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Characterization and primary processes of stentorin and its function in *Stentor coeruleus*

Kim, Il-Hyun, Ph.D.

The University of Nebraska - Lincoln, 1988

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CHARACTERIZATION AND PRIMARY PROCESSES OF STENTORIN AND ITS FUNCTION IN *STENTOR COERULEUS*

by

Il - Hyun Kim

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major : Chemistry

Under the Supervision of Professor Pill-Soon Song

Lincoln, Nebraska

December, 1988

TITLE

CHARACTERIZATION AND PRIMARY PROCESSES OF STENTORIN AND ITS FUNCTION IN STENTOR COERULEUS

BY

Il - Hyun Kim

APPROVED	DATE
Dr. Pill - Soon Song	9-2-88
Dr. Therese Cotton	9-2-88
Dr. Naba Gupta	9-2-88
Dr. Kit Lee	9-2-88

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Il - Hyun Kim, Ph.D.

University of Nebraska, 1988

Adviser: Pill-Soon Song

The unicellular protozoan ciliate, *Stentor coeruleus*, exhibits sensitive responses to visible light. The primary photoreceptor for the photophobic and phototactic responses in this organism is a chromophore containing protein named stentorin. Two forms of the stentorin pigments, stentorin I (strongly fluorescent) and stentorin II (very weakly fluorescent), have been isolated from the pigment granules or from whole cell extracts by a hydroxylapatite and/or a Bio-Gel A-1.5m gel filtration column chromatography. These pigment preparations have been characterized by a variety of techniques, including HPLC, polyacrylamide gel electrophoresis and spectroscopy. The apparent molecular weights of stentorin I and II have been determined to be 82,000 -103,000 and 500,000 - 810,000, respectively. Stentorin I is regarded as a proteolipid which shows anomalous behavior on SDS-PAGE and seems to form oligomeric complexes in solution, and is either a non-functional fraction or serves as an antenna pigment. Stentorin II appears to be the primary photoreceptor whose absorption and fluorescence properties are consistent with the action spectra for the photoresponses of the ciliate to visible light. Stentorin II exhibits typical properties of a membrane protein. The chromophore in stentorin II has an

induced optical activity, most likely due to a protein-chromophore interaction, that is strong enough to withstand mild detergent conditions. This may indicate that the protein-chromophore interaction is of a covalent character. Stentorin II exhibits four fluorescence decay components at various emission wavelengths, pHs and pDs. Investigation of the effects of deuterium oxide and pD, as well as pH, on the fluorescence decay kinetics and time-resolved spectra of stentorins shows that stentorin I exhibits a free chromophore-like peptide behavior, as judged by its accessibility to solvents, whereas stentorin II is a larger molecular assembly composed of several proteins in which the chromophore may be deeply imbedded within the hydrophobic core of the protein. These results are consistent with a model showing proton dissociation as a primary photoprocess in the excited state of stentorin, facilitated by the quarternary structure and/or integrity of the protein assembly of the photoreceptor structure.

