THE OCELLAR PIGMENT OF THE ANTHOMEDUSA SPIROCODON SALTATRIX: DOES ITS PHOTOREDUCTION BEAR ANY PHYSIOLOGICAL, SIGNIFICANCE?

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THE OCELLAR PIGMENT OF THE ANTHOMEDUSA *SPIROCODON SALTATRIX*: DOES ITS PHOTOREDUCTION BEAR ANY PHYSIOLOGICAL SIGNIFICANCE ?[1)2)]

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A number of hydropolyps and hydromedusae react to changes in light intensity. The anthomedusa, *Spirocodon saltatrix* (Tielesius) also responds to shadows by quick pulsation of the bell (Kikuchi, 1947) and slow contraction of tentacles (Hisada, 1956). Scanning the photosensitive site by a glass rod method, Hisada has shown that reactions are mediated through the ocellus, a saucer-like depression which is located at each tentacular base. Each ocellus is lined by pigment cells (Little, 1914), through which a fine process with ciliary apparatus extends into the cup (Eakin and Westfall, 1962). Cells that bear such apparatus are thought to be sensory, though there is no convincing evidence as regards interconnection between the so-called sensory cells and bipolar or tripolar nerve cells.

The main pigment is insoluble in distilled water and in many fat solvents but is extractable from fresh, frozen or lyophilized material with HCl-methanol and cationic or anionic detergents such as cetyltrimethylammonium bromide or sodium cholate but not with non-ionic detergents such as digitonin or triton X (Yoshida, 1963; Sugahara, unpublished). After denaturation, it becomes soluble in a few other solvents (Yoshida, Ohtsuki and Suguri, 1967). The extracted pigment may be reduced with $(NH_4)_2S$, $Na_2S_2O_4$, $NaBH_3$ and ascorbic acid, the color changing from yellow to purple, and re-oxidized with H_2O_2 and $NaNO_3$ (Yoshida and Ohtsuki, 1966). These properties resemble those reported for ommochrome pigments (Butenandt *et al*, 1960) and the medusan ocellar pigment has in fact been identified as an ommochrome of xanthommatine type from its behavior on the thin-layer and paper chromatographs and absorption characteristics in the UV, visible and infra-red regions of the spectrum (Yoshida, Ohtsuki and Suguri, 1967).

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The ommochrome pigments are considered not to be photosensitive. However, the color changes from yellow to purple upon illumination under anaerobic conditions and in a reverse way by admission of air (Yoshida and Ohtsuki, 1966). Changes in redox potential was followed under anaerobic light conditions (Yoshida, 1967; Yoshida, Suguri and Sugahara, unpublished). A Thunberg-Borsook type cell was placed in a specially designed sample chamber with a side window and the whole setting was attached to a spectrophotometer, Shimazu, QV 50. While bleaching light passed through a water filter of 15 cm deep containing $CuSO_4$ was applied from the side window, the potential between a platinum electrode and a KCl-agar-Kaoline mixture filled in a capillary bridge was recorded continuously. At intervals of a few minutes, the bleaching light was shuttered off and the optical density at 440 nm was measured through the other side of the cell. Results are shown in Fig. 1. The two parameters change proportionately to each other, showing that the nature of the reaction is in fact

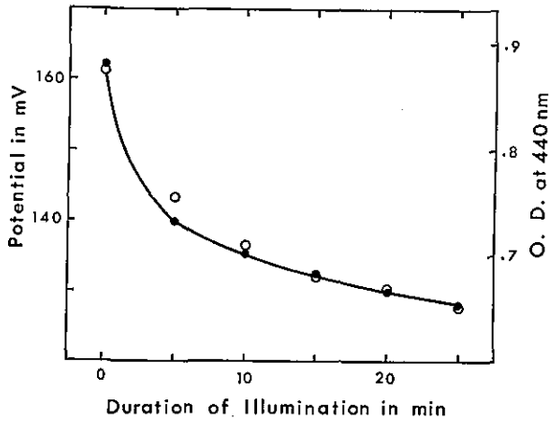

Fig. 1. Changes in the redox potential (solid circles) and the optical density at 440 nm (open circles) of medusan ommochrome pigment dissolved in M/15 phosphate buffer, upon illumination under anaerobic conditions.

a photoreduction.

Does such an anaerobic photoreduction bear a primary importance in photoreception? The answer is not straightforward. First. All the visual pigments known so far possess retinal as a prosthetic group, whose photo-isomerization, instead of light-induced electron transfer, has been established as an important event in the initial process of visual excitation (Weale, 1968). Second. The ommochrome has been considered to function as a filtering pigment in insect ommatidia (Goldsmith, 1965).

The third point concerns the action spectrum (Yoshida and Suguri, unpublished). Changing from a conditioning light (I) to lights of lesser intensities (I'), it was found that reciprocals of reaction times which were calculated from kymograph recordings became a straight line when plotted against the logarithm of I' within limits of a certain range. The following experimental procedure was based on these findings. Medusae were illuminated for a fixed period with white light of a fixed intensity (I). It was changed over instantaneously to one of eight monochromatic lights of varying intensities (I') produced through a series of interference filters and neutral wedges. Relative energy levels of the colored lights were measured with a calibrated photomultiplier and checked against human scoptopic visibility. When reciprocals of the reaction times obtained with each colored light were plotted as above, a family of lines were obtained. All the lines ran parallel, being displaced along the abscissa to varying degrees. The displacement must be brought forth by differing effectiveness of each monochromatic light. For each line, a value of I' corresponding to a fixed point on the ordinate was found on the abscissa. These values were taken as relative energies of monochromatic lights which should produce a constant size of response. After due calculation, an action spectrum based on quantum effectiveness was obtained (Fig. 2). The peak is seen between 480 and 500 nm, which is separated by about 50 nm from the λ max of the oxidized, namely, photoreducible form of the pigment.

