X-ray diffraction studies on light induced structural changes of cephalopod visual cells

Toshiaki HAMANAKA*1, Masatsugu SEIDOU2, Kinya NARITA3, Masanao MICCHINOMAE4, Kazuki ITO5 Katsuzo WAKABAYASHI1 and Yoshiyuki AMEMIYA6

1 Department of Systems and Human Science, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan.
2 Department of General Education, Aichi Prefectural University of Fine Arts and Music, Nagakute, Aichi 480-1194, Japan.
3 Department of Biology, Faculty of Science, Toyama University, Toyama, Toyama 930-8555, Japan.
4 Department of Biology, Faculty of Science, Konan University, Kobe, Hyogo 658-0072, Japan.
5 Institute of Materials Science, University of Tsukuba, Tennodai, Tsukuba 305-8572, Japan.
6 Graduate School of Frontier Sciences, The University of Tokyo, Hongo, Bunkyo, Tokyo 113-8656, Japan.

Introduction
The initial step of the visual process is the absorption of light by the visual pigment. The cephalopod visual pigment is located in microvilli which are cylindrical extensions of the cell membrane, arranged hexagonally within the rhabdome. Previously, the squid retina fixed by glutaraldehyde was used, because this tissue disintegrated within 1 hour of dissection. We have reported that we could succeed in recording the x-ray diffraction pattern from unfixed retina by the use of the synchrotron radiation and a storage phosphor screen, the imaging plate[1]. Also, we have reported the some change of diffraction pattern induced in response to the light stimulation[2].

In the last study[3], we reported that the lattice dimension of hexagonally arranged microvilli decreased upon the light illumination and recovered to the original one in the dark about ten minutes after the light stimulation[2]. In this study, by use of CCD-based x-ray detector, we have tried to follow the change of diffraction pattern after the light illumination.

Experimental
Living, active specimens of the squid, Watasenia scintillans were captured at Toyama Bay of the Japan sea and brought to Tsukuba within several hours. The squids were decapitated and their retinas dissected in dim red light. For the x-ray experiment, a 1-mm thick slice of retina was kept in an artificial seawater chamber with Mylar windows at 4 C. Schematic diagram of a slice of squid retina was shown in the previous report[1]. The artificial seawater containing D-glucose was oxygenated and gently circulated through the sample chamber during the experiment. Blue light emitted LED was used for light stimulation (465nm in wavelength).

X-ray experiments have been performed with a mirror-monochromator optics (the Muscle Diffractometer) at BL-15A1[4]. The wavelength of the radiation was 0.150 nm. The sample-to-detector distance was 2335 mm. X-ray diffraction intensity was recorded with a CCD-based x-ray detector system[5]. X-ray diffraction data were successively taken with the same sample in the dark and/or light. The exposure time was 1 seconds and each recording finished within 30 minutes after decapitation.

Results and Discussion
The present study confirmed the change of the lattice dimension upon the light illumination observed in the previous studies. However, the lattice dimension became irreversibly larger than the original one after the recovery and the quality of the diffraction pattern deteriorated concomitantly. The similar change was also seen in the unilluminated retina. These results suggest that the decrease of the lattice dimension in response to the light stimulation may be related to the light-dark adaptation of visual cell. But the increase of the lattice dimension may correspond to the disintegration of the tissue by the radiation damage and/or the autolysis.

References

* hamanaka@bpe.es.osaka-u.ac.jp