FEBS Letters xxx (2014) xxx–xxx

journal homepage: www.FEBSLetters.org

Chemical activation of the cyanobacterial orange carotenoid protein

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ARTICLE INFO

Article history:
Received 23 September 2014
Revised 20 October 2014
Accepted 22 October 2014
Available online xxxx

Edited by Richard Cogdell

Keywords:
Orange carotenoid protein
Non-photochemical quenching
Hofmeister series

ABSTRACT

The effects of the Hofmeister series of ions on the activation of the orange carotenoid protein (OCP) from the inactive orange form to the active red form were tested. Kosmotropes led to lower OCP activation, whereas chaotropes led to greater OCP activation. Concentrations of thiocyanate exceeding 1.5 M dark activate the orange carotenoid protein to its red form. This chemically activated OCP was studied by UV–vis and circular dichroism spectroscopies. The chemically-activated OCP quenches the fluorescence of phycobilisomes in vitro, to a level comparable to that of the light-activated OCP.

1. Introduction

Light availability to photosynthetic organisms changes rapidly throughout the day due to changes in the sun's position, cloud movement, sunflecks, and sun patches [1]. Intermittent periods of high light can over-saturate photosynthetic capacity. If left unregulated, excess absorbed photons can produce reactive oxygen species, resulting in cell damage or death [2,3]. Photosynthetic organisms have evolved multiple strategies to prevent oxidative damage from light stress, including decreasing antenna size and non-photochemical quenching [4]. NPQ provides a rapid valve, on a timescale of seconds to minutes, to dissipate excess absorbed light. The mechanisms of NPQ have been described for cyanobacteria, algae, and plants. Members of the light-harvesting complex (LHC) superfamily initiate NPQ upon sensing low luminal pH in plants and algae [5,6]. Cyanobacteria, however, sense rapid exposure to high light using a blue-light photosensor, called the orange carotenoid protein (OCP) [7,8].

The orange carotenoid protein is a 35 kDa protein involved in photoprotection in many cyanobacteria [9,10]. It functions as a sensor of light intensity, using the carotenoid 3′-hydroxyechinenone as a chromophore [11,12]. Upon exposure to extended periods (minutes) of high light, inactive, orange OCP (OCP^O) becomes the active, red form (OCP^R) [12], and rapidly quenches the phycobilisome antenna complex in vitro [13]. When the OCP gene is disrupted in *Synechosystis* sp. PCC 6803, no quenching of the phycobilisome is observed [7]. Overexpression of OCP leads to increased fluorescence quenching in *Synechosystis* [12]. Overexpressed OCP primarily binds echinenone due to limiting amounts 3′-hydroxyechinenone in the cell [14]. Echinenone-OCP is able to photoconvert and function similar to 3′-hydroxyechinenone–OCP. Deleting the *crtO* gene, required for the production of echinenone and 3′-hydroxyechinenone, results in OCP binding zeaxanthin [14]. Zeaxanthin-OCP appears yellow and is not photoactive [14]. As expected, the *ΔcrtO* stain lacking photoactive OCP fails to quench the phycobilisome [14]. These experiments have been confirmed by in vitro reconstitution of OCP with purified phycobilisomes in which only the red form is competent to quench [13]. The photoconversion properties of OCP are critical to its function as a photoprotective protein.

The conformational changes associated with OCP activation are significant [15]. OCP has two domains held together by a linker region. In the crystal structure, the N-terminal domain consists of two four-helix bundles, whereas the C-terminal domain is a member of the nuclear transport factor 2 superfamily [16]. Structural changes occurring between the N- and C-terminal domain occur upon activation. The active form appears to have a more open conformation and greater solvent exposure to 3′-hydroxyechinenone. Light-induced Fourier transform infrared (FTIR) difference spectroscopy showed that α-helices are less rigid and the β-sheets are more compact in the active form [12]. Time-dependent mass spectroscopy-based carboxyl footprinting of OCP in the red and

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http://dx.doi.org/10.1016/j.febslet.2014.10.024
0014-5793/

orange forms revealed increased surface exposure in the red form of the interface between the N- and C-terminal domains [17]. Furthermore, the glycine ethyl ester modification of carboxyl groups in aspartic acids (residues E174, E244, E258, E261, E262) and glutamic acids (residues D6, D19, D304, D311) used in footprinting inhibited photoactivation and deactivation of OCP [17]. This suggests that the interface is critical for activation and relaxation of OCP. Native-mass spectrometry of the orange and red forms revealed differences in oligiomerization [15]. The inactive, orange form contains a ~1:1 mixture of monomers and dimers, whereas the active red form was mostly monomeric [15]. These conformational changes are critical for OCP function [13]. OCP^R readily binds to purified phycobilisomes in vitro, whereas OCP^O does not bind [13].

Studying the various forms of OCP in vitro is challenging [18]. OCP^O is susceptible to photoconversion during laser experiments, and OCP^R undergoes rapid reversion to OCP^O in the dark at ambient temperatures [12]. Additionally, the quantum yield of photoconversion is very low (~0.03) [12]. Thus, studying OCP^R requires constant illumination. Previous researchers have frozen OCP to stabilize both forms [18,19]. However, this is incompatible with many experiments. Since OCP activation results in monomerization and conformational changes, we decided to test the effects of the Hofmeister series on OCP activation.

