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[SHORT COMMUNICATION]

Direct Control of Pigment Aggregation and Dispersion in Tilapia Erythrophores by Light

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ABSTRACT—Innervated and denervated erythrophores of the tilapia, *Oreochromis niloticus*, responded directly to light of defined wavelength by pigment aggregation or dispersion, although similar effects did not occur in melanophores. In spectral regions between 400-440 nm and 550-600 nm, pigment aggregation took place, while the dispersion response was accelerated at wavelengths between 470-530 nm. The progress of the aggregation or dispersion response depended on the light intensity. These results may imply the coexistence of three kinds of visual pigments in tilapia erythrophores.

INTRODUCTION

It is generally known that pigment aggregation and dispersion in fish chromatophores are controlled by nervous and endocrine systems, although chromatophores often respond directly to light in larval and/or young fish (Fujii and Oshima, 1986; 1994). In some fish species however, chromatophores of "adult" fish also have a light-sensitivity; such as pigment dispersion in melanophores of the dark chub, *Zacco temmincki*, (Iga and Takabatake, 1983) and in leucophores of the medaka, *Oryzias latipes* (Ohta and Sugimoto, 1980), pigment aggregation in medaka xanthophores (Kawai, 1989; Oshima *et al*., 1998) and longer-wavelength light-reflecting response of motile iridophores of the neon tetra, *Paracheirodon innesi* (Lythgoe and Shand, 1982; Nagaishi and Oshima, 1989). The most effective wavelength that induced melanosome dispersion in *Zacco* melanophores was 525 nm (Naora *et al*., 1988), while pigment aggregation within medaka xanthophores was effectively caused by light with wavelengths between 410 and 420 nm (Oshima *et al*., 1998). Very recently, we found that erythrophores of adult tilapias respond rapidly to light even if the luminous intensity is low, and that the direction of pigment migration changes when the peak wavelength of incident light is varied. Until now, light-sensitive chromatophores have been thought to respond to light only by one directional movement of light-absorbing or -scattering organelles. In the present study, the responsiveness of these interesting erythrophores to light with various defined peak wavelengths is analyzed, and the significance of these responses is reported.

MATERIALS AND METHODS

Two species of the tilapia, *Oreochromis niloticus* and *O. mossambicus*, of either sex, 10-20 cm and 10-15 cm in body length, respectively, were used. Since erythrophores are present only in the skin tissue of fins, the tail fin was excised and split in a physiological solution of the following composition: NaCl 125.3 mM, KCl 2.7 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.8 mM, D-glucose 5.6 mM, Tris-HCl buffer 5.0 mM (pH 7.2). Denervated chromatophores were obtained by making a transverse incision with a razor blade across two fin-rays together with their interradial membrane near the base of the tail fin (Fujii, 1959). This operation was followed by the appearance of a dark band ("Parker's band"). Since they are disconnected from cell bodies, the chromatic nerves in time inevitably lose their function. In the present study, experiments on denervated chromatophores were made on fish operated on 4 days earlier. After the nonresponsiveness of denervated chromatophores to isotonic K^+-rich solution (128 mM) was ascertained (cf. Fujii, 1959), they were used for the experiments.

The split-fin preparation was attached, epidermis side down, to a holder with two glass fibers, one side of which had been attached to a coverslip with epoxy adhesive. The holder was then turned over and placed in a perfusion chamber with a narrow trough filled with physiological saline (Oshima and Fujii, 1984). To observe chromatophore responses to light, the chamber was placed on the stage of a light microscope (ORTIPHOT-2, Nikon) equipped with a halogen lamp (12V, 100W) as a light source. Using various band pass filters (BP-40, 43, 44, 45, 47, 48, 50, 52, 53, 55, 58, 60; Kenko), light with a defined wavelength was applied to the preparation. As an example, the spectral transmittance of the BP-50 filter (λ_{max} = 498.5) is shown in Fig. 1. The light intensity was changed by neutral density filters and was measured using a thermocouple gauge and a micro volt meter (AM-1001, Ohkura electric). Irradiations were conducted in a darkroom at room temperature between 23-26°C, and skin preparations were perfused continuously with fresh saline.

