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[RAPID COMMUNICATION]

Blue Chromatophores in Two Species of Callionymid Fish

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ABSTRACT—Dendritic chromatophores that contained blue pigmentary organelles were found in the bluish parts of the skin of two callionymid species, the mandarin fish, *Synchiropus splendidus*, and the psychedelic fish, *S. picturatus*. We named these novel cells "cyanophores" and the organelles "cyanosomes". In response to various stimulatory cues, the cyanophores responded by the aggregation or dispersion of cyanosomes. In addition to their role in the revelation of bluish hues, these cyanophores may participate in the changes in shades of blue of these gorgeous fish.

INTRODUCTION

Melanophores, erythrophores, xanthophores, leucophores and iridophores are the names given to the chromatophores that are involved with the integumental coloration of poikilothermic vertebrates [1, 3, 4]. These chromatophores are primarily responsible for the dark, red, yellow, whitish and iridescent tones of the skin, respectively. Very often, the combined effects of many kinds of chromatophore give rise to coloration that could not be achieved by chromatophores of a single type [3]. To date, no bluish chromatophores have been reported. The bluish hues of animals have been explained mainly by the presence of iridophores, which contain piles of thin, flat, light-reflecting purine crystals [4, 5]. The multilayered thin-film interference phenomenon occurring within these piles has been shown to be primarily responsible for reflecting light at shorter wavelengths. However, studying brightly colored skins of two species of marine teleost species, we recently found novel chromatophores that contain blue organelles, which appear to be directly responsible for the bluish shade of the skin. In this communication we report our preliminary results.

MATERIALS AND METHODS

The mandarin fish, *Synchiropus splendidus*, and the psychedelic fish, *S. picturatus*, belonging to the family Callionymidae (Perciformes), were obtained from local dealers. Pieces of skin excised from various parts of the trunk and the pectoral, abdominal, dorsal and caudal fins were immersed in a physiological solution for teleosts (in mM: NaCl, 125.3; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.8; D-(+)-glucose, 5.6; Tris-HCl buffer, 5.0; pH 7.2), and subjected to morphological and physiological examination. When pieces of fin were used for physiological tests, split-fin preparations [2] were made. Both standard transmission illumination and dark-field incident illumination were used for observation of the chromatophores in the skin. Conventional transmission electron microscopy was used for the examination of structural details. The photoelectric method that was used to record the motile responses of chromatophores was fundamentally the same as that described previously [6]. Sometimes, K^+-rich saline solution was employed to induce the aggregation of pigment. In such cases, the concentration of Na^+ ions was reduced so that the solution remained isosmotic to the standard saline.

RESULTS AND DISCUSSION

Standard transmission light microscopy revealed that, in the dermis of the bluish portions of both callionymid fish, a number of blue-colored chromatophores were present together with a few chromatophores of other types (Fig. 1**a-d**). Resembling other common chromatophores, the blue chromatophores were dendritic cells, although the number of dendrites was rather small as compared to that of dendrites of most melanophores observed to date in teleosts. When viewed under epi-illumination, these cells also appeared bluish in color. In the skin, there were also a few melanophores that resulted in black coloration and, in fact, they served as very good controls for comparisons of colors between the bluish and the black cells (Fig. 1**a-d**).

Even under the light microscope, numerous bluish organelles were recognizable within the cells. Electron microscopy on both species of fish revealed that the bluish chromatophores contained many characteristic pigmentary organelles in which fibrous material was visible (Fig. 2). Being enclosed by a limiting membrane, the organelles were approximately 0.5 μm in diameter but were very irregularly shaped. The thin, flat crystals that are always found in iridophores [4, 5] were not found anywhere in the cytoplasm. Thus, these blue cells generate bluish coloration without the participation of the multilayer thin-film interference phenomenon. In other words, the absorption of light by the

