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Spectral Sensitivity of Melanophores in the Primary Color Response of the Rose Bitterling, *Rhodeus ocellatus ocellatus*

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Abstract Melanophores of young *Rhodeus ocellatus ocellatus* have the ability to respond by melanosome dispersion to the direct action of visible light. The effective wavelength within visible light region for inducing melanosome dispersion was investigated using melanophores located around the base of the dorsal fin of young fish set on the stage of a light microscope. The melanophores were exposed to light of various wavelengths (420–680 nm) but of the same intensity by placing interference filters under the condenser diaphragm. The most effective wavelength was about 420 nm. Longer wavelengths were less effective for the induction of melanosome dispersion.

Generally, light induces a chromatophore response by acting on the cells directly or by acting on the eye of the animal. The former is called "the primary color response" and the latter "the secondary color response". In the latter, light stimulation is mediated by the nervous and/or hormonal systems. As the nervous and/or hormonal controls are usually dominant over the direct action of light on the chromatophores, the primary color response may often be hidden by the secondary color response. For that reason, reports on the primary color response of chromatophores for eye bearing animals are fewer than those for non-eye bearing animals (cf. Parker, 1948; Fujii and Oshima, 1986).

The direct action of light induces melanosome dispersion in melanophores (the primary color response) of young *Rhodeus ocellatus ocellatus* (Ohta, 1983). The body on the developing young fish is slightly translucent, so its tissues would seem to be severely susceptible to influences of light. The existence of integumentary melanophores with dispersed melanosomes may be effective in protecting the inner tissues from harmful components of light. Therefore, we can postulate that a plausible function of melanosome dispersion in the primary color response is to block harmful light from animal tissues. However, this would not explain melanosome aggregation (*Fundulus heteroclitus*, Spaeth, 1913; *Xiphophorus maculatus*, Wakamatsu et al., 1980). Irradiation with shorter wavelength light near the ultraviolet range is speculated to be harmful for animal tissues. Investigation of the relationship between the primary color response and effective wavelength, therefore, may provide a clue as to the functional significance of the primary color response. The present report was conducted to establish the effective wavelength for inducing melanosome dispersion of melanophores of young *R. ocellatus ocellatus*.

Materials and methods

Mature eggs and spermatozoa were separately expressed from female and male rose bitterling, *Rhodeus ocellatus ocellatus*, into a physiological saline (Yamamoto, 1941) by pressing their abdomens with fingers. The eggs were transferred into sperm-suspended freshwater. The fertilized eggs were kept in an incubator (Sanyo, MIR) at 25°±1°C. Melanophores of young fish (larval stage) 15–24 days after the insemination were used as materials. Under dark conditions, the melanosomes within the melanophores remained in an aggregated state. The young fish were put onto a hollow slide glass with some cotton fibers soaked with water at the bottom. Head and tail parts of the young fish were covered by the cotton fibers. The hollow slide glass was set on the stage of a light microscope (Olympus, EC). The young fish were kept under dark conditions below about 10 lx except during an illumination exposure. The melanophores around the base of the dorsal fin were illuminated by transmitted light (Olympus, LSD, tungsten lamp, 6V, 30W) from the microscope. The strength of the light from the tungsten lamp on the stage of the microscope was measured

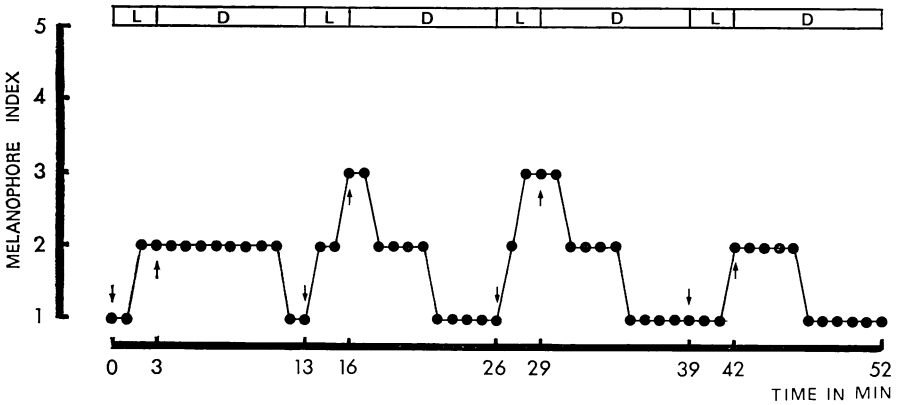

Fig. 1. A typical response of a melanophore to repeated exposure to white light (about 1,200 lx) and darkness. L, light; D, darkness.

