Short Communications

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Minimizing thermal gradient problems during precession photography of protein crystals. By C. Thaxton and E. Lattman, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154, USA

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An air-stream cooling device and a new crystal mounting technique minimize thermal-gradient problems during precession photography of proteins.

Many investigators cool protein crystals during X-ray diffraction studies. The appropriate method depends upon the camera and X-ray generator being used, and upon the specimen itself. Some workers place the diffraction unit in a cold room, an arrangement which complicates camera alignment and X-ray machine maintenance. Rotating-anode X-ray generators run less reliably in the cold. The most common alternative, dating from Kaufman & Fankuchen (1949), is to blow a stream of cold air at the crystal. With this method of cooling one often encounters convection within the capillaries in which protein crystals are usually mounted.

In experiments where the capillaries are filled with mother liquor (as with tropomyosin crystals, see below), currents develop that dislodge the crystal from the capillary wall. In the usual semi-dry mounting, distillation may cause the crystal to dry out or dissolve (Finch & Klug, 1959; Petsko, 1975).

To minimize convection, a laminar air flow is usually directed coaxially with the capillary, which is kept as short as possible. This procedure provides uniform cooling and thus avoids thermal differentials which are responsible for convection across and along the capillary. The geometry of precession-camera motion, however, makes uniform cooling difficult when the capillary is not coaxial with the dial rotation axis, since then the capillary will precess about the air-stream axis. In addition to allowing transverse gradients, the motion may obtrude the capillary through the cold isothermal zone in front of the delivery nozzle into warmer regions of the air stream. This latter problem is most serious with conventional coolers which have small diameter (~ 1 cm) nozzles, even when the capillary is short. Distillation is not so difficult a problem on an oscillating camera, where only small deviations from collinearity occur during any one photograph, or on a four-circle diffractometer.

Crystals of the muscle protein tropomyosin are 95°, water by weight and are therefore very fragile (Cohen, Caspar, Parry & Lucas, 1971). They are grown in quartz capillaries at 4 °C and then mounted on the precession camera for study. The crystals must be kept cool while on the camera in such a way that they are not dislodged from the capillary wall, either by convection currents or other forces. We have developed a precession-camera cooler that minimizes these currents by producing a large, laminar flow of cold air. Much lower temperatures could be achieved by using gas from a liquid cryogen generator, but system components would have to be suitably adapted. Details of a technique similar to ours for generating and conditioning air have been given by Marsh & Petsko (1973). The novel

Table 1. Cross-sectional diameters of isothermal cooling zones

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<th>Cooling Cone No.</th>
<th>Cross-Sectional Diameter in mm at Each Distance in cm from Nozzle Exit</th>
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Fig. 1. Air delivery nozzle of Plexiglass construction. (A) Two concentric cylinders (4.5 and 6 cm inside diameters) with 2 mm wall and 16 cm long. (B) Annular cavity filled with polyurethane insulating foam to bulk density of 2 lb ft⁻³ (~ 0.032 g cm⁻³). (C) Ring plugs. (D) Air intake manifold fitted with polyvinylchloride hose connector, 3 8 inch (~9 mm) inside diameter. (E) Thermistor monitor probe for digital thermometer. (F) Four brass screens, two in front of the probe and two behind which are 100 mesh (1600 openings cm⁻²). The number and position of the screens affect system performance significantly, and we have determined these by trial. Each brass screen is rotated a few degrees relative to the previous one to further widen the laminar core. Air velocity: 400 cm s⁻¹. Order of magnitude screen Reynolds number is 100, based on a kinematic viscosity coefficient for air of 0.1 cm² s⁻¹. Unobstructed path from F to exit (~ 10 cm) allows turbulence to dissipate after air is chopped finely by screens.

Fig. 2. Isothermal cooling cones. Zones with temperature differentials of 0.2, 0.5 and 1°C are marked respectively as cones 1, 2 and 3. Cones were determined by yz⁻mapping of the volume in front of the nozzle with a thermistor head sensor for a digital thermometer with 0.1°C accuracy. The precession motion does not significantly affect the cone.
features of the new cooler are the use of higher flow rates and the design of the air delivery nozzle. The flow is 14.5 ft³ min⁻¹ at 70 p.s.i. and at 4°C through a large diameter (4.5 cm) nozzle with four screens (Young, 1966; Frenz et al., 1969) inset to ensure laminar flow. The cooling cone from this nozzle envelops the specimen capillary even during the complicated motion of a precession camera. The stream is sufficiently large and isothermal that it can make angles of at least 30° with the axis of a capillary 4 cm long without crystals being dislodged. Fig. 3 shows the delivery nozzle of the cooler mounted on the precession camera.

Protein crystals are not usually photographed submerged in mother liquor. In the conventional mounting a crystal is held to the wall of the capillary by a thin film of liquid, and a drop of mother liquor is placed elsewhere in the capillary to stabilize the humidity. When one must cool a crystal mounted in this way, distillation within the capillary is often encountered and crystals either dry out or dissolve. We have found this occurs more frequently when the mother liquor has low ionic strength.

A simple way of eliminating this problem in many cases is to place the crystal as close as practical to the sealed end of the capillary, and to fill the free volume below it with mineral oil. Cylindrical sections of capillary sealed with epoxy resin to form a flat bottom are more suitable, because of absorption problems, than the usual flame-sealed, tapered capillaries. The very small volume in which the crystal is placed restricts the distillation, and the exposed end of the capillary promotes uniform cooling. We have found that our large-flow cooler is preferable to conventional coolers in maintaining uniform cooling of specimens when quite large excursions from collinearity are encountered on precession.

When it is necessary to keep the crystal more centrally located in the capillary, distillation is more probable. To minimize distillation here the free volume of the capillary above and below the crystal can be filled with mineral oil. Further, the entire capillary can be shielded from the air stream by surrounding it with a thin, closed cylindrical sheath of mylar or quartz. Immersing the sheath in the cooling cone thus simulates cold-room environment for the capillary. By suitably adapting a goniometer head the sheath can be made to rotate, which further minimizes distillation by minimizing the crosswise differential in temperature. Thus use of the large-flow cooler with either the stationary or rotating sheath also relaxes the usual requirement of coaxial air delivery.

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References