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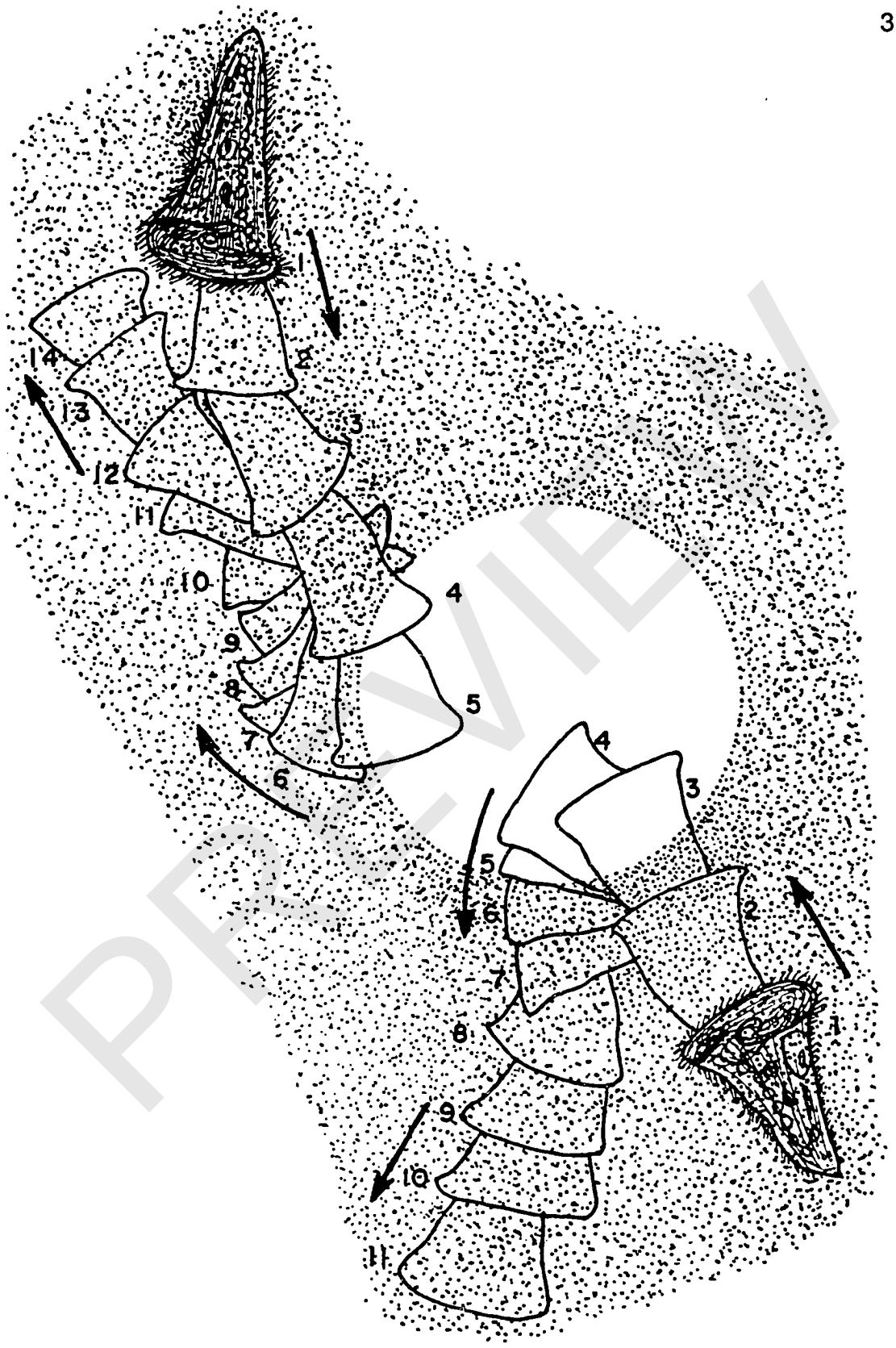
INTRODUCTION AND STATEMENT OF PURPOSE

The large blue-green heterotrich protozoan, *Stentor coeruleus* , is an aneural unicellular ciliate. The biological and behavioral characteristics of this protozoan are interesting in many respects, as can be seen from a detailed book by Tartar (1).

One of the most interesting forms of behavior of *Stentor coeruleus* is the response to light. The light response of *Stentor coeruleus* was first reported by Holt and Lee in 1901 (2). Later, extensive studies of this phenomenon were done by Jennings (3) and Mast (4) who stated that *Stentor coeruleus* cells tend to congregate in the shady or dark areas of the culture. It has been shown that any one or a combination of three light-induced movement phenomena (photokinesis, photophobic responses, and phototaxis) can be responsible for the accumulation of microorganisms in a specific light (or dark) zone (5). *Stentor coeruleus* exhibits two distinct light responses, a step-up photophobic response, i.e., light- avoiding swimming, and negative phototaxis, i.e., swimming away from the light source and in a direction parallel to the direction of light propagation (6,7). A video recording of the photophobic response by single cells has been obtained. A frame of this recording is shown in Fig. 1.

The light microscopy and electron microscopy investigations of

Figure 1. The step-up photophobic response of *Stentor coeruleus* , as it encounters the lighted area (700 W/m^2).
The position of the cells has been traced every 0.24 sec from a video recording.



the fine structure in *Stentor* provided varying reports of its anatomy (8-16). Fig. 2 shows an electron microscope photograph of the organism, which possesses numerous cilia. Cilia comprising the oral membranelles are longer than the body cilia which are organized in rows. The organism is propelled by the motion of these cilia. However, the mechanism of photosensory transduction in *Stentor coeruleus* has remained a mystery until late 1977 when Song and his colleagues began to investigate it (17). Several aspects of the photosensory transduction mechanism of this interesting ciliate have since been reported (6,7,18,19,20). The chromoprotein stentorin serves as the primary photoreceptor for the step-up photophobic and negative phototactic responses in *Stentor coeruleus* (6,7,21,22). The photoreceptor molecules for these light-avoiding behaviors of the organism occur in pigment granules (23). It is well known that *Stentor coeruleus* synthesizes blue-green pigments which are localized in vesicles (granules) close to the cell membrane (24). Each of the spherical pigment granules is surrounded by a membrane (25,26). Initial isolation and characterization of the chromoprotein stentorin showed at least two spectroscopically distinguishable forms, stentorin I with a strongly emitting chromophore, and stentorin II with an extremely weak fluorescent chromophore. The stentorin chromophore was tentatively identified as hypericin-derived (Fig. 3) and possibly covalently bound to a protein (18). As sufficient amounts of the cells of *Stentor* have not been readily obtainable and the chromoprotein(s) were found to be anomalous in their chromatographic behaviors, little progress on the further characterization of stentorins I and II has been made during the past

Figure 2. Scanning Electron Microscope photographs of *Stentor coeruleus*. (Cambridge S4-10 Stereoscan)

a. whole cell b. cilia on oral pouch

c. cilia on oral pouch d. body cilia

[Courtesy of Dr. Kit Lee and Mr. Scott Florell.]

