1991). Among side chains, the most highly hydrated appear to be Asp and Glu, whose COO⁻ groups bind, on average, two water molecules each (Baker & Hubbard, 1984; Thanki *et al.*, 1988). On the other hand, the best-ordered water sites are created by residues whose side chains simultaneously make hydrogen bonds to other protein atoms (His, Asp, Asn, Arg) or may be sterically restricted (Tyr, Trp).

The distributions of water molecules around protein groups follow the geometrical patterns expected from simple bonding ideas (Baker & Hubbard, 1984; Thanki *et al.*, 1988). Interactions with NH groups are linear, and those with C=O groups show a preferred angle of ∼130° at the oxygen-atom acceptor, consistent with interaction with an oxygen-atom lone pair; restriction to the peptide plane is not very strong, however. Although the distributions around polar side chains generally follow the expected patterns (Thanki *et al.*, 1988), there is little evidence of ordered water clusters around non-polar groups. This may be because water clusters need to be 'anchored' by hydrogen bonding to polar groups to be seen crystallographically.

22.2.6. Hydrogen bonding in nucleic acids

Hydrogen bonding by purine and pyrimidine bases is, together with base stacking, a major determinant of nucleic acid structure. With so many hydrogen-bonding groups, there are many potential modes of interaction between bases (Jeffrey & Saenger, 1991). Those that are actually found in DNA and RNA structures are, however, much more restricted in number, at least based on presently available experimental data.

22.2.6.1. *DNA*

DNA structure is dominated by the prevalence of duplex structures and hence by the classic Watson–Crick hydrogen-bonding pattern of A–T and G–C base pairs. This hydrogen-bonding pattern is not affected by whether the double helix has A-form, B-form, or Z-form geometry. Other hydrogen-bonding modes in DNA are probably very rare, arising only as a result of mutations (which produce mismatches), chemical modifications, such as methylation, or other disturbances, such as the binding of drugs or proteins so as to alter DNA conformation. Mismatches can give stable hydrogen bonding but at the expense of local perturbations of the DNA structure.

22.2.6.2. *RNA*

In contrast to DNA, RNA molecules generally form single-stranded structures, which are correspondingly much more complex and less regular. This means that catalytic and other activities can be generated in addition to their information-carrying roles. Current knowledge of detailed RNA three-dimensional structure is limited to transfer RNAs and several ribozymes, including a large ribosomal RNA domain (Cate *et al.*, 1996). Even from this small sample, however, it is clear that a great diversity of hydrogen-bonding interactions exists; RNA molecules contain regions of double-helical structure, often with classical Watson–Crick A–U and G–C base pairing, but these regions are interspersed with loops and bulges and tertiary interactions between the various secondary-structural (double-helical) elements. These interactions include many unconventional base pairings (*e.g.* see Fig. 22.2.6.1).

Some RNA structural motifs may prove to be of widespread general importance in RNA molecules. One example is a sharp turn with sequence CUGA in the hammerhead ribozyme that exactly matches turns in tRNAs (Pley *et al.*, 1994). Another is the GNRA tetraloop structure (N = any base, R = purine). This loop has a well defined structure, stabilized by hydrogen bonding and stacking involving its own bases, and it also presents further hydrogen-bonding groups that can dock into 'receptor' structures in other parts of the RNA molecule. This results in triple or quadruple base interactions (Fig. 22.2.6.1) that tie different parts of the RNA structure together; the parallel with hydrogen-bonding side chains in proteins is very strong. The 2′-hydroxyls of ribose groups are also used in some of these interactions (Fig. 22.2.6.1). Further ribose interactions involve interdigitated ribose groups that line the interfaces between adjacent helices such that pairs of riboses interact by hydrogen bonding through their 2′-hydroxyl groups, forming 'ribose zippers' As many more RNA structures are determined experimentally, it is likely that more hydrogen-bonding motifs will be recognized, and their full role in RNA structure can be better assessed than at our present, imperfect state of knowledge.

22.2.7. Non-conventional hydrogen bonds

The vast majority of hydrogen bonds in biological macromolecules involve nitrogen and oxygen donors exclusively. Nevertheless, several other interactions have all the characteristics of hydrogen bonds and clearly contribute to structure and stability where they occur.

22.2.7.1. *C—H···O hydrogen bonds*

Sutor (1962) first summarized evidence for C—H···O hydrogen bonds following earlier suggestions by Pauling (1960), and current evidence has been nicely summarized in several recent articles (Derewenda *et al.*, 1995; Wahl & Sundaralingam, 1997). The energy of C—H···O hydrogen bonds has been generally estimated as ∼0.5 kcal mol⁻¹ (about 10% of an N—H···O interaction) but may be higher, especially in hydrophobic environments. It also depends on the acidity of the C—H proton, with methylene (CH₂) and methyne (CH) groups being most favourable.

A number of examples of C—H···O hydrogen bonds can be found in nucleic acid structures (Wahl & Sundaralingam, 1997). The best known is that between the backbone O5′ oxygen and a purine C(8)—H or pyrimidine C(6)—H, when the bases are in the *anti* conformation. Another example is given by a U–U base pair, in which the two bases form a conventional N(3)—H···O(4) hydrogen bond and a C(5)—H···O hydrogen bond.
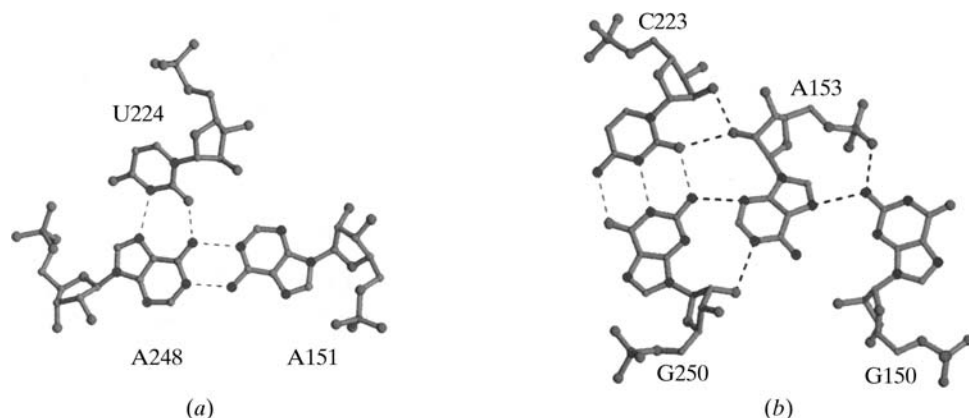

