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Novel function of pigeon iron–sulfur protein for the magnetoreception predicted by SAXS analysis with a permanent magnetic device

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1 Introduction

Magnetoreception, which is a sense to perceive the magnetic field information, is used for many animal behaviors (migratory, homing, feeding, breeding, etc.) [1]. Cryptochrome (CRY) in the retinal cell of some animal species can act as a quantum sensor to detect the angle of the geomagnetic field [2,3]. However, partner proteins for CRY, such as CRY receptors that transmit magnetic information from CRY to perception and scaffold proteins that assist the magnetoreception function of CRY molecules, have not yet been identified. If CRY molecules are not anchored in retinal cells by assistant substances, CRY molecules will be randomly oriented, rotated and diffused, and the detected angle of the magnetic field will differ for each CRY molecule. In such situations, the angle of the magnetic field line would not be perceived correctly.

On the other hand, Qin *et al*. found that the iron–sulfur (Fe–S) cluster assembly homolog 1 of pigeon (clISCA1) interacts with CRY (clCRY4) and the clCRY4/clISCA1 complex tends to be orientated by weak magnetic fields (40 μT–1 mT) [4]. These results suggest that the ISCA1 may be one of the candidates of CRY partners. In the previous study, we clarified that clISCA1 molecules in a clISCA1 solution form columnar oligomers under the existence of Fe-S clusters, and these columnar oligomers in a 29.3 mg/mL clISCA1 solution elongate due to the self-assembly induced by applying the static magnetic field (<1 T) [5,6]. Recently, we further investigated the magnetic response of the molecular behavior of clISCA1 at lower magnetic fields (<180 mT) and a lower clISCA1 concentration (6.9 mg/mL). In this report, we summarize our observations using SAXS and previous studies. A novel function of clISCA1 for the magnetoreception can be presumed from this summary.

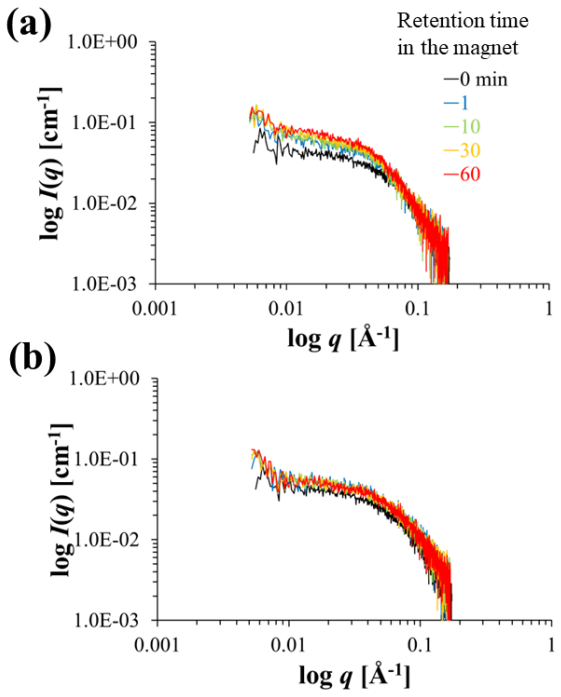

Fig. 1. Time course (0 – 60 min) of SAXS curves of 6.9 mg/mL clISCA1 solution with applying the external static magnetic field. (a) the condition A with 180 mT. (b) the condition B with –40 mT.

