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X-ray diffraction study on the light induced structure change of squid visual cell

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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 studies[3,4], 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 the present 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 caputured 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[5]. The wavelength of radiation was 0.150nm. The sample-to-detector distance was 2050mm. X-ray diffraction pattern was recorded with a CCD-based x-ray detector system. The time resolved x-ray diffraction data were successively taken on the same sample in the dark and light. The first frame contained a diffraction pattern in the dark and the data of the second one was taken during or after the light illumination. The following frames were obtained in the dark after a light flash. The exposure time was 0.3 or 0.5 seconds and the duration of a light flash was 0.2-1 seconds. The interval between successive frames was 150 or 300 seconds.

Results and Discussion

X-ray diffraction pattern showed the change of lattice dimension upon the light illumination. In general, the lattice dimension increased vertically after a light flash, but decreased in the horizontal direction. However the lattice dimension did not show the recovery within the present measuring time and the significant deterioration of diffraction pattern proceeded gradually.

The change of the lattice dimension in response to the light stimulation may be related to the visual excitation. The intensity of light flash might be too strong to observe the recovery of the lattice dimension within the present observation time. The deterioration of diffraction quality may correspond to the disintegration of the tissue by the radiation damage.

References

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