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10.2. Cryocrystallography techniques and devices

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10.2.1. Introduction

The ability to collect X-ray data from macromolecular crystals at cryogenic temperatures has played a key role in the more widespread and effective use of crystallography as a tool for biological research. Radiation-damage rates are greatly reduced at low temperature, often by orders of magnitude, making it possible to work with crystals that would otherwise diffract too weakly or decay too quickly. In particular, this radiation protection is essential for the full use of increasingly powerful synchrotron-radiation sources, and the coupling of the two permits the investigation of ever larger macromolecular complexes and the collection of higher-resolution data from nearly all samples. Cryogenic data collection has also allowed efficient experimental phasing using multiple-wavelength methods, as well as preservation of unstable samples and trapping of transient enzyme intermediates, an area that will undoubtedly continue to gain in importance.

In this chapter, practical aspects of cryocrystallography are discussed, with an emphasis on techniques and devices for crystal preparation and handling. A number of prior reviews covering these and other aspects of macromolecular cryocrystallography are available (Hope, 1988, 1990; Watenpaugh, 1991; Rodgers, 1994, 1997; Abdel-Meguid *et al.*, 1996; Garman & Schneider, 1997; Parkin & Hope, 1998). Radiation-damage protection at low temperature, which is not well understood, has also been discussed (Henderson, 1990; Gonzalez & Nave, 1994; Rodgers, 1996; Garman & Schneider, 1997), and the principles and operation of cryostats for sustained cooling during data collection are described elsewhere (Rudman, 1976; Hope, 1990; Garman & Schneider, 1997).

10.2.2. Crystal preparation

Macromolecular crystals are intimately associated with bulk aqueous solution. It surrounds them and penetrates them as solvent-filled channels, which typically account for 30–80% of the crystal volume. A key goal, therefore, of any procedure for cooling these samples to cryogenic temperatures is to prevent the formation of hexagonal crystalline ice. Ice formation, because of the associated increase in specific volume, inevitably disrupts the order of the macromolecular crystals and renders them useless for data collection. The principle that underlies current methods is that sufficiently rapid cooling causes the formation of a rigid glass before ice nucleation can occur. The high viscosity of the glass then prevents subsequent rearrangement into an ordered lattice. Early attempts to flash cool macromolecular crystals were made by Low *et al.* (1966), Haas (1968), and Haas & Rossmann (1970).

With samples as large as macromolecular crystals, however, it is not possible to achieve the high cooling rates necessary to prevent ice formation in water and most aqueous crystallization solutions. The most general method for overcoming this problem is to equilibrate the crystal with a solution containing a cryoprotective agent that slows ice nucleation and allows the formation of a glassy solid with attainable cooling rates. A list of cryoprotectants used successfully with macromolecular crystals is shown in Table 10.2.2.1. Typically, these cryoprotectants are included in the established stabilization or harvest solution at concentrations that range from 6–50%. Glycerol is frequently chosen for initial trials and appears to be a widely applicable cryoprotectant for both salt and organic precipitants. Concentrations of glycerol necessary to prevent ice formation in a number of typical crystallization solutions have been tabulated (Garman & Mitchell, 1996). Other compounds such as ethylene glycol and small sugars fall into this same class. Crystallization precipitants such as 2-methyl-2,4-pentanediol (MPD) or polyethylene glycol (PEG) can often simply be increased in concentration to provide sufficient cryoprotection. Ethanol, methanol and MPD are useful in relatively low-salt conditions. The listed stereoisomer of butanediol is a particularly effective cryoprotectant and can be used where other components of the solution, such as high salt, may limit the amount of cryoprotectant that can be added. Limitations owing to salt in the crystallization mix can also be overcome by transferring the crystals to a solution containing an organic precipitant before or during the introduction of the cryoprotectant (Singh *et al.*, 1980; Ray *et al.*, 1991; Wierenga *et al.*, 1992). Combinations of cryoprotectants have been used where a single cryoprotectant alone did not permit successful flash cooling.

Table 10.2.2.1. *List of cryoprotectants used successfully for flash cooling macromolecular crystals*

See the text as well as Rodgers (1994, 1997), Abdel-Meguid *et al.* (1996), and Garman & Schneider (1997) for additional details.

(2*R*,3*R*)-(−)-Butane-2,3-diol	2-Methyl-2,4-pentanediol
Erythritol	Polyethylene glycol 400
Ethanol	Polyethylene glycol 1000–10 000
Ethylene glycol	Propylene glycol
Glucose	Sucrose
Glycerol	Xylitol
Methanol	

It is rare that a crystal can be transferred without damage directly to a solution containing full-strength cryosolvent. Usually, the cryoprotectant must be introduced slowly to reduce stress on the crystal lattice. Methods of introducing cryoprotectant-containing solutions are listed in Table 10.2.2.2. Techniques such as serial transfer through increasing cryoprotectant concentrations or dialysis are preferred. They allow the crystal to equilibrate with the cryosolvent, leading to reproducible crystal quality and unit-cell dimensions after flash cooling. Also, with equilibrium methods, the solution conditions can be altered in an attempt to control any crystal damage associated with the flash-cooling process itself. The best scheme for serial transfer must be determined empirically. Equilibration time at each step depends on a number of factors (size of the crystals, solvent content, viscosity of the solution) but can be as rapid as less than a minute for small cryoprotectants or as long as hours for large polymers (Bishop & Richards, 1968; Fink & Petsko, 1981; Ray *et al.*, 1991). Typically, 5% increments in cryoprotectant concentration with equilibration times of 15 min or longer at each step are used in initial trials. The step size is then decreased if damage occurs. Dialysis can be done conveniently in the small buttons used for crystallization. These are available with chamber

Table 10.2.2.2. *Methods for introducing the cryoprotectants needed for flash cooling*

(1) Serial transfer into increasing strengths of cryoprotectant
(2) Dialysis
(3) Growth in cryoprotectant
(4) Brief transfer before flash cooling
(5) Direct transfer into full-strength cryoprotectant

sizes as low as 5 µl and allow a piece of dialysis membrane to be stretched and held securely over the opening. Dialysis times range from 1 to 24 h depending on the size of the cryoprotectant and the viscosity of the solution.