Fourth. Microspectrophotometric analyses were made on fresh pigment cells prepared by frozen section at 20μ (Yoshida, unpublished). Scanning area was 10X

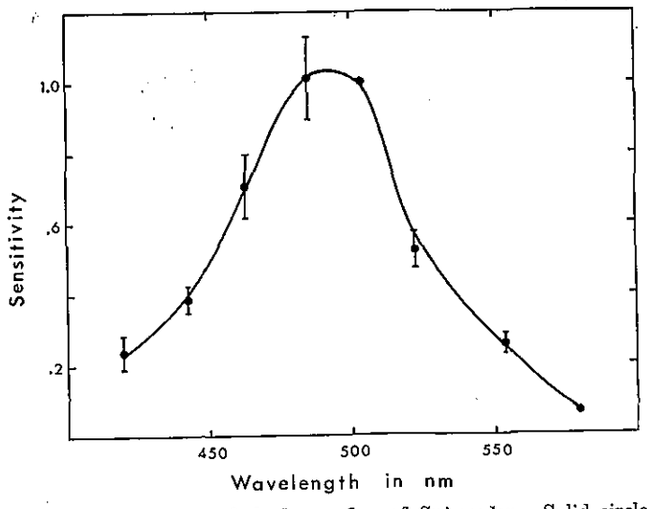

Figure 2. Action spectrum of shadow reflex of *Spirocodon*. Solid circles, averages of 7 series of experiments, normalized at 504 nm. Vertical bars indicate the range of standard deviation.

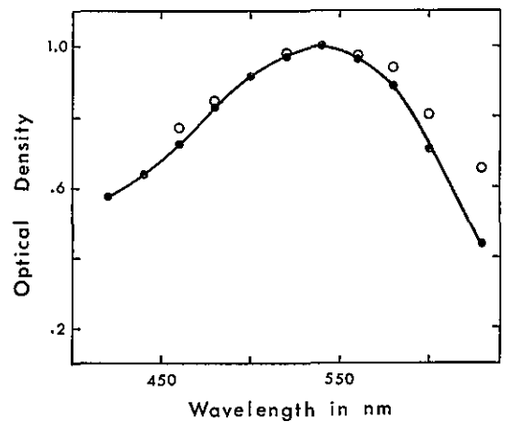

Fig. 3. Absorption spectrum of a single pigment cell, normalized at 540 nm. Solid circles, measured in darkness. Open circles, measured under room light.

20μ and results are shown in Fig. 3. The peak is located at 540 nm, again not at 440 nm. The curve resembles closely that reported by Strother (1968) on insect screening pigments. He maintains that the red-shfit in λmax is due to the pigment being a protein complex *in vivo* but it is also possible that the position of the peak reflects the predominance of reduced pigment *in vivo*.

Fifth. Pigment granules are found electronmicroscopically as rather large, scattered masses (Kawaguti, unpublished[1]), differing from the general appearance of photosensory structures (Eakin and West-fall, 1962). Sixth. The time course of the photoreductive reaction is too slow to account for the quick response of the bell pulsation and a relatively large amount of photon is required for completion of the photoreduction.

A search for C_{20}- or C_{40}- carotenoids may be a more orthodox approach. Preliminary analyses were made using about 2,000 medusae[2] at a time (Yoshida and Sugahara, unpublished). Excised ocelli were kept frozen until use. After lyophilization, sonication or homogenization, pigments were extracted with acetone and then transferred to petroleum ether by dilution of the acetone with an equal volume of water. The extract was washed with water and dried over anhydrous sodium sulphate. Chromatographic separations were made on columns of either activated or de-activated alumina, eluting the pigment with petroleum ether containing acetone at various concentrations. Each fraction was examined

1. Information of unpublished work was supplied to me by Professor Kawaguti, to whom I am greatly indebted.
2. This amounts to about half a million ocelli. We are indebted to Mr. Ohtsu and Mrs. Yoshida for their help in excising ocelli.

spectrophotometrically by Hitachi EPS-3T. Several series of such experiments were performed on both dark and light adapted animals. With these extensive analyses, it was not possible to detect either retinal or retinol. Composition of C_{40}-carotenoids in ocelli was compared with that in tentacles. The two resembled very closely; in other words, no carotenoid that is specific to ocelli was detectable.

Thus far, therefore, the pigment that is proved to be photosensitive and that is found specifically in ocelli, is the photoreducible pigment. What this means should be worked out in future.

Summary

The redox potential and the optical density of the ocellar pigment of *Spirocodon*, an ommochrome of xanthommatine type (λmax; 440 nm), decreased proportionately to each other under anaerobic conditions. Taking into account the action spectrum of shadow reflex (λmax; about 490 nm), microspectrophotometric data (λmax; 540 nm) and some others, the physiological significance of such a photoreduction was critically discussed. It was also noted that no retinal nor retinol was detectable from ocelli and no carotenoid that is specific to ocelli was detected.

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