2 Experiment

The construction of clISCA1 was described previously [5]. Approximately a 40 μL sample solution containing 6.9 mg/mL clISCA1, 20 mM Tris-HCl buffer (pH 8), 150 mM NaCl and 10 mM 3-mercapto-1,2-propanediol was used for SAXS measurements. The wavelength used was 1.55 Å. The sample-to-detector distance was 3 m. The clISCA1 solution was injected into a sample cell with a 2 mm path length. A sample cell was inserted into a periodic Nd–Fe–B permanent magnetic circuit set in the BL-10C [7] as shown in Fig.5a of ref. [5] and Fig.2a of ref. [6]. Two different conditions of the magnetic field (condition A and condition B) were generated in the clISCA1 solution by changing the position of a sample cell in a magnetic circuit and the gap (1~2.5 cm) of a magnetic circuit [7]. For the condition A, the difference of the magnetic flux densities in the range from 160 to 180 mT was generated in a sample cell, and the magnetic flux density at beam center was adjusted to 180 mT. For the condition B, the difference of the magnetic flux densities in the range from –20 to 100 mT was generated in a sample cell, and the magnetic flux density at the beam center was adjusted to –40 mT. SAXS data during the application of the external static magnetic field for 60 min were collected using a PILATUS3 2M detector (Detectris) at 20 ˚C. X-ray exposure time to collect one SAXS curve was 1min. Buffer scattering was subtracted from each curve to yield the sample scattering curves $I(q)$s using the program *SAngler* ver.2.1.64 [8].

Since clISCA1 forms columnar oligomers, the average cross-sectional radius of gyration (R_c) was evaluated using the program *SCÅTTER* ver.4 with equations (1) and (2) [9].

$$I(q) = I_c(q)/q \quad (1)$$
$$I_c(q) = I_c(0)exp(-R_c^2 q^2/2) \quad (2)$$

$I_c(q)$ denotes the scattering curve of the cross-section of the particle. q is the magnitude of the scattering vector. The Guinier region of $I_c(q)$ can be approximated by the equation (2). The R_c and $I_c(0)$ values can be evaluated from a linear fit to the cross-sectional Guinier plot ($\ln\{q \bullet I(q)\}$ vs. q^2 plot). $I(q)$s were further converted to the distance distribution function $P(r)$s by Fourier transform using the program *GNOM* in *ATSAS* ver.3.1.3 [11]. The maximum diameter (D_{max}) of the clISCA1 columnar oligomer was estimated from the $P(r)$ function satisfying the condition $P(r) = 0$ for $r > D_{max}$.

3 Results and Discussion

Fig.1a and 1b show the time course of SAXS curves of clISCA1 for the conditions A and B, respectively. In both Fig.1a and 1b, the scattering intensities at $q < 0.01$ Å^{-1} of $I(q)$s increased with the retention time of the clISCA1 solutions in the magnet. Since these changes in $I(q)$s was not due to protein aggregation by radiation damage [3,4], the result indicated that the oligomerization of clISCA1 molecules was magnetically induced. Fig.2a and 2b show $P(r)$ functions obtained from SAXS curves shown in Fig.1a and 1b, respectively. The profiles of $P(r)$s in Fig.2a and 2b were changed from a double peak (0 min) to a tailed peak (60 min) according to retention time of the clISCA1 solution in the magnet, suggesting that the particles in the clISCA1 solution changed from a dumbbell shape (probably dimer or more) to a columnar shape. D_{max} of the clISCA1 oligomer increased from 73 to 113 Å for the condition A and from 73 to 108 Å for the condition B (Fig.3). Conversely, R_c during 60 min of retention time in the magnetic was almost constant (17±2 Å for the condition A and 13±1 Å for the condition B) as shown in Fig.3. These results indicated that the cross-section diameter of the columnar clISCA1 oligomer does not change, but the columnar oligomer's long axis elongates by applying the external static magnetic field [5].

With a retention time of 0 min in the magnet (under the natural geomagnetic condition), D_{max} measured in this study (73 Å) was smaller than that measured using 29.3 mg/mL clISCA1 solution in the previous study (158 Å) [5]. This result indicates that the length of the clISCA1 columnar oligomer depends on both the magnetic field condition and the clISCA1 concentration. However, the tendency to elongate magnetically the clISCA1 columnar oligomer shown here was very similar to the result in the previous study [5,6]. Therefore, the present results demonstrate that the translational diffusion and oligomerization of clISCA1 are magnetically responsive under conditions closer to the natural environment than in previous studies [5,6].

