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A simple technique for consistently obtaining large single crystals of hen egg-white lysozyme in a concentration gradient of $NiCl_2$

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A simple protocol is reported for consistently obtaining large single crystals of hen egg-white lysozyme (HEWL) greater than 2 mm in the longest dimension. Comparative crystallization experiments with different sources of HEWL showed that the simple addition of glass capillaries, 'crystal hangers', reduced excessive nucleation and resulted in large single crystals regardless of the commercial source of HEWL.

1. Introduction

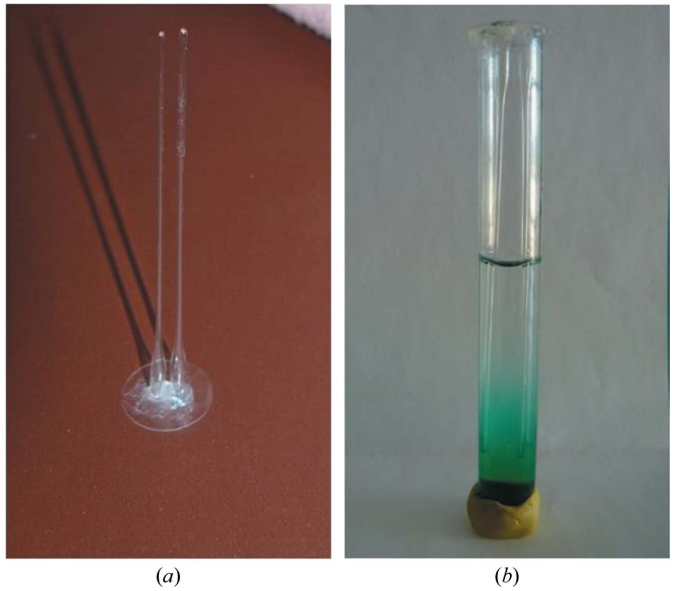
HEWL is the most widely used and extensively studied protein, serving as a benchmark both in protein crystal growth (PCG) and in protein structural analysis using X-ray and neutron diffraction methods. In spite of the ever-decreasing crystal-volume requirement in X-ray crystallography, there remains a demand for much larger crystal volumes (0.1–1.0 mm^3) in neutron crystallography (Blakeley *et al.*, 2008). To supply 2 mm-size crystals of HEWL routinely for the testing of a neutron single-crystal diffraction beamline, we became interested in the $NiCl_2$ concentration-gradient method, which has been used to grow HEWL crystals within two weeks, far exceeding 1.5 mm and even reaching 4 mm in the longest dimension, for X-ray topography (Tachibana *et al.*, 2003) and for neutron diffraction (Niimura *et al.*, 1997). Although the original method was reported in both a conference abstract (Ataka & Katsura, 1992*a*) and an institutional journal (Ataka & Katsura, 1992*b*), their circulations seem to be limited to Japanese or collaborative laboratories. Moreover, we found that its reproducibility depended on the commercial source of HEWL. Here, we report comparative crystallization studies with different sources of HEWL, and show that the simple addition of capillaries reduces excessive nucleation and results in consistently large single crystals of HEWL exceeding 2 mm in the longest dimension.

2. Experimental

Comparative PCG experiments were conducted at 296 K on HEWL obtained from two commercial sources (Sigma–Aldrich Corporation, USA, and Seigakaku Corporation, Japan). Seigakaku provide HEWL as a lyophilized powder obtained after six recrystallizations (Seikagaku 100940). Sigma–Aldrich provide HEWL as a lyophilized power obtained after three recrystallizations (Sigma L7651 and L6876). All HEWL solutions were prepared from lyophilized powders dissolved at a final concentration of 50 mg ml^{-1} in 50 m*M* Na acetate buffer (pH 4.5). All solutions were filtered through a 0.22 µm Sartorius filter. Powdered $NiCl_2$ (1 g) was placed in the bottom of a vertically held test tube of 100 mm in length, and an aqueous solution of HEWL was carefully applied on top (Ataka & Katsura, 1992*b*). The salt dissolved within a few hours and started to diffuse upwards. Capillaries (Hampton HR6-152, HR6-160, HR6-170) were attached to a cover-slip (Hampton HR3-239) and inserted into the test tube, sealed by grease, as shown in Fig. 1. Control PCGs without capillaries, and test PCGs with varying numbers of capillaries (1–5), capillary diameters (0.1–1.5 mm) and amounts of protein (60–400 mg), were prepared.

