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 Cα—C1 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 Cα—Cβ bond, whereas the ring flips about the Cα—Cβ 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 Cα—Cβ 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 \(\leq 1.0\) Å and activation volumes of about 50 Å\(^3\) (Wagner, 1980), and involving concerted displacement of numerous groups of atoms (Wang, 1975.1).

![Figure 19.7.5.1](image)

180° ring flips of tyrosine and phenylalanine about the Cα—Cβ bond. On the left, the atom numbering is given and the \(x\) \(x\) rotation axis is identified with an arrow. The drawing on the right presents a view along the Cα—C1 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).

References