Although it is technically difficult to clarify the role of the magnetic orientational property of clCRY4/clISCA1 oligomer and the magnetic response of clISCA1 in the vicinity of geomagnetic fields (<0.1 mT), clISCA1 certainly responds to the external magnetic field. Since the clISCA1 oligomer binds two types of Fe–S clusters ([2Fe–2S] and [3Fe–4S] cluster) and mononuclear Fe atoms [12,13], the magnetism of clISCA1 oligomer should be regulated by the synergistic effect of

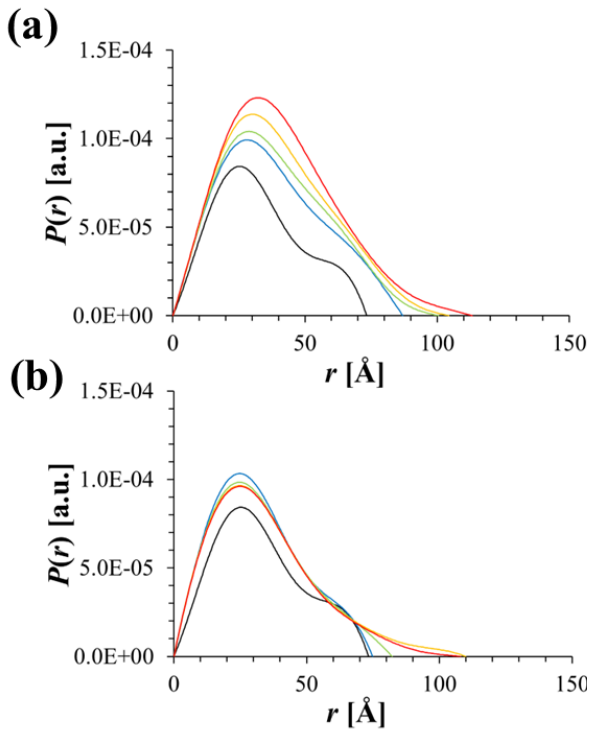

Fig. 2. $P(r)$ functions of clISCA1 obtained by Fourier transform from $I(q)$s of (a) Fig. 1a and (b) Fig.1b.

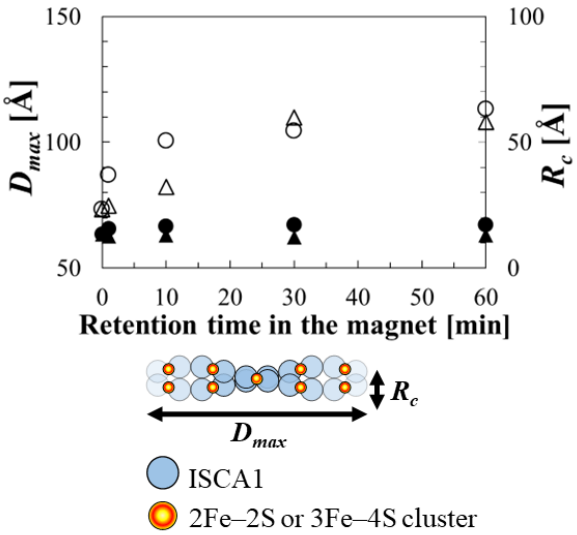

Fig. 3. Time course of D_{max} and R_c of the clISCA1 columnar oligomer in the magnetic circuit. In the upper figure, open circles and open triangles indicate D_{max} of the condition A and the condition B, respectively. Closed circles and closed triangles indicate R_c of the condition A and the condition B, respectively. The lower figure shows the schematic drawing of the clISCA1 oligomer connected by Fe–S clusters.

mononuclear Fe atoms, [3Fe–4S] / [2Fe–2S] mixing binding and the redox state of Fe–S clusters [5,12,13]. For example, $[2Fe–2S]^{1+}$ (containing one Fe(II) and one Fe(III)) and $[3Fe–4S]^{0}$ (containing one Fe(II) and two Fe(III)s) become paramagnetic states. Conversely, $[2Fe–2S]^{2+}$ (containing two Fe(III)s) and $[3Fe–4S]^{1+}$ (containing three Fe(III)s) become a diamagnetic state [14].