Another technique, growth of the crystals directly in cryoprotectant solutions, is particularly convenient and effective. In some cases, the primary precipitant, MPD for example, may provide cryoprotection if the concentration used in crystallization is sufficiently high. More commonly, however, additives such as glycerol are included in the crystallization buffer. An advantage of this technique is that crystals can be mounted directly from the crystallization drop, eliminating potential damage in transferring to a harvest or cryoprotective solution.

When it is not possible to identify a cryosolvent compatible with the crystals, a brief exposure to the cryoprotective solution may allow successful flash cooling. Apparently, the water in crystal solvent channels is constrained sufficiently to prevent nucleation, and simply exchanging the external aqueous solution with cryosolvent provides protection. The 'quick dunk' in the cryosolvent may be as short as a few seconds, and for some crystals it is possible to combine this technique with prior equilibration in lower, non-damaging concentrations of cryoprotectant. The same principle of preventing ice formation in the external solution forms the basis of an alternative technique developed by Hope (1988). Here, the external solution is replaced by a hydrocarbon oil before flash cooling.

Finding suitable cryoprotection conditions is a trial-and-error process. Two problems must be overcome: the cryoprotectant must be introduced without significant damage to the crystal, and damage during the flash-cooling process must be minimized. A scheme for systematically determining conditions for flash cooling is given in Fig. 10.2.2.1. In order to assess the effect of subsequent manipulations, it is important first to establish the resolution and rocking curve of the crystals under normal harvest conditions. Then one or a few cryoprotectants can be added to the harvest solution under conditions that allow equilibration with the crystal. The minimum concentration of cryoprotectant necessary to prevent ice formation can be determined by flash cooling candidate solutions using the loop-mounting technique described in Section 10.2.3. A sufficient concentration of cryoprotectant will result in a transparent glass upon cooling, while too low a concentration will produce opaque microcrystalline ice. A solution of cryoprotectant 2–3% above this minimum value should be used to allow for the added volume and therefore slower cooling when the crystal is present. If the crystals crack or dissolve in a cryosolvent, then the cryoprotectant should be introduced more slowly, the solution conditions (precipitant concentration, ionic strength, pH) altered, or the cryoprotectant eliminated from consideration.

The diffraction quality of crystals that show no visible sign of damage should be assessed at the crystal-growth temperature, and solution conditions should be altered if there has been a significant loss in resolution or an increase in rocking-curve width. For crystals that are incompatible with a wide range of cryosolvent conditions, quick-dunk and oil-coating techniques should be considered. Limited cross-linking (with glutaraldehyde, for example) can sometimes stabilize crystals for the introduction of cryoprotectant or improve stability during flash cooling.

When conditions that result in little or no damage have been identified, the crystals should be flash cooled and the diffraction assessed again. The formation of even small amounts of microcrystalline ice can be detected after flash cooling as characteristic powder rings at low-order spacings of 3.90, 3.67, 3.44 Å. If ice forms, a greater concentration of cryoprotectant must be used. An increase in the rocking-curve width of the crystal at this stage is common, probably due to the thermal stress on the lattice or changes in solution properties on cooling. If this increase is more than 50%, or if any loss of resolution occurs, solution conditions should be altered and the process repeated. The concentration of the cryoprotectant can be increased and different cryoprotectants tested. Other solution parameters, as noted above, can also be adjusted in an attempt to decrease the damage from flash cooling. In addition, different flash-cooling techniques (discussed below) can be tested to determine whether they produce less damage. Suitable cryosolvent conditions are usually established after a few trials, and even in difficult cases it has generally proven possible to find acceptable conditions by continuing to refine solution parameters.

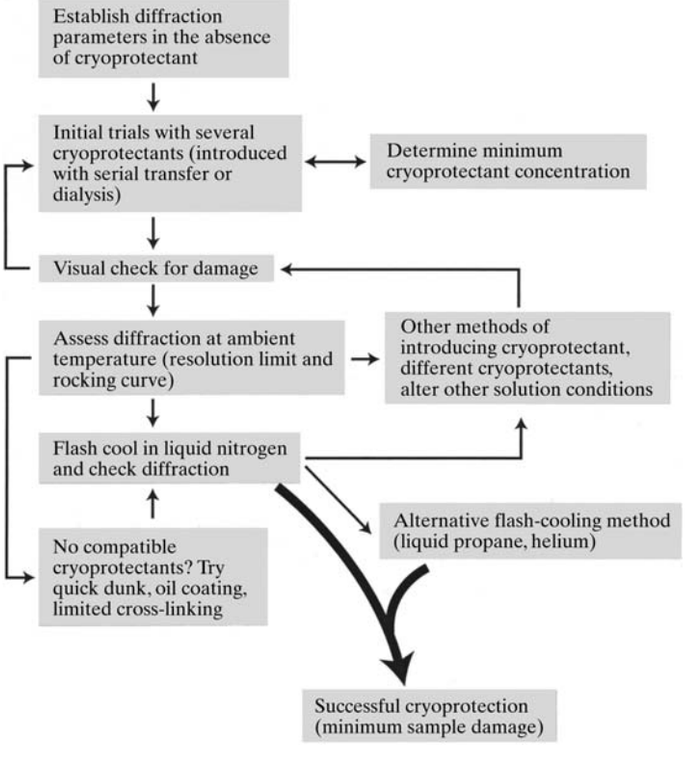

Fig. 10.2.2.1. Recommended pathway for optimizing cryoprotectant conditions and flash cooling.

