Protein Crystallography by MAD Method Using 35 keV X-Rays

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The Structural Biology I beamline (BL41 XU) is characterized by a wide energy range of available X-rays from 6.5 keV to 37.5 keV (M. Kawamoto et al., Rev. Sci. Instrum., in press). The ultra-high energy X-rays over 30 keV, provided as third harmonics emission of the SPring-8 standard undulator, are expected to be useful for multi-wavelength anomalous dispersion (MAD) phasing around K absorption edges of iodine (33.2 keV) and xenon (34.6 keV). Xenon is a rare gas atom that can be introduced into many protein crystals by pressurization. Iodine is a monovalent anion that is recently used as a heavy atom for phasing. In order to study the ability of the MAD method using ultra-high energy X-rays, we tried to perform MAD experiments with Xe and iodine derivatives of hen egg-white lysozyme crystals (tetragonal, $P4_12_12$).

The Xe derivative crystal was prepared using a specially designed chamber at 30 atm. The crystal was subsequently frozen with liquid ethane and stored in liquid nitrogen. Three data sets were collected for MAD phasing at X-ray energies of 22.96, 34.64, and 34.62 keV, based on XAFS spectroscopy with the Xe derivative. The binding sites of Xe were clearly found in dispersive and anomalous difference Patterson maps. The phasing calculation was carried out using SHARP at 2.5 Å resolution, and the phase was improved and extended by solvent flipping method using SOLOMON to 1.5 Å resolution. The electron density distributions were sufficient enough to identify each amino acid and to build a whole model of lysozyme molecule. For the iodine derivative prepared by co-crystallization, three data sets were also collected around K absorption edge for MAD phasing. The quality of electron density map was similar with that from the Xe derivative and the molecular modeling is in progress on the Iodine electron density map.

Identification of hydrogen atoms of protein at a low temperature.

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We collected X-ray diffraction data from a crystal of chitinase A1 using the Mar CCD diffractometer at several wavelengths. The data collected at a wavelength of 0.5 Å gave the best resolution. We processed and integrated this data with the program MOSFLM, and then scaled together multiple observations of reflections with the program SCALA. As shown in Table 1, an atomic resolution data was obtained. We are now refining the structure with the program REFMAC5. The current refinement statistics are summarized in Table 2.

Table 1. Summary of data collection and processing statistics

Data collection	
No. of crystals used	1
Temperature(K)	1.0 X 10^2
Exposure time per frame(sec)	10
Oscillation range per frame(deg)	1
Total oscillation range(deg)	180
Detector distance(mm)	146
Wavelength(Å)	0.5
Space group	*P*1
Unit-cell dimensions	*a*=43.007Å *b*=46.839Å *c*=55.700Å *α*=109.293° *b*=95.455° *g*=116.680°
Data reduction	
Resolution range (Å)	50.637 – 1.100
$R_{merge}(I)$	0.055(0.213)†
Completeness	0.981(0.973)†
<*I*/s>	8.3(3.0)†
Multiplicity	2.0 (2.0)†
No. of molecules per asymmetric unit	1

†Values in parentheses are for the highest resolution shell(1.16 – 1.10 Å) of the reciprocal space spherically divided to 10 bins.

Table 2. Current refinement statistics

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REFINEMENT.
  PROGRAM     : REFMAC 5.0
  AUTHORS     : MURSHUDOV,VAGIN,DODSON

    REFINEMENT TARGET : MAXIMUM LIKELIHOOD

 DATA USED IN REFINEMENT.
  RESOLUTION RANGE HIGH (ANGSTROMS) :   1.07
  RESOLUTION RANGE LOW  (ANGSTROMS) :  50.32
  DATA CUTOFF             (SIGMA(F)) : NONE
  COMPLETENESS FOR RANGE        (%) :  97.20
  NUMBER OF REFLECTIONS             : 132700

 FIT TO DATA USED IN REFINEMENT.
  CROSS-VALIDATION METHOD       : THROUGHOUT
  FREE R VALUE TEST SET SELECTION : RANDOM
  R VALUE      (WORKING + TEST SET) :  0.14639
  R VALUE          (WORKING SET) :  0.14544
  FREE R VALUE                   :  0.16403
  FREE R VALUE TEST SET SIZE   (%) :  5.1
  FREE R VALUE TEST SET COUNT      :  7125