When the external static magnetic field is applied to a sample solution, the ferromagnetic, ferrimagnetic, superparamagnetic, paramagnetic, and antiferromagnetic particles in a solution gather at a high magnetic intensity location. Conversely, the diamagnetic particles gather at a low magnetic intensity location. Therefore, the clISCA1 oligomers formed at low and high magnetic field intensities would have different magnetic states, namely diamagnetic and nondiamagnetic states, respectively. More specifically, the oligomerization ability of clISCA1 in the nondiamagnetic state may be improved at high magnetic flux density locations, such as high-latitude regions of the Earth (Fig.4).

Moreover, clCRY4 may recognize the nondiamagnetic clISCA1 oligomers, since the clCRY4/clISCA1 complex behaves like a ferrimagnetic material [4] and the clISCA1 oligomer bound to both Fe–S clusters and mononuclear Fe atoms behaves as a paramagnetic substance averagely at 300 K [12]. Taken together, the amount of clCRY4 molecules anchored on the clISCA1 oligomer will increase at high-latitude regions, which may increase the amount of functional clCRY4 molecules as compass magnets. As a result, the signal intensity of the magnetic information transmitted from clCRY4 to perception may be changed depending on the geomagnetic field intensity. If clISCA1 acts as a scaffold for clCRY4, pigeon may be able to perceive not only the angle of the magnetic field line but also the magnetic flux density reflecting the latitude.

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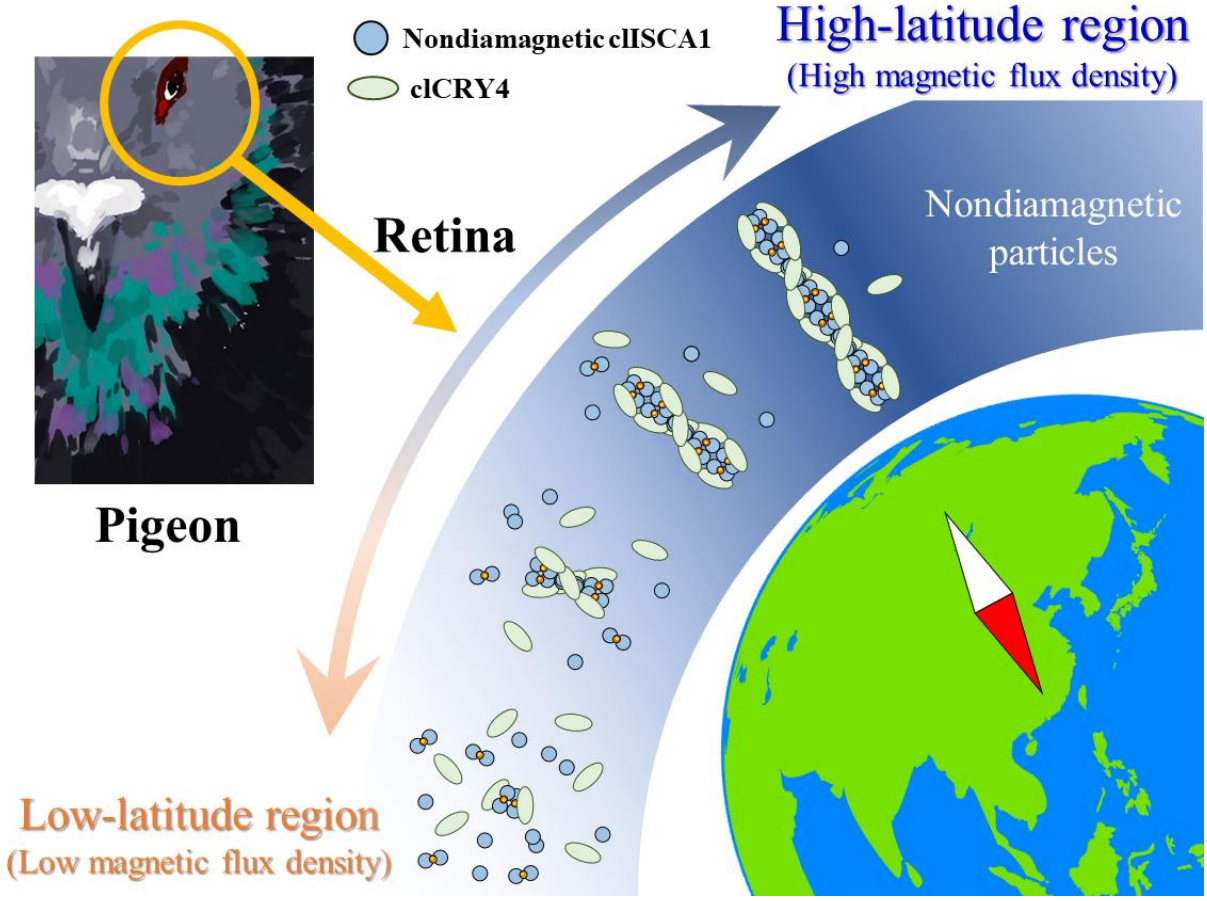

