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

Protein	γB-Crystallin	γD-Crystallin	Ceruloplasmin
Space group	$P4_12_12$	$P2_12_12_1$	$P3_221$
Cell parameters (Å)	57.5 × 98.0	57.8 × 70.0 × 117.3	213.9 × 85.6
Molecular weight (kDa)	21	21	132
Z	8	4	6
V_M (Å^3 Da^{-1})	1.93	5.65*	4.95
$V_{\rm solv}$ (%)	36	78*	75
Resolution (Å)	1.5	2.0	3.1

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

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 Å^3 Da^{-1} for the 116 distinct crystal forms considered by Matthews with median and most common values of 2.61 and 2.15 Å^3 Da^{-1}, respectively) is essentially independent of the volume of the asymmetric unit. Matthews further defines the quantity $V_{\rm prot}$, the fraction of the crystal volume occupied by the protein:

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

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

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

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

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

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.

references