The Hofmeister series classifies ions based on their capacity to "salt in" or "salt out" proteins [20,21]. Typically, the anionic series is written as $SO_4^{2-} > PO_4^- > F^- > Cl^- > NO_3^- > I^- > ClO_4^- > SCN^-$, with those on the left result in "salting out" and those on the right result in "salting in" of proteins. Ions that "salt out" are called kosmotropes, whereas those that "salt in" are called chaotropes [20]. Previous studies have shown that high phosphate, a kosmotrope in the Hofmeister series, results in poor activation of OCP [13]. Here, we show that high kosmotropes concentrations result in decreased activation, whereas chaotropes have the opposite effect. Furthermore, high concentrations of thiocyanate are shown to activate OCP even in the dark, where it remains stable and functional in its red form.

2. Results

We anticipated that the Hofmeister series would alter the rate of OCP activation. To test this, we diluted OCP^O into 100 mM MOPs (pH 7.0) buffers containing 500 mM sodium salts of fluoride, chloride, iodide, or thiocyanate (Fig. 1). We found that the chaotropes sodium thiocyanate and sodium iodide activated OCP much quicker than the kosmotrope sodium fluoride. Sodium chloride was found to be intermediate between the sodium fluoride (a kosmotrope) and sodium iodine and sodium thiocyanate (chaotropes). Sodium chloride is generally regarded as the salt that separates the kosmotropes and chaotropes in the Hofmeister series [20]. Thus, the OCP activation rate follows the Hofmeister series. This is in agreement with previous work showing that phosphate, a kosmotrope, slows OCP activation [13].

Since OCP was most strongly activated by thiocyanate, we further tested different concentrations of thiocyanate on OCP activation in the dark (Fig. 2). We observed that OCP activation occurs in the dark at sodium thiocyanate concentrations somewhere between 1 and 1.5 M. Careful observation indicated that OCP turns into the red form immediately upon mixing with high concentrations of thiocyanate. Chemically activated OCP has essentially identical absorption peak and shape to those of light activated OCP (data not shown). Lower concentrations of thiocyanate increased the activation rate, but required light for full activation. Partial activation may be present at concentrations of 0.5 and 1.0 M as some loss of the 465 nm band occurs with the appearance

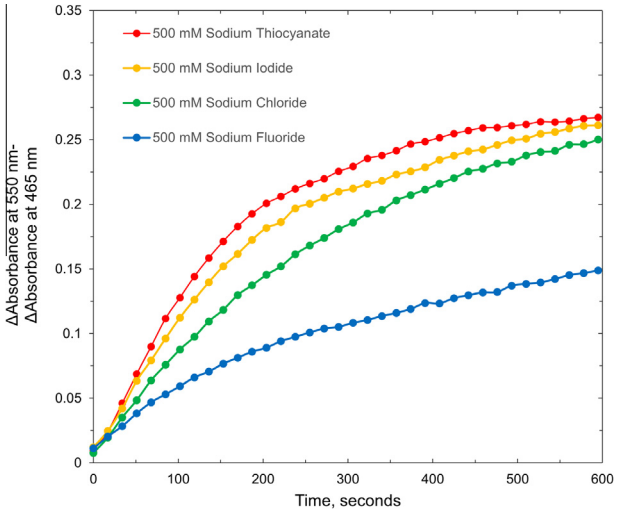

Fig. 1. Time course of OCP activation for different sodium salts. Time points are represented by circles for each salt. Lines are included for easier visualization. OCP was illuminated by blue light using a band pass filter (220 μmol photons m^{-2} s^{-1}, 350–540 nm). The gain of absorbance at 550 nm with the loss of absorbance at 465 nm was used as a measure of OCP activation.

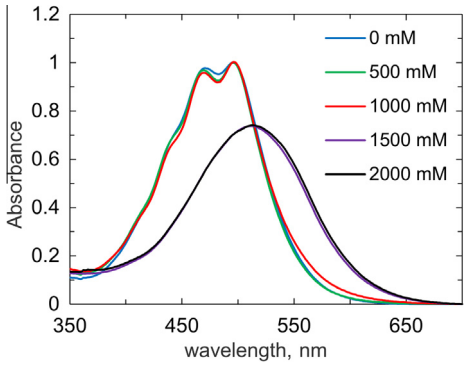

Fig. 2. Chemical activation of OCP. OCP was diluted into sodium thiocyanate concentrations ranging from 0 to 2000 mM in the dark. Spectra shown are after 30 min of incubation.

of a slight shoulder around 510 nm (Fig. 2), while critical activation must occur between 1.0 and 1.5 M (Fig. 2). Chemical activation at 1.5 M thiocyanate is not immediate, and takes around 40 min for full activation (Supplementary Fig. 1). It should be noted that chemically active OCP remains in the red form at ambient temperatures in the dark for at least 5 days, in contrast to the strong light activated OCP that are prone to damage and degradation.

The chemically activated OCP allowed us to study the red form in the absence of constant illumination or freezing. This allowed us to perform circular dichroism (CD) experiments on OCP (Fig. 3). 3′-hydroxyechinenone is nearly symmetric, and thus, normally CD inactive [22]. However, the binding of 3′-hydroxyechinenone to the protein introduces asymmetry into the chromophore, giving rise to a CD signal. The CD spectra of the OCP^O has been previously measured [22]. Our spectra are nearly identical, except for additional fine structure previously unresolved. The CD activity of the red form is significantly lower, suggesting a lowering of asymmetry upon activation and less protein–chromophore interactions. The truncated red form of OCP previously described [23] also has a lowered CD activity [22], but a different overall shape.

We tested the chemically activated OCP to determine if it can quench phycobilisomes in vitro. Gwizdala [13] demonstrated that light activated OCP can be mixed with purified phycobilisomes to

Please cite this article in press as: King, J.D., et al. Chemical activation of the cyanobacterial orange carotenoid protein. FEBS Lett. (2014), http://dx.doi.org/10.1016/j.febslet.2014.10.024