FEBS Letters 353 (1994) 301–304

FEBS 14681

A cytoplasmic domain is required for the functional interaction of SRI and HtrI in archaeal signal transduction

Monika Krah, Wolfgang Marwan, Dieter Oesterhelt*

Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany

Received 15 September 1994; revised version received 20 September 1994

Abstract Phototaxis in the archaeon *Halobacterium salinarium* is mediated by a stable complex of the photoreceptor sensory rhodopsin I and its transducer HtrI, which relays the light stimulus to the signalling pathway. Removal of the cytoplasmic signalling domain of HtrI eliminated the SRI-specific motor response to light stimulation and led to the loss of the spectroscopically detectable physical interaction of SRI and HtrI. A similar phenotype was obtained by deleting part of a cytoplasmic loop located between the second transmembrane helix of HtrI and the signalling domain. These results indicate that the photochemical behavior of sensory rhodopsin I is not determined by interaction with the transmembrane helices of HtrI per se but functionally coupled to the signalling domain. It is proposed that light excitation of SRI results in a conformational change of the transducer which is conducted by the cytoplasmic loop, an extra module not found in the eubacterial transducer homologues, and activates the signalling domain.

Key words: Archaea; Halobacterial transducer for sensory rhodopsin I; Phototaxis; Signal transduction

1. Introduction

Sensory rhodopsin I (SRI), the first photoreceptor isolated from prokaryotes [1], is a retinal-containing seven helix transmembrane protein [2] that mediates the antagonistic phototactic responses towards orange and away from UV light [3–5]. Despite its structural homology to the family of the eukaryotic seven helix receptors, signal transduction does not work via a G-protein coupled cascade. Instead, photoexcitation of SRI is relayed to the cellular signalling pathway by physical interaction with the methyl-accepting transducer HtrI [6], a truncated archaeal homologue of the eubacterial chemoreceptors [7,8]. The transducer is composed of two membrane-spanning α-helices and a large cytoplasmic part that carries the signalling domain, putative methylation sites and an extra loop that is not found in eubacterial MCPs. Reversible methylation is thought to be the molecular mechanism for adaptation by switching off the signalling activity of HtrI. This assumption is based on the sequence homology to the eubacterial MCPs [9], the fact that light stimulation is followed by a release of methanol from the cell [10–12], and the finding that photosensory adaptation occurs by receptor deactivation (Marwan et al., unpublished results).

In vivo, SRI forms a stable, stoichiometric complex with HtrI. Photocycling rate and absorption maximum of SRI dramatically depend on whether or not the molecule is bound to HtrI [13]. Expression of SRI in excess of HtrI results in two spectroscopically distinguishable pools of bound and free SRI. Because the two pools do not exchange on the time scale of the SRI photocycle, the free SRI, although photochemically active, can not function in determining the swimming behaviour of the cell [13]. Here we analyse the role of the different domains of HtrI in its complex formation with SRI and signal transmission from SRI to the signal transduction chain.

*Corresponding author. Fax: (49) (89) 8578 3557.