10.2.3. Crystal mounting

A mounting technique suitable for flash cooling should allow for rapid heat exchange by providing a large surface area and a minimum of extraneous material that must be cooled. The technique should also subject the crystals to little mechanical stress and should result in a relatively compact sample that can be immersed in the narrow gas stream used to maintain the temperature during data collection. The glass capillary tubes conventionally used to mount macromolecular crystals are not well suited to flash-cooling procedures since they insulate the sample, reducing cooling rates, and their bulk interferes with cryogenic equipment. A number of alternative mounting methods used for flash cooling are shown in Fig. 10.2.3.1. Crystals can be affixed directly to thin glass fibres with cement or grease (Haas & Rossmann, 1970; Dewan & Tilton, 1987), or they can be scooped up on thin glass spatulas, a procedure first used in conjunction with the oil-coating method described in Section 10.2.2 (Hope, 1988). A loop-mounting technique introduced by Teng (1990) has proven the most generally applicable, however, and has become the method of choice. Here, the crystal is held suspended in a thin film of cryosolvent formed in a small loop.

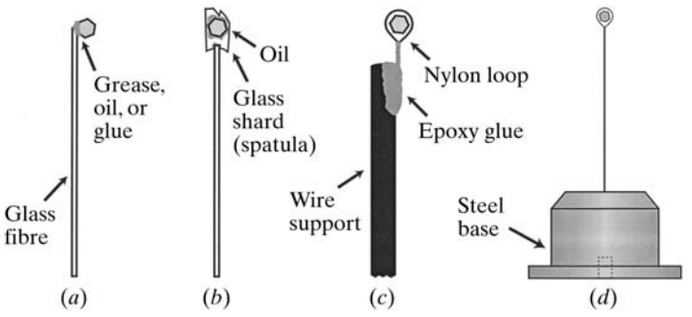

Fig. 10.2.3.1. Different crystal mounts for flash cooling and cryogenic data collection. (*a*) Crystal mounted on a thin glass fibre with adhesive, grease, or oil. (*b*) Crystal placed in a hydrocarbon oil and then scooped onto a thin glass shard. (*c*) Crystal suspended in a film of aqueous solution within a nylon loop. The loop is attached to a thin (∼0.25 mm diameter) wire support. (*d*) A diagram of the entire loop-mount assembly. The base is made of plain steel or a magnetic alloy and has two holes, one for the wire post and one for a locating pin, which reproducibly positions the assembly on the goniometer.

The technique is quick and straightforward, remarkably gentle to the crystal, and provides a large surface area for cooling.

The loops are generally formed from nylon fibre, although glass wool is useful for larger versions because its rigidity keeps them from collapsing under the surface tension of the suspended film. Both types of fibres should have a diameter of approximately 10 µm. This small cross section reduces absorption and scattering from the material itself and also minimizes the thickness of the film in the loop. Several methods of making the loops have been described in detail (Rodgers, 1997; Garman & Schneider, 1997), and nylon loops of different sizes are available commercially. The loop is usually glued to a thin metal wire or other heat-conductive post. The ability to conduct heat rapidly is required to minimize ice formation at the point where the wire or post exits the cold gas stream of the cryostat, which occurs in some orientations of the loop assembly. This post is in turn attached to a steel base, which is used with the magnetic transfer system described below.

Crystals are placed in the loop as shown in Fig. 10.2.3.2. They can be mounted directly from the crystallization drop or after harvesting into any convenient container. Under a stereomicroscope, the crystal is teased to the surface of the solution, usually with the loop itself. Once at the surface, the crystal is carried through the interface by first resting it on the bottom of the loop and then moving the assembly vertically to pull it out of the solution. A practiced experimentalist can usually capture the crystal in the first few tries. The plane of the loop should be kept near the vertical to increase the chance of catching the crystal and to minimize the amount of liquid drawn up with it. An alternative technique is to use a small pipette to place the crystal and a drop of cryosolvent into the loop and then draw off the excess solution with filter paper. In either case, it can be difficult to form a film in the loop with solutions high in organic solvent due to the lack of surface tension. For these solutions, adding PEG up to a few per cent usually allows a stable film to form. Fig. 10.2.3.3 is a photograph of a crystal mounted in a nylon loop. If the diameter of the loop is chosen so that it just accommodates the crystal, mounting is easier and the amount of extra scattering material in the X-ray beam is reduced. Also, asymmetric crystals can then be oriented relative to the assembly by preforming the loop into the appropriate shape.

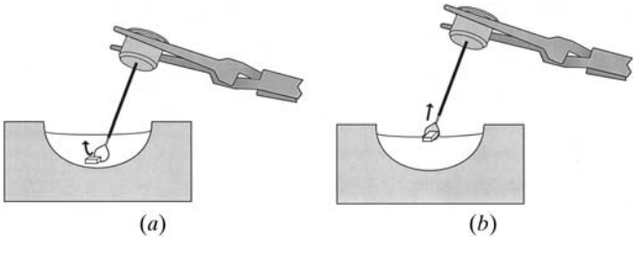

Fig. 10.2.3.2. Mounting a crystal in a loop. (*a*) While viewing with a stereomicroscope, the crystal is teased to the surface of the liquid using the loop. (*b*) It is then drawn through the interface and into the loop. The sizes of the loop and crystal have been exaggerated. Reproduced with permission from Rodgers (1997). Copyright (1997) Academic Press.

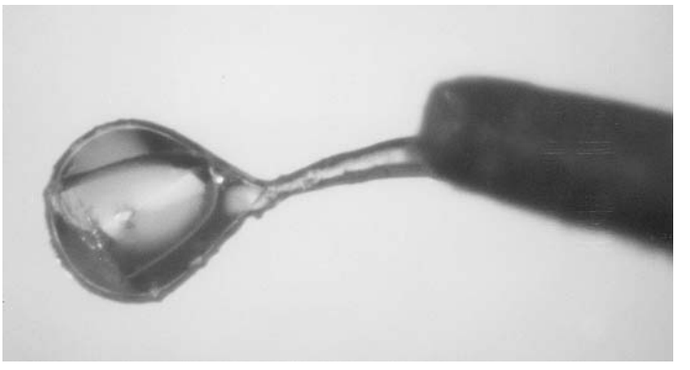

Fig. 10.2.3.3. Photograph of a flash-cooled crystal mounted in a nylon loop. The wire post holding the loop is visible on the right. Reprinted from Rodgers (1994) with permission from Elsevier Science.