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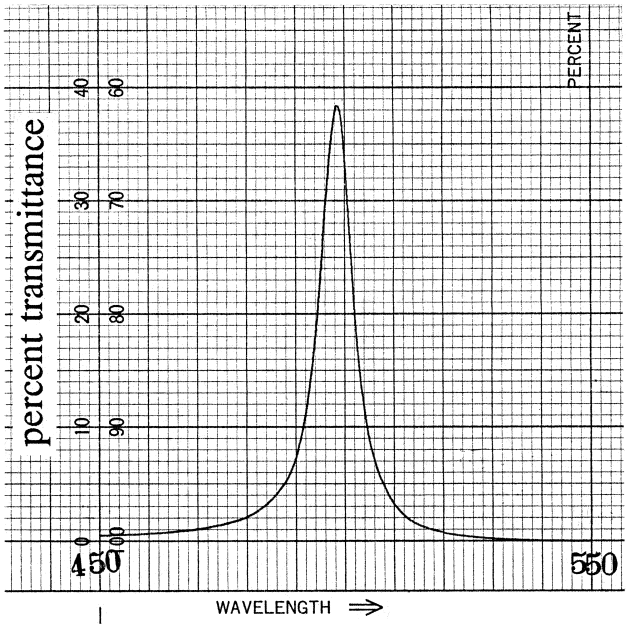

Fig. 1. Spectral transmittance of the BP-50 band pass filter.

RESULTS AND DISCUSSION

Innervated or denervated erythrophores of the Nile tilapia *O. niloticus* were always light-sensitive irrespective of the body length, but in contrast, their melanophores never responded to light. These results indicate that light directly affects tilapia erythrophores. In *O. mossambicus*, however, the light sensitivity differed widely among erythrophores, although the mode of response was similar to that in erythrophores of *O. niloticus*. Therefore, innervated or denervated tail-fin preparations of the Nile tilapia were mainly used in the present experiments.

Aggregation responses occurred in spectral regions between 400-440 nm and 550-600 nm. Fig. 2 indicates erythrophore aggregation responses to light at peak wavelengths of 400 nm (Row A) and 600 nm (Row C), which were the most effective in inducing the aggregation response. In contrast, dispersion response was elicited at wavelengths between 470-530 nm, the most effective wavelength being near 500 nm (Fig. 2, Row B). At 450 nm, a low level of aggregation and/or dispersion took place.

As light intensity decreased, aggregation or dispersion responses were gradually delayed (Figs. 3 and 4), although most erythrophores responded to light even at very low intensity. When erythrophores, whose pigment granules had been aggregated by light at 400 nm or 600 nm, were kept in the dark, pigment dispersion gradually occurred, although it took longer than 10 min. In split-fin preparations kept in complete darkness for 1 hr, all erythrophores assumed a dispersed state similar to melanophores. In contrast, under room light or nor-

