15A/1999G328

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

Toshiaki HAMANAKA*[1], Masatsugu SEIDOU[2], Kinya NARITA[3], Masanao MICHINOMAE[4], Yasunobu SUGIMOTO[1], Katsuzo WAKABAYASHI[1] and Yoshiyuki AMEMIYA[5]
[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]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 retinas fixed by glutaraldehyde was used, because this tissue disintegrated within 1 hour of dissection. It has been reported that we could succeed in recording the x-ray diffraction pattern from unfixed retina by 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 previous study[3], we showed 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. In this study, we have tried to follow the change of diffraction pattern after the light illumination by use of a CCD-based x-ray detector

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 sea water containing D-glucose was oxygenated and gently circulated through the sample chamber during the experiment. Blue light emitted LED was used for light stimulation (465 nm in wavelength).

X-ray experiments have been performed with a mirror –monochromator optics (the Muscle Diffractometer) at BL-15A1[4]. The wavelength of radiation was 0.150 nm. The sample-to-detector distance was 2092 mm. X-ray diffraction intensity was recorded with a CCD-based x-ray detector system[5]. X-ray diffraction data were successively taken on the same sample in the dark and /or light. The exposure time was 1 seconds usually and 40 ms for time resolved experiments and each recording finished within 30 minutes after decapitation.

Results and Discussion

The present study confirmed the change of 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. Sometimes the increase of the lattice dimension started at the early stage after the light stimulation. The similar increase was also observed 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 visual excitation. But the increase of the lattice dimension may correspond to the disintegration of the tissue by the radiation damage and/or the autolysis. Time resolved experiment with 40 ms time slices could not indicate significant changes of diffraction pattern due to partly the low signal to noise ratio.

References

[1] T. Hamanaka et al., PF Activity Report, No.6 124 (1988).
[2] T. Hamanaka et al., PF Activity Report, No.7 131 (1989)
[3] T. Hamanaka et al., PF Activity Report, No.17(Pt.B) 241 (1999)
[4] Y. Amemiya et al., Nucl. Instrum. Methods, 208, 471 (1983)
[5] Y. Amemiya et al., Rev. Instrum. Methods, 66, 2290 (1995)

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