FEBS Letters 581 (2007) 368–374

Photometrical analysis with photosensory domains of photoreceptors in green algae

Takatoshi Kagawa[a,*], Noriyuki Suetsugu[a,b]

[a] *Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai 1-1-1, Tsukuba 305-8572, Japan*
[b] *Division of Photobiology, National Institute for Basic Biology, Okazaki 444-8585, Japan*

Received 22 November 2006; revised 14 December 2006; accepted 15 December 2006

Available online 11 January 2007

Edited by Richard Cogdell

Abstract **Chloroplast photoorientation in the green alga *Mougeotia scalaris* is controlled by blue and red light. The properties of the LOV domains of phototropin A and B were consistent with previous data of action spectra and photoreceptor lifetime for blue light-mediated photoorientation. The LOV domains of the neochromes did not bind flavin, while the domains of neochrome 2 contributed to multimer formation. The absorption spectra of the neochrome phytochrome photosensory domain with phytochromobilin were very similar to the action spectra for red light-induced photoorientation. These results indicate that phototropin and neochrome work as the blue and red photoreceptors involved in photoorientation.**

Keywords: Chloroplast photoorientation; Neochrome; Phototropin; Phytochrome; Phytochromobilin; *Mougeotia scalaris*

1. Introduction

Each cylindrical cell of the filamentous green alga *Mougeotia scalaris* contains one giant ribbon-shaped chloroplast and these have been used as material for photobiological analysis for a long time [1]. The most studied physiological response is chloroplast photoorientation. The chloroplast orientates towards incident light of weak or medium intensity. The orientation movement is mainly controlled by red light (R) irradiation [2], but blue light (B) irradiation also plays a role in the induction [3,4].

In higher plant cells with many chloroplasts, light-induced chloroplast relocation is induced mainly by B irradiation. Weak and strong light irradiation induce the accumulation and avoidance of chloroplasts, respectively [5]. It has been shown in the dicot *Arabidopsis thaliana* that the photoreceptors phototropin 1 (phot1) and 2 (phot2) are involved in the accumulation response but only phot2 is responsible for avoidance. Phototropin consists of two light sensitive, light-oxygen-voltage (LOV) domains at the N-terminus and a serine/threonine kinase domain at the C-terminus. Each LOV domain binds one flavin mononucleotide. In the fern *Adiantum capillus-veneris*, two phototropins have been identified and *Ac*phot2 has been found to mediate the avoidance response [6]. Four phototropins in the moss *Physcomitrella patens* act as photoreceptors in chloroplast relocation movement [7]. Although two phototropin genes, *MsPHOTA* and *MsPHOTB*, have so far been isolated in *M. scalaris*, their involvement in chloroplast photoorientation and their photochemical properties remain to be elucidated.

In several non-seed plants such as ferns, mosses and green algae, R- as well as B-induced chloroplast movement is known. In *A. capillus-veneris*, R-induced chloroplast relocation is controlled by phytochrome 3 (*Ac*phy3) which is a chimeric protein of a phytochrome photosensory region and a full length phototropin domain [8]. In *P. patens*, phytochrome-dependent chloroplast relocation movement is observed [9]. Four conventional phytochrome genes have been isolated, but no aberrant phytochrome-like sequences such as *Ac*phy3 had been found [10]. The R-induced chloroplast movement was reduced in *photA2photB1photB2* triple disruptants [7]. Together, these results indicate that the movement is mediated by conventional phytochromes through phototropins [7]. In *M. scalaris*, chloroplast photoorientation also shows typical R/FR photoreversibility, indicating phytochrome dependency. One conventional phytochrome (*Ms*phy1) and two neochromes (*Ms*neo1 and *Ms*neo2) which consist of a phytochrome photosensory region and phototropin-like sequences [11], have been identified. Since action spectra for chloroplast photoorientation are similar to the difference spectra of *Ms*neo1 and transient expression of both *MsNEO1* and *MsNEO2* cDNAs rescue an *Ac*phy3 mutant, it has been suggested that the photoreceptors for R-induced chloroplast photoorientation may be neochromes [11].

Recently, it was established that recombinant phytochromes with phytochromobilin (PΦB) were expressed in *E. coli* cells expressing both heme oxygenase and PΦB synthase [11,12]. Using this expression system, the recombinant phytochrome with chromophore can be analyzed photometrically. In this paper, in order to characterize the candidate photoreceptors for B- and R-mediated chloroplast photoorientation in *M. scalaris*, we performed spectral analysis of photosensory domains of several photoreceptors, two phototropins (*Ms*photA and *Ms*photB), two neochromes (*Ms*neo1 and *Ms*neo2) and one phytochrome (*Ms*phy1), using the heterologous expression system in *E. coli*.

*Corresponding author. Fax: +81 29 853 6270.
E-mail address: kagawa@agbi.tsukuba.ac.jp (T. Kagawa).

Abbreviations: B, blue light; CaM, calmodulin; FR, far-red light; LOV domain, light-oxygen-voltage domain; Neo1-PL, photosensory domain of neochrome 1; Neo2-PL, photosensory domain of neochrome 2; PhotA-L, LOV domains of phototropin A; PhotB-L, LOV domains of phototropin B; Phy1-P, phytochrome photosensory domain of phytochrome 1; PΦB, phytochromobilin; R, red light

0014-5793/$32.00
doi:10.1016/j.febslet.2006.12.041

2. Materials and methods

2.1. Genes

cDNAs of *PHOTA* (Accession Number AB206963), *PHOTB* (AB206964), *NEO1* (AB206961), *NEO2* (AB206962) and *PHY1* (AB206965) of *Mougeotia scalaris* were used.

