10. CRYOCRYSTALLOGRAPHY

10.1. Introduction to cryocrystallography

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10.1.1. Utility of low-temperature data collection

10.1.1.1. *Prevention of radiation damage*

Since about 1985, low-temperature methods in biocrystallography have moved from stumbling experimentation to mainstay production techniques. This would not have happened without good reason. A brief discussion of the advantages of data collection at cryogenic temperatures is given.

Biocrystals near room temperature are sensitive to X-rays and generally suffer radiation damage during data measurement. Often this damage is so rapid and severe that a number of different crystals are needed for a full data set. On occasion, damage is so rapid that data collection is impossible. Crystal decay is typically accompanied by changes in reflection profiles and cell dimensions, which alter the positions of diffraction maxima, exacerbating the problem of changing diffraction intensities. The use of more than one crystal almost always introduces inaccuracies. Intensities from a crystal near the end of its usable life will have decay errors. Individual samples of biocrystals frequently have measurable differences in structure; merging of data will result in an average of the structures encountered, with some loss of definition. Crystals cooled to near liquid-N_2 temperature typically show a greatly reduced rate of radiation damage, often to the extent that it is no longer an issue of concern. The protection from radiation damage was noted early on. Petsko (1975) observed numerous cases of this effect. A noteworthy example is the successful prevention of radiation damage to crystals of ribosome particles (Hope *et al.*, 1989).

Radiation damage appears to be related to the formation of free radicals. At sufficiently low temperature, two effects can influence the rate of damage: movement of the radicals is hampered, and the activation energy for reaction is not available. A revealing observation has been described by Hope (1990). A crystal that had been exposed to synchrotron radiation for many hours at 85 K showed no overt signs of radiation damage. However, while the crystal was being warmed toward room temperature, it suddenly turned black and curled up like a drying leaf. More commonly, crystals turn yellow under X-ray irradiation, and bubbles and cracks appear on warming. The rate of free-radical formation would be affected little by temperature, so that when sufficient mobility and activation energy become available, the stored radicals will react.

10.1.1.2. *Mechanical stability of the crystal mount*

The mechanical stability of samples is also of concern. Crystals mounted in capillaries and kept wet have a tendency to move, giving rise to difficulties with intensity measurements. A crystal at cryotemperature is rigidly attached to its mount; slippage is impossible.

10.1.1.3. *Effect on resolution*

The effects on radiation damage and mechanical stability are clear-cut, and provide the main reasons for using cryotechniques. Resolution can also be affected, but the connection between temperature and resolution is neither simple nor obvious. If low resolution is the result of rapid radiation damage, lowering the temperature can lead to much improved resolution. However, if low resolution is mainly caused by inexact replication from one unit cell to another, lowering the temperature may have little effect on resolution. If the mosaic spread in the crystal increases upon cooling, resolution may even deteriorate.

In a model proposed by Hope (1988), a relationship between resolution r and temperature T is given by

$$r_2 = r_1[(B_0 + bT_2)/(B_0 + bT_1)]^{1/2}.$$

Here r_1 is the resolution at T_1, r_2 is the resolution at T_2, B_0 is the value of B at $T = 0$ and b is a proportionality constant. The underlying assumption is that for any given temperature, the temperature factor [*i.e.* $\exp(-B\sin^2\theta/\lambda^2)$] at the resolution limit has the same value; thus the effects of scattering factors and Lp factors are ignored. We see that if B_0 is the predominant term, lowering T will not have much effect, whereas for small B_0 (a relatively well ordered structure) the effect of T on r can be large. For example, if the room-temperature resolution is 1.5 Å, the resolution at 100 K can be around 1 Å, but if the room-temperature resolution is around 3 or 4 Å, little change can be expected. A qualitative assessment of these effects was clearly stated by Petsko (1975).

10.1.2. Cooling of biocrystals

10.1.2.1. *Physical chemistry of biocrystals*

Crystals are normally brought from room temperature to the working, low temperature by relatively rapid cooling, either in a cold gas stream, or by immersion in a cryogen such as liquid nitrogen or liquid propane. One goal of the procedure is to avoid crystallization of any water present in the system, whether internal or external to the crystal. Ice formation depends on the formation of nuclei. Nuclei are formed either by homogenous nucleation, *i.e.* in bulk liquid, or by heterogeneous nucleation, *i.e.* at the surface of a phase other than the liquid. Although data pertaining to biocrystals are scarce, indications are that internal nucleation, whether homogenous or heterogeneous, is not common. Proteins that induce nucleation at mild supercooling are known, so presumably there exist regions in these proteins which help to prearrange water molecules so that they readily form ice nuclei. There are also proteins that hinder nucleation. At present there is no basis for predicting the outcome of cooling for any given protein crystal. Only in a statistical sense can one be reasonably confident that a given macromolecule will not promote the freezing of water.

Vali and coworkers (Götz *et al.*, 1991; Vali, 1995) have provided a quantitative treatment of ice nucleation that can serve as a guideline. They observe that the absolute rate of formation of nuclei increases with the volume of water and with decreasing temperature. The probability p that a volume of water will freeze during a time span t is given by

$$p = J(T)Vt,$$

where $J(T)$ is the nucleation rate at temperature T. Based on empirical data, $J(T)$ is given by

$$J(T) = 6.8 \times 10^{-50} \exp[3.9(273 - T)],$$

where J is in m^{-3} s^{-1} and T is in K. Note that $J(T)$ increases by a factor of 50 per K. As a practical limit, bulk water cannot be cooled

references