Functional expression of His-tagged sensory rhodopsin I in *Escherichia coli*

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Abstract Sensory rhodopsin I (SRI) from *Halobacterium salinarum* was functionally expressed in *Escherichia coli* and subsequently purified to homogeneity using a C-terminal His-tag anchor. Yields of 3–4 mg SRI/l cell culture can be obtained. The absorption and photocycle properties of SRI were similar if not indistinguishable from those of the homologously expressed SRI. A global fit analysis of the photocycle data and the calculation of the spectra of states provided strong evidence for the existence of an N-like intermediate.

Key words: Archaeal phototaxis; Photocycle; *Halobacterium salinarum*

1. Introduction

The plasma membrane of the archaeal *Halobacterium salinarum* contains four retinal proteins, the light driven ion pumps bacteriorhodopsin (BR) and halorhodopsin (HR) as well as the two photoreceptors sensory rhodopsin I (SRI) and II (SRII) (reviewed in [1,2]). SRI mediates an attractant response to orange light and in a two photon process with additional blue light a repellent answer of the bacteria [3]. The second pigment SRII is a photophobic receptor which absorbs maximally at 490 nm [4,5]. The interplay of the two receptors enables the cell to avoid cell damaging blue light and to seek optimal light conditions for the ion pumps BR and HR.

Despite their different functions all archaeabacterial rhodopsins share a common architecture. They consist of seven transmembrane helices and contain the chromophore all-*trans* retinal covalently linked via a protonated Schiff base to a conserved lysine residue. Stimulated by light the pigments undergo similar photocycle reactions which encompass an all-*trans* to 13-*cis* isomerisation of the retinal, charge transfer steps, as well as conformational changes of the protein.

Recent progress in the heterologous expression of archaeal rhodopsins provides a facile access to the photophobic receptor pSRII and the chloride pump pHR from *Natronobacterium pharaonis* [6,7]. In this report we describe the expression of SRI from *H. salinarum* in *Escherichia coli* and its high yield purification via a C-terminal His-tag. The heterologously expressed protein displays an absorption spectrum which is indistinguishable to that of SRI isolated from *H. salinarum*.

2. Materials and methods

2.1. Plasmids and strains

For DNA manipulation *E. coli* strain XL1 and for gene expression *E. coli* BL21 (DE3) was used. The *sop1* gene was amplified via PCR from the plasmid pSopFS-Eco (kindly provided by D. Oesterhelt) [8]. By using the primer 5′-TGGTGCGCCATGGACGCCGTCGCAA-3′ and 5′-GGGGACGGAATTCTCGTCCGCCGCC-3′ an *Nco*I-site at the N-terminus and an *Eco*RI-site at the C-terminus was introduced. The stop-codon TGA was replaced by GAG which encodes Glu. The PCR product was ligated into the *Nco*I/*Eco*RI digested vector PET27bmod which provides the C-terminal 6×His tag [7].

2.2. Expression and purification

The protein was expressed according to [6] and [7]. Transformed *E. coli* cells BL21 (DE3) were grown in LB medium (50 mg/l kanamycin) at 37°C. At an OD_{578} of 0.6 IPTG (1 mM) and all-*trans* retinal (10 μM) were added. The cells were harvested after a 2 h induction period by centrifugation. The blue coloured pellet was washed with 4 M NaCl, 50 mM MES pH 6.0, resuspended in the same buffer and the cells were disrupted in a microfluidizer (Microfluidics Corporation, Newton, MA, USA). Cell debris was removed by low spin centrifugation (5000×*g*, 45 min, 4°C). For solubilisation of the membranes 2% (w/v) DM was added and the suspension was incubated for 1.5 h at 4°C. The solubilised membranes were isolated by high spin centrifugation (100 000×*g*, 1 h, 4°C) and incubated with Ni-NTA agarose (Quiagen, Hilden, Germany) for 1 h at 4°C. Thereafter, the Ni-NTA resin was applied to a chromatography column and washed excessively with 4 M NaCl, 50 mM MES pH 6.0, 20 mM imidazole, 0.05% w/v DM to remove unspecifically bound proteins and excess retinal. Impurities from cytochromes were first eluted with the above buffer between pH 5.4 and pH 5.6 before SRI was isolated at pH 4.0. The sample was immediately titrated to pH 6.0.

