

Synchrotron White-Beam X-Ray Topography of Ribonuclease S Crystals

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Beamline: X19C

Introduction: A number of x-ray topographic studies of protein crystals have appeared in the literature, mainly applied to crystals of tetragonal lysozyme. Protein crystals tend to suffer severe and rapid radiation damage from the intensity of a white synchrotron beam. Possibly this is the reason that monochromatic radiation has more often been used for their topography, although monochromatic synchrotron radiation topographs may be dominated by contours of orientation. In this study the optimum conditions for the white-beam topography of ribonuclease S crystals and their useful lifetime in the beam were determined.

Methods and Materials: Ribonuclease S crystals with a width of 1mm or smaller were received in sealed capillaries and mounted on X19-C's goniometer. A 1mm² white beam was filtered through a 2.7mm thick plate of aluminum and allowed to fall on the samples. Laue patterns were recorded for crystal alignment on 5x7" sheets of medical x-ray film in 15sec exposures placed perpendicular to the incident beam at a specimen-to-film distance of 20cm. Topographs were recorded on 8x10" sheets of Kodak Industrex SR-1 film in 180sec exposures. The films were protected from radiation scattered from the incident beam's passage through air by a lead plate with a 4mm wide hole mounted upstream from the sample. Low Bragg angles (ca. 0.5°) required a 3mm wide square of lead to be taped to the film cassette as a beam stop.

Results: Fig. 1 shows the quality of topographs that may be obtained with this level of orientation control. They show some of the characteristics that have been reported in the literature for lysozyme crystals: a featureless bulk in the case of Fig. 1(a) and (c) [1], and sectorial contrast in Fig. 1(b) [2]. Among the features from the literature of lysozyme that were not observed in these crystals were gross asterism and misorientation across subgrain boundaries [3], and the presence of a macromosaic structure whose individual mosaic blocks are resolvable by x-ray topography [4]. In a few of the RNase S crystals, as in Fig. 1(d), dark contrast surrounded the point of crystal nucleation, indicating the presence of strain in that part of crystal formed during early stages of growth. While the quality of the crystals varied somewhat, all showed an outline faithful to the crystal's shape, possibly including the shadow of an edge between two crystal facets, but otherwise with little topographic detail. A uniform topograph, free of dark contrast indicating stress, is usually considered indicative of a protein crystal with a low mosaicity.

Conclusions: With appropriate filtration of the white beam, careful attention to background noise reduction and crystal alignment using fast film, indexed synchrotron white-beam topographs of protein crystals could be recorded with definition and contrast equal to that of monochromatic-beam topographs reported in the literature; without damaging the samples appreciably during the experiment.

References:

- [1]. I. Dobrianov, K. D. Finkelstein, S. G. Lemay and R. E. Thorne, "X-ray Topographic Studies of Protein Crystal Perfection and Growth," *Acta Cryst.*, **D54**, 922, 1998.
- [2]. O. Vidal, M. C. Robert, B. Arnoux and B. Capelle, "Crystalline Quality of Lysozyme Crystals Grown in Agarose and Silica Gels Studied by X-ray Diffraction Techniques," *J. Cryst. Growth*, **196**, 559, 1999.
- [3]. K. Izumi, S. Sawamura and M. Ataka, "X-ray Topography of Lysozyme Crystals," *J. Cryst. Growth*, **168**, 106, 1996.
- [4]. Z. W. Hu, B. R. Thomas and A. A. Chernov, "Laboratory Multiple-Crystal X-ray Topography and Reciprocal-Space Mapping of Protein Crystals: Influence of Impurities on Crystal Perfection," *Acta Cryst.*, **D57**, 840, 2001.

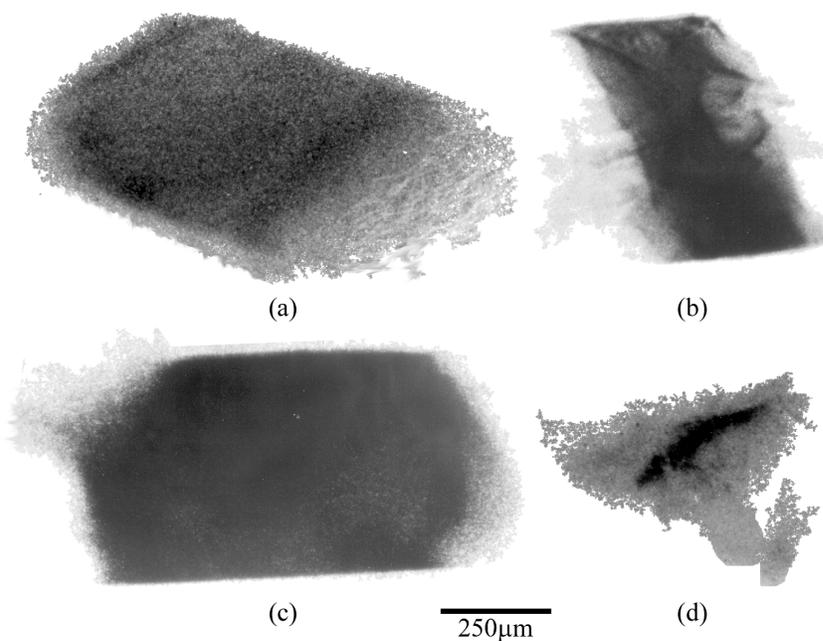


Figure 1. Synchrotron white-beam x-ray topographs of RNase S crystals. $g = 10\bar{1}1$, $\lambda = 0.63 \text{ \AA}$.