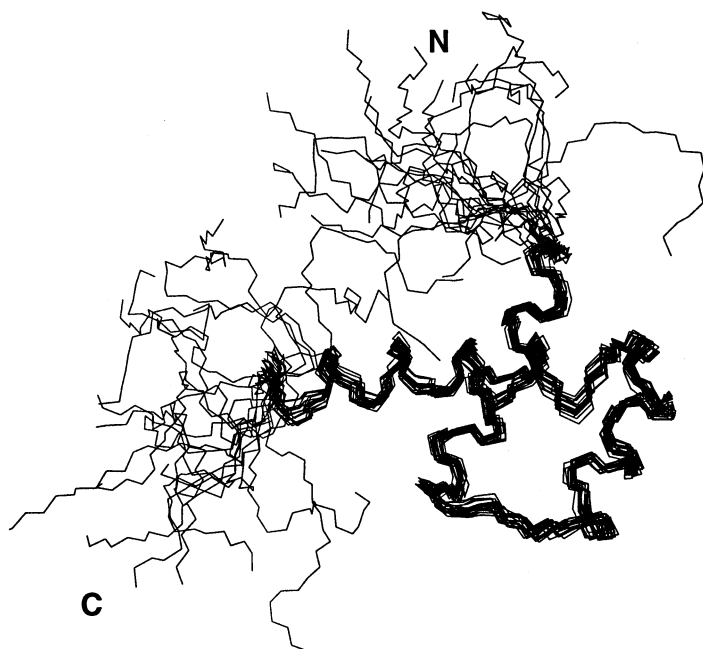19. OTHER EXPERIMENTAL TECHNIQUES

Fig. 19.7.2.3. Polypeptide backbone of 19 energy-refined conformers selected to represent the NMR solution structure of the *Antennapedia* homeodomain. From residues 7 to 59, the structure is well defined by the NMR data. The chain-terminal segments 0–6 and 60–67 are disordered, and additional NMR studies showed that these terminal segments behave like 'flexible tails'. [Drawing prepared with the atomic coordinates from Qian *et al.* (1989).]

contrasts with the well defined core. For nucleic acids, the 'global folds', for example, formation of duplexes, triplexes, quadruplexes, 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 connectivity to look for in specified polypeptide segments may be derived. The X-ray structure may also tentatively be used as a starting reference to resolve ambiguities in the assignment of NOE distance constraints (Güntert *et al.*, 1993), which may help to reduce the number of cycles needed to collect the input data for a high-quality structure determination (Fig. 19.7.2.1).

An attractive possibility for combined use of X-ray and NMR data has in the past arisen in situations where both a low-resolution crystallographic electron-density map and a secondary-structure determination by NMR are available. As mentioned before, NMR secondary-structure determination in proteins usually derives directly from the backbone resonance assignments (Wishart *et al.*, 1991; Wüthrich *et al.*, 1984) and does not depend on the availability of a complete three-dimensional structure. Since the 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}H–^{1}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; in an alternative analysis of the NMR data, surface hydration has been characterized by reduced diffusion rates of the water, without specifying individual hydration sites; Brüschweiler & Wright, 1994). Diffraction experiments, on the other hand, probe the total fraction of time that a water molecule spends in a particular hydration site, but they are insensitive to the residence time at that site on any particular visit. Furthermore, while hydration water molecules in protein crystals are observed in discrete surface sites that are not blocked by direct protein–protein contacts, the entire surface of a protein in aqueous solution is covered with water molecules. Overall, the NMR view of hydration in solution, which has also been rationalized with long-time molecular-dynamics simulations (*e.g.* Billeter *et al.*, 1996; Brunne *et al.*, 1993), is largely complementary to crystallographic data on hydration.

19.7.5. NMR studies of rate processes and conformational equilibria in three-dimensional macromolecular structures

Similar to the aforementioned hydration studies, information on intramolecular rate processes in macromolecular structures cannot usually be obtained from the standard protocol for NMR structure determination (Fig. 19.7.2.1), but results from additional experiments. The complementarity of such NMR information to crystallographic data is well illustrated by the 'ring flips' of phenylalanine and tyrosine (Wüthrich, 1986). The observation of these ring-flipping motions in the basic pancreatic trypsin inhibitor (BPTI)

(Wüthrich & Wagner, 1975) was a genuine surprise for the following reasons. In the refined X-ray crystal structure of BPTI, the aromatic rings of phenylalanine and tyrosine are among the side chains with the smallest temperature factors. For each ring, the relative values of the B factors increase toward the periphery, so that the largest positional uncertainty is indicated for carbon atom 4 on the symmetry axis through the C^β—C^1 bond, rather than for the carbon atoms 2, 3, 5 and 6 (Fig. 19.7.5.1), which undergo extensive movements during the ring flips. Theoretical studies then showed that the crystallographic B factors sample multiple rotation states about the C^α—C^β bond, whereas the ring flips about the C^β—C^1 bond seen by NMR are very rapid 180° rotations connecting two indistinguishable equilibrium orientations of the ring. The B factors do not manifest these rotational motions because the populations of all non-equilibrium rotational states about the C^β—C^1 bond are vanishingly small. The ring-flip phenomenon is now a well established feature of globular proteins, manifesting ubiquitous low-frequency internal motions with activation energies of $60-100$ kJ mol^{-1}, amplitudes of $\gtrsim 1.0$ Å and activation volumes of about 50 Å^3 (Wagner, 1980), and involving concerted displacement of numerous groups of atoms (Fig. 19.7.5.1).
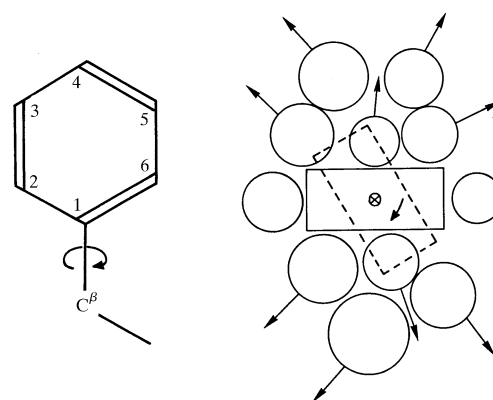

Fig. 19.7.5.1. 180° ring flips of tyrosine and phenylalanine about the C^β—C^1 bond. On the left, the atom numbering is given and the χ^2 rotation axis is identified with an arrow. The drawing on the right presents a view along the C^β—C^1 bond of a flipping ring in the interior of a protein, where the broken lines indicate a transient orientation of the ring plane during the flip. The circles represent atom groups near the ring, and arrows indicate movements of atom groups during the ring flip (Wüthrich, 1986).

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