2.3. Laser-flash photolysis

The photocycle experiments were done according to [9].

3. Results and discussion

3.1. Expression and purification

The expression of SRI in *E. coli* follows the same method developed for pSRII, pHR and BR [7]. For SRI a high expression level is observed as indicated by the deep blue colour of the harvested cells. The purification takes advantage of the His-tag and yields about 3–4 mg SRI/l cell culture. The purity of the sample is greater than 95% as judged from the ratio of the chromophore absorption at 587 nm to the protein peak at 280 nm (Fig. 1) which was determined to be 1.9.

3.2. Absorption properties

The absorption spectrum of SRI measured at two different pH values is presented in Fig. 1. The spectra are essentially identical to those already described for the homologously ex-

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Abbreviations: DM, dodecylmaltoside; IPTG, isopropylthiogalactoside; MES, morpholinoethanesulfonic acid; SRI, sensory rhodopsin I; SRII, sensory rhodopsin II

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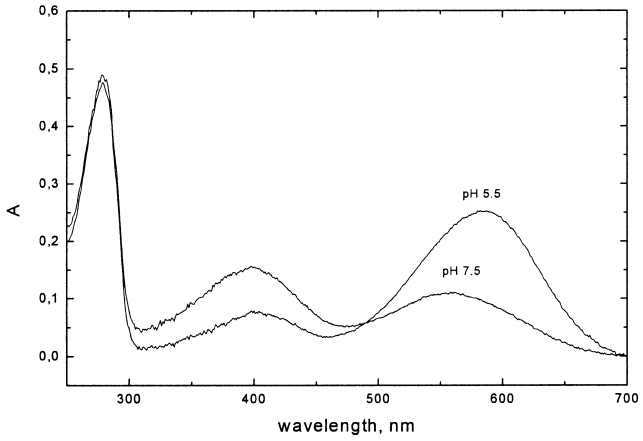

Fig. 1. UV/VIS absorption spectrum of SRI at pH 5.5 and pH 7.

pressed protein [10]. The maximum of the main chromophore band shifts from 587 nm at low pH to 550 nm at alkaline pH conditions. This transition which is only partially reversible has an isosbestic point at 500 nm and reflects the deprotonation of the SB counter-ion Asp-76 with a pK_a of 7.2 [11,12]. Concomitantly with these changes, the amplitude of the band at around 400 nm increases which might be assigned to the deprotonated Schiff base [10].

3.3. Photocycle kinetics

After photoexcitation of SRI the transient absorption changes have been recorded at pH 4.0, 5.5 and 6.0 and a spectral range from 350 to 700 nm (Fig. 2). The broad negative (around 580 nm) and positive (around 400 nm) absorptions monitor the decay and reformation of the initial resting state and the formation and decay of the SRI_{380} species (M-state), respectively. A red shifted O-like state as observed in all other archaeal rhodopsins could not be detected.

As previously reported the photocycle kinetics are considerably affected by the proton concentration [13–15]. For example, the amplitude of the SRI_{380} species (M-intermediate, trace 1 at 380 nm) declines with a decreasing pH (Fig. 2). At pH 4.0 almost no M-state could be detected (Fig. 2C). It has been argued, that this observation is due to the pH dependent shift of SRI_{550} to SRI_{587}. The latter species which does not accumulate S_{380} becomes dominant at lower pH [13,16].

The rate constants provided by the global fit are generally in agreement with published data [14,15] (see Table 1 for a comparison of the data) although the published data vary considerably. However, as can be depicted from Table 1, the formation of M is accomplished in ms whereas the reformation of the original ground state occurs in the second range. Since the photocycle is quite sensitive to the pH of the buffer the discrepancies between the published data might be due to the different sample preparations (e.g. solubilisation) and/or buffer conditions. It should be noted that within the dead time of the instrumental setup the M-intermediate is already present indicating the presence of another rate constant in the μs time range as reported in [17]. A biphasic formation of SRI_{380} has also been observed by Haupts et al. [15].

