Comparative Biochemistry and Physiology, Part A 156 (2010) 416–421

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Spectral sensitivity of light-induced hatching and expression of genes mediating photoreception in eggs of the Asian tadpole shrimp *Triops granarius*

Kazuyuki Kashiyama, Chihiro Ito [1], Hideharu Numata [2], Shin G. Goto *

Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan

ARTICLE INFO

Article history:
Received 19 January 2010
Received in revised form 15 March 2010
Accepted 19 March 2010
Available online 25 March 2010

Keywords:
Arrestin
Asian tadpole shrimp
Branchiopoda
Opsin
Phototransduction cascade
Spectral sensitivity
Visual pigment

ABSTRACT

Embryonic development of the Asian tadpole shrimp *Triops granarius* is arrested at an early stage of organogenesis under darkness, whereas exposure to light resumes its development and promotes hatching. To better understand the photoreception systems involved in the light-induced hatching of *T. granarius* eggs, we examined the spectral sensitivity of hatching and the expression of genes involved in photoreception (opsins) and phototransduction (arrestin 2). Hatching of eggs is induced by light of broad wavelengths, from ultraviolet (UV) (395 nm) to red (660 nm), suggesting that visual pigments are involved in photoreception. Next, we focused on opsins which are protein moieties of visual pigments and arrestin 2 which is involved in a phototransduction cascade. Transcripts of several opsin genes and the arrestin 2 gene were detected in *T. granarius* eggs. Thus, we present a possibility that *T. granarius* eggs use visual pigments for light perception in light-induced hatching.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Light induces the hatching of a wide variety of branchiopod crustacean eggs, including that of the Asian tadpole shrimp *Triops granarius* (Pancella and Stross, 1963; Bishop, 1967; Hempel-Zawitkowska, 1970; Sorgeloos, 1973; Takahashi, 1975, 1977; Mitchell, 1990; Horiguchi et al., 2009). *T. granarius* is widely found in the paddy fields that stretch from northern Kyushu to Kanto district in Japan for a short period in summer (Akita, 1976). In summer, *T. granarius* lays eggs in the mud, where they lie desiccated beneath the soil throughout the season when the paddy fields were drained. The desiccated eggs float on the water surface in next spring when the fields are irrigated and plowed, and hatch within a few days. Takahashi (1975) found that hatching of eggs is suppressed in darkness even if the desiccated eggs are submerged, but upon exposure to light they hatch immediately. Horiguchi et al. (2009) revealed that just like desiccated eggs, the hatching of eggs that have not been desiccated is also suppressed by darkness and induced by light (see also Takahashi, 1977). Horiguchi et al. (2009) also found that eggs that had not been desiccated develop irrespective of the presence of light, but they arrest their development at an early stage of organogenesis on days 10–15 at 25 °C under constant darkness. When the eggs maintained at 25 °C for 15 days under constant darkness were exposed to light, they immediately resume their development and hatch (Horiguchi et al., 2009). In addition, histological observations revealed that the nauplius eye has not yet been formed at the early stage of organogenesis. Nevertheless, the embryos at the stage could detect light for developmental resumption (Horiguchi et al., 2009). This light responsiveness is considered to be responsible for maintaining the univoltine life cycle of *T. granarius* in paddy fields; the dark muddy environment of paddy fields suppresses hatching during summer when the paddy fields are still filled with water, and thereafter the eggs spend the season when the paddy fields are drained as desiccated eggs in a dormant state, and water and light by irrigation and plowing in next spring induce their hatching.

Although the ecological significance of light-induced hatching in branchiopod crustaceans is reported (Takahashi, 1975; Horiguchi et al., 2009), little information is available on the photoreception system that mediates the light-induced hatching (Van der Linden et al., 1985, 1986). To better understand the photoreception systems involved in the light-induced hatching of *T. granarius* eggs, we determined the spectral sensitivity of hatching and examined the expression of genes involved in photoreception (opsins) and phototransduction (arrestin 2). Opsins are protein moieties of visual pigments, and arrestin 2 contributes to the arrest of the phototransduction cascade (Nathans et al., 1986; Dolph et al., 1993; Zuker, 1996).

* Corresponding author. Tel.: +81 6 6605 2573; fax: +81 6 6605 2522.
E-mail address: shingoto@sci.osaka-cu.ac.jp (S.G. Goto).
[1] Present address: Institute of Zoology, University of Regensburg, 93040 Regensburg, Germany.
[2] Present address: Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan.