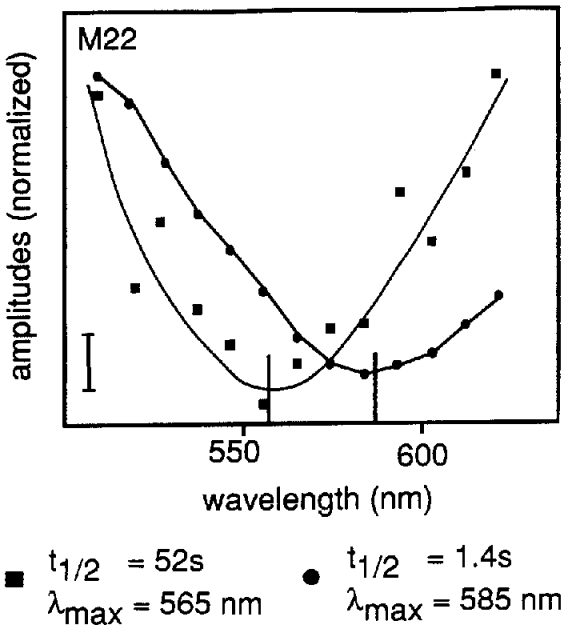
2. Materials and methods

The construction of pHtrNovo started from the plasmid pHtrSop, which carries the *htrI–sopI* operon with flanking sequences and a mutated halobacterial *gyrB* gene conferring resistance to novobiocin [5]. The 2 kb *Eco*RV–*Spe*I fragment carrying downstream sequences of the *sopI* gene was removed by partial digestion and the remaining vector fragment was ligated after an end-filling reaction with the Klenow fragment of *E. coli* DNA polymerase. The resulting plasmid pHtrNovo carries the *htrI-sopI* operon with a 282 bp flanking sequence in the upstream region and 106 bp in the 3'-region. Site-specific mutagenesis was performed by using recombinant PCR [17]. Vent DNA polymerase (New England Biolabs, Beverly, MA) was used to prevent unwanted mutagenesis through misincorporations during PCR. A specific part of the *htrI* gene was deleted by a two-step PCR protocol resulting in the fusion of an upstream and a downstream fragment flanking the region to be deleted. Using oligonucleotides that contained add-on sequences at their 5'-ends that overlap with the sequence to be joined, mirrored ends in each of the two primary PCR fragments were obtained. The two separate PCR fragments which overlap with their add-on sequences were combined and re-amplified, yielding the desired product. The plasmids, pHtrΔSD and pHtrΔLoop were constructed by replacing a 1.5 kb *Spe*I–*Pst*I fragment of pHtrNovo by mutagenized *htrI* sequences. The oligonucleotides used for site-directed mutagenesis and sequencing were synthesized on an automatic DNA synthesizer (Applied Biosystems, 381A). The following oligonucleotides were employed for site-directed mutagenesis:

Htr-Spe.seq:
5'-GGCCCCAACCTTATGGAGGG-3',
Htr-Pst.rev:
5'-GGCGACGGTGAACGACGCC-3':
Htr-ΔLoop.seq:
5'-GGAGATCGCCGCCACGGGGGACCTCACCC-3'
Htr-ΔLoop.rev:
5'-CCCCCGTGGCGGCGATCTCCTTGATGCTGG-3'
Htr-Nhe.seq:
5'-CGAGATCACGCTAGCGGAGGAAACGCAGGACC-3'
Htr-Nhe.rev:
5'-CCTCCGCTAGCGTGATCTCGGCGACCTGGTCG-3'

Transformation of *H. salinarium* was performed as described in [18]. Novobiocin was used at a concentration of 2 $\mu g \cdot ml^{-1}$ in liquid medium and at 1 $\mu g \cdot ml^{-1}$ for agar plates.

SSDI 0014-5793(94)01068-4



3. Results and discussion

HtrI dramatically influences the photocycling rate and the absorption maximum of SRI at physiological pH [13,14]. In intact cells the photochemically produced UV-absorbing intermediate of free SRI decays thermally to the initial state with a half-life of 52 s. By complex formation with HtrI, the decay is accelerated by two orders of magnitude to 1.4 s. In parallel, the absorption maximum of the initial state of SRI is red-shifted by complex formation. We measured the thermal decay of the UV-absorbing intermediate in intact cells of strain M22 that overproduce SRI and therefore contain free, in addition to complexed, SRI. The absorption difference maximum of the

←

Fig. 1. Difference absorption spectra of complexed and free SRI in intact cells of the SRI overproducing strain M22. Cells were suspended to a density of OD_{590} = 6 in normal growth medium suplemented with 1% arginine as a fermentative energy source. Recovery of the initial state was recorded following a 12 s irradiation with 580 nm using a spectrophotometer as described in [16]. The data points represent the normalized amplitudes of light-induced absorbance changes at different wavelength. The bar corresponds to an absorbance change of 1 mOD_{585} for the fast component and 7 mOD_{565} for the slow component.