Contrary to published results we find at pH 5.5 a second very slow phase of $t = 18$ s. The amplitude of this component is rather low and its absolute spectra is identical to the ground state (data not shown). It seems unlikely that this species

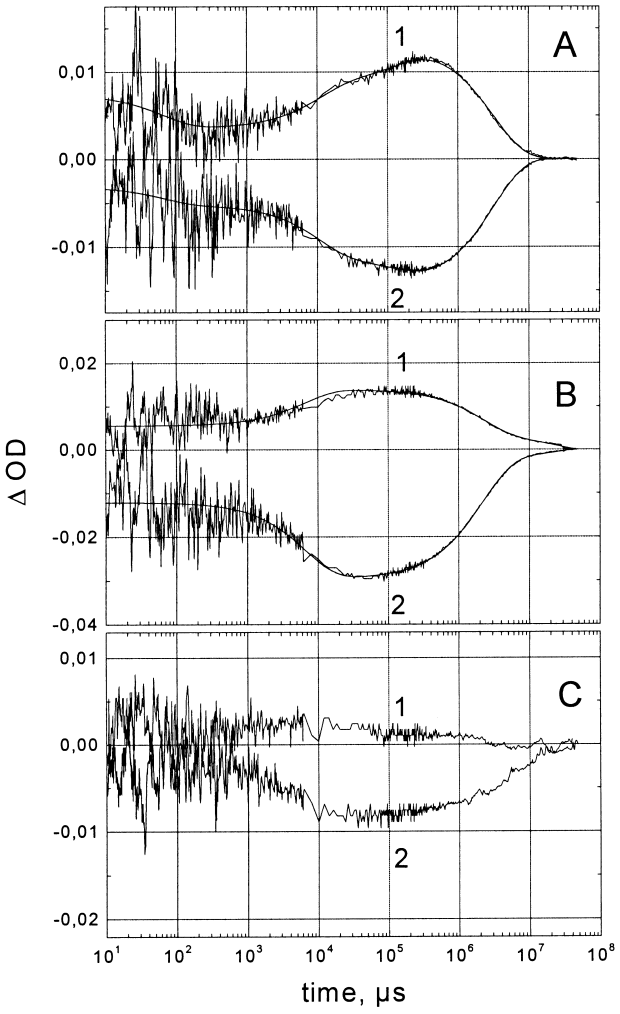

Fig. 2. Transient absorption changes after photoexcitation of SRI at 20°C: Traces at wavelengths characteristic for SRI_{380} (trace 1: 380 nm) and SRI_{550}/SRI_{580} (trace 2: 580 nm) are depicted at pH 6.0 (A), 5.5 (B) and 4.0 (C) (4 M NaCl, 50 mM MES, 0.05% w/v DM). Thin solid lines in (A) and (B) represent the results of the global fit.

belongs to the native photocycle because the baseline shifted minimally during data acquisition due to bleaching of the sample. The program accounted for this shift with an additional component.

3.4. Absolute spectra of kinetic states

Assuming a photocycle model in which the measured first order rate constants are assigned to a scheme of irreversible

Table 1
Comparison of published SRI photocycle rate constants

	Buffer	M-formation	M-decay
This report	pH 5.5	4 ms	1.5 s
	pH 6.0	7 ms; 100 ms	2.2 s
Olson et al. [19]	pH 5.5	n.d.[a]	214 ms; 1.26 s
Bogomolni and Spudich [20]	pH 6.8	270 μs	750 ms
Haupts et al. [15]	pH 5.5	20 μs; 400 μs	600 ms
	pH 6.8	30 μs; 400 μs	1.3 s
Jung et al. [17]	pH 5.0	10 μs; 11 ms	n.d.[a]
	pH 6.0	11 μs; 17 ms	

[a] not determined.

