace amounts of environmental O₂, whereas FixL and EcDOS operate under aerobic conditions and sense a decrease in O₂ concentrations from normal levels. Because the O₂-binding affinity of YddV (14 μM) is higher than that of other GCSs, it may be that E. coli YddV must work under semi-anaerobic, but not strictly anaerobic, conditions in the large intestine. If it is assumed that only YddV and EcDOS are involved in homeostasis of c-diGMP in E. coli in specific organs (Fig. 2), the two enzymes would function distinctly and synergistically, depending on the O₂ concentration, to regulate the c-diGMP level in response to physiological stimuli.

It should be noted that Mb and Hb function by taking up and releasing O₂ for transport and delivery to respiring mitochondria. PAS sensors discern the presence of O₂ and activate genes related to aerobic metabolism, and GCS sensors respond to hypoxia and activate the associated genes. Therefore, the O₂ affinities for these heme proteins differ to accommodate their functional purposes.

Specific Characteristics of Heme-based Oxygen Sensors, Including Non-GCSs

The increasing availability of information about the biochemical and physicochemical characteristics of heme-based oxygen sensors, including non-GCSs, has made it worthwhile to compare the specific properties of these sensors with those of other heme proteins, such as Hb and Mb (Table 1).

The Heme Fe(II)-O₂ Complex Is Stable with a Low Autoxidation Rate Constant—The autoxidation rate of heme proteins is controlled by several factors (5, 6). The hydrogen bond provided by the neutral imidazole side chain of the distal His residue (E7) plays the most crucial role in the inhibition of Mb heme iron autoxidation. This interaction prevents both dissociation and protonation of bound oxygen. The pH dependence of the rate observed at or above 7.0 may be attributable to protonation of the heme Fe(II)-O₂ complex. In addition, the superoxide (the product of autoxidation of the heme Fe(II)-O₂ complex) dissociation pathway predominantly determines the rate. Moreover, the accessibility of the distal pocket to solvent water molecules increases protonation of the heme Fe(II)-O₂ complex, resulting in an increased rate.

The importance of the heme Fe(II)-O₂ complex in catalytic enhancement or suppression of oxygen sensors is evidenced by the stability of the heme Fe(II)-O₂ complex. In fact, the stability of the heme Fe(II)-O₂ complex of GCSs is such that the spectrum of the heme Fe(II)-O₂ complex does not change for hours to days at room temperature. This stability is reflected in the very low GCS autoxidation rate constants, which are <0.025 min⁻¹ (Table 1). It is reasonable to infer that the heme Fe(II)-O₂ complexes of other oxygen sensors, such as FixL and EcDOS (which contain the heme-bound PAS domain) and DevS (which contains the heme-bound GAF domain), are similarly stable, given that the heme Fe(II)-O₂ complex is the key form of their functions. This stability of the heme-based oxygen sensor is in contrast to that of other heme proteins, such as cytochrome P450, where the O₂ molecule is activated in the heme active site; thus, the heme Fe(II)-O₂ complex is not as stable as that in heme-based oxygen sensors. The hydrogen bond from Tyr, Arg, or Thr plays important roles in stabilizing...
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the heme Fe(II)-O₂ complex in heme-based oxygen sensors, similar to that from the distal His residue (E7) in Hb and Mb (Table 1).

Redox potential would also be expected to contribute to the stability of the heme Fe(II)-O₂ complex and autoxidation rate constant. However, currently available data indicate that this prediction is not always borne out for heme-based oxygen sensors. For example, redox potential values of −17 and 45 mV versus the standard hydrogen electrode for YddV (32) and EcDOS (68), respectively, do not strictly correspond with the associated stability and autoxidation rate constants (YddV, 0.0076 min⁻¹; and EcDOS, 0.005 min⁻¹) of these heme proteins.

NO and CO Exhibit Divergent Functional Regulation, but Selectivity among O₂, NO, and CO Is Determined by a “Sliding Scale Rule”—Other diatomic ligands, such as NO and CO, bind to GCS as well, and it is likely that these are more important during early evolution than O₂ (14–16). As summarized in Table 1, these gas molecules play divergent roles in the regulation of catalytic function. For example, addition of NO to the heme Fe(II) complex inhibits the catalytic activity of YddV and HemDGC, whereas the same treatment enhances BpeGReg activity. Similarly, addition of CO to the heme Fe(II) complex of YddV, BpeGReg, and AgGcHK stimulates the catalytic functions of these enzymes but has no effect on HemDGC. Ligand-dependent functional regulation is similar for non-GCS oxygen sensors. Specifically, FixL, EcDOS, DevS, and DosT accept NO and CO in addition to O₂, although the catalytic activities of the ligand-bound complexes are variable (Table 1).

Regarding ligand specificity/discrimination of GCSs, the Kₐ values of O₂ binding are significantly higher than those of CO binding, except in the case of HemDGC, whereas the same treatment enhances BpeGReg activity. Similarly, addition of CO to the heme Fe(II) complex of YddV, BpeGReg, and AgGcHK stimulates the catalytic functions of these enzymes but has no effect on HemDGC. Ligand-dependent functional regulation is similar for non-GCS oxygen sensors. Specifically, FixL, EcDOS, DevS, and DosT accept NO and CO in addition to O₂, although the catalytic activities of the ligand-bound complexes are variable (Table 1).

The Heme Iron Complex Serves to Suppress Catalysis Because Heme-free Forms Have Sufficient Catalytic Activities—Why the heme iron complex is bound to heme-based oxygen sensors is an interesting question. Contrary to expectations, heme-free forms and heme-binding domain-truncated forms of YddV, AgGcHK, and EcDOS have sufficient catalytic activity (32, 44, 72, 94). Instead of stimulating catalysis, the heme iron complex on the heme-based oxygen sensor serves to suppress catalysis, and oxygen association/dissociation releases this catalytic suppression.

Summary

GCSs appear to represent prototypical heme-based oxygen sensors with a basic similarity to Hb and Mb, with which they share a common genetic ancestry. O₂ binding to the heme iron complex in GCSs significantly enhances associated catalytic activity, including DGC and HK activities, and MCP function; thus, the heme Fe(II)-O₂ complex in GCSs is the physiologically relevant form, similar to the case for Hb and Mb. This characteristic contrasts with that of non-GCS oxygen sensors, where either O₂ dissociation from or association with the heme iron complex stimulates catalysis. The high O₂ affinity for the heme Fe(II) complex, the stability of the heme Fe(II)-O₂ complex, and the role of the Tyr residue (B10) of the distal site in the stability of the O₂ complex are essential contributors to the specific characteristics of GCSs. The sliding scale rule accounts for ligand discrimination of GCSs, as well as other gas sensors (92, 93).

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