



การเตรียมผลึกและศึกษาโครงสร้างเบื้องต้นด้วยรังสีเอกซเรย์ของเอนไซม์ไลโซไซม์ในไข่ขาวของ
ตะพานน้ำพันธุไต้หวัน (*Trionyx sinensis* Wiegmann)

**CRYSTALLIZATION AND PRELIMINARY X-RAY STRUCTURE ANALYSIS OF
THE EGG-WHITE LYSOZYME FROM A TAIWANESE SOFT-SHELLED TURTLE
(*TRIONYX SINENSIS* WIEGMANN)**

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บทคัดย่อ: ไลโซไซม์ที่สามารถทำให้บริสุทธิ์ได้จากไข่ขาวของตะพานน้ำสายพันธุไต้หวัน พบว่ามีน้ำหนัก
โมเลกุลประมาณ 14.8 กิโลดาลตัน เมื่อผ่านการแยกแแถบโปรตีนโดยใช้ 12.5% SDS-PAGE
ผลึกของไลโซไซม์สามารถเตรียมได้ด้วยวิธีที่เรียกว่า sitting drop vapor diffusion โดยใช้ 30% (w/v)
polyethylene glycol 8000 ในบัฟเฟอร์ 0.1 M sodium cacodylate ที่มี pH 6.5 และ 0.2 M
ammonium sulfate เป็นตัวตกตะกอน ผลึกเดี่ยวของไลโซไซม์ที่เตรียมได้นำมาศึกษาโครงสร้างด้วยการ
หักเหของรังสีเอกซเรย์ภายใต้ 2 Å resolution พบว่าผลึกของเอนไซม์อยู่ในรูป orthorhombic ซึ่งมีการ
จัดเรียงตัวของโมเลกุลอยู่ในกลุ่ม P2₁2₁2₁ โดย unit cell ของผลึกมีขนาด ด้าน $a = 37.8$ Å ด้าน
 $b = 55.6$ Å และด้าน $c = 72.2$ Å และมีจำนวนหนึ่งโมเลกุลของเอนไซม์ต่อ asymmetric unit ข้อมูลที่
ได้จากการทดลองเป็นข้อมูลที่สามารถรวบรวมได้ถึง 1.9 Å resolution มี R_{merge} ประมาณ 4.6%
และเป็นข้อมูลที่เหมาะที่จะนำมาใช้ในการศึกษาโครงสร้างของเอนไซม์นี้ โครงสร้างของผลึกเดี่ยว
ของไลโซไซม์จากไข่ขาวของตะพานน้ำสายพันธุไต้หวันที่ได้จากการการศึกษาด้วยรังสีเอกซเรย์ได้ทำการ
หาเฟสเริ่มต้นด้วยเทคนิค Molecular Replacement จากค้นแบบโครงสร้างของไลโซไซม์จากไข่ขาวของ
ไก่ฟ้า (PDB ID 1GHL) เพื่อหาโครงสร้างที่สมบูรณ์ต่อไป

Abstract: Lysozyme has been purified from the egg-white of a Taiwanese soft-shelled turtle.
This soft-shelled turtle's egg-white lysozyme migrated on 12.5% SDS-PAGE at about
14.8 kDa. The lysozyme has been crystallized using the sitting drop vapor diffusion
technique and 30% (w/v) polyethylene glycol 8000 in 0.1 M sodium cacodylate, pH 6.5
containing 0.2 M ammonium sulfate as a precipitant. One of the crystals diffracted X-rays
beyond 2 Å resolution and belonged to the orthorhombic, space group P2₁2₁2₁, with unit cell
dimensions of $a = 37.8$ Å, $b = 55.6$ Å, and $c = 72.2$ Å and one molecule of the enzyme per

asymmetric unit. The data were collected to 1.9 Å resolution with an R_{merge} of 4.6%, suitable for high resolution structure analysis. The single-crystal X-ray structure of lysozyme has been initially phased with the Molecular Replacement technique using pheasant egg-white lysozyme (PDB ID 1GHL) as a search template. Model rebuilding and refinement are in progress.

