Rapid protein crystal structure determination using third-generation synchrotron sources

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Recent developments at third-generation synchrotron sources bring a promise to revolutionize protein structure determination. These instruments provide well-collimated, very bright, stable, and tunable x-ray sources. Synchrotron radiation from the Structural Biology Center's (SBC) undulator beamline was used to collect a multiwavelength anomalous diffraction (MAD) data at 100 K. The four-wavelength experiment was completed to 2.25 Å resolution in 23 minutes on a single crystal of 16 kDa protein containing three SeMet residues. The SeMet-labeled protein from a thermophilic bacteria was produced in the *E. coli* over-expression system that was supplemented with SeMet under conditions known to suppress *E. coli*'s methionine biosynthesis. The result was a 93% incorporation of SeMet into the protein. The protein was purified in two steps and it crystallized readily in the orthorhombic space group $C222_1$ with unit cell parameters a = 62.6 Å, b = 64.8 Å, c = 74.3 Å, and $\alpha = \beta = \gamma = 90^o$. At each wavelength in a single pass, 60 2^o oscillation images were collected in 345 seconds using a mosaic 3 x 3 CCD detector. Data were processed with HKL2000. Two of the three selenium sites were located automatically using the program RSPS in CCP4 suite. These sites were refined with the MLPHARE, which gave an overall FOM of 0.72 for data between 10–2.25 Å. The overall map correlation coefficient of the solvent-flattened map produced with the DM and the map calculated from coordinates of the refined model is 0.82. Further evidence of the high quality of the data was obtained with the program wARP, which was used first to extend phases to 1.7 Å and then automatically trace a complete main chain of the protein with polyalanine/serine. This ultrarapid MAD data collection has recently been successfully executed at the SBC undulator beamline for two other larger protein structures. The high photon brilliance, very fast CCD detector, and newly designed efficient data reduction software combined with a multi-cpu computing environment allows for a new approach to synchrotron data collection. The complete MAD experiment (including data acquisition, processing, and initial phase determination) may take less than a day. The approach provides a basis to revolutionize protein structure determination using x-ray crystallography at the synchrotron facilities.

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