The loop-mounting technique can also be used for data collection above cryogenic temperatures by sealing the loop and pin in a large diameter (3 mm) glass or quartz X-ray capillary (Fig. 10.2.3.4). A guard composed of stiff wax or a plastic plug cemented to the pin helps to guide the capillary over the sample before sealing it to the base with high vacuum grease or a cement low in volatile solvent. Loop mounting can be less damaging for many crystals than capillary mounting, and it results in a more uniform X-ray absorption surface.

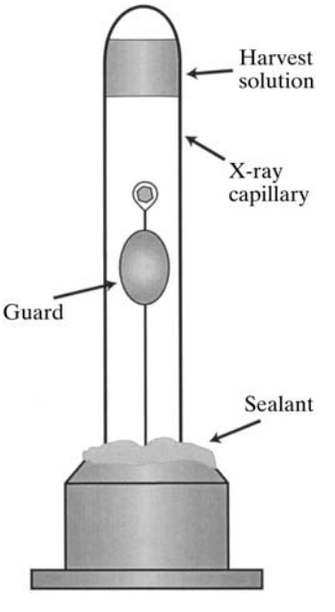

Fig. 10.2.3.4. Arrangement for using the loop-mounting technique at non-cryogenic temperatures.

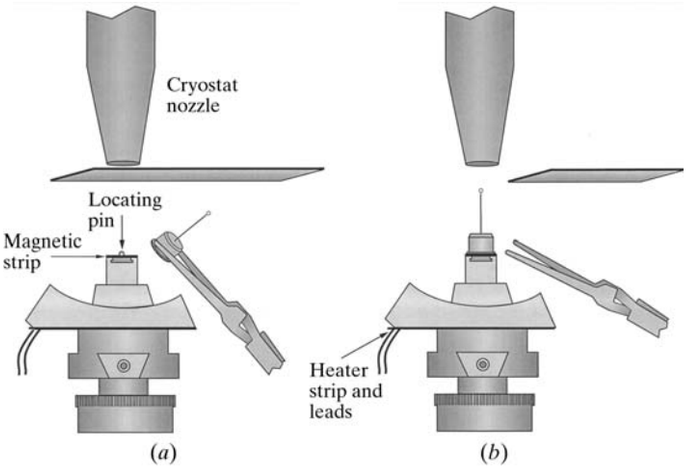

Fig. 10.2.4.1. Flash cooling a crystal in the cold gas stream from a cryostat. (*a*) The cold gas from the nozzle is blocked and the loop assembly placed on the goniometer. A locating pin on the goniometer ensures reproducible positioning of the loop assembly, which is held in place by a magnetic strip. (*b*) The gas stream is then unblocked to rapidly cool the crystal. A heating element in contact with the goniometer keeps it from icing during data collection. Based on a diagram by Rodgers (1997).

10.2.4. Flash cooling

Once mounted in the loop, the crystal must be cooled rapidly to prevent ice formation. A simple and often effective approach (see Hope, 1990; Teng, 1990) is to flash cool the sample in a cryostat gas stream (most frequently nitrogen, but also helium) right on the X-ray camera. This technique has the added advantage of leaving the crystal in position for immediate analysis and data collection. As shown in Fig. 10.2.4.1, the gas stream from the cryostat nozzle is temporarily deflected while the loop assembly is placed on the goniometer of the X-ray camera. The stream is then unblocked, allowing the cold gas to flow over the crystal. Deflecting the cold stream before placing the loop assembly eliminates the risk that the sample will cool slowly and form ice in the warmer outer layers of the gas stream. The arrangement of the cryostat nozzle shown in Fig. 10.2.4.1, with the gas stream coaxial to the loop assembly, is particularly effective. The cooling gas (usually at around 110 K for nitrogen cryostats) flows across both surfaces of the loop, maximizing the rate and evenness of cooling. Other orientations of the nozzle are frequently used, and in those cases the loop should be aligned with one edge pointing at the incoming gas. Note that a heating element, as shown in Fig. 10.2.4.1, is required to prevent icing of the goniometer with the nozzle in the coaxial position.

When handling the loop-mounted crystal before flash cooling, care must be taken to avoid drying the sample. The same characteristics that make the loop mount so effective for flash cooling, a large surface area and a small amount of surrounding solution, also promote a rapid loss of water and any other volatile component. The resulting change in solute concentration can damage the crystal or result in non-isomorphism between crystals. For this reason, every effort should be made to reduce the time required to flash cool the crystal after it is mounted. One key to avoiding delay when flash cooling in the cold stream is a rapid and reliable method of attaching the loop assembly to the goniometer. A magnetic mounting system (Fig. 10.2.4.1) developed by Rodgers (1994, 1997) is frequently used. Here, either a portion of flexible magnetic strip or solid magnet is affixed to the goniometer to hold the ferromagnetic base of the loop assembly. The base is positioned reproducibly by a small locating pin protruding from the goniometer, which mates with the centred hole in the loop base