Introduction: Lysozymes (mucoprotein *N*-acetylmuramyl hydrolase, EC 3.2.1.17) have been isolated from various origins. These lysozymes possess qualitatively the same catalytic activity in their capacity to hydrolyze glycosidic $\beta(1\rightarrow4)$ linkages in the bacterial cell wall. They differ in their primary structure, specific activity, and various physicochemical properties. They are classified into three types: chicken type (C-type), phage type (T4-type), and goose type (G-type). Since only limited information seems to be available on egg-white lysozymes of reptiles which are the nearest ancestors to birds, we have purified an egg-white lysozyme of a Taiwanese soft-shelled turtle and studied its tertiary structure by X-ray analysis. This work is the first report of a high resolution X-ray structure study of the soft-shelled turtle's egg-white lysozyme. The three-dimensional structure of the soft-shelled turtle's egg-white lysozyme is important to evolutionary studies on lysozymes.

Methodology: The eggs of a Taiwanese soft-shelled turtle (*Trionyx sinensis* Wiegmann) were collected from local farms in Thailand. The egg-whites of the collected eggs were pooled and a lysozyme was purified by pH precipitation and cation exchange chromatography. The purity of purified protein and its molecular weight were determined on 12.5% SDS-PAGE. Protein concentration was estimated using the BCA assay. The enzyme was crystallized from aqueous solutions at a concentration of 20 mg/ml. Crystallization conditions were initially screened using the microbatch under paraffin oil method and prepared on a large scale using the sitting drop vapor diffusion technique. A crystal (440 μm x 80 μm x 20 μm) was harvested and diffracted X-rays to 2.0 Å resolution on an Rigaku/MSR R-AXIS IV⁺⁺ detector using a RU-H3R rotating anode generator running at 50 kV and 100 mA, and equipped with Osmic Blue confocal focusing mirrors and a 0.3 mm collimator. Diffraction data were recorded over a 85° rotation of the crystal around the phi axis in 180 diffraction images with a width of 1° per image. The integrated intensity was obtained with the CrystalClear/d*TREK (Pflugrath, 1999). The data were phased using the Molecular replacement technique for structural determination.

Results, Discussion and Conclusion : A soft-shelled turtle's egg-white lysozyme has been reported to be a C-type lysozyme based on its primary structure by Sompong Thammasirirak (2003). It consists of a single peptide chain of 131 amino acid residues with a molecular weight of about 14.8 kDa. The enzyme was crystallized by the addition of 30% (w/v) polyethylene glycol 8000 in 0.1 M sodium cacodylate, pH 6.5 containing 0.2 M ammonium sulfate as a precipitant at 18 °C. The crystals were stable for many months and one of crystals diffracted to a resolution of 2 Å. Three dimensional X-ray data were collected to 1.9 Å with an R_{merge} of 4.6%. This crystal was orthorhombic. Systematic absences were observed for the $h00$ reflections when $h = 2n+1$, for the $0k0$ reflections, when $k = 2n+1$, and for the $00l$ reflections when $l = 2n+1$ which defined the space group as $p2_12_12_1$. The unit cell dimensions were $a = 37.8$ Å, $b = 55.6$ Å, and $c = 72.2$ Å giving a cell volume of 151536 Å³. Assuming one molecule of M_r approx. 14800 per asymmetric unit, the V_M value is 2.56 Å³/Da. The V_M

of the soft-shelled turtle's egg-white lysozyme is significantly higher than other C-type lysozymes e.g. known forms of hen egg-white lysozyme crystals (vary between 1.83 and 2.12 Å³/Da) or for the orthorhombic form of pigeon egg-white lysozyme ($V_M = 2.05 \text{ Å}^3/\text{Da}$). This value indicates that the soft-shelled turtle's egg-white lysozyme has much larger proportion of liquid than other type-C lysozyme crystals. Given that the density of the soft-shelled turtle's egg-white lysozyme crystal is 1.3 g/ml, the volume of the crystal occupied by liquid is 49% as compared with 24-48% in the other lysozyme crystals mentioned above. The single-crystal X-ray structure of lysozyme has been initially phased with the Molecular Replacement technique using pheasant egg-white lysozyme (PDB code 1GHL) as a search template. Model rebuilding and refinement are in progress.

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