Effect of Crystal Size and Cooling Method on Cryoprotection and Data Quality

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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 required to successfully flash cool 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 (J. Appl. Cryst., in press). Comparing 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.

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