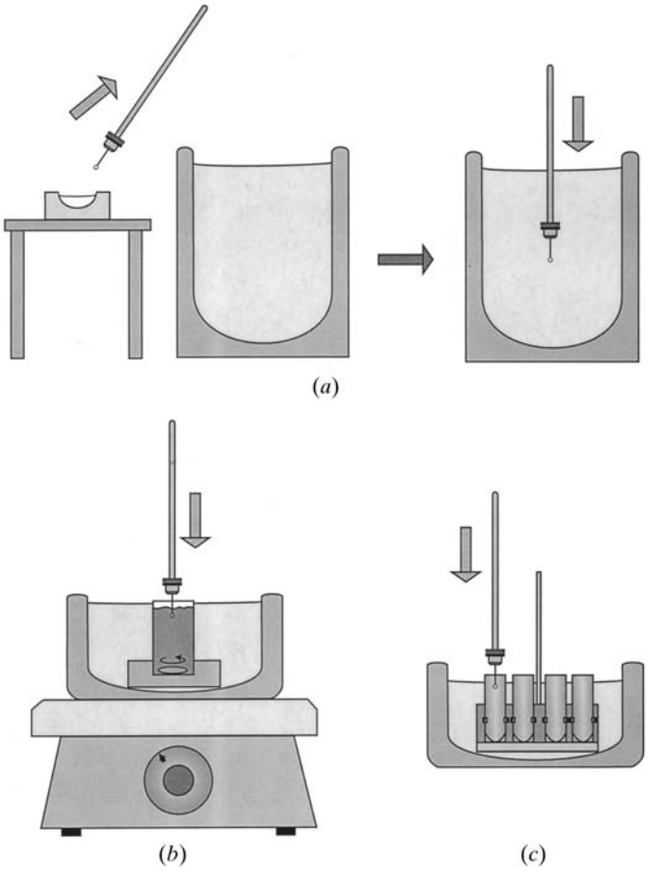

Fig. 10.2.4.2. Flash cooling in a liquid cryogen. (*a*) Cooling in liquid nitrogen. The loop assembly is attached, *via* a magnet mount, to a short rod and the crystal is captured. It is then quickly plunged into a nearby Dewar of liquid nitrogen. (*b*) One method of flash cooling in a liquid cryogen such as propane. The cryogen is placed in a weighted container, which itself stands in a Dewar of liquid nitrogen. The Dewar rests on a stir plate, which mixes the liquid cryogen to ensure a uniform temperature. When the temperature of the cryogen is just above its melting point, the loop assembly is plunged into the liquid. (*c*) A variation on cooling in propane or a similar cryogen. The cryogen is placed into small plastic vials designed for cryogenic storage. Just before the cryogen freezes, the loop assembly is plunged directly into a vial. A holder for the vials allows multiple samples to be prepared sequentially.

(Fig. 10.2.3.1*d*). A second pin or key can be used to specify the orientation of the loop assembly about its axis if necessary.

While flash cooling in the cold stream is convenient, an alternative method, rapidly plunging the crystal into a liquid cryogen, offers several advantages. This technique generally results in more even cooling of both sides of the loop-mounted sample, which may decrease damage due to thermal stress (Haas & Rossmann, 1970). It also reduces the time between mounting the crystal and flash cooling, and it can be used easily in any location – a cold room, for example. Another possible advantage of the liquid-cryogen method is that it produces a higher cooling rate than the cryostat gas stream, at least over much of the temperature range traversed during cooling (Walker *et al.*, 1998; Teng & Moffat, 1998). With increased cooling rates, the percentage of cryoprotectant necessary to prevent ice formation is lower, an advantage when benign cryoprotectant conditions prove difficult to find. Changes in solution dielectric or other parameters may also cause less damage. On the other hand, although cooling may be more even in a liquid cryogen, the overall increase in cooling rate could result in even greater thermal gradients, and therefore greater thermal stress,

across the crystal. Systematic studies are needed to assess the effect of cooling rate on the quality of flash-cooled crystals, but in practice the liquid-cryogen technique has proven effective and is widely used.

Common cryogens for flash cooling are liquid nitrogen, propane, and, to a lesser extent, ethane and some types of Freon. (Another potentially useful cryogen, liquid helium, has not yet been explored for flash cooling macromolecular crystals.) There is some disagreement about relative cooling rates in liquid nitrogen *versus* liquid propane for samples the size of loop-mounted crystals (Walker *et al.*, 1998; Teng & Moffat, 1998), but both cryogens are known to work well for flash cooling. Since liquid nitrogen is simpler to use and safer than propane, it should be considered for initial trials with a new type of crystal. A diagram showing flash cooling with liquid nitrogen is presented in Fig. 10.2.4.2(*a*). The crystal is captured in the loop and quickly plunged into a Dewar filled with liquid nitrogen. Attaching the loop assembly to a short rod equipped with a magnetic mount allows it to be plunged deeply into the liquid nitrogen, which may increase the cooling rate by preventing the build-up of insulating gas around the crystal. To minimize drying of the sample during transfer, the crystal container and the Dewar are located as close as possible. If necessary, drying can be further reduced by using a portable humidifier to add moisture in the area.

Other cryogens, such as propane, can be tested if results with liquid nitrogen are not satisfactory. Two methods for flash cooling in these other cryogens are illustrated in Figs. 10.2.4.2(*b*) and (*c*). In the first (Fig. 10.2.4.2*b*), the liquid cryogen is held in a small container with a weighted base, which is placed in a Dewar of liquid nitrogen to cool the cryogen. The cryogenic liquid is mixed using a magnetic stir bar to ensure a uniform temperature throughout the sample. Since the boiling points of these cryogens are well above their melting points, it is possible in the absence of stirring to have relatively warm, and therefore less effective, cryogen near the top of the container. When a temperature probe indicates that the cryogen is just above its melting point, the crystal is mounted and plunged quickly into the liquid. A variant of this technique (Fig. 10.2.4.2*c*) calls for plunging the loop assembly directly into cryogen-filled plastic vials, which are used for low-temperature transfer and storage of the crystals (see Section 10.2.5). The cryogen is then allowed to solidify around the crystal before it is placed on the X-ray camera or stored for later use. With this technique, it is more difficult to ensure that the temperature of the cryogen is uniform throughout the container. Other mechanisms for flash cooling in liquid cryogens have been described (Hope *et al.*, 1989; Abdel-Meguid *et al.*, 1996), and devices for combining xenon derivatization with flash cooling (Soltis *et al.*, 1997) are available commercially.

