



JOURNAL OF  
APPLIED  
CRYSTALLOGRAPHY

**Volume 53 (2020)**

**Supporting information for article:**

**Hydrogen/deuterium exchange behavior in tetragonal hen egg-white lysozyme crystals affected by solution state**

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## **Crystallization**

Hen egg-white lysozyme was purchased (HWL, Sigma L6876-1G). As a standard protein solution, a 100% D<sub>2</sub>O (Euriso-top, 99.9%) -solvated HWL solution at a concentration of 10 mg/mL was prepared and stored at 4 °C for at least 2 days. After storage, protein solution was concentrated by an ultrafiltration unit (Amicon Ultra, Merck Millipore) to a concentration of 100 mg/mL and stored at 4 °C before use. Crystallization solutions of 3 - 4% sodium chloride in 0.1 M sodium acetate (pD 4.6) prepared with sodium acetate-d3 (Sigma Aldrich, 99 atom % D), acetic acid-d4 (Sigma Aldrich, 99.5 atom % D), and 100% D<sub>2</sub>O, were used. The pD value was measured using a benchtop pH meter (HORIBA pH meter F-71). The sitting-drop vapor diffusion technique was used for crystallization with drop sizes of 2 μL protein + 2 μL crystallization solution. The drop solutions were filtered using a 0.1 μm filter (Ultrafree-MC Centrifugal Filter UFC30VV00, Merck Millipore) to suppress excessive nucleation, and placed on the Fluorinert liquid (Hampton Research) on the sitting drop well to prevent the crystals from sticking to the surface of the crystallization plate. Only one or two crystals were appeared in each batch at room temperature (20 °C) after more than 1-2 weeks with high probability. The crystals were grown by adding mixture solutions of stock protein containing crystallization solutions. In 4 months, after 15 times additions of mixture solution, a crystal large enough for the neutron diffraction work with its volume of 0.4 mm<sup>3</sup> was obtained.

## **Neutron and X-ray diffraction experiments**

The crystals for neutron diffraction were mounted in quartz capillaries with a trace amount of mother liquor, and the neutron data were collected at room temperature with Laue diffractometer CG-4D's IMAGINE installed at the High Flux Isotope Reactor at Oak Ridge National Laboratory.<sup>1-2</sup> The neutron quasi-Laue single crystal diffraction intensities on 12 frames were collected with an exposure time of 20 hours per each frame. The data were processed using LAUEGEN, and the data were normalized and merged by the program LSCALE and SCALA programs.<sup>3-6</sup> The X-ray data were collected using the crystals obtained from the same crystallization conditions used for neutron data collection. The crystals were mounted in soda-glass capillaries, and the X-ray data were collected at room temperature using CuKα radiation from a rotating anode X-ray source with an RAXIS VII imaging plate detector (Rigaku). The oscillation range was 1.0° per image.

A total of 180 images were collected with an exposure time of 1 min. per frame. Diffraction data were processed with the HKL2000 package.<sup>7</sup>

### Structure determination

The X-ray structure was solved by the molecular replacement method using the program Molrep<sup>8</sup> from the CCP4 program suite and the coordinates of lysozyme (PDB Code: 193l.pdb<sup>9</sup>) without waters and ions as a model. The coordinates for H/D exchanged HWL were initially refined against X-ray data with PHENIX.refine within the PHENIX package.<sup>10</sup> At the last stage of the refinement against only X-ray data, water oxygen atoms and ions (Na and Cl) were added in the refined model. After the convergence of R-factors, the X/N joint refinement,<sup>11</sup> which involves maximum-likelihood-based refinement of the coordinates, individual temperature factors, and occupancy of exchangeable H/D atoms in the protein, were carried out using X-ray and neutron data.<sup>11</sup> Deuterium atoms at the N-terminus (Lys1) were added manually. In the structure, all water molecules were assigned to deuterium oxide molecules, because none of the reagents except the HWL protein used in the experimental procedures included hydrogen atoms. For the water oxygen atoms whose nuclear densities were appeared, deuterium atoms were generated and refined by PHENIX.refine, and checked carefully with the consideration of hydrogen bonding networks. For the water oxygen atoms for which nuclear density was not observed, the deuterium atoms were not assigned. The manual model buildings during the refinement procedures were carried out using COOT.<sup>12</sup> The quality of the model was checked using the MolProbity server<sup>13</sup> at <http://molprobity.biochem.duke.edu/> (Chen *et al.*, 2010). Pictures were prepared using the program PyMOL Molecular Graphic System program (Version 1.2r3pre, Schrodinger, LLC). The data collection and the refinement statistics are summarized in Table S1.

