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Insights into heme-based O_2 sensing from structure–function relationships in the FixL proteins

Kenton R. Rodgers *, Gudrun S. Lukat-Rodgers

Department of Chemistry and Molecular Biology, North Dakota State University, Ladd Hall, Fargo, ND 58105-5516, USA

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Abstract

FixL proteins are bacterial heme-containing signal transduction proteins responsible for sensing the O_2 concentration in the organism's environment. In *Sinorhizobium meliloti* FixL is a protein histidine kinase that, together with its response regulator FixJ, constitute an oxygen-sensitive switch for regulation of the organism's nitrogen fixation and microaerobic respiration genes. The O_2 sensitivity of the switch is such that it transitions during the process of symbiosis in alfalfa roots. *Bradyrhizobium japonicum* FixL similarly regulates microaerobic and anaerobic respiration genes during symbiosis in soybean roots. FixLs responds to low oxygen concentrations with increased autophosphorylation activity of their kinase domains. The phosphorylated FixL provides a phosphoryl group to FixJ within a FixLJ complex. The phosphorylated FixJs are transcriptionally active toward their target genes. The FixL kinase domain is inhibited when the heme in FixL is oxygenated. Kinetic and thermodynamic studies of ligand binding to both ferrous and ferric FixLs have shown a generally low affinity for ligands relative to myoglobins. These relatively low ligand affinities are attributable almost completely to diminished rates of ligand binding. The heme and its environment in liganded and unliganded FixLs have been characterized by UV–visible spectroscopy, resonance Raman spectroscopy, EXAFS, and X-ray crystallography. These studies have revealed that in the purified proteins, the heme is converted from a six-coordinate low spin state to a five-coordinate high spin state upon O_2 release. Comparisons of spectroscopic and structural characteristics of deoxyFixL with oxy-FixL, met-FixL–CN, FixL–CO, and FixL–NO complexes indicate that distal affects in the heme pocket are, at least in part, responsible for communicating the ligation state of the heme to the kinase domain. The mechanisms by which ligand binding events are communicated from the heme to the kinase domain involves propagation and/or amplification of the ligation-coupled conformational transitions of the heme and its immediate protein environment. More recently, time-resolved experiments examining the non-equilibrium, ligand-coupled dynamics initiated by O_2, CO, and NO photolysis from the corresponding FixL complexes have begun to shed light on the landscape of the switching coordinate. Current thinking and understanding of the mechanism for signal transduction in the FixLJ systems are discussed in the context of these physical investigations.

Keywords: FixL; Oxygen; Sensor; Heme; Kinase; Regulation

1. Introduction

The past 20 years or so have borne witness to a myriad of studies focused on protein-based sensing of the diatomic gas molecules, CO, NO, and O_2. Oxygen sensing mechanisms fall into two principal classes. One takes advantage of the thermodynamic oxidizing power of O_2 to affect oxygen atom transfer (i.e., α-ketoglutarate dependent prolyl hydroxylases, PHDs/HPHs [1–3]) or multi-electron transfer (i.e., flavin binding PAS proteins [4], Aer [5–7] and NifL [8,9], the iron sulfur-containing protein FNR [10,11]) dependent changes in protein

* Corresponding author. Tel.: +1 701 231 8746; fax: +1 701 231 8831.
E-mail address: kent.rodgers@ndsu.nodak.edu (K.R. Rodgers).

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structure and/or conformation. The other relies on reversible O_2 binding and the ability to transduce the free energy change associated with making or breaking of the Fe–O_2 bond to drive structural reorganization of the protein. The O_2-binding sensors are heme-*b* containing proteins, some of which comprise PAS domains (i.e., FixLs [12,13], *Ec*Dos [14,15], *Ax*PDEA1 [16,17]), and others do not (i.e., the globin coupled sensor *Bs* HemAT [18–20]). This review describes the biophysical characterization of FixLs and the contributions of these studies to the current understanding of their signal transduction mechanism.