10.2.5. Transfer and storage

Crystals flash cooled in a liquid cryogen must be placed for data collection in the cold gas stream of a cryostat without any substantial warming. One common transfer method (Rodgers, 1994, 1997) is shown in Fig. 10.2.5.1. Once the loop assembly has been plunged into the Dewar of liquid nitrogen, it is inserted into a small plastic vial of the type normally used for cryogenic storage, ensuring that the sample remains below the liquid surface during the operation. There is then sufficient liquid nitrogen in the vial to keep the sample cold as it is transferred to the cryostat gas stream. Again, the magnetic mounting system is used to reduce the time required for transfer. For X-ray cameras with vertical spindles, as shown in Fig. 10.2.5.1, some means of pointing the magnetic mount downward is required to prevent the nitrogen from spilling out of the vial. The goniometer illustrated has a detachable arc extension (Engel *et al.*, 1996; Litt *et al.*, 1998) that provides this capability. When the loop assembly is attached to the magnet, the vial is quickly withdrawn, exposing the crystal to the gas stream. The arc slide can then be returned to the normal position and the arc extension removed.

Another device (Mancia *et al.*, 1995) for achieving the correct transfer geometry is shown in Fig. 10.2.5.2. This 'flipper' mechanism can be extended to permit transfer of the crystal. The device is then rotated about the hinge to reorient the loop assembly for data collection. The hinge is positioned so that rotation does not translate the crystal, keeping it in the cold stream during reorientation.

When cooling in other liquid cryogens such as propane, the same cryovial transfer system is used. Flash cooling in cryovials (Fig. 10.2.4.2*c*) permits direct transfer using the magnetic mounting system. Usually, the liquid cryogen has been solidified in the vial, and it is allowed to melt at least partially before placing the crystal on the goniometer. Any remaining solid then melts and drips away (although it is often necessary to remove the last drop on the crystal with filter paper). When cooling in a larger volume of cryogen (Fig. 10.2.4.2*b*), the crystal can be 'hopped' rapidly from the cooling cryogen to the surrounding vat of liquid nitrogen. A drop of cryogen transfers with the crystal, keeping it from warming. The loop assembly can then be placed in a cryovial and transferred to the goniometer.

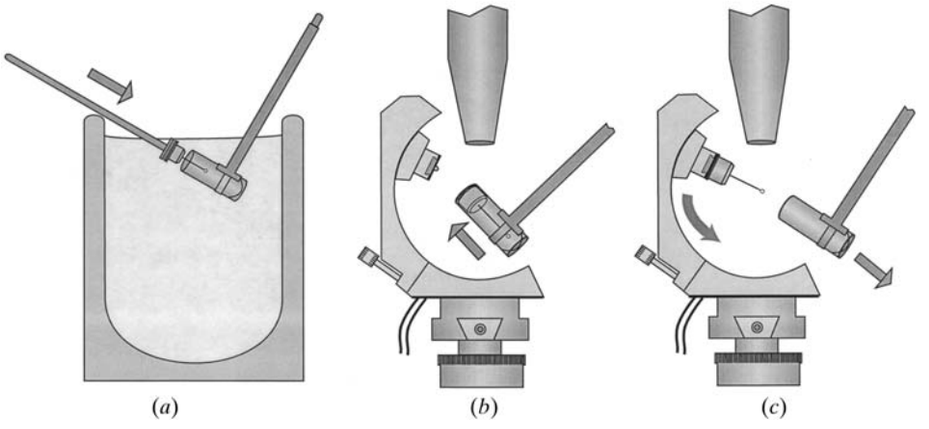

Fig. 10.2.5.1. Transfer of the flash-cooled crystal and loop assembly to a goniometer using a cryovial. (*a*) The loop assembly with a flash-frozen crystal is placed in the vial, which is held by a rod-shaped tool. The operation is carried out beneath the surface of the liquid nitrogen in a Dewar. (*b*) The loop assembly is transferred to the goniometer using the magnetic mounting system. (*c*) The vial is withdrawn, exposing the crystal to the cold gas stream. With this arrangement of goniometer and cryosystem nozzle, it is necessary to use a device that allows the magnetic mount to point downward. Here, a detachable arc extension provides this ability. After crystal transfer, the arc slide can be returned to the normal position and the extension removed.

Another device that does not use cryovials has been introduced (Parkin & Hope, 1998) to facilitate transfer from liquid nitrogen. The device consists of a split metal cup attached to handles that allow the cup to be opened and closed. When closed, the two halves of the metal cup form a cavity that can accommodate and grasp the loop assembly. As shown in Fig. 10.2.5.3, the loop assembly is inserted after first cooling the tongs in liquid

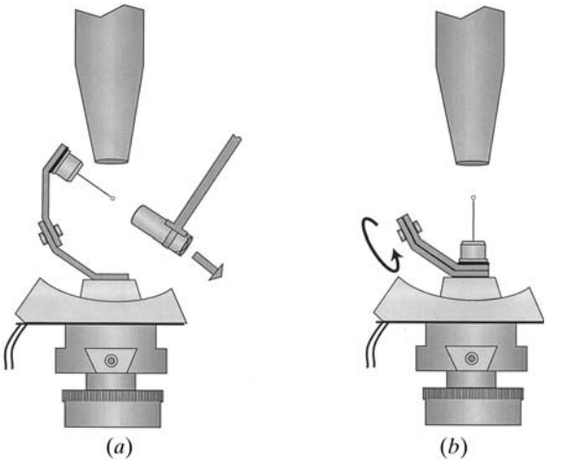

Fig. 10.2.5.2. Transfer using an alternative device for achieving the correct magnetic mount orientation. (*a*) A hinged mechanism is extended to orient the magnetic mount downward, and the loop assembly is attached. (*b*) The mechanism is rotated about the hinge to place the mount in the normal orientation for data collection.