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2010.03.012

2. Materials and methods

2.1. Animals

From mid-June through mid-July of 2004–2006, *T. granarius* shrimps were collected from the paddy fields of Kishiwada city (34.5°N, 135.4°E) in Osaka prefecture, Japan, and were maintained in dechlorinated tap water at 25 ± 1 °C under 16 h light: 8 h dark cycles in the laboratory. They were fed on Tetramin or Tetraplankton (Tetra Werke, Melle). According to the previous study (Horiguchi et al., 2009), mature females with full egg pouches were transferred individually into a vessel in which a polyethylene net was placed on a plastic dish, and the vessel was kept under darkness to avoid unnecessary light exposure to eggs. Eggs laid within 3 h under darkness were collected under white fluorescent light (FL40SW or FL30SW, Panasonic Corporation, Kadoma) within 1 h, and were maintained in dechlorinated tap water at 25 ± 1 °C under constant darkness for 15 days. Horiguchi et al. (2009) found that the eggs maintained under darkness for 15 days are light-sensitive, and nauplii appeared within 24 h when the eggs were exposed to constant white light (3 W m^{-2}). Thus, in the present study, we used the same experimental schedule to collect nauplii as that of Horiguchi et al. (2009).

2.2. Hatching induced by exposure to monochromatic light

Horiguchi et al. (2009) found that more than 60% of eggs that are maintained under constant darkness at 25 °C hatch within 24 h, and more than 80% within 5 days, more than 90% within 15 days, when the eggs were exposed to constant white light (3 W m^{-2}). According to these results, in the present study eggs maintained for 15 days under constant darkness were exposed to constant monochromatic light for 5 days (Fig. 1, upper panel). Monochromatic illumination was produced by ultraviolet (UV) light emitting diode (LED) (SDL-5N3CUV-A, λ_{max} = 395 nm, SANDER, Taibai), blue LED (SLP-0137A-51, λ_{max} = 470 nm, Sanyo Electric., Osaka), yellow LED (TLOH180P, λ_{max} = 583 nm, Toshiba, Tokyo), and red LED (SLP-838A-37 S1, λ_{max} = 660 nm, Sanyo Electric.). The photon flux density was measured using a portable spectroradiometer (LI-1800; LI-COR, Inc., Lincoln, USA), and set by neutral density filters (Lee Filters, Andover) and by changing the distance between the light source and plate containing the eggs. In the present study, photon flux densities were adjusted to 7.9×10^{13}–8.7×10^{13}, 45.5×10^{13}–48.2×10^{13}, and 93.9×10^{13}–104.8×10^{13} photons s^{-1} cm^{-2} at each of wavelengths of 395, 470, and 660 nm, and 0.9×10^{13}, 7.2×10^{13}, and 35.7×10^{13} photons s^{-1} cm^{-2} at of 583 nm.

After exposure to monochromatic light, the eggs were maintained under white light (3 W m^{-2}) for 15 days (a strong stimulus inducing hatching) to see the total number of eggs that could hatch. Because the percentage of hatching largely varied among egg batches, the response rate was calculated by dividing the number of eggs hatched during treatment by the total number of eggs hatched after 15 days of white light exposure. As a control (control 1), some eggs of the same batch used in the monochromatic light exposure were exposed to white light (3 W m^{-2}) for 20 days, and response rates on days 15 and 20 were calculated (Fig. 1, middle panel). In another control (control 2), some eggs of the same batch were continuously maintained under darkness for an additional 5 days and then transferred constant light conditions for 15 days. The response rate on day 20 in the control 2 was calculated (Fig. 1, lower panel). Response rates were compared by Tukey-type multiple comparisons for proportions (Zar, 2009). We also calculated the photon flux density that produces 50% response rate, by logarithmic approximation. Each experiment was replicated twice (for UV and yellow light exposures) or 3 times (for blue and red light exposures). The numbers of eggs used in each experiment were 20–79.