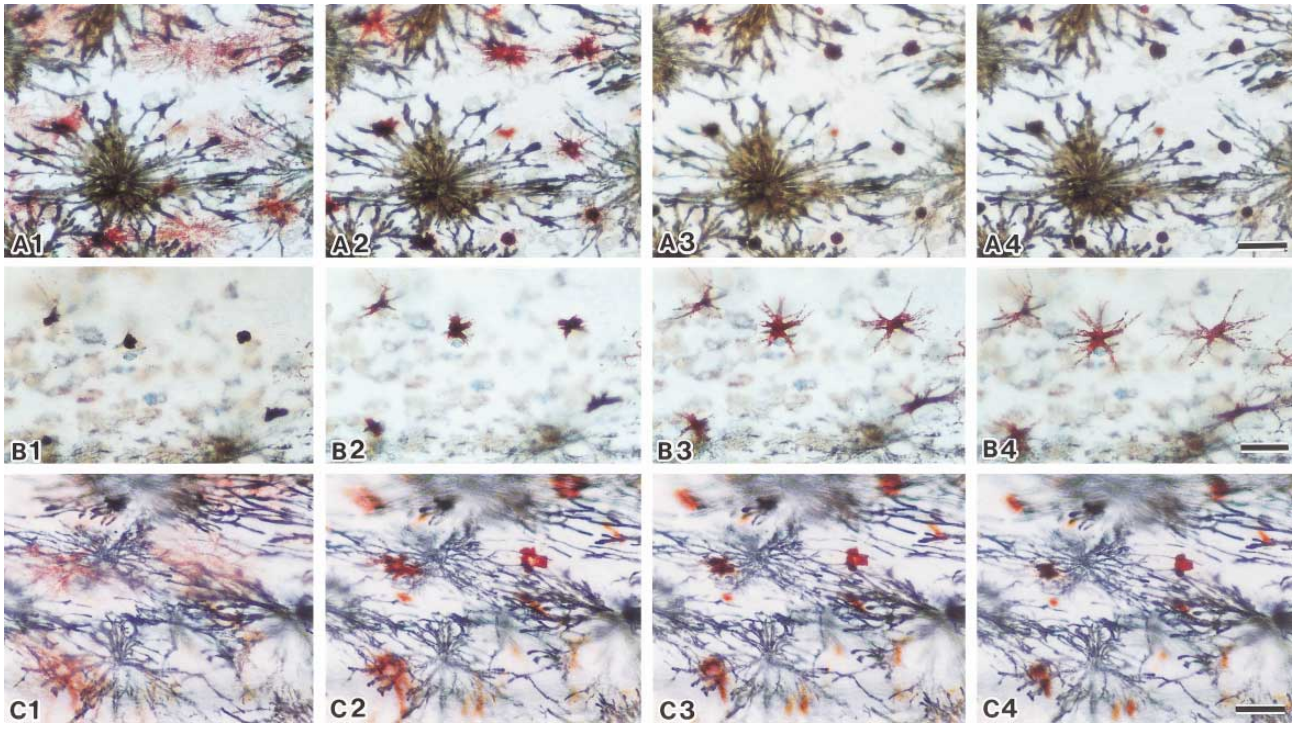

Fig. 2. Responses of erythrophores to light at wavelengths of about 400 nm (upper row), 500 nm (middle row) or 600 nm (lower row). (A1) Erythrophores equilibrated in physiological saline in the dark (control). (A2, A3 and A4) 1-min, 3-min and 5-min irradiation, respectively, at 400 nm (light intensity: 2.5 μW/mm^2). (B1) Erythrophores whose pigment granules had been aggregated by light with a peak wavelength of 400 nm (control). (B1, B2 and B3) 1-min, 3-min and 5-min irradiation, respectively, at 500 nm (intensity: 2.5 μW/mm^2). (C1) Erythrophores equilibrated in physiological saline in the dark (control). (C2, C3 and C4) 1-min, 3-min and 5-min irradiation, respectively, at 600 nm (intensity: 2.0 μW/mm^2). Denervated preparations of *O. niloticus* were used. Scale bars indicate 50 μm.