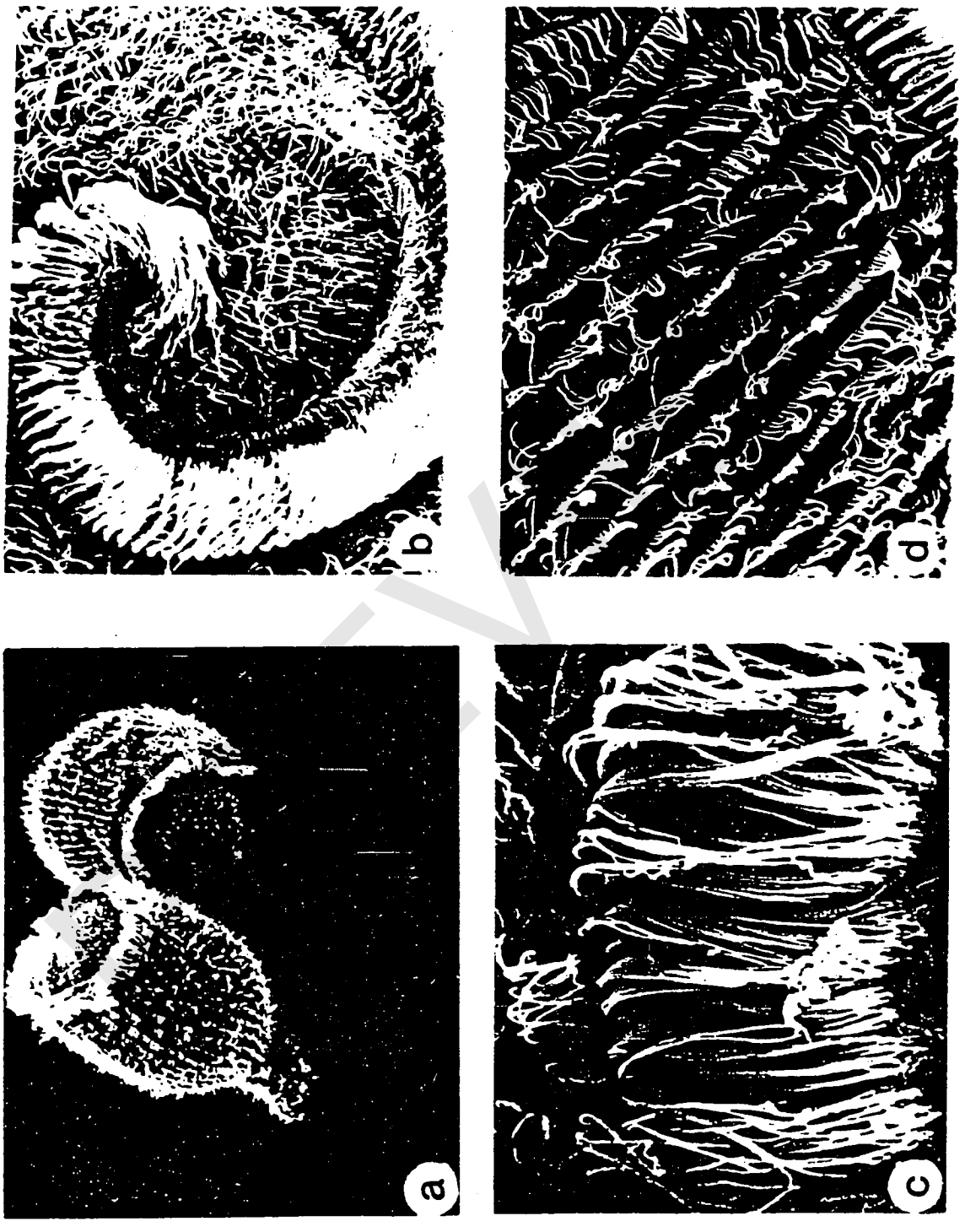


Figure 3. Absorption spectrum of hypericin dissolved in absolute ethanol.

Inset : Two possible structures for the stentorin chromophore. Other structures having peptide linkage elsewhere and involving more than one -OH group are also possible. The most likely linkage, which must be readily hydrolyzable, is the ester linkage with a carboxylic group of the apoprotein.