5. Effect of Magnetic Fields on L-Threonine Dehydrogenase (Video 12)

<Explanation>

We investigated the effect of magnetic fields on the growth of crystals while a protein by the name of L-threonine dehydrogenase (TDH), extracted from archaea and having heat-resistant enzyme, is crystallized in a strong magnetic field generated by a superconducting magnet.

The video shows images of the TDH crystallization process photographed at intervals of 30 minutes. Crystallization was conducted by the hanging drop method. A strong magnetic field of flux density 8T was applied in the direction from the recess of the screen to the front.

The TDH crystal comes in a spindle shape, and the *C*-axis of the crystal is oriented perpendicularly to the direction of the magnetic field. This video shows a scene of experiments conducted before the knowledge becomes clear that TDH is oriented perpendicularly to the direction of the magnetic field. Therefore, it may be somewhat difficult to discern whether TDH is really oriented in conjunction with the magnetic field. This is one of the rare videos that successfully show the scene of TDH crystal growth in the magnetic field.

The continuous photos below show the orientation of crystals when magnetic fields of (A) 0 T, (B) 2 T, (C) 4 T, and (D) 10 T are applied to *native*-TDH. It is obvious that the crystals are oriented perpendicularly to the direction of magnetism in a magnetic field of 4 T or above.

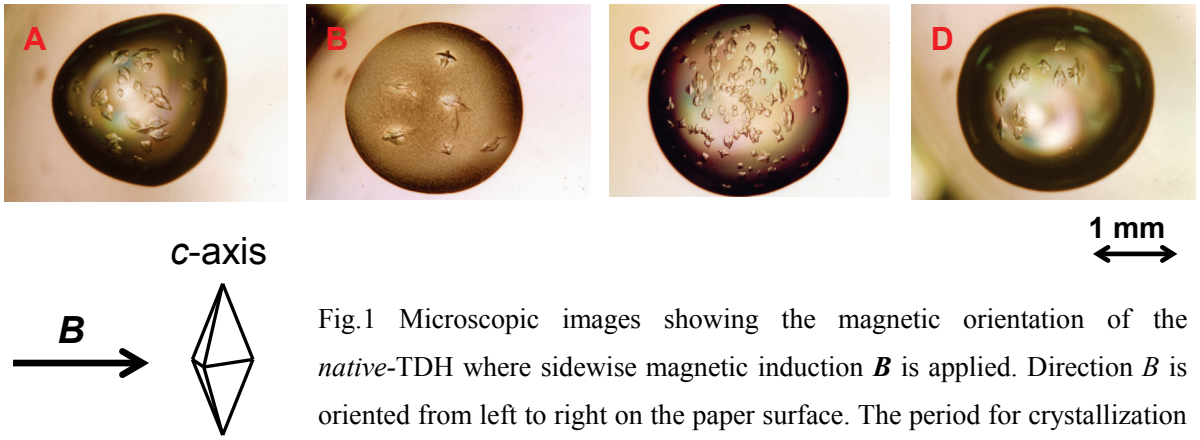

Fig.1 Microscopic images showing the magnetic orientation of the *native*-TDH where sidewise magnetic induction ***B*** is applied. Direction *B* is oriented from left to right on the paper surface. The period for crystallization is 3 days, and the temperature is 5°C.

<Complementary Explanation>

For *native*-TDH, a zinc atom is placed on its molecular surface. In this study, Zn^{2+} ions were removed with a chelating agent, and genetically modified TDH was produced by replacing them with Co^{2+}, Ni^{2+}, and Gd^{3+} ions having a greater magnetic susceptibility so that an effect similar to the magnetic field can be examined. As shown in the Refs. [1-2], the *modified*-TDH crystals, replaced with Co^{2+} and Gd^{3+}, exhibited more remarkable magnetic orientation than the *native*-TDH, and that orientation was caused at the 2T level.

In regards to the *modified*-TDH crystals, the crystallization was conducted by tilting the superconducting magnet in the horizontal direction. In order to get the magnet tilted in the horizontal direction, substantial

modification of the empirical equipment was needed. Therefore, there are no videos that have recorded the process of crystal growth. Photos (A) ~ (D) in Fig. 1 show microscopic images taken shortly after the specimen was taken out of the magnet bore.

<Place of execution>

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<Research papers>

[1] S. Maki, and M. Ataka,
"High quality crystallization of protein by use of strong magnetic fields (*in Japanese*) ",
JAEA-Conf 2006-008, Report on JAERI's Reimei Research Program April 1, 2005 - March 31, 2006, Advanced Science and Research Center, Japan Atomic Energy Agency, pp.155-161 (2007).

[2] S. Maki, K. Ishikawa, and M. Ataka,
"Orientation of growing crystals of Co- or Gd-containing L-threonine dehydrogenase by magnetic fields",
Journal of Crystal Growth **311**, pp. 4725-4729 (2009).