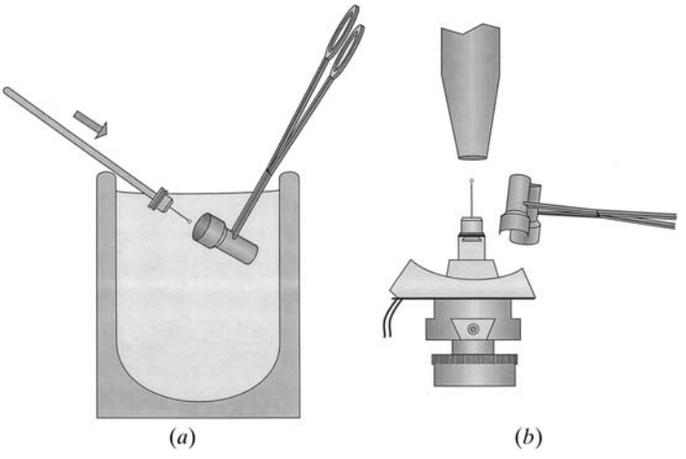

Fig. 10.2.5.3. Transfer using tongs. (*a*) The crystal is inserted into a split metal cup that allows the base to be held securely. (*b*) The tongs are inverted and used to place the loop assembly on the magnetic mount. The jaws of the tongs are then opened to separate the halves of the cup, and the tongs are withdrawn.

nitrogen. The thermal mass of the tongs prevents warming as the crystal is then placed on the goniometer. The tongs are opened and removed to expose the crystal to the gas stream.

Any of these transfer procedures can be reversed in order to return the loop assembly to liquid nitrogen without thawing the crystal. The assembly and cryovial can then be placed in a Dewar designed for long-term storage. Some opening should be present in the loop-assembly bases, or the cryovials should be notched, to allow free movement of liquid nitrogen. The vials are conveniently held and organized using aluminium canes, which take up to five samples and have tabs that hold the loop assemblies in place. For even more secure long-term storage, loop assemblies with threaded bases that screw into the cryovials are available.

The ability to store samples for long periods of time permits a number of crystals to be flash cooled under consistent conditions, which can be important for maintaining isomorphism, and crystals can also be stockpiled for later data collection at a synchrotron X-ray facility. In fact, crystals should be prescreened for quality in the laboratory before synchrotron data collection to make efficient use of time on the beam line. Finally, crystals that degrade in growth or harvest solutions, or that contain macromolecules in unstable or transient states, can be conveniently preserved by flash cooling and storage in liquid nitrogen.

References

10.1

Bald, W. B. (1984). *The relative efficiency of cryogenic fluids used in the rapid quench cooling of biological samples. J. Microsc.* **134**, 261–270.

Bellamy, H. D., Phizackerley, R. P., Soltis, S. M. & Hope, H. (1994). *An open-flow cryogenic cooler for single-crystal diffraction experiments. J. Appl. Cryst.* **27**, 967–970.

Douzou, P., Hui Bon Hoa, G. & Petsko, G. A. (1975). *Protein crystallography at sub-zero temperatures: lysozyme–substrate complexes in cooled mixed solutions. J. Mol. Biol.* **96**, 367–380.

Esnouf, R. M., Ren, J., Garman, E. F., Somers, D. O'N., Ross, C. K., Jones, E. Y., Stammers, D. K. & Stuart, D. I. (1998). *Continuous and discontinuous changes in the unit cell of HIV-1 reverse transcriptase crystals on dehydration. Acta Cryst.* D**54**, 938–953.

Fu, Z.-Q., Du Bois, G. C., Song, S. P., Harrison, R. W. & Weber, I. T. (1999). *Improving the diffraction quality of MTCP-1 crystals by post-crystallization soaking. Acta Cryst.* D**55**, 5–7.

Garman, E. F. & Mitchell, E. P. (1996). *Glycerol concentrations required for cryoprotection of 50 typical protein crystallization solutions. J. Appl. Cryst.* **29**, 584–587.

Götz, G., Mészáros, E. & Vali, G. (1991). *Atmospheric particles and nuclei*, p. 142. Budapest: Akadémiai Kiadó.

Hope, H. (1988). *Cryocrystallography of biological macromolecules: a generally applicable method. Acta Cryst.* B**44**, 22–26.

Hope, H. (1990). *Cryocrystallography of biological macromolecules at ultra-low temperature. Annu. Rev. Biophys. Biophys. Chem.* **19**, 107–126.

Hope, H., Frolow, F., von Böhlen, K., Makowski, I., Kratky, C., Halfon, Y., Danz, H., Webster, P., Bartels, K. S., Wittmann, H. G. & Yonath, A. (1989). *Cryocrystallography of ribosomal particles. Acta Cryst.* B**45**, 190–199.

Hui Bon Hoa, G. & Douzou, P. (1973). *Ionic strength and protein activity of supercooled solutions used in experiments with enzyme systems. J. Biol. Chem.* **248**, 4649–4654.

Mitchell, E. P. & Garman, E. F. (1994). *Flash freezing of protein crystals: investigation of mosaic spread and diffraction limit with variation of cryoprotectant concentration. J. Appl. Cryst.* **27**, 1070–1074.

Parkin, S. & Hope, H. (1998). *Macromolecular cryocrystallography: cooling, mounting, storage and transportation of crystals. J. Appl. Cryst.* **31**, 945–953.

Petsko, G. A. (1975). *Protein crystallography at sub-zero temperatures: cryoprotective mother liquors for protein crystals. J. Mol. Biol.* **96**, 381–392.

Post, B., Schwartz, R. S. & Fankuchen, I. (1951). *An improved device for X-ray diffraction studies at low temperatures. Rev. Sci. Instrum.* **22**, 218–220.

Ryan, K. P. (1992). *Cryofixation of tissues for electron microscopy: a review of plunge cooling methods. Scanning Microsc.* **6**, 715–743.

Schreuder, H. A., Groendijk, H., van der Lan, J. M. & Wierenga, R. K. (1988). *The transfer of protein crystals from their original mother liquor to a solution with a completely different precipitant. J. Appl. Cryst.* **21**, 426–429.

Travers, F. & Douzou, P. (1970). *Dielectric constants of alcoholic-water mixtures at low temperature. J. Phys. Chem.* **74**, 2243–2244.

10.1 (*cont.*)

Vali, G. (1995). In *Biological ice nucleation and its applications*, edited by R. E. Lee Jr, G. J. Warren & L. V. Gusta, pp. 1–28. St Paul: APS Press.