Fig. 22.2.6.1. Hydrogen-bonding interactions in RNA tertiary structure. In (*a*), a triple base interaction is shown. In (*b*), G150 and A153 of a GAAA tetraloop participate in multiple hydrogen-bond interactions involving bases, riboses and phosphate. Reprinted with permission from Cate *et al.* (1996). Copyright (1996) American Association for the Advancement of Science.

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In proteins, two groups are regarded as being particularly significant (Derewenda *et al.*, 1995). These are the $C^\epsilon H$ of His side chains and the methylene H atoms of the main-chain α-carbon atoms. C—H$\cdots$O hydrogen bonds involving His side chains have been found for the active-site His residues of proteins of the lipase/esterase family and in other proteins (Derewenda *et al.*, 1994). The $C^\alpha H$ atoms appear to provide much more widespread C—H$\cdots$O hydrogen bonding, however, especially in β-sheets, where they are directed towards the 'free' lone pairs of the main-chain C=O groups. C—H$\cdots$O hydrogen bonds may thus play a previously unrecognised role in satisfying the hydrogen-bond potential of C=O groups. In general, Derewenda *et al.* (1995) find a significant number of C$\cdots$O contacts that meet the criteria for C—H$\cdots$O hydrogen bonds; the H$\cdots$O distance peaks at 2.45 Å (C$\cdots$O 3.5 Å), which is less than the van der Waals distance of 2.7 Å, and the angles indicate that the H atoms are directed at the acceptor lone-pair orbitals.

22.2.7.2. *Hydrogen bonds involving sulfur atoms*

Sulfur atoms are larger and have a more diffuse electron cloud than oxygen or nitrogen, but are nevertheless capable of participating in hydrogen bonds. Given that the radius of sulfur is $\sim$0.4 Å greater than that of oxygen, hydrogen bonds can be assumed if the distance H$\cdots$S is less than $\sim$2.9 Å, or S$\cdots$O(N) is less than $\sim$3.9 Å, providing the angular geometry is right. In proteins, the SH group of cysteine can be a hydrogen-bond acceptor or donor, whereas the sulfur atoms in disulfide bonds and in Met side chains can act only as acceptors.

The clearest example of hydrogen bonding involving Cys residues is given by the NH$\cdots$S hydrogen bonds in Fe-S proteins (Adman *et al.*, 1975); here, peptide NH groups are oriented to point directly at the S atoms of metal-bound Cys residues, with H$\cdots$S distances of 2.4–2.9 Å. Similar NH$\cdots$S hydrogen bonds are found in blue copper proteins, involving the Cys ligands. In these cases, the cysteine sulfur is deprotonated and therefore more negative, making it a stronger hydrogen-bond acceptor, and it is likely that hydrogen bonding to cysteine S$^-$ atoms is common. A large survey of Cys and Met side chains in proteins has given evidence of both N—H$\cdots$S and S—H$\cdots$O hydrogen bonds involving the SH groups of Cys side chains (Gregoret *et al.*, 1991). In particular, Cys residues in helices frequently hydrogen bond to the main-chain C=O group four residues back in the helix in SH$(n)\cdots$O$(n-4)$ interactions analogous to those seen for Ser and Thr residues in helices. On the other hand, O—H$\cdots$S or N—H$\cdots$S hydrogen bonds to the S atoms of Met or half-cystine side chains, although they do exist, are rare (Gregoret *et al.*, 1991; Ippolito *et al.*, 1990).

22.2.7.3. *Amino-aromatic hydrogen bonding*

Surveys of protein structures have shown that aromatic rings (of Trp, Tyr, or Phe) are frequently in close association with side-chain NH groups of Lys, Arg, Asn, Gln, or His (Burley & Petsko, 1986). Energy calculations further suggest that where an N—H group, as donor, is directed towards the centre of an aromatic ring, as acceptor, a hydrogen-bonded interaction with an energy of $\sim$3 kcal mol^{-1} (about half that of a normal N—H$\cdots$O or O—H$\cdots$O hydrogen bond) can result (Levitt & Perutz, 1988). Whether the close associations observed by Burley & Petsko can truly be regarded as hydrogen bonds has been controversial, however. Mitchell *et al.* (1994) have analysed amino–aromatic interactions and shown that by far the most common form of association between sp^2 nitrogen atoms and aromatic rings involves approximately plane-to-plane stacking, which cannot represent hydrogen bonding. There is still, however, a significant number of cases where the H atoms of N—H groups are directed towards aromatic rings, and these represent genuine hydrogen bonds (Mitchell *et al.*, 1994). It is clearly essential to consider the donor–acceptor geometry, both distances and angles, before assuming an amino–aromatic hydrogen bond; the N$\cdots$ring distance should be less than $\sim$3.8 Å, and N—H$\cdots C$ angle greater than 120°, where C is the ring centre (Mitchell *et al.*, 1994).