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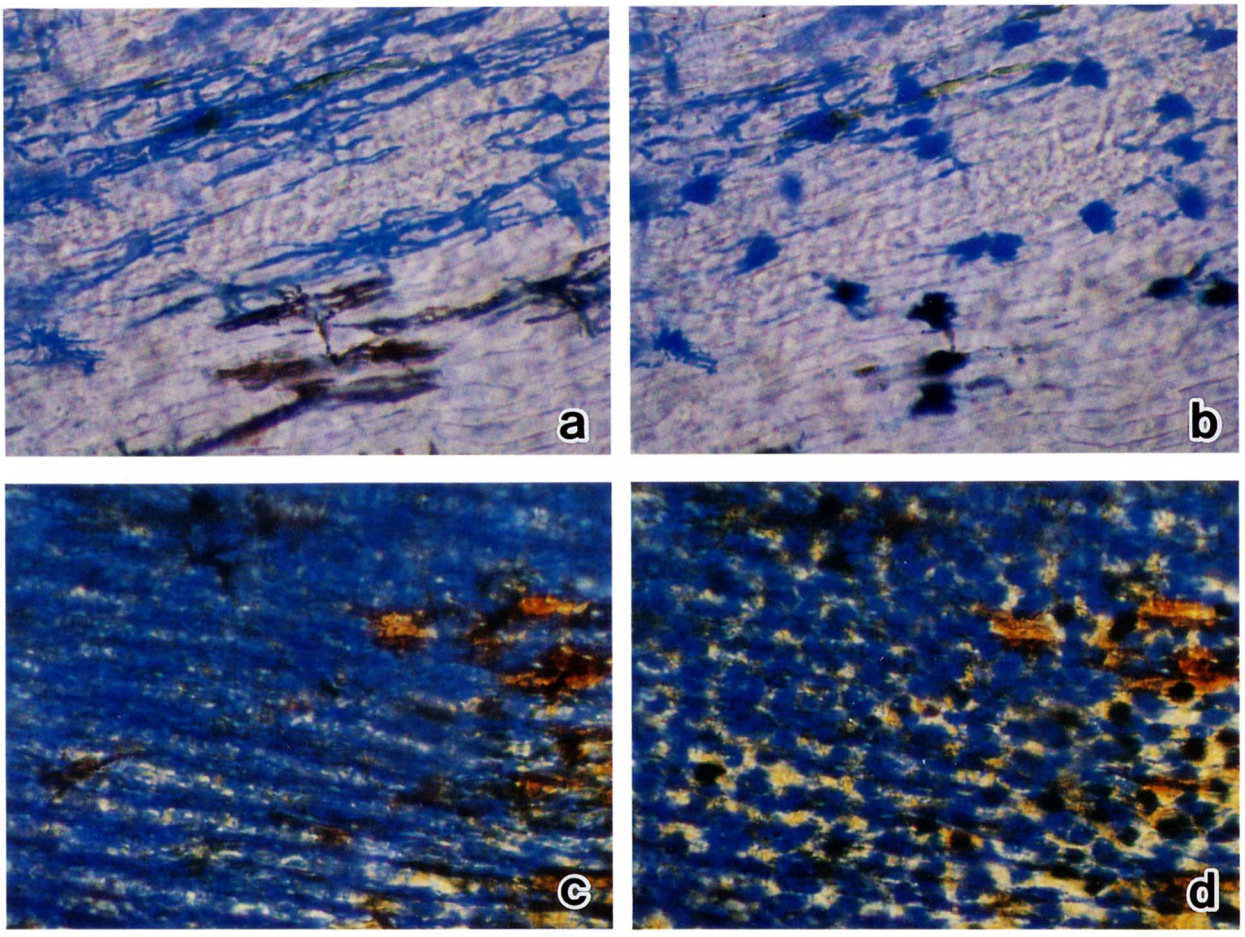

FIG. 1. Photomicrographs showing the blue chromatophores of the mandarin fish, *Synchiropus splendidus*. Standard transmission optics. **a**: Part of a split pectoral fin, equilibrated in physiological saline. **b**: The same area as in **a**, 3 min after application of saline prepared with 50 mM K^+. Black melanophores are visible in the lower, middle regions of **a** and **b**. **c**: Part of a split abdominal fin, equilibrated in physiological saline. **d**: The same area as in **c**, 3 min after application of saline prepared with 50 mM K^+. Melanophores and erythrophores are visible on the right in **c** and **d**. ×300.

pigmentary granules is the sole mechanism responsible for coloration. We propose that these novel chromatophores be called "cyanophores" and that the blue organelles be called "cyanosomes".

In response to various stimulatory cues, the cyanosomes in the cells of both species aggregated or dispersed within the cells, but the rate of these responses was rather low. Figure 3 shows a typical photoelectric recording of the motile responses of the cyanophores in a portion of the split pectoral fin of a mandarin fish. Equilibration of the piece of skin in physiological saline resulted in the dispersion of the pigmentary organelles in the cyanophores. When the irrigating medium was changed to norepinephrine solution, the gradual aggregation of organelles occurred. Alpha-melanophore stimulating hormone (α-MSH) accelerated the dispersion of pigment in the standard saline. K^+-rich saline was also effective in inducing the aggregation of pigment. These observations indicate that the physiological properties of the cyanophores resemble those of the various light-absorbing chromatophores studied to date in many species of teleosts [4].