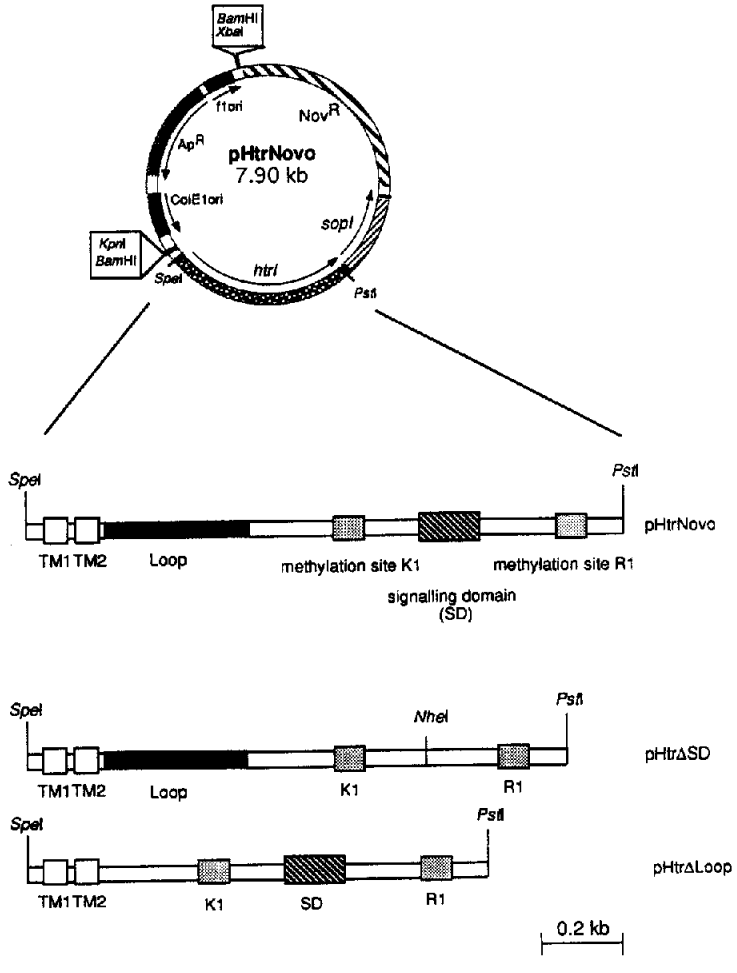


Fig. 2. The *htrI* mutagenesis vector pHtrNovo and its derivatives pHtrΔSD and pHtrΔLoop. The putative functional domains of the *htrI* gene, the two transmembrane helices (TM1, TM2), the cytoplamic loop (loop), the methylation sites (K1 and R1) and the signalling domain (SD), are indicated in each of the constructs.

