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Primary processes in heme-based sensor proteins ☆

Ursula Liebl, Jean-Christophe Lambry, Marten H. Vos *

Laboratory for Optics and Biosciences, CNRS, Ecole Polytechnique, 91128 Palaiseau, France
INSERM U696, 91128 Palaiseau, France

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ABSTRACT

A wide and still rapidly increasing range of heme-based sensor proteins has been discovered over the last two decades. At the molecular level, these proteins function as bistable switches in which the catalytic activity of an enzymatic domain is altered mostly by binding or dissociation of small gaseous ligands (O_2, NO or CO) to the heme in a sensor domain. The initial "signal" at the heme level is subsequently transmitted within the protein to the catalytic site, ultimately leading to adapted expression levels of specific proteins. Making use of the photolability of the heme-ligand bond that mimics thermal dissociation, early processes in this intra-protein signaling pathway can be followed using ultrafast optical spectroscopic techniques; they also occur on timescales accessible to molecular dynamics simulations. Experimental studies performed over the last decade on proteins including the sensors FixL (O_2), CooA (CO) and soluble guanylate cyclase (NO) are reviewed with an emphasis on emerging general mechanisms. After heme-ligand bond breaking, the ligand can escape from the heme pocket and eventually from the protein, or rebind directly to the heme. Remarkably, in all sensor proteins the rebinding, specifically of the sensed ligand, is highly efficient. This "ligand trap" property possibly provides means to smoothen the effects of fast environmental fluctuations on the switching frequency. For 6-coordinate proteins, where exchange between an internal heme-bound residue and external gaseous ligands occurs, the study of early processes starting from the unliganded form indicates that mobility of the internal ligand may facilitate signal transfer. This article is part of a Special Issue entitled: Oxygen Binding and Sensing Proteins.

1. Introduction

Sensor proteins allow for physiological responses to environmental stimuli on the cellular level, such as light, hypoxia, or the presence of specific signaling molecules. A wide range of such proteins exists, which in general contain a sensor domain and an enzymatic moiety. Stimulus-induced alteration of the sensor domain leads to modification of the catalytic activity. The sensor domain may carry a cofactor and one way to classify the sensor proteins is by their cofactor composition. In this review we focus on sensor proteins that carry a heme-group in the sensor domain. Functionally, these proteins can also be considered as a group, as they mostly sense small diatomic gaseous ligands [1–4], in particular NO, CO or O_2; in addition they may also act as sensor of ambient redox potential. However, the catalytic functions associated with these proteins vary largely. They include kinase activity, formation as well as breakdown of cyclic di-GMP, and direct DNA transcription. Directly, or via a cascade of intermittent steps, these proteins eventually influence the expression levels of specific other proteins as a response to the external stimuli.

NO [5], CO [6,7] and O_2 [8] are important signaling molecules and are often involved in metabolic functions. Analysis of the binding parameters for NO, CO and O_2 (in decreasing affinity order) suggests a "sliding scale rule", which allows prediction of the affinity of one ligand, if the K_D for the other ligand(s) is known, with deviations resulting from stereochemical interactions with the surrounding protein elements [9]. In all heme-based sensor proteins, the binding or dissociation of these molecules to the heme iron induces a perturbation of the heme environment that eventually propagates to the enzymatic domain, where the catalytic function is modified. Understanding the functioning of sensor proteins on the molecular level requires determining the signal propagation pathway *through the protein interior*. This is a challenging task: while structural models of the static endpoint configurations (for heme-based sensors containing unliganded and ligand-bound heme) may be available by X-ray crystallography, the short lived intermediates that define the pathway are generally hard to characterize.

Individual heme-based gas sensors in principle act as bistable switch proteins Fig. 1 depicts a general scheme for their switching mechanism and information transmission from the sensor domain to the catalytic domain. The switching from one state to the other is initiated by ligand binding to, or dissociation from, the heme. These are well-defined biochemical events that *intrinsically* take place on the timescale of the vibrations of the chemical bonds, typically tens

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* Corresponding author at: LOB, Ecole Polytechnique, 91128 Palaiseau, France. Tel.: +33 169335066.
E-mail address: marten.vos@polytechnique.edu (M.H. Vos).

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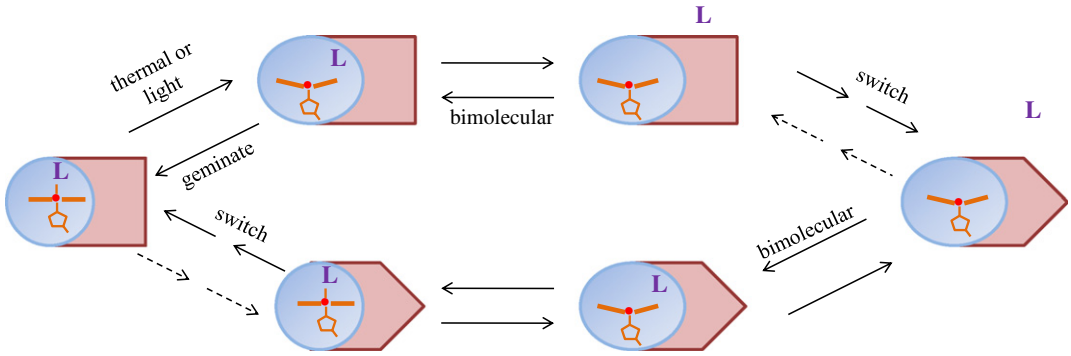