When the skin specimens were treated with an alkaline solution (e.g., 1 N NaOH), the bluish tone faded. However, the chemical nature of blue pigment remains to be determined.

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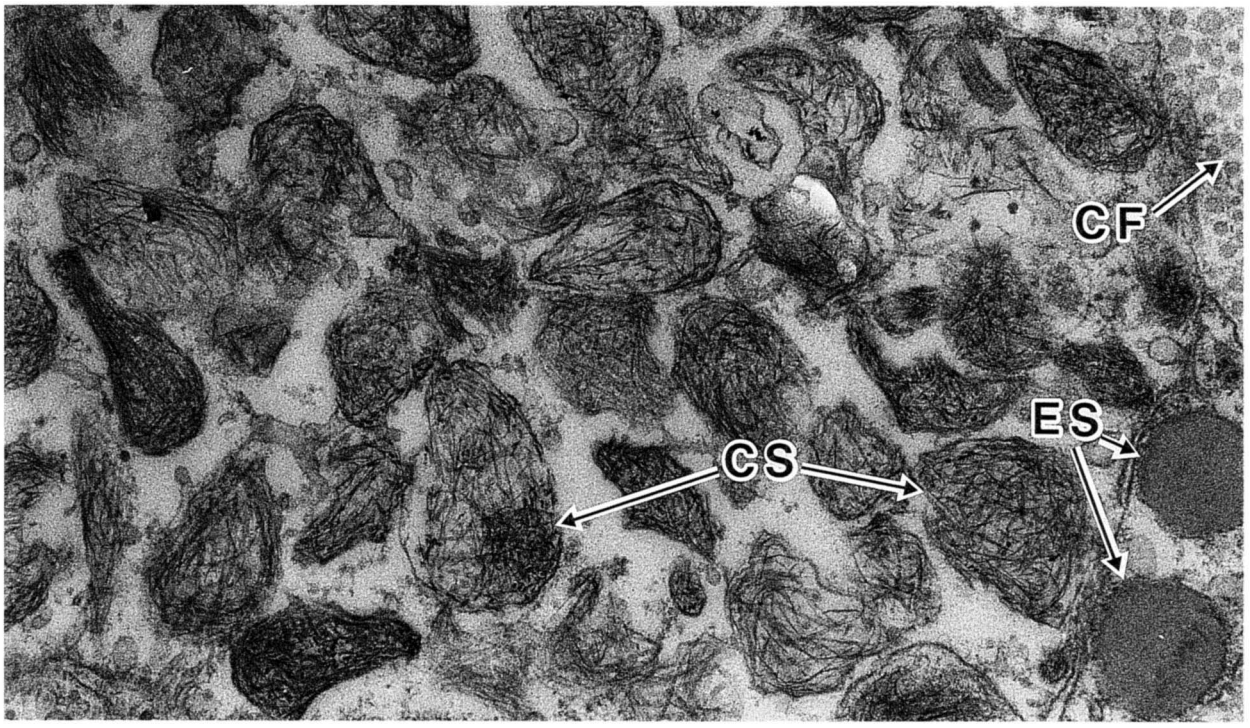

FIG. 2. Electron micrograph of part of a blue chromatophore in the dermis of the abdominal fin of a mandarin fish. Densely distributed pigmented organelles (cyanosomes, CS) are visible. CF, collagen fibrils in the extracellular matrix; ES, erythrosomes in an adjacent erythrophore. ×48,000.

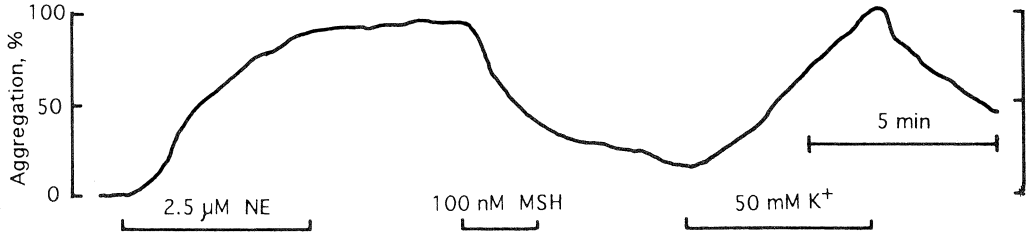

FIG. 3. Typical photoelectric recording of the responses of several cyanophores, located within a circular area of 150 μm in diameter, in a split pectoral fin of a mandarin fish. Norepinephrine (NE) and K^+-rich saline aggregated cyanosomes, while α-MSH caused their dispersion.