Fig. 4. A schematic drawing of the predicted mechanism of clCRY4/clISCA1 complexation in the geomagnetic field, assuming that the clISCA1 molecules are in the nondiamagnetic states. For example, the magnetic flux densities at the Earth's surface of the geomagnetic north and south poles are 65 – 67 μT, which are approximately three times higher than that near the equator (22 μT) [15–17]. The nondiamagnetic clISCA1 may polymerize at high-latitude regions and bind to clCRY4 molecules.

References
[1] S. Johnsen, K.J.Lohmann, *Nat Rev Neurosci*. **6**, 703–712 (2005).
[2] T. Ritz, *et al*, *Biophys J*. **78**, 707–718 (2000).
[3] H. Mouritsen, T. Ritz, *Curr Opin Neurobiol*. 15, 406–614 (2005).
[4] S. Qin, *et al*. *Nat Mater*. **15**, 217–226 (2016).
[5] S. Arai, *et al*., *Protein Sci*. **31**, e4313 (2022).
[6] S. Arai, *et al*., *Photon Factory Activity Report* 2021, #**39**, 29 (2022).
[7] M. Hirai, *et al*., *J Appl Crystallogr*. **36**, 520–524 (2003).
[8] Shimizu N, *et al*., *AIP Conf Proc*. 1741, 050017 (2016).
[9] S. Förster, *J Appl Crystallogr*. **43**, 639–646 (2010).
[10] Z. Guo, *et al*., *Sci Rep*. **11**, 23941 (2021).
[11] K. Manalastas-Cantos, et al., *J Appl Crystallogr.* **54**, 343–355 (2021).
[12] Y. Zhou, *et al*., *Zool Res.* **44**, 142–152 (2023).
[13] Z. Guo, *et al*., *Sci Rep.* **11**, 23941 (2021).
[14] T. Iwasaki, *et al*., *J Am Chem Soc*. **131**, 13659–13667 (2009).
[15] G. Schubert, K.M. Soderlund, *Phys. Earth Planet. Inter*. **187**, 92, (2011).
[16] Geomagnetism Frequently Asked Questions, National Centers For Environmental Information, National Oceanic And Atmospheric Administration, https://www.ngdc.noaa.gov/geomag/faqgeom.shtml#What_are_the_magnetic_elements
[17] The Earth's Magnetic Field: An Overview, British Geological Survey, http://www.geomag.bgs.ac.uk/education/earthmag.html

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