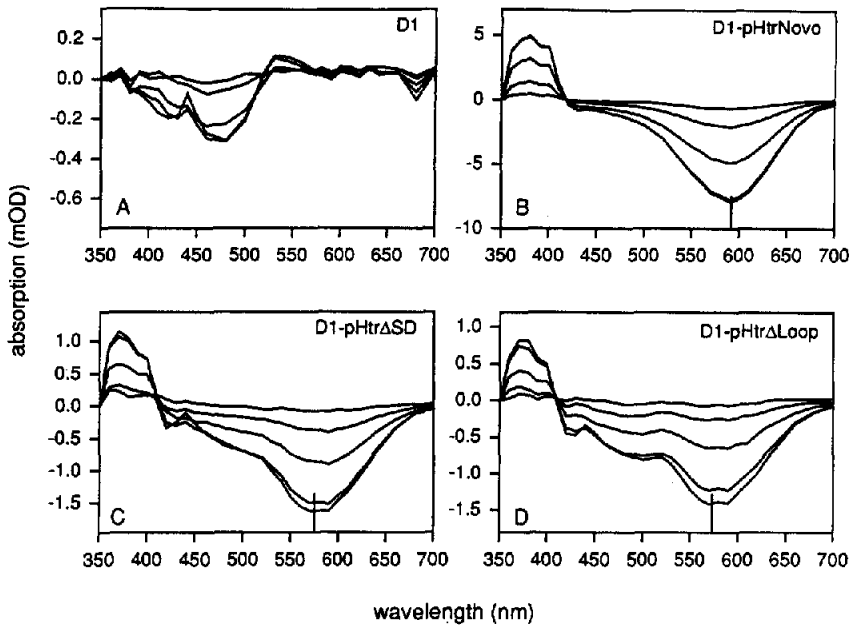


Fig. 3. Light-induced difference spectra recorded from halobacterial membranes from the (A) SRI⁻/HtrI⁻ mutant D1 [6,15] and the D1 mutant transformed with the *htrI-sopI* expression vectors (B) pHtrNovo, (C) pHtrΔSD and (D) pHtrΔLoop. The samples were prepared and measured as described in [13] and normalized with respect to the total protein concentration. The individual curves of each panel represent the absorption difference recorded in 0.5 s intervals under continuous irradiation with white light.

slow component (free SRI) was 565 nm, and that of the fast component (complexed SRI) was 585 nm at pH 7.5 (Fig. 1). Only SRI bound to HtrI can trigger behavioural responses whereas free SRI is physiologically inactive [13]. Therefore, we have three observable parameters to study the functional interaction of SRI and the site-specific mutated HtrI: (i) signalling efficiency of HtrI by measuring behavioral responses of the cells to light stimulation, (ii) absorption maximum, and (iii) photocycling rate of SRI as measured spectroscopically.

In wild-type cells the *htrI* and the *sopI* genes (encoding HtrI and SRI, respectively) are transcribed as a bicistronic message [6]. A genomic fragment containing the two genes together with flanking regions was cloned to give the shuttle vector pHtrNovo. Site-specific mutations were introduced into the HtrI gene by PCR and the wild-type sequence replaced by the mutated fragment (Fig. 2). The SRI and HtrI-deficient strain D1 [15] was transformed with pHtrNovo or the derived mutated plasmids pHtrΔSD or pHtrΔLoop. Transformants were