2.2. Recombinant polypeptide purification and spectroanalysis

For expression of the recombinant polypeptides of PhotA-L (49–399), PhotB-L (36–373), Neo1-PL (1–1067), Neo2-PL (1–1056) and Phy1-P (1–580), the cDNAs were amplified by PCR in such as way as to add restriction enzyme sites at both ends that enabled easy introduction into pCAL-n-EK with six his-tag sequences. After construc-

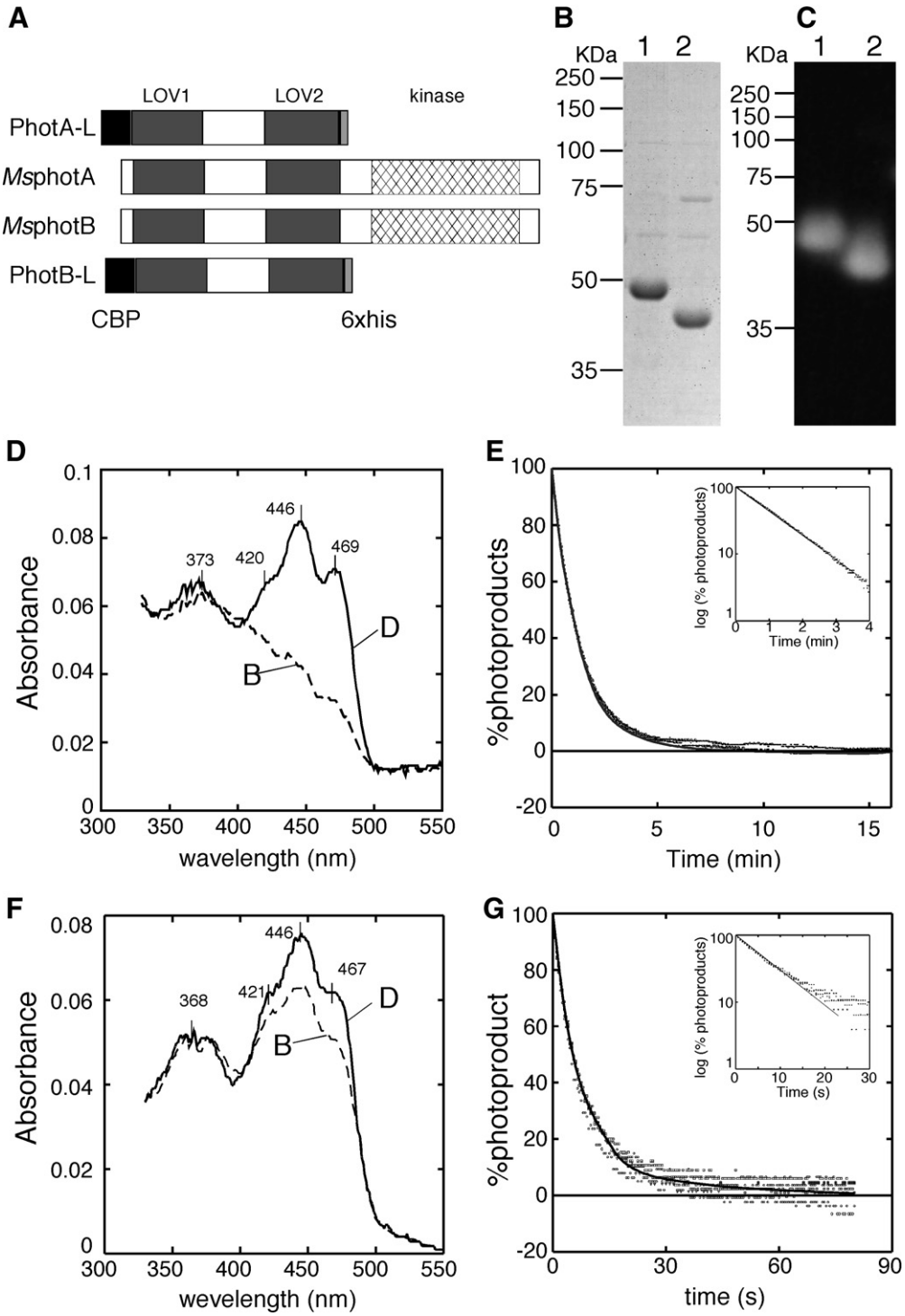

Fig. 1. Spectral analysis of LOV domains in *Ms*photA and *Ms*photB. (A) Schematic drawing of *Ms*photA, *Ms*photB and recombinant polypeptides (PhotA-L, PhotB-L). (B, C) CBB staining (B) and immunoblotting with anti-his antibody (C) of purified recombinant polypeptides of PhotA-L (lane 1) and PhotB-L (lane 2). (D) Absorption spectra of PhotA-L before (D) and after (B) B irradiation. (E) Time course of % photoproducts of PhotA-L after the B irradiation was turned off. Inset, single exponential plot in panel E. Absorptions at 450 nm were measured after the irradiation with saturated B. (F) Absorption spectrum of PhotB-L before (D) and after (B) B irradiation. (G) Time course of % photoproducts of PhotB-L after the B irradiation was turned off. Inset, single exponential plot in panel G. Other details are the same as (E).