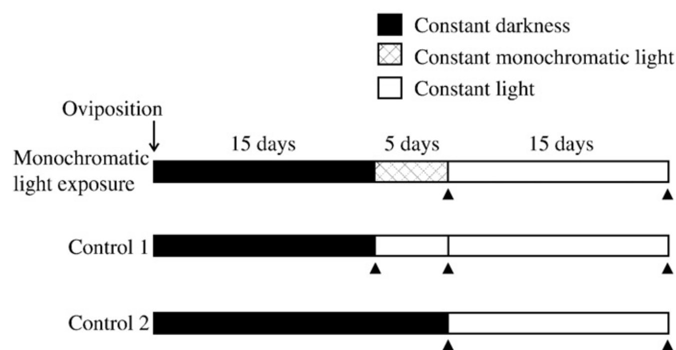

Fig. 1. Experimental schedules of monochromatic light exposure and controls (1 and 2). The numbers of hatching were counted at the days indicated by triangles.

2.3. RNA isolation and cDNA synthesis

Total RNA was extracted with TRIzol Reagent (Invitrogen, Carlsbad) according to the supplier's instruction and was dissolved in water. Genomic DNAs were digested with deoxyribonuclease I (Amplification Grade; Invitrogen).

For reverse transcription-polymerase chain reaction (RT-PCR), single-strand cDNA (ss cDNA) was synthesized with Oligo $(dT)_{12–18}$ (Invitrogen) and M-MLV reverse transcriptase (Invitrogen). The Super SMART PCR cDNA Synthesis Kit (BD Biosciences, San Jose) was used for rapid amplification of cDNA ends (RACEs) to synthesize double-strand cDNA (ds cDNA).

2.4. Clone development, 3′ and 5′ RACEs, and sequencing

Total RNA was extracted from the compound eyes and the ocelli of a few adults of both sexes. PCR was conducted using 0.5 U of Platinum *Taq* DNA polymerase (Invitrogen) in reactions containing 1× PCR Buffer minus Mg^{2+} (as formulated by Invitrogen), 0.2 mM of dNTP mixture, and 1.5 mM of $MgCl_2$.

Arrestin 2 (*Arr2*) fragment was obtained by PCR using ss cDNA, Arr-F1 and Arr-R1 primers. PCR conditions comprised 3 min at 95 °C, 40 cycles of 15 s at 95 °C, 15 s at 48 °C, and 40 s at 72 °C. For RACEs of *Arr2*, the first PCR was performed with ds cDNA and a gene-specific primer (TgArr-F and -R for 3′ and 5′ RACEs, respectively) using the SMART RACE cDNA Amplification Kit (BD Biosciences). PCR conditions comprised 5 cycles of 5 s at 94 °C and 30 s at 72 °C; 5 cycles of 5 s at 94 °C, 10 s at 70 °C, and 30 s at 72 °C; 25 cycles of 5 s at 94 °C, 10 s at 68 °C, and 30 s at 72 °C. The second (nested) PCR was performed with 0.05% of the first PCR product and the same gene-specific primer according to the supplier's instructions. PCR conditions comprised 3 min at 95 °C and 37 cycles of 15 s at 95 °C, 15 s at 65 °C, and 40 s at 72 °C.

As a control for RT-PCR, *Ribosomal protein L32* (*RpL32*) was used. The fragment was obtained by PCR with ss cDNA and RpL32-F1 and RpL32-R1 primers. PCR conditions comprised 3 min at 95 °C and 32 cycles of 15 s at 95 °C, 15 s at 50 °C, and 30 s at 72 °C. For 3′ and 5′ RACEs, ds cDNA and a gene-specific primer (TgRpL32-F and -R, respectively) were used. PCR conditions comprised 5 cycles of 5 s at 94 °C and 1 min at 72 °C; 5 cycles of 5 s at 94 °C, 10 s at 70 °C, and 1 min at 72 °C; 25 cycles of 5 s at 94 °C, 10 s at 68 °C, and 1 min at 72 °C.

PCR and RACE products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and ligated into the pGEM-T Easy Vector (pGEM-T Easy Vector Systems, Promega). Plasmids were purified with the Wizard Plus SV Minipreps DNA Purification System (Promega) and sequenced on an ABI PRISM 310 Genetic Analyzer with Big Dye Terminator v1.1 or v3.1 Cycle Sequence Kit (Applied Biosystems, Foster City, CA, USA). To eliminate the possibility of *Taq* polymerase errors, 4 clones derived from PCR products, 4–5 clones from 3′ RACE products, and 5–7 clones from 5′ RACE products were sequenced.

Download English Version:

https://daneshyari.com/en/article/1972955

Download Persian Version:

https://daneshyari.com/article/1972955

Daneshyari.com