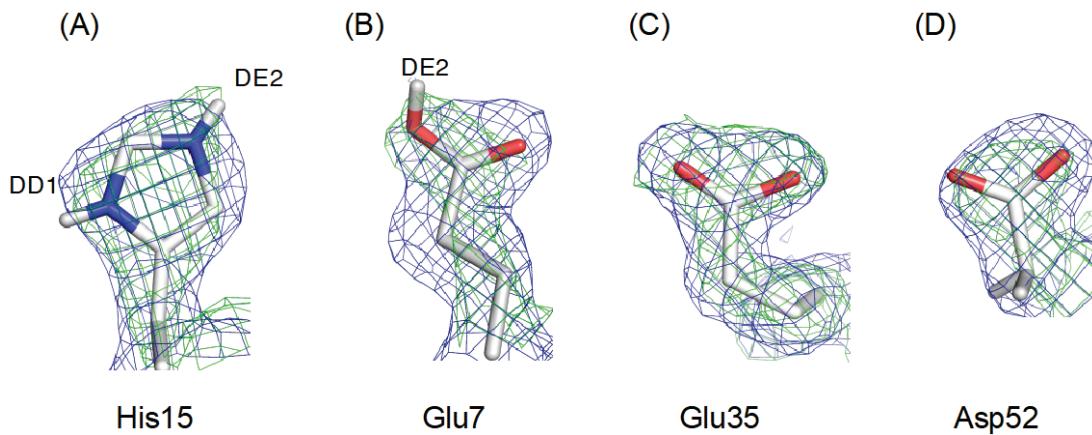
**Table SI.** Data statistics and refinement parameters

| PDB code   | Neutron<br>6K8G | X-ray         |
|--|-----------------|---------------|
| Data collection                                    |                 |               |
| Wavelength (Å)                                     | 2.8-4.5         | 1.54          |
| Resolution (Å)                                     | 34.12 – 2.00    | 100.0 – 2.00  |
| (outer shell)                                      | (2.11-2.00)     | (2.07-2.00)   |
| Measurements                                       | 47,204          | 202,913       |
| Unique reflections                                 | 7,338           | 8,051         |
| $R_{\text{merge}}^{\text{a}}$                      | 0.163(0.290)    | 0.059 (0.118) |
| Redundancy   | 6.4 (5.1)       | 25.2 (22.0)   |
| Completeness (%)                                   | 87.8 (79.5)     | 93.3 (65.7)   |
| $I/\sigma(I)$                                      | 5.5 (3.1)       | 63.5 (26.2)   |
| Unit Cell  |                 |               |
| Space group  | $P4_32_12$      | $P4_32_12$    |
| Cell dimensions (Å)                                |                 |               |
| $a$  | 79.1            | 79.1          |
| $c$  | 37.8            | 37.8          |
| No. of molecules per asymmetric unit               | 1               | 1             |
| Refinement   |                 |               |
| Resolution range (Å)                               | 100.0 – 2.0     | 100.0 – 2.0   |
| No. of reflections                                 | 7,337           | 8,026         |
| $R$ free set (%)                                   | 5               | 5             |
| $R_{\text{work}}/R_{\text{free}}^{\text{b,c}} (%)$ | 0.225 / 0.255   | 0.132/ 0.180  |
| No. of non-H/D atoms                               |                 |               |
| Protein  | 997             |               |
| Solvent molecules                                  | 87              |               |
| Ions (Na, Cl)                                      | 2               |               |
| No. of deuterium atoms                             |                 |               |
| Protein  | 262             |               |
| Solvent  | 152             |               |
| Average $B$ factors (Å <sup>2</sup> )              |                 |               |
| Non-H/D atoms protein/ others                      | 22.2/34.9       |               |
| Deuterium atoms protein/ others                    | 33.3/41.2       |               |
| Rmsd bond length (Å)                               | 0.013           |               |
| Rmsd bond angles (deg.)                            | 1.320           |               |
| Ramachandran plot                                  |                 |               |
| favored (%)  | 98.4            |               |
| outliers (%)                                       | 0.0             |               |

<sup>a</sup> $R_{\text{merge}} = \sum (|I - \langle I \rangle|) / \sum (I)$ <sup>b</sup> $R = \sum |F_{\text{obs}}| - |F_{\text{calc}}| / \sum |F_{\text{obs}}|$ <sup>c</sup> $R_{\text{work}}$  is calculated from a set of reflections in which 5% of the total reflections have been randomly omitted from the refinement and used to calculate  $R_{\text{free}}$ .