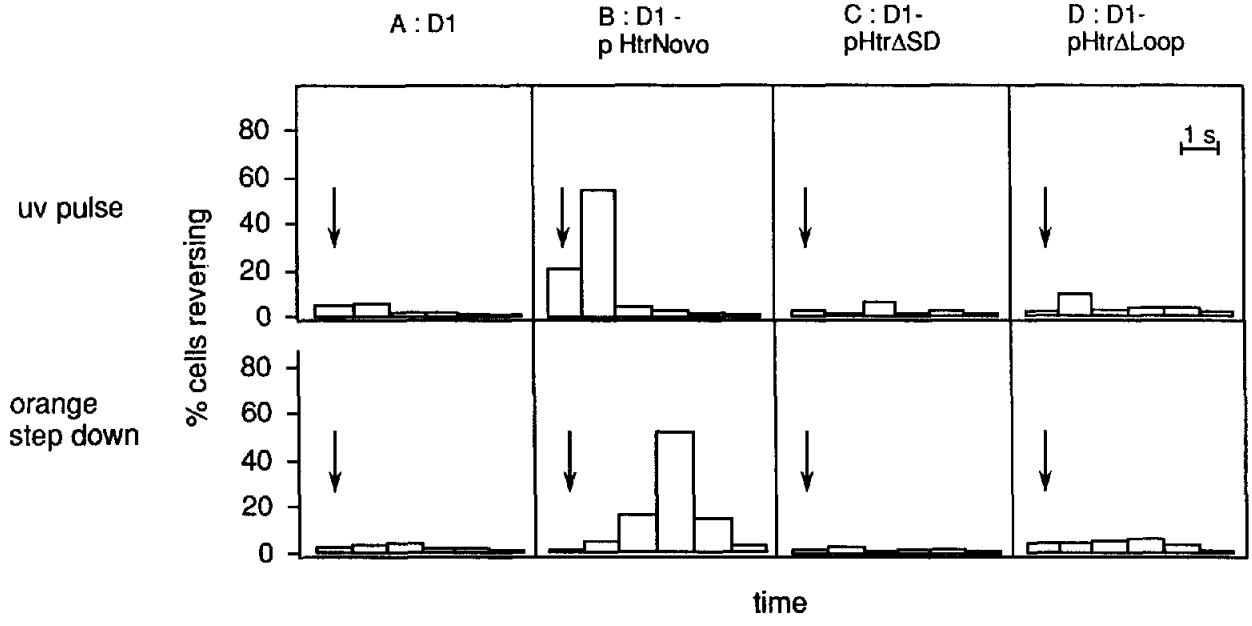


Fig. 4. Behavioural responses of the cells to UV pulse and orange light step down stimulation. Stimulus application is marked by an arrow. Control experiments made sure that light stimuli were saturating. Cells were prepared and measured as described in [6].

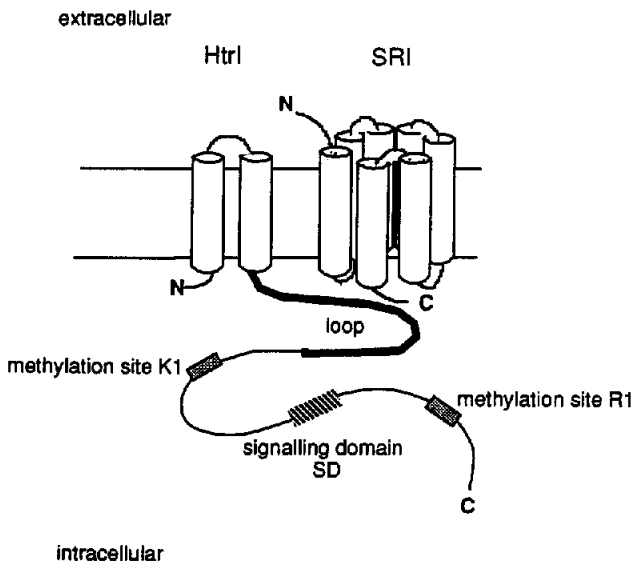


Fig. 5. Model of HtrI and its putative functional domains investigated in this study.

selected that are resistant to novobiocin. The vectors were integrated into the genome by a single crossover. The sequence of the incorporated *htrI* gene was verified by genomic sequencing and the expression of *sopI* was assayed spectroscopically and that of *htrI* by Western blotting using a polyclonal antibody. The expression of the two proteins correlated quantitatively in each clone tested (data not shown).

Cells transformed with the wild-type sequence expressed SRI and HtrI, which formed a functional complex as demonstrated spectroscopically and by the SRI-dependent photophobic responses (Figs. 3A,B and 4A,B). Deletion of the signalling domain of HtrI abolished any detectable response to orange light step down or UV light step up (Fig. 4C). This is consistent with the prediction that this domain activates downstream elements of the signal transduction pathway which activate or inhibit switching of flagellar rotation. Surprisingly, the same mutation also caused a loss of the spectroscopically detectable interaction of HtrI with SRI, that is, the absorption maximum of SRI was blue-shifted and the thermal decay of the UV-absorbing intermediate was slow (Fig. 3C and Table 1). Deletion of part of the cytoplasmic loop also destroyed both the light-induced behavioural responses and the spectroscopically detectable interaction with SRI (Figs. 3D and 4D).

