

Supplementary Materials for

Protein Crystallization by Drying

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Preliminary three-dimensional structure of a lysozyme molecule from crystals obtained simply by drying

Data sets of the other HEWL crystals obtained by drying in a polyimide tube (Hagitec Co., Ltd., PIT-LS-3.0) were collected by synchrotron X-ray crystallography (SPring-8, BL-44XU). The HEWL structure was determined by molecular replacement using the Molrep program from Collaborative Computational Project Number 4 software for macromolecular X-ray crystallography graphical user interface (CCP4i) [1] with the structure of lysozyme (Protein Data Bank (PDB) entry 193L [2]) as a model. In this model, a sodium ion was detected as a part of the unit cell. The model was refined using Refmac5 from the CCP4i and manually fitted with winCoot0.7 [3]. We finally refined the model until $R_{\text{factor}} = 0.2845$ and $R_{\text{free}} = 0.3059$. Data collection statistics of the HEWL crystal obtained by drying is shown in Table S1. Although structure data has not been deposited in the PDB, we have found a change (1.5 Å) in the position of the hydroxyl oxygen of Ser 72 around a Na⁺ site in the HEWL molecule (Figure S1) and the loss of the Na⁺ ion, the same as previously reported in a precipitant-free HEWL structure [4]. The three-dimensional structures of the crystals differed slightly from those obtained by salting-out methods as a whole.

References

1. Collaborative Computational Project, Number 4. The CCP4 suite: programs for protein crystallography. *Acta Cryst. D*, **1994**, 50, 760-763.
2. Vaney, M.C.; Maignan, S.; Ries-Kautt, M.; Ducruix, A. High-resolution structure (1.33 Å) of a HEWL lysozyme tetragonal crystal grown in the APCF apparatus. Data and structural comparison with a crystal grown under microgravity from SpaceHab-01 mission. *Acta Cryst. D*, **1996**, 52, 505-517.
3. Emsley, P.; Lohkamp, B.; Scott, W.G.; Cowtan, K. Features and development of Coot. *Acta Cryst. D*, **2010**, 66, 486-501.
4. Suzuki, Y.; Tsuge, H.; Hondoh, H.; Kato, Y.; Uehara, Y.; Maita, N.; Hosokawa, K.; Ueta, S. Precipitant-free lysozyme crystals grown by centrifugal concentration reveal structural changes. *Cryst. Growth Des.* **2018**, 18, 4226-4229.

Table S1. Data collection statistics of the HEWL crystal obtained by drying.

Diffraction Source	BL44XU, SPring-8
Detector	Dectris Eiger X 16
Crystal-detector distance (mm)	150 mm
Rotation range per image (°)	1.0
Total rotation angle (°)	180
Exposure time per image (s)	1
Wave length (Å)	0.9
Attenuator	Al 2.1 mm
Temperature (K)	298
Space group	$P4_32_12$
a, b, c (Å)	79.3, 79.3, 38.0
α, β, γ (°)	90, 90, 90
Mosaicity (°)	0.03
Resolution range (Å)	56.07 – 1.44 (1.44 – 1.42)
Total number of reflections	183,978 (11,172)(13771)
Number of unique reflections	21,185 (1,108)
Completeness (%)	91.1 (96.8)
Multiplicity	8.7 (10.1)
R_{meas}	0.160 (0.995)
Mean $I / \sigma(I)$	10.2 (2.1)
Overall B factor from Wilson plot (Å ²)	14.9

Values for the outer shell are given in parentheses.

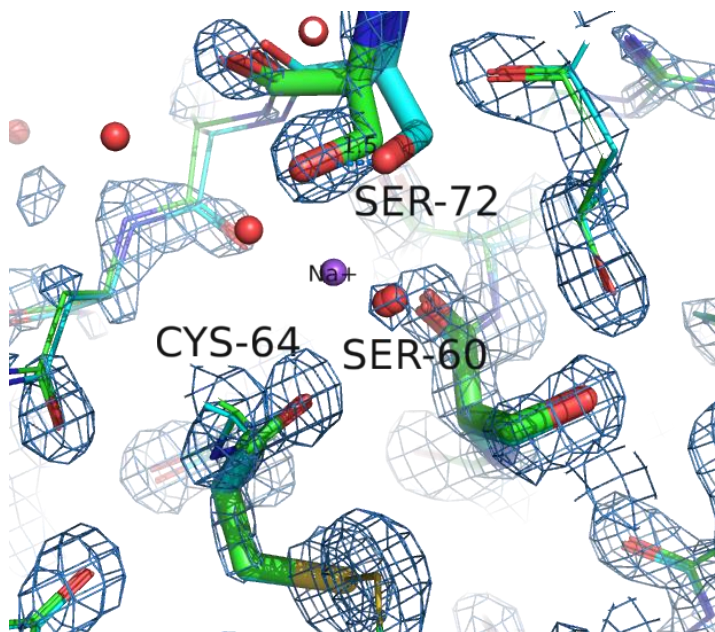


Figure S1. Alignment of our preliminary HEWL structure (green sticks) with the structure of 193L (blue sticks). Na⁺ is existed only in the 193L structure. The position of the hydroxyl oxygen in our structure is significantly separated (1.5 Å) from that of the 193L structure. Electron density of our preliminary HEWL structure is shown as mesh (contoured at 0.8660 eÅ⁻³).