Preparation and Crystallization of Perdeuterated T4 Phage Lysozyme for Neutron Diffraction

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T4 phage lysozyme (T4L) is an endoacetylmuramidase that degrades the murein of the bacterial cell wall by cleavage of the α-1,4-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine [1]. We previously reported that the substitution of the catalytic Thr26 to the nucleophilic His converts the wild type (WT) T4L from an inverting to a retaining glycosidase, in which the β-configuration of the substrate is retained in the product [2]. It was also found that the Thr26His mutant T4L can catalyze the transglycosylation reaction more effectively than hydrolysis although the WT T4L has no transglycosidase activity. The Thr26His mutant T4L is the first glycosidase in which the His residue was used as a catalytic residue and the first engineered enzyme acquired the transglycosylation activity. To clarify the role of the substituted His26 on transglycosylation and its relationship to the neighboring acidic residue Asp20 by neutron crystallography, the perdeuterated recombinant proteins of the WT and Thr26His mutant T4L were prepared for crystallization in this study. The use of perdeuteration would reduce the crystal-size requirement by one order of magnitude and enable better visualization of the protonation and hydration states of the catalytic residues. The perdeuterated forms were produced in *Escherichia coli* cells cultured in deuterated rich media. After purification, macroseeding was performed to grow large crystals by transferring individual crystals to hanging drops comprising a 1:2 mixture of the protein solution (50 mg/mL) and the crystallization buffer (1.8 M Na/K phosphate (pD 6.6), 0.1 M NaCl, 0.1 M Tris-DCl (pD 7.0) and 0.1 M 1,6-hexanediol). A crystal of Thr26His mutant T4L with a volume of 0.1 mm^3 was grown after one month. Preliminary neutron-diffraction experiment at the research reactor FRM-II (Munich, Germany) at 100 K gave diffraction spots beyond 2.5 Å resolution for 1.5 hour exposure.

References

[1] R. Kuroki, L. H. Weaver, B. W. Matthews, *Nat. Struct. Biol.* **2**, 1007-1011 (1995).
[2] R. Kuroki, L. H. Weaver, B. W. Matthews, *Proc. Natl. Acad. Sci. USA*, **96**, 8949-8954 (1999).