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Dual role for a bacteriophytochrome in the bioenergetic control of *Rhodopsdeudomonas palustris*: Enhancement of photosystem synthesis and limitation of respiration

Mila Kojadinovic [a], Aurélie Laugraud [b], Laurie Vuillet [c], Joël Fardoux [c], Laure Hannibal [c], Jean-Marc Adriano [a], Pierre Bouyer [a], Eric Giraud [b], André Verméglio [a,*]

[a] *CEA Cadarache, DSV/IBEB/SBVME/LBC, UMR 6191 CNRS/CEA/Univ Aix-Marseille, Saint-Paul-lez-Durance, F-13108 France*
[b] *Université Lyon 1, Pôle Rhone-Alpin de Bioinformatique, UMR 5558, Laboratoire de Biométrie et Biologie Evolutive, 43 boulevard du 11 novembre 1918, Villeurbanne, F-69622, France*
[c] *Laboratoire des Symbioses Tropicales et Méditerranéennes, IRD, CIRAD, AGRO-M, INRA, UM2. TA A-82/J, Campus de Baillarguet, 34398 Montpellier Cedex 5, France*

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Abstract

In the purple photosynthetic bacterium *Rhodopseudomonas palustris*, far-red illumination induces photosystem synthesis via the action of the bacteriophytochrome *Rp*BphP1. This bacteriophytochrome antagonizes the repressive effect of the transcriptional regulator PpsR2 under aerobic condition. We show here that, in addition to photosystem synthesis, far-red light induces a significant growth rate limitation, compared to cells grown in the dark, linked to a decrease in the respiratory activity. The phenotypes of mutants inactivated in *Rp*BphP1 and PpsR2 show their involvement in this regulation. Based on enzymatic and transcriptional studies, a 30% decrease in the expression of the alpha-ketoglutarate dehydrogenase complex, a central enzyme of the Krebs cycle, is observed under far-red light. We propose that this decrease is responsible for the down-regulation of respiration in this condition. This regulation mechanism at the Krebs cycle level still allows the formation of the photosynthetic apparatus via the synthesis of key biosynthesis precursors but lowers the production of NADH, i.e. the respiratory activity. Overall, the dual action of *Rp*BphP1 on the regulation of both the photosynthesis genes and the Krebs cycle allows a fine adaptation of bacteria to environmental conditions by enhancement of the most favorable bioenergetic process in the light, photosynthesis versus respiration.

Keywords: Bacteriophytochrome; Photosynthesis; Respiration; Alpha-ketoglutarate; Regulation; *Rhodopseudomonas palustris*

1. Introduction

Due to their extraordinary metabolic versatility, photosynthetic bacteria can grow with or without oxygen, via aerobic or anaerobic respirations and photosynthesis. To benefit from this flexibility and take advantage of available resources, these bacteria have to rapidly adapt and respond to variations in their environment. This requires sensing changes in light and oxygen concentration since the regulation between photosynthesis and respiration is of prime importance for their energetic fitness (for review see [1–3]). This regulation occurs at two different levels.

Short-term interactions between the electron carriers enable photosynthetic bacteria to utilize the most favorable bioenergetic process in the light, i.e. photosynthesis. Consequently, light strongly inhibits respiratory activity [4] by two non-exclusive mechanisms. The first is an indirect effect of the light-induced proton motive force on complexes involved in the respiratory activity in particular at the level of complex I [5–7]. The second mechanism involves a direct competition between electron carriers common to both photosynthetic and respiratory chains (ubiquinone, the cytochrome bc_1 complex or the cytochrome c_2) [8,9].

On the transcriptional level, regulations of the synthesis of the bioenergetic chains in purple bacteria involve several redox and light sensors [3,10,11]. The sensor kinase RegB and its

* Corresponding author. Fax: +33 442254701.
E-mail address: avermeglio@cea.fr (A. Verméglio).

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response regulator RegA are responsible for the global transcriptional control of aerobic and anaerobic metabolic processes in response to the redox state of the cell (for review see [12]). In addition, specific aerobic regulators of the expression of photosynthesis genes have been described in several photosynthetic bacteria. The transcriptional factors PpsR (or CrtJ) act as repressors of the synthesis of the photosynthetic apparatus under high aeration in *Rhodobacter* (*Rb*.) *sphaeroides* [13], *Rb. capsulatus* [14], and *Rhodopseudomonas* (*Rps.*) *palustris* [15,16]. A more complex role has however been reported in the case of *Rubrivivax gelatinosus* where this transcriptional factor represses some photosynthesis genes or activates others [17]. The aerobic photosynthetic bacterium *Bradyrhizobium* ORS278 possesses two distinct proteins, PpsR1 and PpsR2, which have opposite effect on the synthesis of the photosynthetic apparatus, the O_2-sensitive PpsR1 being an activator whereas the O_2-insensitive PpsR2 acts as a repressor [10,18].

To date, two types of photoreceptors have been documented as regards to the light regulation of the synthesis of the photosynthetic apparatus of purple bacteria. (i) AppA, a blue light receptor, represses the photosynthetic genes expression in *Rb. sphaeroides* [19–21]. (ii) A bacteriophytochrome, present in *Rps. palustris* (*Rp*BphP1) and *Bradyrhizobium* (*Br*BphP), has been shown to trigger the synthesis of the entire photosynthetic apparatus under aerobic conditions by antagonizing the repressing effect of PpsR2 [10,15,22]. In addition to this bacteriophytochrome, two others, *Rp*BphP2 and *Rp*BphP3, act in tandem in *Rps. palustris* to control the synthesis of LH4 complexes [23,24], characterized by a single absorption band in the near infra-red at 800 nm [25].

