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Leading Opinion

The role of protein assembly in dynamically tunable bio-optical tissues☆

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ABSTRACT

Cephalopods are nicknamed the "masters of disguise" for their highly evolved camouflage mechanisms, including the hallmark ability to rapidly change the color and reflectance of their skin. Previously, reflectin proteins were identified as the major biomaterial component of iridosomes [1], specialized light-reflecting architectures that contribute intense structural color to squid skin, eyes, and organs [2–5]. Supramolecular assembly of reflectin has been recognized as a key property in the protein's function [6]. Here, we report the first cloning and expression of a specific reflectin protein found in the responsive iridophore cells of the squid *Loligo pealeii*, which are unique in their ability to switch on/off and change color. We demonstrate that these iridophores can be chemically tuned to reflect the entire visible spectrum. By examining recombinant reflectin, we show that this dynamic optical function is facilitated by the hierarchical assembly of nanoscale protein particles that elicit large volume changes upon condensation. These findings provide insight into the design and synthesis of biomaterials for complex, responsive function in optical applications.

1. Introduction

Living organisms can be viewed as complex responsive material systems in which environmental stimuli prompt responses such as movement, growth, camouflage, temperature modulation, and adaptive metabolic changes. Emerging chemical synthesis and fabrication techniques have sought to mimic the dynamic nature of biological materials [7,8]. In particular, soft materials such as polymers and hydrogels have been explored as self-actuating materials that undergo mechanical deformation when subjected to environmental stresses [9–12]. In biological systems, however, actuation is driven by molecular interactions that are amplified to deliver relevant mechanical forces [13]. Examples include the activation of myosin for muscle contraction [14], the opening of plant stomata [15], and cell adhesion [16], in which chemical stimulation at the molecular level regulates a variety of functions ranging from motility to transpiration. In most cases, proteins are the molecular machines that elicit actuation by changing conformation. To carry out a physiologically relevant response, organisms rely on hierarchical biomaterials organization that spans the nanoscale to the macroscale. From a synthetic perspective, understanding this multiscale nature is necessary for designing biological or bio-inspired materials that are capable of complex, self-regulating, and dynamic functions.

We report on an exotic, tunable structure found in the dermis of loliginid squid, where hierarchical protein assembly drives the dynamic color response of specialized light-reflecting cells called iridophores, shown in Fig. 1A. These cells contain highly aligned membrane-enclosed platelets of protein-based material that are thought to function as intracellular Bragg reflectors [2], as shown schematically in Fig. 1B. Alternating lamellae of protein (high refractive index material) and extracellular space (low index material) are periodic and spatially separated by the bilayer membranes (Fig. 1C–E). Cell color is defined by coherent Bragg reflection from this multilayer structure and can span the entire visible spectrum.

In loliginids, dermal iridophore cells can undergo reversible color and reflectance changes and are thought to serve both as dynamic camouflage [5] and as a visual channel of communication [17]. While this has prompted much interest in the materials

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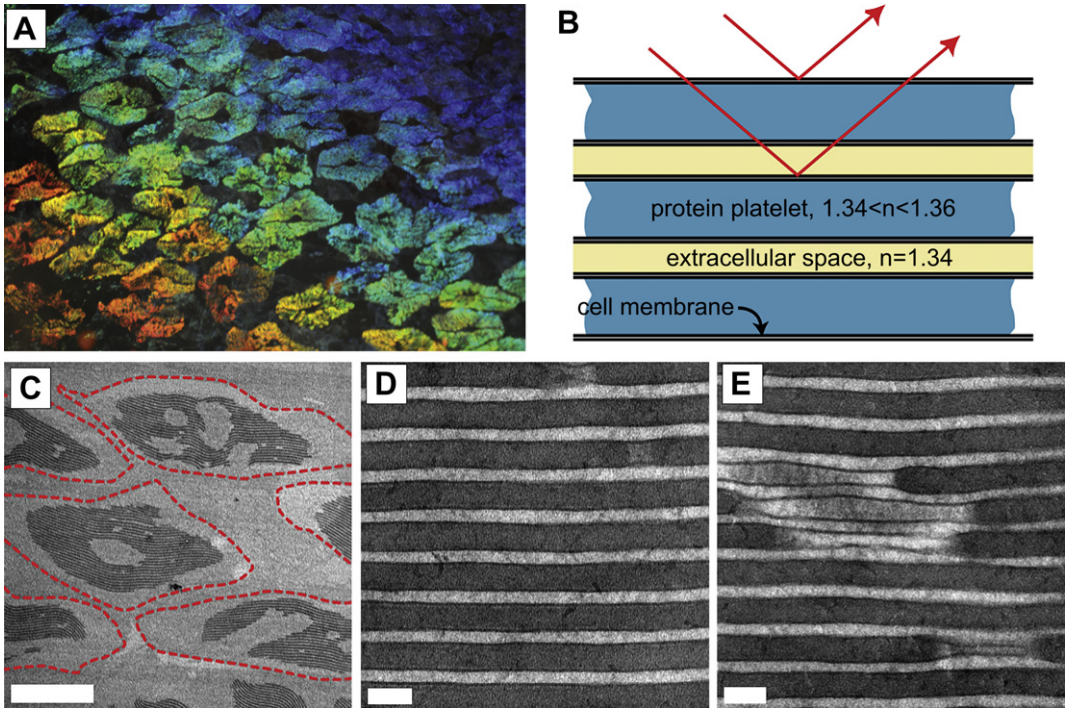