Fig. 1. General scheme of intra-protein signal transmission in heme-based sensors. L represents the sensed ligand. The heme domain is depicted as a blue circle; changes in the domain upon ligand binding/dissociation to the heme as changes in ellipticity. The catalytic domain is depicted as Indian red rectangles (ligand sensing configuration) and pentagons (non-sensing configuration). The scheme is a minimal scheme; for instance pathways that induce switching prior to migration of the ligand out of the sensor domain (upper pathway) or prior to ligand binding to the heme (lower pathway) are also possible.

of femtoseconds ($1\ \text{fs} = 10^{-15}\ \text{s}$). Subsequently, they lead to rearrangements of the environment of the heme-ligand system. The propagation of a perturbation within a protein can proceed fast, in principle with the speed of sound, i.e., on the order of 1 nm/ps. The initial, "mechanical", rearrangement of the structure can therefore occur on the timescale of picoseconds. These rearrangements will be deterministic for further steps that can involve thermally activated processes such as ligand migration within the protein and hydrogen bond breaking, and consequently occur on longer time scales. Also, the initial steps can be very sensitive to molecular regulation processes of the signaling. Characterization of the primary processes occurring on the picoseconds time scale is therefore important for a full comprehension of the switching mechanism.

In practice, the events following *binding* of a ligand cannot be followed on a timescale faster than microseconds, because of the intrinsic dead-times of mechanical mixing techniques [10]. However, heme-groups have the fortuitous property that axial ligands can in principle be photodissociated. Therefore, in solutions or crystals of heme-based sensor proteins, ligand dissociation can be synchronized by employing ultrashort (femtosecond) light pulses. Using suitably delayed pulses of electromagnetic radiation, the subsequent changes can then be monitored [11,12]. Thus, heme-ligand dissociation, which physiologically occurs thermally (stochastically), can be synchronized in this way. This property makes heme-based sensor proteins convenient model systems for determining mechanisms of mechanically driven intra-protein signaling pathways (naturally, light sensors are also ideally suited for such optical studies; yet their early transitions can be electronic in nature).

With the advent of advanced time resolved spectroscopic and crystallographic techniques, heme-proteins have always been rapidly and extensively used to study the general mechanisms of protein and ligand dynamics on a wide range of timescales, especially using the oxygen storage protein myoglobin as a model system. This large body of data serves as a reference for the study of sensor proteins, which have an intrinsically different physiological function, but in common with myoglobin that they bind ligands to heme. However, in sensor proteins, specific long range functional structural changes in the protein are expected upon formation or dissociation of the heme-ligand bond and therefore these techniques potentially can yield more extensive functionally relevant information. Spectroscopic techniques that are suitable for studying the primary processes in heme-based sensors include ultrafast transient absorption spectroscopy (especially for heme-ligand interaction and binding kinetics), transient resonance Raman spectroscopy (including impulsive Raman spectroscopy [13]), that monitors heme structural changes, and transient infrared spectroscopy, that monitors the ligand-protein interaction and in principle protein structural changes. Time-resolved crystallography, that associates impulsive ligand dissociation and full structural information [14], is in principle highly suitable to follow intra-protein signaling; accordingly the sensor domain of FixL is one of the very few systems studied with this technique, although not yet on a fast timescale [15]. Combining these experimental approaches with molecular dynamics simulations, that typically cover the same picosecond time range, allows reliable modeling of the early intermediate states.

Here we note that photodissociation of a heme-ligand bond does not necessarily lead to release of the ligand out of the heme pocket. (Partial) recombination of the dissociated heme-ligand pair can occur prior to ligand escape ("geminate recombination"), typically occurring on the picoseconds to nanoseconds timescale [11] (Fig. 1). This phenomenon, while highly informative in itself and potentially physiologically relevant in tuning the switch response time, may reduce the effective quantum yield of ligand release and in some cases limits the practical applicability of the photodissociation technique to the very early processes in sensor proteins.

During the last decade a number of studies on the primary processes appeared, covering a rapidly increasing number of heme-sensor proteins. Here we provide a non-comprehensive overview of these studies.

2. The "prototypical" sensor protein FixL

The best studied heme-sensor protein is the homodimeric rhizobial oxygen sensor FixL that regulates microaerobic adaptation and restricts the expression of nitrogen fixation and denitrification genes to hypoxic conditions [16]. FixL contains an N-terminal heme-binding PAS domain, where oxygen binding and initial sensing occur, and a C-terminal histidine kinase domain. The presence of saturating oxygen concentrations fully inhibits kinase activity, whereas in the absence of O_2 it catalyzes phosphorylation of the transcription factor FixJ, eventually triggering hypoxia-specific gene expression [2].

Although physiologically an oxygen sensor, FixL can also bind other diatomic ligands; CO and NO display very modest kinase inhibition [17]. In FixL, the unliganded and oxygenated forms of the heme are (high-spin) five-coordinate and (low-spin) six-coordinate, respectively [18]. The corresponding absorption spectra, the heme-geometry of the oxycomplexes [19], as well as the strength of the bond between the heme-iron and O_2 [20] in FixL are very similar to those of the oxygen-storage protein myoglobin (Mb). However, the ligand-binding properties are very different [21]. The oxygen affinity and oxygen-binding rates are considerably lower in FixL [22], which in air is only ~80% oxygen-saturated, in agreement with its sensor function. The affinity of CO is even further reduced with respect to that of myoglobin, leading to unusually weak ligand discrimination between CO and O_2 [22]. The very low affinity for these ligands has been argued to arise from heme-iron confinement on the proximal side through tension transmitted via the proximal histidine rather than steric hindrance effects on the distal side [23,24], although the inverse has also been proposed [21].