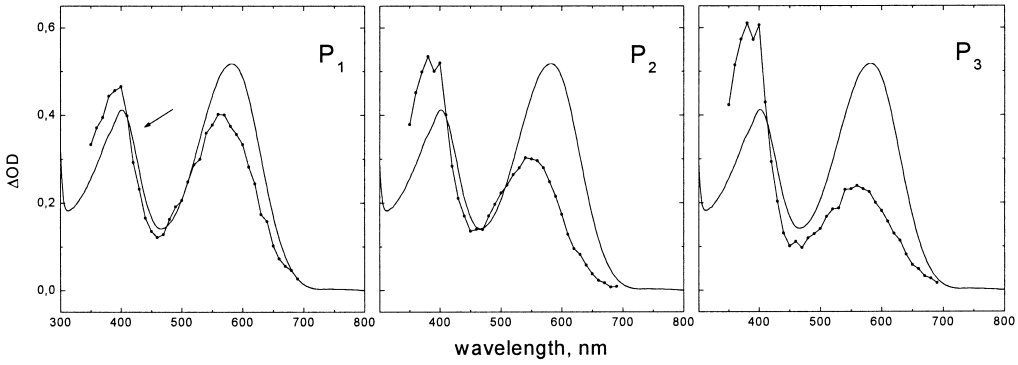

Fig. 3. Absolute spectra of kinetic states (P_1–P_3) provided by the global fit (the rate constants are given in Table 1). The calculation covering the time interval from 1 μs to 50 s was based on the set of data recorded at pH 6.0 and 20°C (4 M NaCl, 50 mM MES, 0.05% w/v DM). For comparison, the spectra of the original ground state are shown (thin solid line) in each panel.

transitions, the absolute spectra of the kinetic states can be calculated [9]. The corresponding spectra are depicted in Fig. 3. All intermediates (P_1, P_2 and P_3) are characterised by two absorption maxima at around 380 nm and at 560 nm which indicates that the corresponding kinetic states consists of two fast equilibrating species. Taking their spectral position into account the underlying components can be assigned to the archetypical M- and L-like states. The irreversible transition from P_1 to P_2 shifts the rapid equilibrium between L and M. On the other hand, the decay of P_3 occurs only with $\tau_3 = 2.2$ s which represents the last step in the photocycle, the reprotonation of the Schiff base concomitantly with the reformation of the original resting state. Taking this chronological sequence into account one can assign the 560 nm absorption of P_3 to an N like but not to an L-like spectral state. The photocycle of SRI at pH 6.0 could then be described by the following scheme:

$$\mathrm{SRI} \xrightarrow{\mathrm{hv}} [\mathrm{L} \leftrightarrow \mathrm{M}] \xrightarrow{7\ \mathrm{ms}} [\mathrm{L} \leftrightarrow \mathrm{M}] \xrightarrow{100\ \mathrm{ms}} [\mathrm{M} \leftrightarrow \mathrm{N}] \xrightarrow{2.2\ \mathrm{s}} \mathrm{SRI}$$

Another point should be mentioned. The spectra of the M-like state form an isosbestic point with the spectrum of the ground state at about 410 nm (Fig. 3, indicated by the arrow). This observation implies that the photo-excitation of SRI at 532 nm not only induces a depletion of the main chromophore band at 580 nm but also of the band with its maximum at 400 nm. This observation might be explained by a rapid equilibrium between SRI_{400} and SRI_{550}/SRI_{590}, a proposal which was put forward by Krebs et al. [10]. Already in P_1 this spectral feature is present, indicating that this equilibrium has an intrinsic rate constant at least in the μs time range.

It should be noted, that the elucidation and interpretation of the absolute spectra possess some ambiguities which certainly need to be analysed in more detail. First, the experimental data are at least a superposition of the two independent photocycles of SRI_{590} and SRI_{550}. Second, branching reactions as discussed in [18] might lead to a systematic error in the calculation of the absolute spectra which assumes sequential first order reactions.

Summarising, the data of the absorption and the photocycle properties of SRI expressed in *E. coli* are in agreement with previously published results from homologously expressed SRI. It can be concluded that neither the *E. coli* expression system nor the C-terminal His-tag alter the functionality of SRI. Therefore, the *E. coli* expression system represents an attractive alternative for the preparation of SRI and its site directed mutants as well as for the facile incorporation of retinal analogues.

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