FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Light-induced switching of HAMP domain conformation and dynamics revealed by time-resolved EPR spectroscopy

Daniel Klose [a], Natalia Voskoboynikova [a], Ioan Orban-Glass [a], Christian Rickert [a], Martin Engelhard [b], Johann P. Klare [a], Heinz-Jürgen Steinhoff [a,*]

[a] Department of Physics, University of Osnabrück, Barbarastr. 7, 49076 Osnabrück, Germany
[b] Max-Planck-Institute for Molecular Physiology, Otto-Hahn-Str. 11, 44227 Dortmund, Germany

ARTICLE INFO

Article history:
Received 25 June 2014
Revised 30 August 2014
Accepted 3 September 2014
Available online xxxx

Edited by Miguel De la Rosa

Keywords:
Phototaxis
Chemotaxis
Chemoreceptor
HAMP domain
Spin labeling
Coiled-coil signaling

ABSTRACT

HAMP domains are widely abundant signaling modules. The putative mechanism of their function comprises switching between two distinct states. To unravel these conformational transitions, we apply site-directed spin labeling and time-resolved EPR spectroscopy to the phototactic receptor/transducer complex *Np*SRII/*Np*HtrII. We characterize the kinetic coupling of *Np*HtrII to *Np*SRII along with the activation period of the transducer and follow the transient conformational signal. The observed transient shift towards a more compact state of the HAMP domain upon light-activation agrees with structure-based calculations. It thereby validates the two modeled signaling states and integrates the domain's dynamics into the current model.

1. Introduction

The propagation of signals across cellular membranes or along extended scaffolding proteins is often governed by switching the signal transmitting proteins or their complexes in a stable and robust manner between on- and off-states characterized by different conformations and/or dynamics in the underlying structural ensemble [1,2]. Among such systems, coiled-coil signal transduction complexes in bacterial and archaeal photo- and chemotaxis, show a remarkable combination of properties in their performance [3]. These systems cluster into extended hexagonal arrays [4] together with the subsequent kinase CheA and adapter proteins CheW. Via a two-component system with the response regulators CheB and CheY [5], they bias the rotation direction of the flagellar motor [5]. Thereby they mediate the change in cell motility, taxis, in response to external stimuli such as light in phototaxis or chemicals in chemotaxis, with a high gradient sensitivity of down to ~1% over the length of the bacterial cell, and simultaneously maintain a linear output dependence over five orders of magnitude in ligand concentration or light intensity while integrating the various signals and their possibly opposite effects, i.e. both attracting and repelling stimuli [3].

The photoreceptor–transducer complexes and the chemoreceptors, that are homologous to the transducers [6], contain two types of coiled-coil structures. The extended cytoplasmic signaling, adaptation and kinase control domain [7,8] forms a four helical coiled-coil consisting of a dimer in which each monomer contributes two anti-parallel supercoiled helices. The second coiled-coil domain, called HAMP domain [9], is a signaling module found in over ~5500 proteins [10].

This four-helical bundle is made up by a dimer, featuring a parallel arrangement of two shorter helices from each monomer linked by an 11 residue connector. HAMP domain functions in terms of signal inversion or amplification are still under debate [7] as is the conformation of the domain's signaling state. Different conformational states have so far only been observed on distinct HAMP sequences, yet a conformational transition between two such states upon signaling [11] is the current working hypothesis.

In the phototaxis system of the archaea *Natronomonas pharaonis* studied here (Fig. 1), light activates the retinal chromophore in a separate receptor protein [12], here *Np*SRII, which forms a complex

* Corresponding author.
E-mail address: hsteinho@uos.de (H.-J. Steinhoff).

http://dx.doi.org/10.1016/j.febslet.2014.09.012
0014-5793/

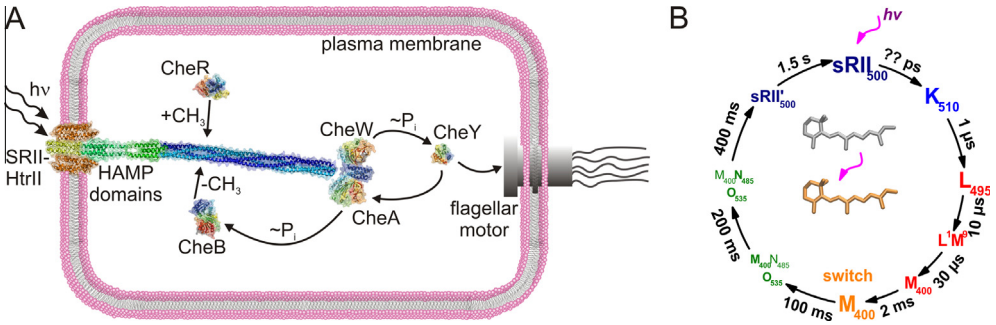

Fig. 1. (A) Two component phototaxis system of *N. pharaonis* with *Np*SRII/*Np*HtrII as the receptor/transducer complex that controls the subsequent kinase CheA. CheA in turn activates the response regulators CheY and CheB by phosphorylation, and CheY-P subsequently alters the rotation of the flagellar motor. A second feedback mechanism called adaptation tunes the transducer's sensitivity via reversible methylation by CheB and CheR [37]. (B) *Np*SRII photocycle, determined by the transient changes in optical absorption spectrum of the retinal chromophore [33]. It undergoes a series of transitions between intermediate states upon activation, fast kinetics populate the signaling M-state from where the receptor relaxes back to the ground state in <1 s.

with its cognate transducer, the extended coiled-coil signaling protein *Np*HtrII [13,14]. In the *Np*SRII/*Np*HtrII complex, helix F of *Np*SRII moves outward in response to photoisomerization of the retinal and in turn induces a downstream conformational change in the tightly coupled transmembrane helix TM2 of *Np*HtrII [13–15]. This conformational change comprises a rotation of TM2 by ~10–15° along with a piston-like displacement towards the cytoplasmic side by ~1 Å [16–18].