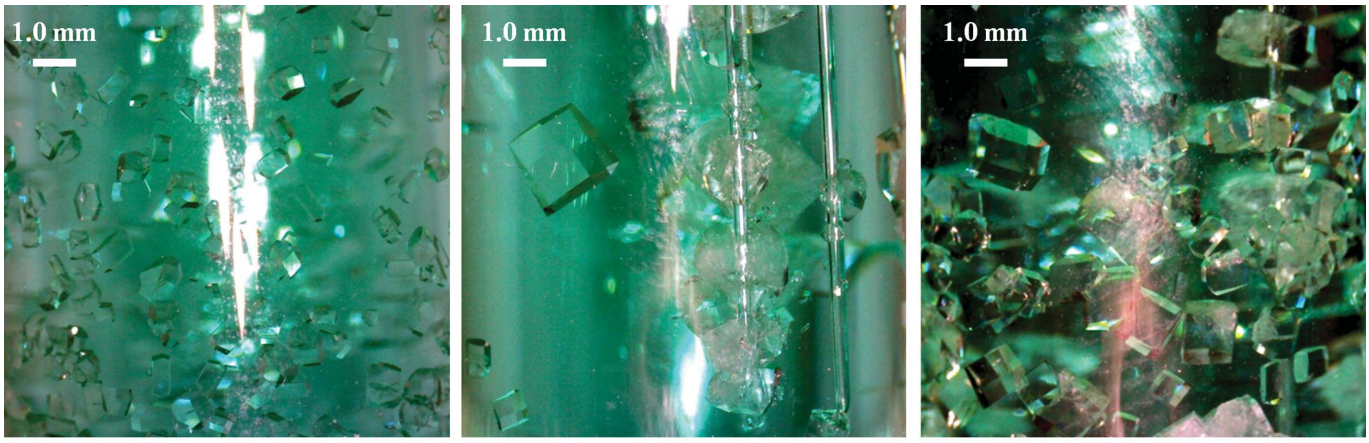
3. Results

The results of the comparative PCG experiments with the varying conditions described above are summarized in Table 1. In both control and test PCGs, crystals started to grow only in a region of 15–40 mm from the bottom of the test tube. In control PCGs of Seikagaku HEWL, a few crystals of 2–4 mm in length were obtained after two weeks at a height of about 23–25 mm, consistent with the original report (Ataka & Katsura, 1992*b*). In the control PCG of Sigma



(*a*) (*b*)

Figure 1
(*a*) Capillaries attached to a cover-slip at the bottom. (*b*) Concentration gradient of $NiCl_2$ with capillaries attached to a cover-slip at the top.



(*a*) (*b*) (*c*)

Figure 2
(*a*) Crystals obtained from Sigma HEWL without capillaries. (*b*) Crystals obtained from Sigma HEWL with capillaries. (*c*) Crystals obtained from Seikagaku HEWL without capillaries.

Table 1
Summary of PCG experiments, giving the size of the crystals in the longest dimension.

The numbers of experiments performed are given in parentheses.

Commercial source	Seikagaku		Sigma	
Product number	100940	L 7651	L 6876	L 6876
Lot No.	E00301	027K14051	059K1658	129K1863
Control PCG without capillaries	2–4 mm (2)	<1 mm (2)	≤1 mm (4)	<3 mm (4)
Test PCG with capillaries	2–4 mm (3)	1–2 mm (2)	2–3 mm (4)	3–4 mm (4)
Time of crystal growth	Within 2 weeks	7–10 d	7–10 d	5–7 d

HEWL, many small crystals were distributed on the wall of the test tube and no single crystal exceeded 1 mm in length in two cases of the Sigma products. In the test PCGs of Sigma HEWL, the crystals consistently grew larger than those in the control PCGs within the same period. We observed that many crystals appeared around the capillaries in all test PCGs. The 'best' yield of large crystals was in the test PCG with two capillaries of 1.0 mm diameter or three capillaries of 0.5 mm diameter per tube. The amount of applied protein solution did not contribute in any significant manner. We found that the inserted capillaries induced crystal nucleation around them and suppressed nucleation on the walls of tubes. Hence, we obtained HEWL crystals of 2–4 mm in length regardless of its commercial source, as shown in Fig. 2.

4. Discussion

Comparison of the PCGs of different HEWL stocks without any purification indicates that crystal size depends on its commercial source as well as its production lot. Such inconsistency due to different commercial sources has been reported in nucleation kinetic studies (Parmar *et al.*, 2007). It is also known that nucleation induced by dust, hair, eyelashes, denatured protein molecules *etc.* in unfiltered crystallization solutions abets crystallization (Chayen, 2009). In the test PCGs, the inserted capillaries seemed to act as 'artificial' nucleation sites that induce crystallization around them, leading to the suppression of nucleation on the walls of the tube with the result of increased crystal sizes. Hence, the protocol developed here for crystallization of HEWL in $NiCl_2$ provides a consistent method for obtaining large single crystals of HEWL. The use of the simple 'crystal hanger' device significantly increases the size of the crystals that can be obtained with HEWL purchased from Sigma and makes it comparable to that obtained with HEWL purchased from Seikagaku. Hence, the requirement for crystals with volumes greater than 1.0 mm^3 for neutron diffraction experiments can be achieved routinely.

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