Small angle neutron scattering analysis of magnetoreceptor protein

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Cryptochrome 4 (Cry4) in some avian species could act as a quantum senser to detect the inclination angle of geomagnetic field, the function of which attributes the magnetic sensitivity of spins of unpaired electrons in radical pair (RP) in CRY generated by blue light irradiation. The excited state of Cry4 induced by blue light irradiation returns to the ground state for about 24 hours [1]. However, the structural characteristics of Cry4 during the return from the excited state to the ground state remain unclear.

Since the energies of incident neutron beams used for SANS are about 5-6 orders of magnitude lower in eV than that of X-rays in the same wavelength range, SANS may be an ideal method for investigation of the structural characteristics of Cry4 while reducing the artifacts of the radiation damage and the radical generation. In this study, SANS measurement was conducted to inspect the structural change from the excited state to the ground state for Cry4a (ErCry4a) from European robin, a representative magnetosensory animal.

The ErCry4a solution was prepared using 100% D_2O buffer. Before the SANS measurements, a 2.8 mg/mL ErCry4a solution in a sample cell (Fig.1) was irradiated with blue light (454 nm) from a fiber output LED source FOLS-01 (CRAFT CENTER SAWAKI) for 10 min to excite ErCry4a. After the irradiation of blue light, the windows of the sample cell were covered with aluminum foil to shield it from light (Fig. 1). SANS measurement was carried out at 20 ˚C. The wavelength of the incident neutron beam was 6 Å. The beam slit size was 15 mmφ. Time-course measurement was performed by alternating between two different sample-to-detector distances (4 m and 1 m) with the different neutron beam exposure times (60 min for 4m and 30 min for 1 m). Raw data (2D-SANS data) were converted to 1D-SANS curves by circular averaging to remove the beam stop and other undesirable parts of the images. A blank cell and buffer scatterings were subtracted from each curve to obtain the sample scattering curves $I(q)$s. $I(q)$s obtained from two different sample-to-detector distances were merged. Several examples of $I(q)$s during the time-course measurement are shown in Fig. 1. The scattering intensity at $q < 0.03$ Å^{-1} gradually increased over 24 hours, indicating that the association of ErCry4a proceeded during the return from the excited state to the ground state. Since this result was different from that recently obtained using ErCry4a solution in H_2O buffer by the size exclusion chromatography analysis with controlling the light condition [2], we are planning to inspect heavy water isotope effects on the molecular structure and behaviour of ErCry4a.

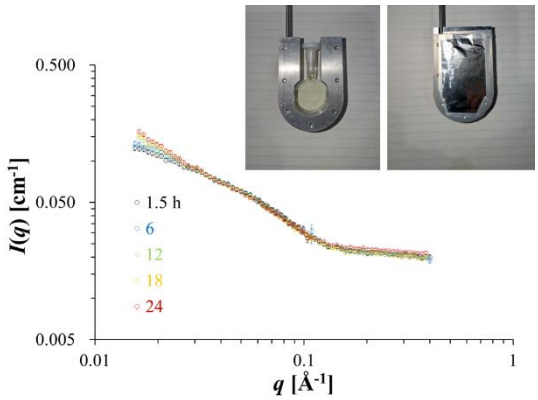

Fig. 1. Neutron scattering curves $I(q)$s of ErCry4a, which are shown in different colors for each time period after shading. Photos show the sample cells before shading (left) and after shading (right).

[1] R. Watari, *et al*., J Biol Chem, **287**, 42634 (2012).
[2] S. Arai, *et al*., Biochem Biophys Res Commun. **737**, 150513 (2024).