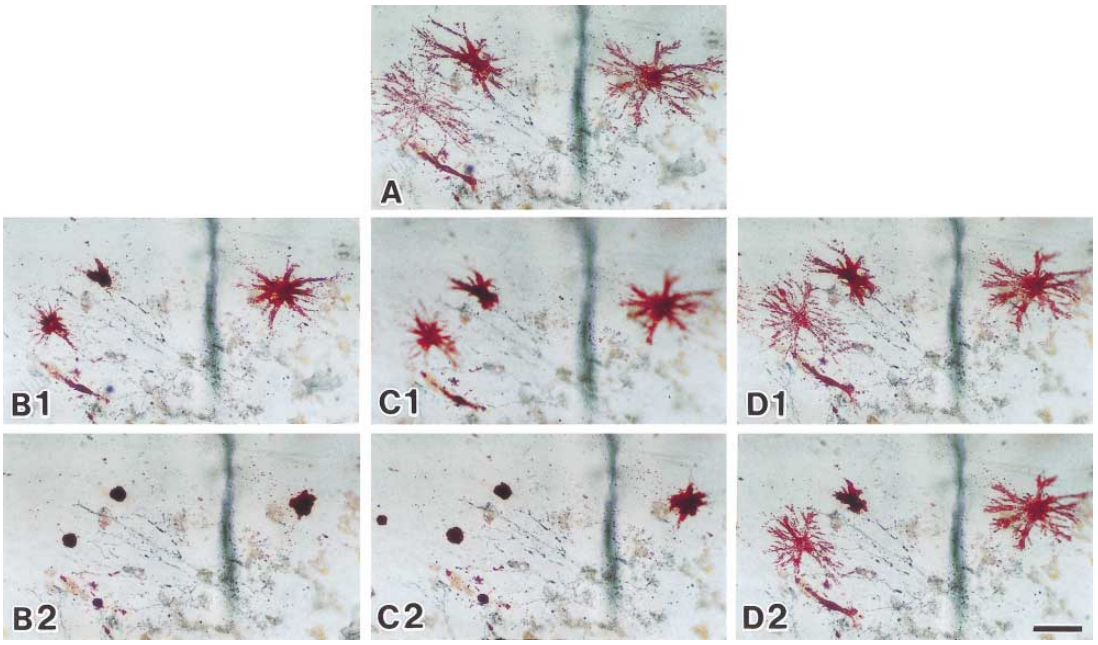

Fig. 3. Microphotographs showing the relationship between the levels of aggregation response and the intensity of light with a peak wavelength of about 430 nm. (A) Erythrophores equilibrated in physiological saline in the dark (control). (B1 and B2) 1-min and 5-min irradiation, respectively, at an intensity of 2.6 μW/mm^2. (C1 and C2) 1-min and 5-min irradiation, respectively, at an intensity of 0.65 μW/mm^2, after erythrophores had been redispersed to the control level shown in (A). (D1 and D2) 1-min and 5-min irradiation, respectively, at an intensity of 0.16 μW/mm^2, after erythrophores had been redispersed to the control level shown in (A). Innervated preparation of *O. niloticus* was used. Scale bar indicates 50 μm.