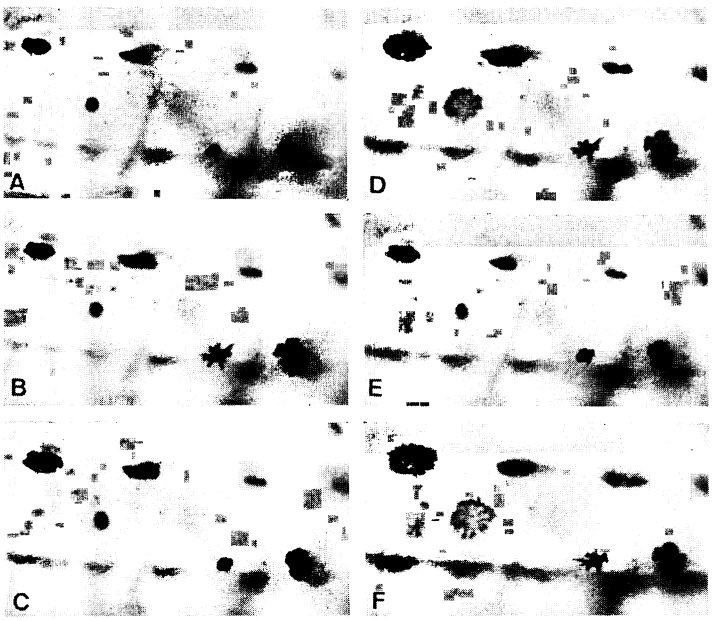

Fig. 2. Micrographs of melanophores reacting to repeated exposure to white light (about 1,200 lx) and darkness. A, initial darkness; B, successive three min illumination; C, successive ten min darkness; D, successive three min illumination; E, successive ten min darkness; F, successive three min illumination. ×90.

by the same method as in a previous report (Ohta and Sugimoto, 1980). To investigate wavelength effects, light with wavelengths in a limited range was obtained by placing various interference filters (Koshin Kohgaku, L type) under the condenser diaphragm of the microscope. The energy of the light passing through the different filters was adjusted to almost the same value by increasing or decreasing the voltage of the illumination apparatus. The light was measured with an optical power meter (Ando Electronic Co. OPM-120) and the value was about 0.2 mW/cm^2 at the site of the microscopic mirror.

The melanophore response of the young to the illumination was expressed by the Melanophore Index (MI; Hogben and Slome, 1931).

Results

1. Responses of melanophores to illumination. In the young fish under dark conditions, most of the melanophores maintained the melanosomes in an aggregated state. Three min illumination with white light (about 1,200 lx on the stage of the microscope) and 10 min darkness were repeated 4 times on melanophores with aggregated melanosomes. The results are shown in Figs. 1 and 2. Upon the first exposure to illumination, melanosomes in the melanophores dispersed in all cases (15). The time required to initiate melanosome dispersion after illumination averaged 67.5±14.2 sec (means±standard error). In most cases, the MI changed from 1 to 2 or 3.

During the first dark period, the MI of melanophores did not change or decreased only slightly. Thereafter, the melanophores responded by dispersion and aggregation of melanosomes to the repetition of illumination and darkness, respectively. The extent of MI change was from 1 to 3.

2. The spectral sensitivity of melanophores for light-response. The aggregated melanophores of the young fish maintained under dark conditions were illuminated by lights having wavelengths from about 420 to 680 nm. The most effective wavelength for melanosome dispersion was about 420 nm. Illumination at this wavelength for 3 min induced melanosome dispersion from 1 to 2–3 (MI) in 8 of 10 cases (Fig. 3). Thereafter the melanophores responded by aggregation and dispersion of the melanosomes to three cycles of alternating 10 min darkness and 3 min illumination. The change in the MI was 1–3.

Illumination with 500 nm light was not effective for melanosome dispersion except for one of 10 cases (Table 1). In only one case, the MI changed from 1 to 2 upon the first illumination. However, the melanophores failed to respond to subsequent illumination at the wavelength. The same results were also obtained in illumination of 580 nm light. The illumination of 680 nm light was not effective for melanosome dispersion in all cases (Table 1).

3. Alternative illumination with lights of different wavelength. Light of the same wavelengths as in the above experiment was used for illumination. Results similar to the typical example shown in Fig. 3 were obtained in 8 of 10 cases. Light of 500 to 680 nm wavelength was not effective for induction of melanosome dispersion. However, illumination at 420 nm wavelength following that at 500 to 680 nm wavelength induced melanosome dispersion with a change in MI from 1 to 4.

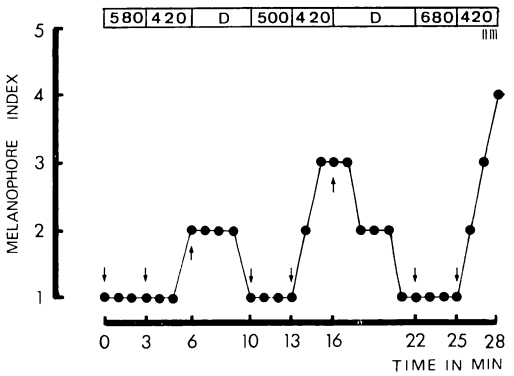

Fig. 3. A typical response of a melanophore to illumination with light of various wavelengths. D, darkness.

