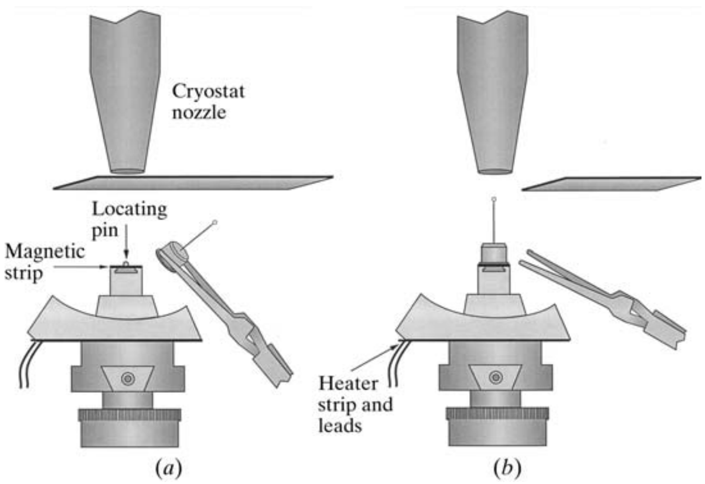

Figure 10.2.4.1
Flash cooling a crystal in the cold gas stream from a cryostat. (*a*) The cold gas from the nozzle is blocked and the loop assembly placed on the goniometer. A locating pin on the goniometer ensures reproducible positioning of the loop assembly, which is held in place by a magnetic strip. (*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).

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.

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.

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. 10.2.3.1*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

  references