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# Photochemical chromophore isomerization in histidine kinase rhodopsin HKR1

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ABSTRACT

**Histidine kinase rhodopsin 1 is a photoreceptor in green algae functioning as a UV-light sensor. It switches between a UV-absorbing state (Rh-UV) and a blue-absorbing state (Rh-Bl) with a protonated retinal Schiff base (RSB) cofactor in a mixture of 13-*trans*,15-*anti* and 13-*cis*,15-*syn* isomers. The present spectroscopic study now shows that cofactor-protein assembly stabilizes the protonated 13-*trans*,15-*anti* RSB isomer. Formation of the active photoswitch requires the photoinduced conversion to Rh-UV. The transitions between the Rh-Bl isomers and the deprotonated 13-*cis*,15-*anti* and 13-*trans*,15-*syn* isomers of Rh-UV proceed via multiple photoisomerizations of one or simultaneously two double bonds.**


## 1. Introduction

Microbial rhodopsins are widespread photoreceptors in prokaryotes and lower eukaryotic groups [1]. They utilize light as a source of energy or information to drive ATP synthesis or to trigger physiological responses. Among them, the well-studied bacteriorhodopsin (BR) that serves as a light-driven proton pump was, for a long time, considered to be a prototype for all other microbial retinal proteins [2]. With increasing interest in this class of proteins, driven by actual and potential applications of microbial rhodopsins such as channelrhodopsin in optogenetics [3], research in this field was revitalized and more retinal proteins were discovered in various organisms. Structure and function of these novel photoreceptors are far from being understood but many of their representatives display a photochemical behavior and mechanistic pattern of the retinal chromophore that differ substantially from BR [4].

We have recently reported on a member of a novel subfamily of histidine-kinase rhodopsins. Histidine kinase rhodopsin I (HKRI) found in the green alga *Chlamydomonas reinhardtii* [5] has been suggested to exert a function in adaptation of behavioral responses to the UV irradiation. HRK1 is a modular protein that comprises a photosensory rhodopsin, a histidine kinase, a response regulator and a putative guanylyl cyclase domain. The photosensor was found to exist in two parent states absorbing at 379 nm (Rh-UV) and 486 nm (Rh-Bl), which can be converted into each other by light of appropriate wavelengths. In this sense, HKR1 acts as a bimodal photoswitch. This contrasts the properties of other retinal proteins running through a unidirectional photoinduced reaction cycle. The color change associated with photoswitching of HKR1 is related to the protonation state of the Schiff base function that provides the linkage of the retinal chromophore with a Lys side chain of the protein. Resonance Raman (RR) spectroscopy has shown that in the Rh-UV state, the retinylidene chromophore is deprotonated and adopts a 13-*cis*,15-*anti* conformation as the M412 state of BR [2,5]. Photoconversion to Rh-Bl by UV irradiation, however, affords a mixture of 13-*trans*,15-*anti* and 13-*cis*,15-*syn* conformers, both carrying a protonated Schiff base as revealed by RR spectroscopy and retinal extraction experiments [5]. This structural heterogeneity of Rh-Bl is reminiscent of the dark-adapted form of BR, which results from the thermal chromophore isomerization of the light-adapted form [6,7].

We are therefore interested in exploring whether thermal retinal isomerization steps also take place in HKR1 and to analyze

*Abbreviations:* BR, bacteriorhodopsin; HKR1, histidine kinase rhodopsin 1; RSB, retinal Schiff base; RR, resonance Raman; Rh-Bl and Rh-UV, blue- and UV-absorbing form of the HKR1photosensor; Rh-Dark, initial dark state of the HRK1 photosensor

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their potential interference with photochemical isomerization routes. In this work we have employed UV–vis absorption and RR spectroscopy to analyze the various isomerization reactions from the cofactor-protein assembly to the transitions between Rh-Bl and Rh-UV.

## 2. Materials and methods

### 2.1. Protein expression

The used DNA-construct was a humanized *C. reinhardtii* HKR1 rhodopsin fragment (amino acids 1–265, accession number: AAQ16277) in a pPICz-vector including a C-terminal c-myc-tag and a 12x-histidine-tag. Heterologous protein expression was performed in the methylotrophic yeast *Pichia pastoris* strain 1168H and was induced by the addition of 2.5% methanol to the growth medium containing 5 μM *all-trans* retinal. Details of protein expression and purification procedures were described earlier [5]. The protein was solubilized in HEPES-buffer pH 7.4 with 0.03% dodecyl maltoside. For the analysis of Rh-Dark all expression and purification steps were performed under red dim light and under strict exclusion of any visible light source.

### 2.2. UV–vis absorption spectroscopy

Steady state absorption spectra were recorded in a Cary 300 Bio Spectrophotometer (Varian Inc., Darmstadt, Germany) at 20 °C with a resolution of 1.6 nm. The Rh-Dark sample was measured without light exposure except the spectrophotometer light (bandwidth: 1 nm, scan rate: 1454.546 nm/min). Formation of Rh-UV was induced by a 2 min illumination of either Rh-Dark or Rh-Blue with different light emitting diodes (LED, Nichia Corporation, Tokushima, Japan, 0.12 W), emitting at 470 nm (blue light), 530 nm (green light), or 590 nm (orange light). Rh-UV was converted to Rh-Bl by a 2 min illumination with a 0.11 W UV-LED (380 nm). Spectra were measured immediately after illumination and after a period of dark adaptation (minutes, hours and days) at room temperature. To ensure equal purification and buffer conditions of the three species, spectra were obtained from the same sample by the above described illumination procedures.