The sequencing of the complete genome of *Rps. palustris* CGA009 has revealed the presence of 3 other putative bacteriophytochromes and a large number (451) of putative regulatory and signaling genes, corresponding to 9.3% of its genome as compared to the usual 5–6% for free-living bacteria [26]. This makes *Rps. palustris* an excellent model to study the adaptability of a photosynthetic bacterium in response to environmental changes.

In the present work, we demonstrate that *Rp*BphP1, in addition to its up regulation of the photosynthetic units, down-regulates the respiratory activity under far-red illumination. This regulation also involves the transcriptional regulator PpsR2. We propose that this down-regulation of the respiratory activity is linked to a decrease in the expression of the alpha-ketoglutarate dehydrogenase complex, a central enzyme of the Krebs cycle. This regulation at the Krebs cycle level both decreases respiratory activity but still allow the synthesis of key biosynthesis precursors for the formation of the photosynthetic apparatus. This mechanism allows a fine adaptation of bacteria to environmental conditions by enhancement of photosynthesis activity versus respiration, i.e. the most favorable bioenergetic process in the light.

2. Materials and methods

2.1. Bacterial strain and growth conditions

The WT strain CEA001 and the various mutants of *Rps. palustris* were grown in a modified photosynthetic medium (PM) [27] by addition of 20 mM ammonium malate as additional carbon and nitrogen sources, and growth factors containing vitamins in proportion used in Hutner medium [28]. Bacteria were grown either in the dark or under 770 nm illumination provided by light emitting diodes (100 μmol of photon/s/m^2, half-peak bandwidth of 25 nm). When indicated, additional actinic illumination was provided by light emitting diodes (875 nm, 1500 μmol of photon/s/m^2, half-peak bandwidth of 25 nm). Pure N_2 and air were mixed using mass-flow controller (Brooks) in order to obtain the appropriate O_2 tension ranging from 1% and 21%. The gas mixtures were flushed in the gas phase of Erlenmeyer flasks of 250 ml. To obtain a good equilibrium between the gas and the liquid phases, the Erlenmeyer flasks contained only 50 ml of growth medium and were shaken at 140 rpm. Cells, inoculated at an initial concentration of OD_{660}=0.1, were collected at various times ranging from 8 h to 160 h for absorption spectra recording, respiratory activity determination, RNA extraction and alpha-ketoglutarate dehydrogenase complex activity determination.

2.2. Absorption spectra and photosystem synthesis measurements

Absorption spectra of intact cells of the WT strain and of the various mutants were recorded with a Cary 50 spectrophotometer. The relative amount of photosystem was determined by integrating the Qy bacteriochlorophyll absorption bands between 700 and 950 nm for suspension of intact cells collected at different times and growth conditions.

2.3. Bacterial growth

Bacterial growth was determined by recording the optical density at 660 nm (OD_{660}) in function of time. This wavelength was chosen because it only depends upon the bacteria light scattering and not to the presence of bacteriochlorophyll molecules which do not absorb in this spectral range. Correlation between OD_{660} and bacterial concentration was determined by plating serial dilutions of the bacterial suspensions and counting the CFU/ml.

2.4. Respiratory activity measurements

The respiratory activity of intact cells was determined using a Clark electrode (Hansatech, Great Britain). Prior to the measurements the cells were harvested and suspended in fresh modified PM medium. The respiratory activity of each strain was normalized to the same number of cells (determined by recording the OD_{660}). The total cytochrome oxidase activity was tested by the addition of tetramethylparaphenylene diamine (TMPD) at a saturating concentration of 100 μM.

2.5. Construction of RpBphP1, RpBphP5, RpBphP6 and ppsR2 mutant strains

The construction of the *RpBphP1*, and *ppsR2* mutants of *Rps. palustris* CEA001 strain has been previously described [15]. The *RpBphP5.CEA001* null mutant was obtained by inserting the *lac*Z-Km^r cassette directly into the unique *Bam*H1 site of the *RpBphP5* gene. To create the *RpBphP6. CEA001* null mutant, a *Sal*I fragment of 39-bp inside the gene was deleted and replaced by the *lac*Z-Km^r cassette of pKOK5 [29]. These constructs were introduced into the pJQ200 suicide vector [30] and delivered by conjugation into the corresponding *Rps. palustris* strain as described [15]. Double recombinants were selected on sucrose and confirmed by PCR.

2.6. RNA extraction

Bacterial cells were collected during dark and 770 nm exponential phases (between 20 h and 25 h of growth corresponding to $OD_{660\ nm}$=0.6–0.9 for dark-grown bacteria, $OD_{660\ nm}$=0.4–0.5 for 770 nm grown bacteria). Two volumes of RNAprotect™ Bacteria Reagent (Qiagen GmbH, Hilden, Germany) were added to one volume of bacterial culture for RNA stabilization. Cells were harvested (5000×*g*, 10 min, room temperature) and kept at −80 °C. Total RNA extraction was performed from 10^9 bacteria using the Nucleospin RNA II kit (Macherey Nagel, Düren, Germany) following the manufacturer's procedures except for the first lysis step. Indeed, *Rps. palustris* cells being resistant to lysozyme digestion, cells were resuspended in RA1 buffer from the Nucleospin