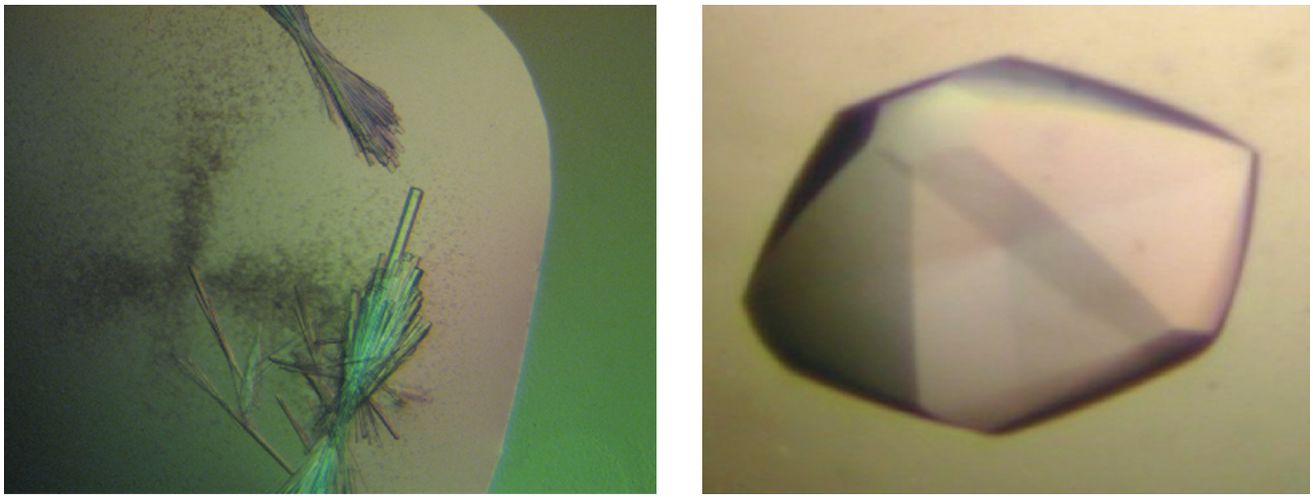

bad crystal good crystal

Figure 2.1. Examples of crystallization trials. Proteins can aggregate in many ways but usually crystallize in only one form. The example on the left shows protein aggregation without forming a crystal (cloudy areas). Aggregation may be random protein association or denaturation of the protein under the conditions of the crystallization trial. Also shown is formation of crystals that do not diffract, possibly because they are too small in two dimensions or because they crystallized in a disordered fashion. The example on the right shows a beautifully formed protein crystal. On occasion, even such a lovely crystal may show poor or no diffraction because of internal systematic lattice disorder. In this case, the crystal diffracts to 0.9Å resolution.

DATA COLLECTION

Once crystals are obtained, they can be tested for their ability to diffract x-rays and data can be collected. There is a fundamental principle about diffraction that allows it to be transformed into structural information: the diffraction pattern of an object is the Fourier transform of the object (for details, see Stout & Jensen, 1989; Blow, 2002; Rhodes, 2006).[4–6] Conversely, the inverse Fourier transform of the diffraction pattern will give a model of the object.

In principle, a single object will diffract x-rays. Diffraction depends on the interaction of electromagnetic radiation with an object and the scattering of that radiation. Other scattering methods also exist, such as the scattering of neutrons from nuclei, but at present they constitute only a very tiny fraction of the diffraction experiments done today. For proteins or other organic molecules, x-rays are the electromagnetic radiation of choice because the typical wavelength of an x-ray is 0.15 nm, the approximate distance of bond lengths in such molecules. Consequently, it should be possible to detect such distances using x-ray diffraction.

Ideally, a single molecule should suffice for such an experiment. However, the use of a single molecule results in such a low intensity of the scattered beam that it is too weak to be measured by any detector available today. Consequently, scattering from many molecules is required to obtain a signal strong enough to measure. For a scattered beam to be measurable, somewhere in the vicinity of 10^{15} molecules are required. Not only are a large number of molecules required, but also they all have to be in the same or a limited number of orientations that repeat in a regular pattern in three dimensions. That is the definition of a crystal in which the repeating unit that builds the crystal is the unit cell. When scattering comes from a crystal it is called diffraction. The fundamental principle of diffraction of a crystal of a protein states that the Fourier transform of the electron distribution of the protein in the crystal is the diffraction pattern and the inverse Fourier transform of the diffraction pattern is the electron density of the protein.

Just as the protein is three-dimensional, so is the diffraction pattern. In addition, the diffraction pattern mirrors the symmetry of the arrangements of the unit cells in the crystal and the protein in the unit cell. These arrangements are defined in terms of space groups and asymmetric units. The asymmetric unit is the smallest unit from which a unit cell can be constructed and represents the minimum number of independently determined structures in a crystal. Thus, an asymmetric unit may contain as few as one example of a protein or as many as twelve or more. Sometimes such arrangements make it possible to determine the oligomeric state of a protein, particularly if one subunit is not identical to another (Figure 2.2).

Because of the three-dimensional character of a diffraction pattern, a reflection, the effect of a diffracted beam on a detector, may lie closer to the center of the pattern or further away from it. The identity of a reflection is defined by its Miller indices, its position on a three-dimensional grid, starting with zero at the center and moving outward. The angles the diffracted beams make with the direction of the incident beam determines the level of information obtainable from them. The larger the angle, the more precise the information carried relative to the distances within the