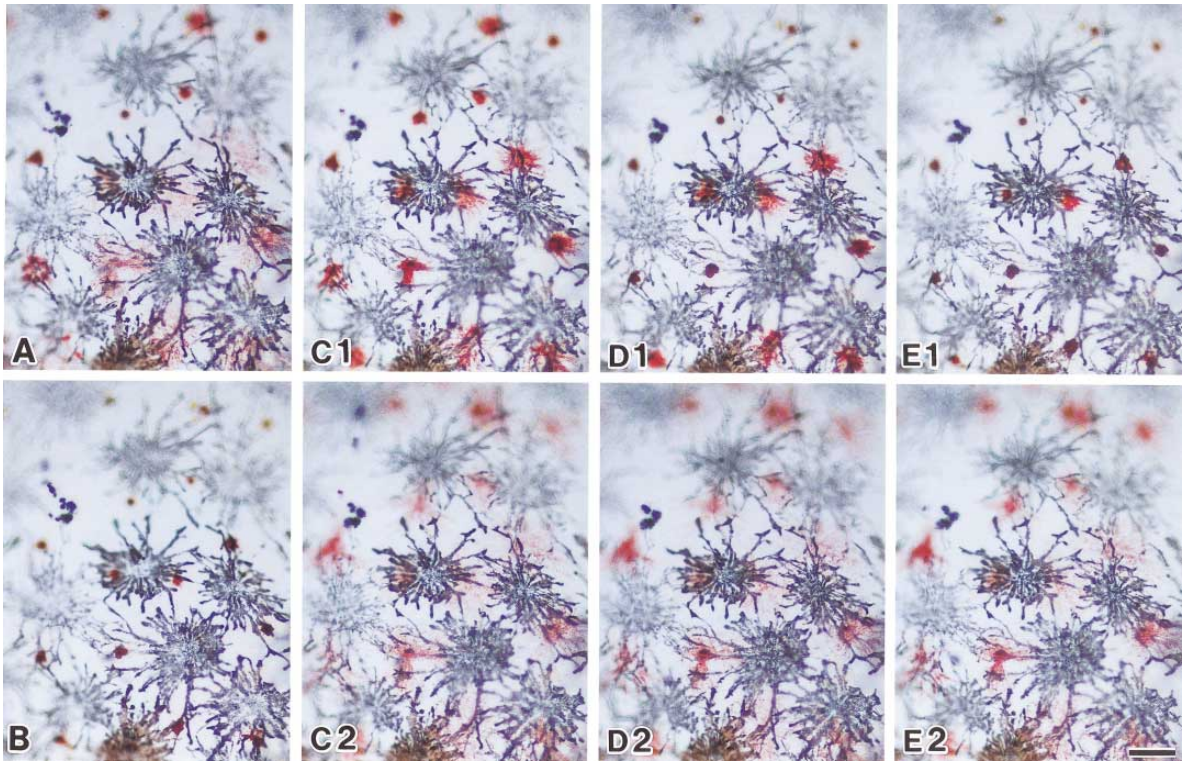

Fig. 4. Microphotographs showing the relationship between the levels of dispersion response and the intensity of light with a peak wavelength of about 500 nm. (A) Erythrophores equilibrated in physiological saline under normal microscopic illumination. (B) Erythrophores whose pigment granules were aggregated by light with a peak wavelength of 400 nm (control). (C1 and C2) 1-min and 5-min irradiation, respectively, at an intensity of 2.2 μW/mm^2. (D1 and D2) 1-min and 5-min irradiation, respectively, at an intensity of 0.55 μW/mm^2, after erythrophores had been reaggregated to the control level shown in (B), using the BP-40 band pass filter. (E1 and E2) 1-min and 5-min irradiation, respectively, at an intensity of 0.14 μW/mm^2, after erythrophores had been reaggregated to the control level shown in (B). Innervated preparation of *O. niloticus* was used. Scale bar indicates 50 μm.

mal microscopic illumination, there were delicate differences in the states of pigment distribution among erythrophores, whereas melanophores under the same conditions maintained a pigment-dispersed state, as shown in Fig. 4A. Some erythrophores fully dispersed their pigment granules, while others aggregated them to various degrees. Fully dispersed erythrophores seemed to be more sensitive to light with a peak wavelength of near 500 nm (see Fig. 4 C1 and D1), but even in the aggregated erythrophores, light at about 500 nm effectively elicited the dispersion response (see Fig. 4 C2, D2 and E2).

From these results, it seems clear that at least three kinds of visual pigments (400 nm-, 500 nm- and 600 nm-sensitive ones) may coexist in erythrophores. Their distribution ratio might prescribe the responsiveness of each erythrophore to environmental light. In the solar spectrum, maximum intensity is detected near 500 nm. In addition, the intensity of light that reaches the earth's surface is higher in spring and summer, when many erythrophores appear in the fins of male tilapias, a phenomenon called "the nuptial coloration". Therefore, in those spawning seasons, the sun's rays may effectively induce the dispersion response in many erythrophores, resulting in an augmentation of red skin color. In the tilapia, prolactin, a pituitary hormone, also induces pigment dispersion, but only in erythrophores and xanthophores, and erythrophores are more sensitive to prolactin in the spring and summer (Oshima *et al*., 1996; Oshima and Nakamura, 1997). Thus, regulation of erythrophore movements are more complex than those of melanophores, which probably contribute significantly to the revelation of the vivid nuptial coloration. However, in natural conditions, the state of pigment distribution within erythrophores might vary subtly in response to environmental light, since there are actually striking differences in the spectral distribution of light in natural water according to the amount of chlorophyll and the dissolved products of natural decay that it contains (cf. Lythgoe and Partridge, 1989). Therefore, the ethological significance of bidirectional movements of pigment granules in response to light should be further studied.

Recently, five cDNA fragments encoding putative visual pigments were isolated from the retina of teleost fish and those genes were shown by *in situ* hybridization to be respectively expressed in different photoreceptor cell types (Hisatomi *et al*., 1997). Moreover, immunocytochemical studies have reported that opsin-based visual pigment is located within neon tetra iridophores (Lythgoe *et al*., 1984). The possible coexistence of visual pigments in tilapia erythrophores also will be examined.

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