139f Effect of Protein Crystal Size, Cooling Method and Soak Time in Cryoprotectant Solutions on Cryoprotection and X-Ray Data Quality

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Structure determination of proteins is accomplished by X-day diffraction techniques, from which biological function of the macromolecule can be elucidated. X-ray data collection for protein crystals is typically performed at cryogenic temperatures to mitigate radiation damage by ionizing X-rays. Most common method of data collection is using a cold nitrogen stream at a temperature of around 100 K. Protein crystals are composed of large fractions of liquid with wide channels (~ 10-100 Å) filled with the crystallizing solution. When using low temperatures for X-ray data collection, the solution in the protein crystal channels and mother liquor surrounding the crystal must be vitrified (transformed into an amorphous state). With crystalline ice formation, the solution within protein channels would expand, destroying the protein crystal lattice. Therefore protein crystals are ‘flash cooled', rapidly brought to cryogenic temperatures, in a cryogenic stream or by plunging in liquid nitrogen before data collection. ‘Cryoprotectants' such as glycerol are used to reduce cooling rates required to achieve vitrification. D-xylose isomerase crystals, with glycerol as cryoprotectant, were flash cooled in the cold nitrogen gas of an Oxford 700 Series Cryostream at 100 K and by plunging in liquid nitrogen. X-ray diffraction data were measured with a Saturn CCD on a Rigaku FR-E X-ray source, processed with Rigaku's CrystalClear 1.3.6 software and crystal quality was assessed at various glycerol concentrations. The minimum glycerol concentration required to successfully flash cool (as indicated by absence of ice) crystals of variable size was determined. The glycerol requirement was found to be a strong function of crystal size. This agrees with our conclusions of earlier studies using different size loops with glycerol added to Hampton Screen solutions [1]. Comparison of the results obtained with gas cooling with those obtained by plunging in liquid nitrogen suggests that liquid nitrogen does not give significant improvement in cooling rates as expected. This is most likely due to film boiling. In general, data quality of gas cooled crystals was better than that of liquid plunged crystals. Comparisons also were made using a ‘slush' of partially frozen nitrogen. Early experiments with nitrogen slush suggest faster cooling rates as compared to those obtained with liquid and gaseous nitrogen. Large crystals were soaked in glycerol solutions for different times to determine minimum soak time required for near complete diffusion of cryoprotectant solutions. This time can be estimated through a simple calculation of a ‘penetration' time. The soak time was found to have a significant effect on success of flash cooling and quality of diffraction data.

[1] Chinte, U., Shah, B., DeWitt, K., Kirschbaum, K., Pinkerton, A. A., and Schall, C., J. Appl. Cryst. **38** , 412-419 (2005).