## Quality of neutron density map

The 2Fo-Fc nuclear density maps, which were calculated using the model proteins with deprotonated His15, showed that a hydrogen atom bound to NE2 of His15 was exchanged to deuterium (DE2 in Fig. S1A) with its site occupation of 0.60, whereas the other, which bound to ND1 (DD1 in Fig. S1A), had an occupancy of 0.49 (Fig. S1A). The side chain of His15 was flipped from that of 1io5.pdb<sup>14</sup> or 1lzn.pdb<sup>15</sup>. The 2Fo-Fc nuclear density maps also showed the protonation state of Glu7 residue, which carboxyl group was protonated in the structure of 1lzn.pdb<sup>15</sup> (Fig. S1B). The atomic occupancy of the deuterium bound to OE2 in Glu7 (DE2 in Fig. S1B) was 0.61. On the contrary, the protonation state of Glu35, whose carboxyl group was analyzed by NMR method<sup>16</sup> and also protonated in the structure of 1lzn.pdb, was not determined in this experiment, because its neutron-scattering length density is insufficient for strict positioning of deuterium (Fig. S1C). The side chain of Asp52 appears not to be protonated in this neutron-scattering length density map (Fig. S1D).



**Figure S1.** H/D-omitted X-ray and the neutron density maps. The 2Fo-Fc X-ray density map (blue, 1.4  $\sigma$ ), and the 2Fo-Fc neutron-scattering length density map (green, 1.4  $\sigma$ ) are shown. The amino acid residues (see text) are illustrated as white sticks models. A. Maps for His15. B. Glu7. C. Glu35. D. Asp52.

## References

1. Meilleur, F., Munshi, P., Robertson, L., Stoica, A. D., Crow, L., Kovalevsky, A., Koritsanszky, T., Chakoumakos, B. C., Blessing, R., Myles, D. A. A. (2013). *Acta Cryst.* **D69**, 2157–2160.
2. Schröder, G. C., O'Dell, W. B., Myles, D. A. A., Kovalevsky, A., Meilleur, F. (2018). *Acta Cryst.* **D74**, 778–786.
3. Campbell, J. W. (1995). *J. Appl. Cryst.* **28**, 228-236.
4. Campbell, J. W., Hao, Q., Harding, M. M., Nguti, N. D., Wilkinson, C. (1998). *J. Appl. Cryst.* **31**, 496-502.
5. Arzt, S., Campbell, J. W., Harding, M. M., Hao, Q., Helliwell, J. R. (1999). *J. Appl. Cryst.* **32**, 554-562.
6. Weiss, M. S. (2001). *J. Appl. Cryst.* **34**, 130-135.
7. Otwinowski, Z., Minor, W. (1997). *Methods Enzymol* **276**, 307-326.
8. Vagin, A., Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022-1025.
9. Vaney, M. C., Maignan, S., Ries-Kraut, M., Ducruix, A. (1996). *Acta Cryst.* **D52**, 505-517.
10. Afonine, P. V., Grosse-Kunstleve, R. W., Echols, N., Headd, J. J., Moriarty, N. W., Mustyakimov, M., Terwilliger, T. C., Urzhumtsev, A., Zwart, P. H., Adams, P. D. (2012). *Acta Cryst.* **D68**, 352-367.
11. Afonine PV, Mustyakimov M, Grosse-Kunstleve RW, Moriarty NW, Langan P, Adams PD (2010) Joint X-ray and neutron refinement with phenix.refine. *Acta Cryst* D66:1153-1163.

12. Emsley, P., Cowtan, K. (2004). *Acta Cryst.* **D60**, 2126-2132.
13. Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., Richardson, D. C. (2010). *Acta Cryst.* **D66**, 12-21.
14. Niimura, N., Minezaki, Y., Nonaka, T., Castagna, J. C., Cipriani, F., Høghøj, P., Lehmann, M. S., Wilkinson, C. (1997). *Nat. Struct. Biol.* **4**, 909-914.
15. Bon, C., Lehmann, M. S., Wilkinson, C. (1999). *Acta Cryst.* **D55**, 978-987.
16. Delepierre, M., Dobson, C. M., Karplus, M., Poulsen, F. M., States, D. J., Wedin, R. E. (1987). *J. Mol. Biol.* **197**, 111-130.