 NUMBER OF NON-HYDROGEN ATOMS USED IN REFINEMENT.
  ALL ATOMS            :    4028

 B VALUES.
  FROM WILSON PLOT           (A**2) : NULL
  MEAN B VALUE      (OVERALL, A**2) :  8.666

 ESTIMATED OVERALL COORDINATE ERROR.
  ESU BASED ON R VALUE                            (A):  0.030
  ESU BASED ON FREE R VALUE                       (A):  0.031
  ESU BASED ON MAXIMUM LIKELIHOOD                 (A):  0.029
  ESU FOR B VALUES BASED ON MAXIMUM LIKELIHOOD (A**2):  0.574

CORRELATION COEFFICIENTS.
  CORRELATION COEFFICIENT FO-FC      :  0.977
  CORRELATION COEFFICIENT FO-FC FREE :  0.971

 RMS DEVIATIONS FROM IDEAL VALUES          COUNT    RMS     WEIGHT
  BOND LENGTHS REFINED ATOMS         (A):  3306 ; 0.014 ; 0.021
  BOND LENGTHS OTHERS                (A):  2808 ; 0.001 ; 0.020
  BOND ANGLES REFINED ATOMS    (DEGREES):  4499 ; 1.570 ; 1.929
  BOND ANGLES OTHERS           (DEGREES):  6568 ; 0.768 ; 3.000
  TORSION ANGLES, PERIOD 1     (DEGREES):   410 ; 5.137 ; 3.000
  TORSION ANGLES, PERIOD 3     (DEGREES):   513 ;16.788 ;15.000
  CHIRAL-CENTER RESTRAINTS       (A**3):   478 ; 0.099 ; 0.200
  GENERAL PLANES REFINED ATOMS       (A):  3742 ; 0.008 ; 0.020
  GENERAL PLANES OTHERS              (A):   670 ; 0.002 ; 0.020
  NON-BONDED CONTACTS REFINED ATOMS  (A):   799 ; 0.380 ; 0.300
  NON-BONDED CONTACTS OTHERS         (A):  2802 ; 0.222 ; 0.300
  H-BOND (X...Y) REFINED ATOMS       (A):   614 ; 0.258 ; 0.500
  H-BOND (X...Y) OTHERS              (A):     1 ; 0.244 ; 0.500
  SYMMETRY VDW REFINED ATOMS         (A):    20 ; 0.367 ; 0.300
  SYMMETRY VDW OTHERS                (A):   101 ; 0.351 ; 0.300
  SYMMETRY H-BOND REFINED ATOMS      (A):    79 ; 0.255 ; 0.500

 ISOTROPIC THERMAL FACTOR RESTRAINTS.      COUNT    RMS     WEIGHT
  MAIN-CHAIN BOND REFINED ATOMS  (A**2):  2040 ; 0.799 ; 1.500
  MAIN-CHAIN ANGLE REFINED ATOMS (A**2):  3257 ; 1.377 ; 2.000
  SIDE-CHAIN BOND REFINED ATOMS  (A**2):  1266 ; 1.873 ; 3.000
  SIDE-CHAIN ANGLE REFINED ATOMS (A**2):  1242 ; 2.710 ; 4.500

 NCS RESTRAINTS STATISTICS
  NUMBER OF NCS GROUPS : NULL



 TLS DETAILS
  NUMBER OF TLS GROUPS  : NULL


 BULK SOLVENT MODELLING.
  METHOD USED : BABINET MODEL WITH MASK
  PARAMETERS FOR MASK CALCULATION
  VDW PROBE RADIUS   :  1.40
  ION PROBE RADIUS   :  0.80
  SHRINKAGE RADIUS   :  0.80

 OTHER REFINEMENT REMARKS:
 HYDROGENS HAVE BEEN ADDED IN THE RIDING POSITIONS

CISPEP     ALA A   76    PHE A   77                0.00
CISPEP     GLU A  204    TYR A  205                0.00
CISPEP     TRP A  433    GLU A  434                0.00
SSBOND     CYS A   81    CYS A  101
SSBOND     CYS A  345    CYS A  356
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