For the signal propagation by the HAMP domain it was suggested [19] that the two-state thermodynamic equilibrium found for the first HAMP domain in *Np*SRII/*Np*HtrII is shifted upon activation, yet further details as well as signal propagation along the coiled-coil transducer remain largely elusive, including the activation mechanism of the five-domain kinase CheA [20]. Therefore, we characterize the kinetic coupling of *Np*HtrII to *Np*SRII along with the activation period of the transducer and follow the transient conformational signal by time-resolved EPR spectroscopy probing at individual spin-labeled sites along the transducer, here throughout the first HAMP domain. Of the various available models for HAMP domain signaling [21], a recently suggested two state model [11,22] encompassing helix rotation [23] was found to be in agreement with our EPR spectroscopic data integrating changes of the dynamics [24,25] into a unified view. The observed kinetics show a tight receptor–transducer coupling scheme for both states and suggest that the relaxation kinetics contribute to signal amplification already on the level of receptor–transducer dimers.

2. Materials and methods

Details for expression, purification and reconstitution of spin-labeled *Np*SRII/*Np*HtrII complexes into native purple membrane lipids, here performed according to [13,16,18], are given in the Supplementary information, along with details on the transient optical absorption and continuous wave (cw) EPR spectroscopy, both carried out as described in [26].

Time-resolved EPR spectroscopy was performed in cw mode and using lock-in detection on a home-built 9.5 GHz spectrometer equipped with a dielectric resonator (Bruker Biospin, Germany) similar as in [18] with details given in the Supplementary information, along with a detailed description of the data analysis procedure.

HAMP domain modeling in the two states [11] was carried out using Modeller 9.11 Build 8834 [27] and followed by energy minimization in NAMD [28] with the CHARMM27 forcefield [29,30] as detailed in the Supplementary information. The fractional volume calculation was implemented in VMD [31] analogous to the algorithm by Altenbach et al. [32].

3. Results & discussion

3.1. Kinetic correlation shows coupled receptor–transducer activation dynamics

To trace the kinase-activating signal along the extended transducer *Np*HtrII, we use spin labeling in conjunction with EPR spectroscopy to probe *Np*HtrII for conformational and dynamical changes starting from the transmembrane region. In order to establish a correlation to the initial signal, we monitor the photo-induced all-trans to 13-cis isomerization of *Np*SRII's retinal chromophore and its subsequent relaxation back to the ground state by transient optical absorption spectroscopy, which allows following the transitions between the so called photocycle intermediates [12,33]. Conformational changes of *Np*SRII observed during the photocycle, most eminently the outward movement of helix F [13,17], activate *Np*HtrII via the coupling of *Np*SRII's helix F with the transducer helix TM2 [16]. Here we directly observed the propagation of the initial signal along the transducer by spin labeling and EPR spectroscopy [34] and correlate the obtained EPR kinetics to the photocycle kinetics.

TM2 continues as amphipathic sequence 1 (AS1) in the subsequent HAMP domain. Upon activation, TM2 undergoes a combined rotation [16] and piston movement [17,35], which is the conformational and dynamical input signal to the first HAMP domain [15]. The signal in TM2, observed at position V78R1 (R1 denotes the spin label side chain), exhibits a fast (~2 ms, cf. Fig. 1B) off-to-on transition followed by a slower relaxation (100–400 ms, cf. Figs. 1B and S1) back to the ground state.

Using spin labels at different positions in *Np*HtrII's first HAMP domain (Fig. 2A) we determined the relaxation kinetics of the *Np*SRII/*Np*HtrII complex by transient EPR and optical spectroscopy. Together the selected spin labeled sites cover most residues neighboring the heptad interface positions (Fig. 2A), which provide the essential inter-helical interactions stabilizing the four-helical bundle – a packing commonly found in coiled-coil helical structures [36]. Signal-induced changes of either conformation or dynamics of the HAMP domain can be expected to alter these essential interactions [11,23] upon switching between signal on- and off-states. Spin labels attached to residues neighboring the heptad positions, where mutations are tolerated in chemoreceptors [24], serve as reporter groups for alterations in conformation and dynamics.

The optical transients of the studied spin labeled variants, determined for the second half of the photocycle, require three exponential functions for fitting (Table 1 and SI) as depicted in Fig. 2B exemplarily for the mutant A94R1. The three resulting time constants for this mutant exhibit slightly slower kinetics than

Please cite this article in press as: Klose, D., et al. Light-induced switching of HAMP domain conformation and dynamics revealed by time-resolved EPR spectroscopy. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.09.012