Fig. 1. Intracellular structure of dermal loliginid iridophores. (A) Darkfield microscope image of excised skin tissue from Loligo pealeii observed at 40× magnification under white light illumination. Iridophore cells appear tapered and flat in the plane of the tissue, with average sizes ranging between 20–40 μm in width and 2–5 μm in thickness. The dark area in the center of each cell corresponds to the position of the cell nucleus. (B) Schematic showing the components of the intracellular Bragg reflector. (C–E) TEM images of ultrathin cross-sections of green iridophore cells fixed with uranyl acetate. (C) Scale bar = 5 μm. (D) Scale bar = 200 nm. (E) Scale bar = 200 nm. Dark, electron-dense areas correspond to protein-filled lamellae. Cell boundaries are outline in red for clarification.

community for the design of adaptive optical structures, the mechanism for this optical response has not been confirmed. Cooper et al. have suggested that color change results from a change in periodicity of the lamellar protein stacks [4] and this actuation is thought to be an intracellular response. Proteins called reflectins were previously isolated from static (non-color changing) iridophores found in the light organ of the squid *Euprymna scopeles* [1] and a recombinant reflectin protein sequenced from the same species was found to exhibit diverse morphologies under a variety of solvent conditions *in vitro* [6]. Prior to our studies, little else was known about the biomaterials that contribute to responsive (color-changing) iridophores found in cephalopod skin.

We recently cloned, sequenced, and analyzed reflectins extracted from the responsive iridophores of the squid *Loligo pealeii* [18]. One of these proteins, Reflectin A1 (RA1, GenBank accession number FJ824804), is abundant in the skin of the mantle where iridophores are observed to flash on and off in a rainbow of hues in the live squid. Immunohistochemistry confirms the localization of RA1 to the interior of the iridophore platelets (Fig. 2). This result is in agreement with the observation by Crookes et al. for the static iridophores of *E. scopeles* [1]. RA1 has a molecular weight of 43 kDa and contains six characteristic reflectin repeat motifs.[2] As with other proteins in the reflectin family, it is rich in aromatic, methionine, and charged residues and is almost entirely devoid of non-polar hydrophobic amino acids (e.g. A, V, I, and L). Reflectins are basic proteins and, in particular, RA1 has an isoelectric point of 9.1. Izumi and Morse observed that changes in reflectance of responsive iridophores in *L. pealeii* are accompanied by multiple tyrosine phosphorylations of RA1, as observed by mass spectrometry and two-dimensional gel electrophoresis of the proteins followed by quantitative immunoblot analysis [18].

We hypothesize that reflectin serves as a molecular switch for chemomechanical transduction in iridophores, triggering contraction of the intracellular protein-containing lamellae constituting the Bragg reflectors. First, we examine the materials properties of RA1 that are critical for responsive iridophore function. We then demonstrate the spontaneous assembly and hierarchical organization of a full-length recombinant Reflectin A1 (rRA1) protein. We further discuss the significance of dynamic post-translational modification, and how reflectin condensation into supramolecular architectures is relevant to chemical actuation of these microscopic reflectors.

2. Materials and methods

2.1. Squid dissection

L. opalescens were purchased from Outer Banks Commercial Fisheries (Oxnard, CA). *L. pealeii* were purchased from Marine Biological Laboratory (Woods Hole, MA). Live squid were killed by decapitation just prior to dissection, optical measurements, and fixation procedures. The mantle was cut along the ventral surface from anterior to posterior. Internal organs and pen were removed. The mantle was then pinned out flat with the dermal surface upward in a tray of artificial seawater (cf. below) chilled to 4 °C. For optimal optical measurements of iridophores, the dermal layers were left intact after removing the underlying muscle and pinned out flat with the iridophores on the uppermost surface. Artificial seawater was prepared according to the following recipe: 0.5 M sodium chloride, 0.01 M potassium chloride, 0.01 M calcium chloride, 0.012 M magnesium chloride, 100 mM phosphate buffer [19].

2.2. Optical measurements

Reflectance spectra were collected through a darkfield microscope equipped with fiber optic probes and an Ocean Optics USB2000 spectrometer. Tissue samples were immersed in artificial seawater and illuminated with a broadband tungsten/

[2] The characteristic reflectin repeat motif was generally defined by Crookes et al as [M/FD(X)$_5$MD(X)$_5$MD(X)$_{3/4}$] The repeats in Reflectin A1 follow a similar but more stringent motif of [PER(Y/W)MDMSGYQMDMQGRWMD(X)$_3$R(X)$_3$P].