5.1.1.4. *Packing of molecules in crystals*

Growth kinetics of the different faces should be correlated with the structural anisotropy of the intermolecular contacts. It has been found that a judicious mutation of a single surface residue of a protein can markedly affect its solubility and hence crystallizability. This method has been used with great success for crystallizing a retroviral integrase (Dyda *et al.*, 1994).

The relationship between crystal morphology and internal crystal structure was examined in the mid-1950s (Hartman & Perdok, 1955*a*,*b*,*c*). It was shown that the morphology of a crystal is determined by 'chains' of strong intermolecular interactions (hydrogen bonding, van der Waals contacts, molecular stacking) running through the entire crystal. For a crystal to grow in the direction of a strong interaction ('bond'), these bonds must form an uninterrupted chain through the structure, giving rise to the periodic bond chain theory. The stronger the interaction between molecules, the more likely the crystal is to be elongated in that direction. If a bond chain contains interactions of different kinds, its influence on the shape of the crystal is determined by the weakest interaction present in a particular chain. Prominent faces are parallel to at least two high-energy bond chains. This enables a correlation to be made between the crystal lattice and the crystal morphology, based on the fact that direct protein–protein contacts, reinforced by well ordered solvent molecules, are important in determining crystal packing (Frey *et al.*, 1988). Studies of the morphology of tetragonal lysozyme (Nadarajah & Pusey, 1996; Nadarajah *et al.*, 1997) showed that the crystallizing unit is a helical tetramer (centred on the 4_3 crystallographic axes).

5.1.2. Crystal mounting

5.1.2.1. *Introduction to crystal mounting*

Once crystals have been obtained and visually characterized, the next procedure involves the transfer of a selected crystal to an appropriate mounting device so that the crystal may be characterized using X-rays. Macromolecular crystals are generally obtained from and stored in a solution containing the precipitant or precipitants and other substances such as uncrystallized protein or other macromolecules. The object is to mount the crystal in such a way that it is undamaged by cracking, drying out, dissolving *etc.* during this operation. In some cases, the crystal may have been stored in a solution containing volatile solvents. Alternatively, the crystals may have been grown at a temperature lower than room temperature and therefore may require special handling in order to avoid crystal deterioration. In other cases, it may be desirable to prepare the crystal for study at cryogenic temperatures. This section deals with the mounting of crystals for all these conditions and concentrates on the mounting of crystals for diffraction experiments at or just below room temperature. Procedures such as 'flash cooling' are used to reduce radiation damage. Crystal-mounting techniques for cryogenic experiments are covered in detail in Part 10 and are only mentioned briefly here. In general, the most difficult part of mounting macromolecular crystals is the transfer of the crystal from a holding solution to a suitable mount. A capillary or, if cryogenic experiments are to be carried out, a cryoloop should be used.

5.1.2.2. *Tools for crystal mounting*

In order to facilitate the process of mounting macromolecular crystals for X-ray diffraction experiments, it is necessary to have the appropriate tools for the task. Fig. 5.1.2.1 shows a collection of some useful tools for the mounting of crystals. These include a binocular microscope, tweezers (two types), thin glass capillaries, Pasteur pipettes, a heater, paper wicks, and a thumb pump. Other

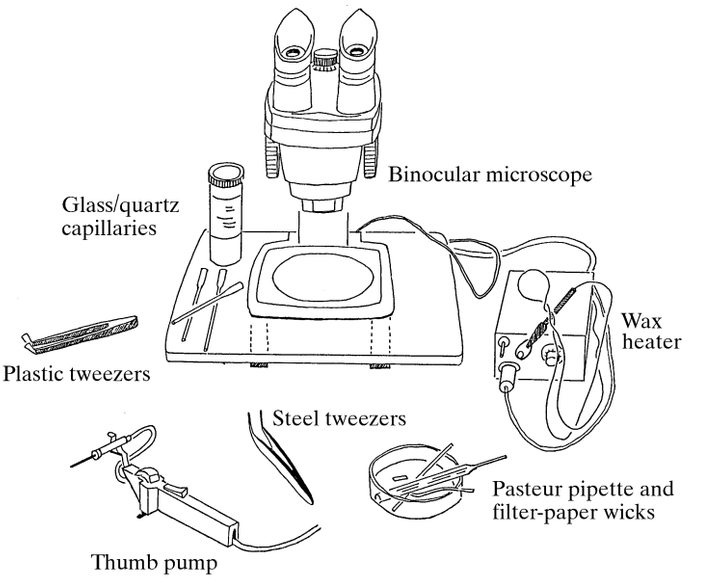

Fig. 5.1.2.1. Tools commonly used for mounting crystals.

useful tools and supplies include surgical scissors, dental wax, latex tubing, light vacuum oil, a cryogenic mounting loop, Plasticine, mounting platforms, mounting pins, absorbent dental points and micropipettes with plastic tips. There are many other items that might be useful, and several variations are found in different laboratories. An important factor in the transfer of crystals from a holding solution to a capillary is that the experimenter needs to feel at ease with the process. The method that will be detailed here has evolved over time and has proved to be a relatively anxiety-free process. Other methods for crystal mounting may be found in the literature (Rayment, 1985; Sawyer & Turner, 1992; McRee, 1993). All of the methods outlined here and in the literature have the same goal, namely, the successful transfer of a macromolecular single crystal to a suitable mount for X-ray data collection.

5.1.2.2.1. *Microscope*

Perhaps the single most important piece of equipment for examining and mounting crystals is a binocular dissection microscope. This should have variable zoom capabilities, and there should be sufficient distance (*e.g.* 5–10 cm) between the objective lens of the microscope and the microscope stage to accommodate the necessary equipment and allow manipulation of the crystals and solutions. A magnification of between 10 and 40 times is probably best in practice. It is also important to ensure that the light source of the microscope is not so intense that it heats the microscope stage, thereby damaging the macromolecular crystals. If the microscope is fitted with crossed polarizers, the quality of the crystals can be assessed.

5.1.2.2.2. *Capillaries*

The capillaries used for crystal mounting are made of thin-walled glass. These capillaries range from 0.1 to 2.0 mm in diameter and have a stated wall thickness of 0.01 mm. In practice, however, the larger the diameter of the capillary, the thinner the glass wall. Therefore, handling of the larger-diameter capillaries is generally very difficult because they are so fragile. Capillaries made of fused quartz are also available, but are not recommended for general use because they produce a higher background with X-rays. Quartz capillaries are not as fragile as the thin-walled glass capillaries, however, and may be useful in experiments where the tensile strength of the capillary is important, for example, when a

references