Discussion

Ohta (1983) described "the primary color response" and found that repeated illumination and darkness induced reversible melanosome dispersion and aggregation in young *Rhodeus ocellatus ocellatus*. The present results verified again these findings and clarified that the wavelength of the light effective for melanosome dispersion was about 420 nm.

The direct response of fish chromatophores to light has been investigated in several species: scale melanophores of *Fundulus heteroclitus* (Spaeth, 1913), *Anoptichthys jordani* (Burgers et al., 1963), *Zacco temmincki* (Iga and Takabatake, 1983), scale leucophores of *Oryzias latipes* (Ohta and Sugimoto, 1980), cultured melanophores of *Xiphophorus*

Table 1. Number of melanophores, within which melanosomes dispersed by the first exposure (three min) to light with 420 to 680 nm wavelength

Wavelength (nm)	No. of melano-phores	No. of responded melano-phores	Change of MI value in responded melano-phores
420	10	8	1–3
500	10	1	1
580	10	1	1
680	10	0	0

maculatus (Wakamatsu et al., 1980), young fish melanophores of *Macropodus opercularis* (Tomita, 1936) and *R. ocellatus ocellatus* (Ohta, 1983). Except in *F. heteroclitus* (Spaeth, 1913) and *X. maculatus* (Wakamatsu et al., 1980), the pigment granules in the chromatophores dispersed in response to direct action of light.

Melanophores of most invertebrates are known to have a primary color response of dispersion of melanosomes upon direct stimulation by light (Weber, 1983). However, the primary color response of melanophores is not uniform (viz., aggregative and dispersive directions of melanosomes) in fishes and amphibians among vertebrates. As research on melanophores in the vertebrates has been conducted under different conditions (viz., scale or cultured melanophores), it is difficult to clarify the cause of the differences in the response. The dispersive response may be related with protection of the inner tissues in the animal body from the light and with thermoregulation (Millott, 1952; Ohta, 1983; Weber, 1983). However, the function of the aggregative response found in some cases is less clear.

The spectral sensitivity of chromatophores has been investigated in a variety of animals from the sea urchin to the amphibian (cf. Weber, 1983). Although the measured sensitivity covers a wide range from 300 to 600 nm, most chromatophores tend to respond to shorter wavelengths of light. In fishes, the wavelength of maximal sensitivity is 185–290 nm in *F. heteroclitus* (Spaeth, 1913), 410 nm in *X. maculatus* (Wakamatsu et al., 1980) and about 420 nm in the present *R. ocellatus ocellatus*. It is an open problem to explain why the direction of melanosome movement is reversed, in spite of a similar range of effective wavelengths. Dispersion of melanosomes upon direct action of light and the above-mentioned effective wavelengths would seem to support a role for dispersion in protection of the inner tissues from harmful effects of short wave light.

Other significant problems in the primary color response are the identity of the photopigment triggering the light action, its site and the various steps from photo reception to movements of pigment granules. The identity and sites of the photopigment remain obscure although a few possible candidates such as pteridines and carotenoid(?) bound to the plasmalemma have been speculated for the sea urchin and fish (Weber and Dambach, 1976; Wakamatsu et al., 1980).

It is now believed that the increase in the intracellular cyclic AMP levels gives rise to melanosome dispersion of lower vertebrates (Fujii and Oshima, 1986). However, light induces melanosome dispersion in *M. opercularis* (Tomita, 1936), *R. ocellatus ocellatus* (Ohta, 1983) and *Z. temmincki* (Iga and Takabatake, 1983) and melanosome aggregation in cultured melanophores of *X. maculatus* (Wakamatsu et al., 1980). Despite of the same stimulation, the direction of melanosome movement is opposite. Therefore, we have to consider a mode of light action which can explain both phenomena (dispersion and aggregation of melanosomes). At present, our knowledge is too scant to discuss any details of the mode of action.

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バラタナゴ黒色素胞の一次反応における波長感受性

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タイリクバラタナゴ, *Rhodeus ocellatus ocellatus*, の仔魚を用い，背鰭の基部付近の体表上黒色素胞における一次反応及びその反応誘起に有効な波長域の検討を行った．暗黒下においては，黒色素胞はほぼ凝集状態を保ち，光照射によりメラノソームの拡散を示した．この反応は可逆的であった．420–680 nm の範囲で，一次反応誘起に最も有効な波長域を調べたところ，これはおよそ 420 nm であった．680 nm の長波長側の光は効果がないことが明らかとなった．

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