### 2.3. Resonance Raman spectroscopy

RR spectra were measured with 514 nm and 413 nm excitation of an $Ar^+$ and $Kr^+$ ion laser, respectively (Coherent, Santa Clara CA, U.S.A.). The Raman signals were detected in a backscattering configuration (180°) via a confocal LabRamHR spectrometer (Horiba, Villeneuve, France), equipped with a liquid-nitrogen cooled CCD detector. The resolution per pixel was between 0.4 $cm^{-1}$, corresponding to a spectral resolution of 1.2 $cm^{-1}$. Typical total accumulation times and laser powers at the sample were 1 h at ca. 1 mW, respectively. Low-temperature experiments were carried out with a Linkam cryostat (Linkam Scientific Instruments, Surrey, UJ), mounted on a computer controlled XY stage (OWIS GmbH, Germany). The cryostat was circularly moved through the laser focus to reduce unwanted photochemical processes. The samples were inserted into the cell under dimmed red light in order to avoid photoactivation before freezing. Further details of the set-up and the measurements were described elsewhere [5]. In all spectra shown in this work, the background was subtracted by a polynomial function. Band fitting and component analysis was carried out as described previously [8].

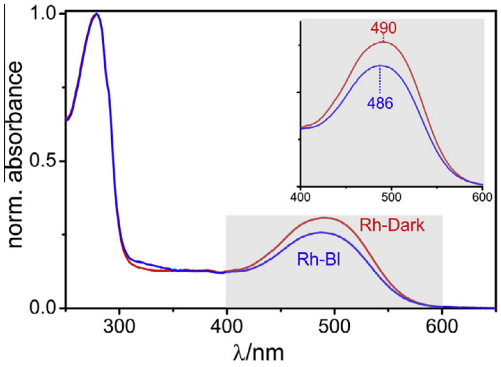


**Fig. 1.** UV–vis absorption spectra of Rh-Bl (blue) and Rh-Dark (red), measured in a HEPES buffer (pH 7.4) at 20 °C. Spectra were normalized to the absorbance maximum at 280 nm. The inset shows the enlarged spectra of Rh-Bl and Rh-Dark in the visible region. The respective absorption maxima are indicated.

## 3. Results and discussion

After purification under daylight conditions, the photosensor of HKR1 is largely in the Rh-UV state, which displays an absorption maximum at 379 nm with poorly resolved vibronic side bands (*vide infra*). In this state, the chromophore adopts a 13-*cis*,15-*anti* geometry with deprotonated Schiff base [5]. As concluded from a RR spectroscopic comparison, even structural details are very similar to the chromophore in the M412 state of BR although the absorption maximum of Rh-UV of HKR1 is shifted further to the UV by ca. 30 nm. Rh-UV can be reversibly converted to the Rh-Bl state that exhibits an absorption maximum at 486 nm (Fig. 1, blue trace), reflecting a mixture of both a 13-*trans*,15-*anti* and 13-*cis*,15-*syn* conformers, both carrying a protonated Schiff base [5].

### 3.1. The Rh-Dark state of HKR1

In case expression, protein-cofactor assembly with all-*trans* (13-*trans*,15-*anti*) retinal, and purification of HKR1 was carried out under strict red-light conditions, the absorption spectrum reveals no indication for Rh-UV but instead displays an absorption spectrum that is similar to that of Rh-Bl albeit not identical (Fig. 1). A careful inspection (Fig. 1, inset) shows a slight red-shift of the absorption maximum to 490 nm accompanied by an increase of the absorbance by nearly 20%. This initial state, denoted as Rh-Dark, is readily converted to Rh-UV upon irradiation with blue light but cannot be recovered photochemically since UV-irradiation affords Rh-Bl (*vide supra*). We thus conclude that the chromophore structure of the initially formed dark state Rh-Dark significantly differs from Rh-Bl.

### 3.2. Resonance Raman spectroscopic analysis of the Rh-Dark state

The irreversible light-induced conversion of Rh-Dark to Rh-UV makes it impossible to measure the RR spectra of Rh-Dark at ambient temperature. Since the photoinduced formation of Rh-UV is associated with thermal relaxation steps, one may reduce depletion of Rh-Dark and Rh-Bl in the exciting laser beam at sufficiently low temperature. This condition is in fact fulfilled at 80 K for Rh-Bl and the low-temperature RR spectrum can be directly compared with a RR spectrum obtained at ambient temperature in a dual-color experiment [5] (Fig. 2A and B). Although at ambient temperature additional UV-irradiation was used to continuously recover Rh-Bl from Rh-UV, a photostationary mixture between Rh-UV and Rh-Bl is established but only the spectrum of the latter species is resonantly enhanced at 514 nm. Compared to this spectrum, at low temperature only minor differences are noted which refer to