The two results demonstrate that the transmembrane helices of HtrI per se are not sufficient for the fast photocycle of bound SRI (Fig. 5). In this case, deletion of the cytoplasmic signalling domain would produce a phenotype of a fast cycling SRI, which can not signal to the flagellar motor. In contrast, the conformational change of the cytoplasmic part of HtrI, caused by the mutation, is transmitted to the binding pocket of the retinal chromophore of SRI. This suggests vice versa that photoisomerization of retinal is transmitted to the signalling domain of HtrI by a conformational change in the cytoplasmic part of the molecule. This conformational coupling may or may not involve the transmembrane helices of HtrI. Consistent with this view is the finding that deletion of part of the cytoplasmic loop that connects the signalling domain to the transmembrane helices of HtrI results in the same combination of phenotypes: loss of signalling activity and altered photochemical properties of SRI. The cytoplasmic loop is not found in the homologous eubacterial methyl-accepting chemotaxis proteins. We propose that this additional structure is involved in transducing the steric movement caused by photoisomerization of the retinal chromophore via conformational changes in SRI to the signalling domain of HtrI (Fig. 5).

Table 1
Half-life time and pH-dependent absorption maximum of SRI co-expressed together with wild-type HtrI or HtrI deleted in the signalling domain or in the cytoplasmic loop

Strain	$t_{1/2}$ (s) (pH 7.5)	Absorption maximum (nm)		
		pH 6.0	pH 7.5	pH 8.7
D1-pHtrNovo	0.8	590	590	590
D1-pHtrΔSD	80	590	565	555
D1-pHtrΔLoop	85	590	565	555

Acknowledgements: We gratefully thank Dr. M. Alam for providing a sample of his anti-HtrI-antibody, Dr. A. Verméglio for help in recording the spectra shown in Fig. 1, and Ms. Heidi Grünberg for skilful technical assistance. We acknowledge Dr. J. Shiozawa and U. Haupts for critical reading of the manuscript. M.K. received a Boehringer Ingelheim doctoral fellowship.

References

[1] Schegk, E.S. and Oesterhelt, D. (1988) EMBO J. 7, 2925–2933.
[2] Blanck, A., Oesterhelt, D., Ferrando, E., Schegk, E.S. and Lottspeich, F. (1989) EMBO J. 8, 3963–3971.
[3] Spudich, J.L. and Bogomolni, R.A. (1984) Nature 312, 509–513.
[4] Marwan, W. and Oesterhelt, D. (1990) J. Mol. Biol. 215, 277–285.
[5] Takahashi, T., Mochizuki, Y., Kamo, N. and Kobatake, Y. (1985) BBRC 127, 99–105.
[6] Ferrando-May, E., Krah, M., Marwan, W. and Oesterhelt, D. (1993) EMBO J. 12, 2999–3005.
[7] Yao, V.J. and Spudich, J.L. (1992) Proc. Natl. Acad. Sci. USA 89, 11915–11919.
[8] Hazelbauer, G.L. (1988) Can. J. Microbiol. 34, 466–474.
[9] Krikos, A., Mutoh, N., Boyd, A. and Simon, M. (1983) Cell 33, 615–622.
[10] Alam, M., Lebert, M., Oesterhelt, D. and Hazelbauer, G.L. (1989) EMBO J. 8, 631–639.
[11] Spudich, E.N., Takahashi, T. and Spudich, J.L. (1989) Proc. Natl. Acad. Sci. USA 86, 7746–7750.
[12] Nordmann, B., Lebert, M.R., Alam, M., Nitz, S., Kollmannsberger, H., Oesterhelt, D. and Hazelbauer, G.L. (1994) J. Biol. Chem. 269, 16449–16454.
[13] Krah, M., Marwan, W., Verméglio, A. and Oesterhelt, D. (1994) EMBO J. 13, 2150–2155.
[14] Spudich, E.N. and Spudich, J.L. (1993) J. Biol. Chem. 268, 16095–16097.
[15] Scharf, B., Pevec, B., Hess, B. and Engelhard, M. (1992) Eur. J. Biochem. 206, 359–366.
[16] Joliot, P., Béal, D. and Frilley, B. (1980) J. Chim. Phys. 77, 209–216.
[17] Higuchi, R., Krummel, B. and Saiki, R.K. (1988) Nucleic Acids Res. 15, 7351.
[18] Cline, S.W., Lam, W.L., Charlebois, R.L., Schalkwyk, L.C. and Doolittle, W.L. (1989) Can. J. Microbiol. 35, 148–152.