

## 3.2. DETERMINATION OF THE DENSITY OF SOLIDS

Table 3.2.3.1. Typical calculations of the values of  $V_M$  and  $V_{\text{solv}}$  for proteins

| Protein                                  | $\gamma$ B-Crystallin | $\gamma$ D-Crystallin           | Ceruloplasmin       |
|--|-----------------------|---------------------------------|---------------------|
| Space group                              | $P4_12_12$            | $P2_12_12_1$                    | $P3_221$            |
| Cell parameters (Å)                      | $57.5 \times 98.0$    | $57.8 \times 70.0 \times 117.3$ | $213.9 \times 85.6$ |
| Molecular weight (kDa)                   | 21                    | 21                              | 132                 |
| $Z$                                      | 8                     | 4                               | 6                   |
| $V_M$ (Å <sup>3</sup> Da <sup>-1</sup> ) | 1.93                  | 5.65*                           | 4.95                |
| $V_{\text{solv}}$ (%)                    | 36                    | 78*                             | 75                  |
| Resolution (Å)                           | 1.5                   | 2.0                             | 3.1                 |

\* In the case of  $\gamma$ D-crystallin, the values for  $V_M$  and  $V_{\text{solv}}$  are abnormally high. A recalculation assuming two molecules per asymmetric unit,  $Z_a = 2$ , gives more reasonable values of  $V_M = 2.83 \text{ Å}^3 \text{ Da}^{-1}$  and  $V_{\text{solv}} = 56 \%$ .

cies of vibration will be related inversely to the square root of the density for small oscillations. The potential accuracy of frequency measurements makes this useful for following density changes in the sample while altering the temperature or pressure; see Rabukhin (1982).

## 3.2.3. Biological macromolecules (By P. F. Lindley)

Biological macromolecules usually present particular difficulties with respect to density measurements and the determination of  $Z_a$ , the number of molecules per asymmetric unit, because of the presence in the crystals of variable amounts of solvent. However, it is often crucially important with respect to a structure determination, particularly using molecular-replacement techniques, to know  $Z_a$ . In many cases,  $Z_a = 1$ , although, as in the case of small molecules, crystals are also found with fractional values of  $Z_a$  when molecular symmetry axes coincide with crystal symmetry axes, or values greater than 1 if there are multiple copies of the molecule in the asymmetric unit. In practice, it is usually necessary only to know the solvent content of the crystals between rather coarse limits in order to distinguish possible values of  $Z_a$ , and this problem has been addressed for globular proteins by Matthews (1968). A rather more precise knowledge of the solvent may be essential for the use of density-modification techniques in phase refinement. Matthews defines a quantity,  $V_M$ , the crystal volume per unit of protein molecular weight (*i.e.* the ratio of the volume of the asymmetric unit determined from X-ray diffraction measurements to the molecular weight of the protein in the asymmetric unit) and shows that  $V_M$  bears a simple relationship to the fractional volume of solvent in the crystal. The range of observed values of  $V_M$  (1.68 to  $3.53 \text{ Å}^3 \text{ Da}^{-1}$  for the 116 distinct crystal forms considered by Matthews with median and most common values of 2.61 and  $2.15 \text{ Å}^3 \text{ Da}^{-1}$ , respectively) is essentially independent of the volume of the asymmetric unit. Matthews further defines the quantity  $V_{\text{prot}}$ , the fraction of the crystal volume occupied by the protein:

$$V_{\text{prot}} = 1.66\nu/V_M,$$

where  $\nu$  is the partial specific volume of the protein in the crystal and for most proteins approximates to  $0.74 \text{ ml g}^{-1}$ . With this approximation,

$$V_{\text{prot}} = 1.23/V_M$$

and, by difference, the fractional volume occupied by the solvent is therefore

$$V_{\text{solv}} = 1 - 1.66\nu/V_M \approx 1 - 1.23/V_M.$$

On this basis, the range of  $V_M$  cited above converts to a solvent content ranging from 27 to 65%, with values near 43% occurring most frequently. For cases where the solvent content appears abnormally low or high in respect of the physical properties of the crystal and the resolution of the diffraction pattern, then some alteration to the value of  $Z_a$  may well be indicated. Some typical examples are given in Table 3.2.3.1. It should be noted that, although the method described above appears to obviate the need to measure the density of crystals, a precise experimental measurement of the crystal density, wherever practical, is always a useful investment.

In a recent development, Kwong, Pound & Hendrickson (1994) have devised an experimental method for the determination of  $Z_a$  using a volume-specific amino acid analysis. The crystal volume is determined from optical measurements of crystals mounted in glass capillaries, and the number of molecules in that volume is determined by amino acid analysis. From the unit-cell volume determined from X-ray measurements and the space-group symmetry,  $Z_a$  can be calculated from the number of molecules per crystal volume. The method requires extreme care to obtain precise measurements of the crystal volume and access to high-performance liquid chromatography and associated equipment for the amino acid analysis.