accuracy of the individual measurements. Initially, NMR structures were calculated using distance-geometry techniques, and subsequently the principles of distance geometry have been introduced into molecular-dynamics programs in Cartesian coordinates (Brünger et al., 1986) or in torsion-angle space (Güntert et al., 1997). Model calculations performed in conjunction with the initial protein structure determinations had shown that NMR structure calculation depends critically on the density of NOE distance constraints, while it is remarkably robust with regard to low precision of the individual distance constraints (Havel & Wüthrich, 1985). For the common presentation of an NMR structure, one considers the result of a single structure calculation as representing one molecular geometry that is compatible with the NMR data. To investigate further whether or not this solution is unique, the calculation is repeated with different boundary conditions, where for each calculation, convergence is judged by the residual constraint violations. All satisfactory solutions, by this criterion, are included in a group of conformers that is used to represent the NMR structure (Fig. 19.7.2.3). The precision of the structure determination is reflected by the dispersion among this group of conformers. In proteins, larger variations are typically observed near the chain ends, in exposed loops and for surface amino-acid side chains, which contrasts with the well defined core. For nucleic acids, the ‘global folds’, for example, formation of duplexes, triplexes, quadruples, or loops, can be well defined by NMR, but because of the short range of the NOE distance measurements, certain ‘long-range’ features, for example, bending of DNA duplexes, may be more difficult to characterize.

19.7.3. Combined use of single-crystal X-ray diffraction and solution NMR for structure determination

The chemical shifts in proteins or nucleic acids cannot be calculated with sufficiently high precision from the X-ray crystal structures to predict the NMR spectra reliably. This limits the extent to which improved efficiency of an NMR structure determination may be derived from knowledge of the corresponding crystal structure. Nonetheless, for resonance assignments with sequential NOEs, information on the type of covalent connections between regular secondary-structure elements can often also be unambiguously determined, this NMR information may be helpful in tracing the electron-density map for the determination of the corresponding X-ray crystal structure (Kallen et al., 1991). Furthermore, since identical global architectures are usually found for corresponding crystal and solution structures of globular proteins, NMR structures have been used to solve the corresponding crystal structure by molecular replacement (e.g. Braun et al., 1989). Considering the ease with which high-quality X-ray data are obtained nowadays once suitable crystals are available, it remains to be seen whether this kind of combined use of data obtained with the two methods will also play a role in the future.

19.7.4. NMR studies of solvation in solution

In NMR structures, the location of hydration water molecules is determined by the observation of NOEs between water protons and hydrogen atoms of the polypeptide chain. In contrast to the observation of hydration water in X-ray crystal structures, this information is not routinely collected within the scope of a standard protocol for NMR structure determination (Fig. 19.7.2.1), but requires additional experiments (Otting et al., 1991). Because the NOE decreases with the sixth power of the $1\text{H}^2\text{H}$ distance, only water molecules in a first hydration layer are typically observed. The NOE intensity is further related to a correlation function describing the stochastic modulation of the dipole–dipole coupling between the interacting protons, which may be governed either by the Brownian rotational tumbling of the hydrated protein molecule or by interruption of the dipolar interaction through translational diffusion of the interacting spins, whichever is faster (Otting et al., 1991). Interior hydration waters are typically observed in identical locations in corresponding crystal and solution structures, but NMR provides additional information on molecular mobility. Completely buried hydration water molecules have thus been found to exchange with the bulk solvent at rates corresponding to millisecond residence times in the protein hydration sites, and measurements with nuclear magnetic relaxation dispersion also revealed exchange rates on the microsecond to nanosecond timescale (Denisov et al., 1996). For surface hydration water, the lifetimes in the hydration sites are typically even shorter, and NMR measures the average duration of these ‘visits’ (Otting et al., 1991;