Walker, L. J., Moreno, P. O. & Hope, H. (1998). *Cryocrystallography: effect of cooling medium on sample cooling rate. J. Appl. Cryst.* **31**, 954–956.

10.2

Abdel-Meguid, S. S., Jeruzalmi, D. & Sanderson, M. R. (1996). *Crystallographic methods and protocols*, edited by C. Jones, B. Mulloy & M. R. Sanderson, pp. 55–87. New Jersey: Humana Press.

Bishop, W. H. & Richards, F. M. (1968). *Properties of liquids in small pores. Rates of diffusion of some solutes in cross-linked crystals of β-lactoglobin. J. Mol. Biol.* **38**, 315–328.

Dewan, J. C. & Tilton, R. F. (1987). *Greatly reduced radiation damage in ribonuclease crystals mounted on glass fibres. J. Appl. Cryst.* **20**, 130–132.

Engel, C., Wierenga, R. & Tucker, P. A. (1996). *A removable arc for mounting and recovering flash-cooled crystals. J. Appl. Cryst.* **29**, 208–210.

Fink, A. L. & Petsko, G. A. (1981). *X-ray cryoenzymology. Adv. Enzymol. Relat. Areas Mol. Biol.* **52**, 177–246.

Garman, E. F. & Mitchell, E. P. (1996). *Glycerol concentrations required for cryoprotection of 50 typical protein crystallization solutions. J. Appl. Cryst.* **29**, 584–587.

Garman, E. F. & Schneider, T. R. (1997). *Macromolecular cryocrystallography. J. Appl. Cryst.* **30**, 211–237.

Gonzalez, A. & Nave, C. (1994). *Radiation damage in protein crystals at low temperature. Acta Cryst*. D**50**, 874–877.

Haas, D. J. (1968). *X-ray studies on lysozyme crystals at −50 °C. Acta Cryst.* B**24**, 604.

Haas, D. J. & Rossmann, M. G. (1970). *Crystallographic studies on lactate dehydrogenase at −75 °C. Acta Cryst.* B**26**, 998–1004.

Henderson, R. (1990). *Cryoprotection of protein crystals against radiation damage in electron and X-ray diffraction. Proc. R. Soc. London Ser. B*, **241**, 6–8.

Hope, H. (1988). *Cryocrystallography of biological macromolecules: a generally applicable method. Acta Cryst.* B**44**, 22–26.

Hope, H. (1990). *Crystallography of biological macromolecules at ultra-low temperature. Annu. Rev. Biophys. Biophys. Chem.* **19**, 107–126.

Hope, H., Frolow, F., von Böhlen, K., Makowski, I., Kratky, C., Halfon, Y., Danz, H., Webster, P., Bartels, K. S., Wittmann, H. G. & Yonath, A. (1989). *Cryocrystallography of ribosomal particles. Acta Cryst.* B**45**, 190–199.

Litt, A., Arnez, J. G., Klaholz, B. P., Mitschler, A. & Moras, D. (1998). *A eucentric goniometer head sliding on an extended removable arc modified for use in cryocrystallography. J. Appl. Cryst.* **31**, 638–640.

Low, B. W., Chen, C. C. H., Berger, J. E., Singman, L. & Pletcher, J. F. (1966). *Studies of insulin crystals at low temperatures: effects on mosaic character and radiation sensitivity. Proc. Natl Acad. Sci. USA*, **56**, 1746–1750.

Mancia, F., Oubridge, C., Hellon, C., Woollard, T., Groves, J. & Nagai, K. (1995). *A novel device for the recovery of frozen crystals. J. Appl. Cryst.* **28**, 224–225.

Parkin, S. & Hope, H. (1998). *Macromolecular cryocrystallography: cooling, mounting, storage and transportation of crystals. J. Appl. Cryst.* **31**, 945–953.

Ray, W. J. Jr, Bolin, J. T., Puvathingal, J. M., Minor, W., Liu, U. & Muchmore, S. W. (1991). *Removal of salt from a salt-induced protein crystal without cross-linking. Preliminary examination of desalted crystals of phosphoglucomutase by X-ray crystallography at low temperature. Biochemistry*, **30**, 6866–6875.

Rodgers, D. W. (1994). *Cryocrystallography. Structure*, **2**, 1135–1140.

Rodgers, D. W. (1996). *Cryocrystallography of macromolecules. Synchrotron Radiat. News*, **9**, 4–11.

Rodgers, D. W. (1997). *Practical cryocrystallography. Methods Enzymol.* **276**, 183–203.

Rudman, R. (1976). *Low-temperature X-ray diffraction.* New York: Plenum Press.

Singh, T. P., Bode, W. & Huber, R. (1980). *Low-temperature protein crystallography. Effect on flexibility, temperature factor, mosaic spread, extension and diffuse scattering in two examples: bovine trypsinogen and Fc fragment. Acta Cryst.* B**36**, 621–627.

Soltis, S. M., Stowell, M. H. B., Wiener, M. C., Phillips, G. N. Jr & Rees, D. C. (1997). *Successful flash-cooling of xenon-derivatized myoglobin crystals. J. Appl. Cryst.* **30**, 190–194.

Teng, T.-Y. (1990). *Mounting of crystals for macromolecular crystallography in a free-standing thin film. J. Appl. Cryst.* **23**, 387–391.

Teng, T.-Y. & Moffat, K. (1998). *Cooling rates during flash cooling. J. Appl. Cryst.* **31**, 252–257.

Walker, L. J., Moreno, P. O. & Hope, H. (1998). *Cryocrystallography: effect of cooling medium on sample cooling rate. J. Appl. Cryst.* **31**, 954–956.

Watenpaugh, K. D. (1991). *Macromolecular crystallography at cryogenic temperatures. Curr. Opin. Struct. Biol.* **1**, 1012–1015.

Wierenga, R. K., Zeelen, J. Ph. & Nobel, M. E. M. (1992). *Crystal transfer experiments carried out with crystals of trypanosomal triosephosphate isomerase (TIM). J. Cryst. Growth*, **122**, 231–234.