Plant growth is essentially limited by the availability of nitrogen which is high in the atmosphere but limited in the earth's crust. The legumes are able to thrive in nitrogen-deficient soils by virtue of their ability to enter into symbiotic relationships with Gram-negative soil-dwelling bacteria from the genus *Rhizobium*, which carry genetic code for the enzymatic machinery necessary to fix nitrogen and to survive under the conditions required for fixation [21]. The symbiotic relationships are initiated upon infection of the plant roots by the bacteria. During symbiosis, the bacterium enters the plant cell and resides therein as an endosymbiont having a plant-derived membrane. Among the plant responses to infection is its expression the O_2-binding heme protein leghemoglobin, which sequesters the O_2 pool and provides for its controlled delivery to the endosymbiont. Under these conditions, the symbiotic bacteriod begins to express high-affinity oxidases and/or denitrification enzymes that support its aerobic respiration under the microaerobic conditions or anaerobic conditions of the root nodule, respectively. It also begins to express nitrogenase along with its associated proteins to catalyze the conversion of N_2 to ammonia.

However, the catalytic turnover of N_2 by nitrogenase is rather slow and is facilitated by considerable expenditure of ATP [22]. Moreover, activated (reduced) nitrogenase [23] and the intermediates having an N–N bond, HN=NH and H_2N–NH_2, are unstable with respect to oxidation by O_2. Given the material and energy commitments to producing and running the nitrogen-fixing machinery, there would be little survival value in constitutive expression of the O_2-sensitive nitrogenase enzyme. Thus it is tightly regulated at the transcriptional level in inverse proportion to $P(O_2)$, to which *Rhizobia* must respond in such a way as to generate signals that can directly or indirectly regulate (a) the cell's ability to respire under conditions of low O_2 tension and (b) its commitment to the energy and materials intensive enterprise of N_2 fixation. Although FixLs are known from a number of other organisms, two have been substantially characterized by multiple biophysical methods; they are the FixLs from *Sinorhizobium meliloti* (*Sm*FixL) and *Bradyrhizibium japonicum* (*Bj*FixL). Thus, the following discussion will be restricted to these two FixL proteins.

*The regulatory roles of Sm*FixL. *Sinorhizobium meliloti* targets alfalfa for symbiosis. In *S. meliloti*, both *fixK* and *nifA* transcription are regulated by the *Sm*FixLJ system [12,13]. FixK regulates transcription of genes whose products, which include a high-affinity cytochrome oxidase, facilitate microaerobic respiration as $P(O_2)$ drops during symbiosis. NifA regulates transcription of the nitrogen fixation genes. Therefore the genes for both microaerobic respiration and nitrogen fixation are under FixLJ control.

*The regulatory roles of Bj*FixL. *Bradyrhizobium japonicum* targets soybean for infection and subsequent symbiosis. Only $fixK_2$ is regulated by *Bj*FixLJ [24,25]. $FixK_2$ regulates the transcription of genes for both microaerobic respiration, and anaerobic respiration wherein NO_3^- is the terminal electron acceptor [24]. Transcription of these genes provides the ability of the organism to transform its respiration mechanism as O_2 tension drops during symbiosis. Since transcription of *nifA* does not appear to be regulated by FixLJ in *B. japonicum* N_2 fixation is not under FixLJ control in this organism.

The current understanding of the regulatory roles played by *Sm*FixL and *Bj*FixL facilitating the symbiotic lifestyles of their respective organisms stands on results from a variety of experimental approaches including microbial genetics, heme ligand binding kinetics, autophosphorylation and phosphotransfer assays, steady state and time resolved spectroscopies, and solution and solid state structural methods. This review focuses primarily on the biophysical studies of the FixLs performed to date and their contribution to our understanding of the mechanism by which FixL transduces changes in the O_2 chemical potential to modulate the autophosphorylation rate of its kinase domain and the transfer of the phosphoryl group to FixJ.

2. FixL proteins

The FixLs are multi-domain, cytoplasmic proteins. *Bj*FixL is soluble [26] and *Sm*FixL contains an N-terminal membrane-spanning domain that localizes it to the cytoplasmic side of the inner membrane [25]. Both proteins comprise a heme-binding sensor domain and a kinase domain. Both the sensor and kinase domains of the FixLs are required for $P(O_2)$ responsive transcriptional regulation. Together with their corresponding FixJs, the FixLs constitute two-component signal transduction systems wherein FixL is kinase active under microaerobic or anaerobic conditions [26]. The phosphorylated histidine kinase of FixL is competent to phosphorylate a conserved aspartic acid in the N-terminal signaling domain of its response regulator, FixJ. Phosphorylation derepresses the C-terminal domain of FixJ, at which point it becomes transcription-