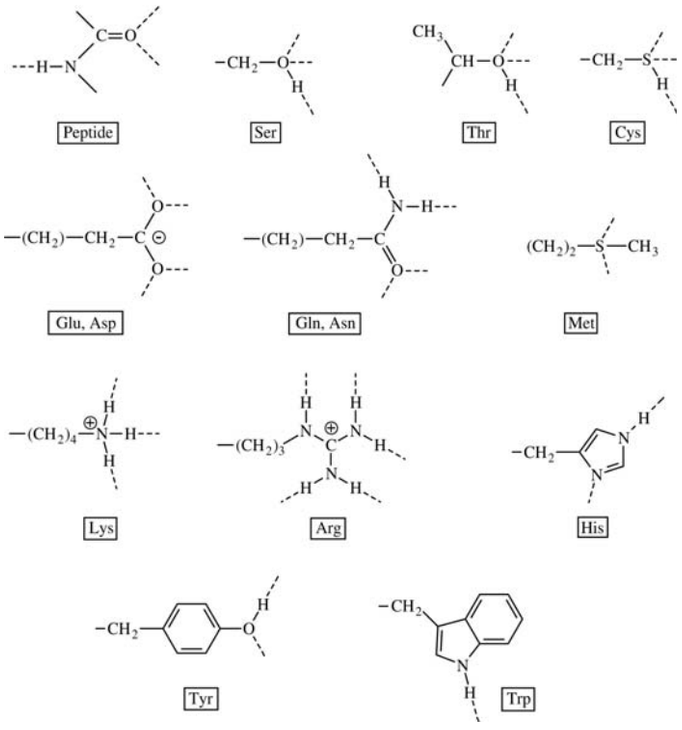

Fig. 22.2.3.1. Hydrogen-bonding potential of protein functional groups. Potential hydrogen bonds are shown with broken lines. Arg, Lys, Asp and Glu side chains are shown in their ionized forms.

recipients of hydrogen bonds from protein side chains in protein–DNA complexes. The sugar residues of RNA have a 2′-OH which can act as both hydrogen-bond donor and acceptor, and the 4′-O of both ribose and deoxyribose can potentially accept two hydrogen bonds.

It is the bases of DNA and RNA that have the greatest hydrogen-bonding potential, however, with a variety of hydrogen-bond donor or acceptor sites. Although each of the bases could theoretically occur in several tautomeric forms, only the canonical forms shown in Fig. 22.2.3.2 are actually observed in nucleic acids. This leads to clearly defined hydrogen-bonding patterns which are critical to both base pairing and protein–nucleic acid recognition. The —NH_2 and >NH groups act only as hydrogen-bond donors, and C=O only as acceptors, whereas the >N— centres are normally acceptors but at low pH can be protonated and act as hydrogen-bond donors.

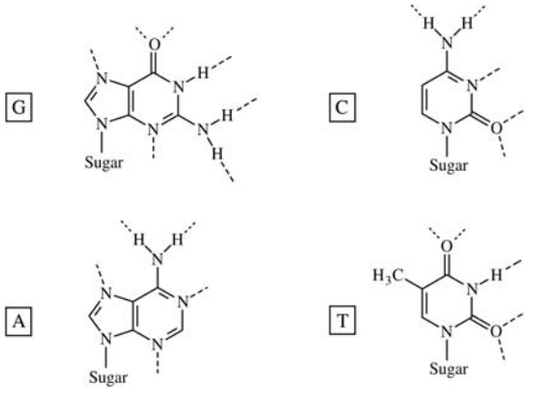

Fig. 22.2.3.2. Hydrogen-bonding potential of nucleic acid bases guanine (G), adenine (A), cytosine (C) and thymine (T) in their normal canonical forms.

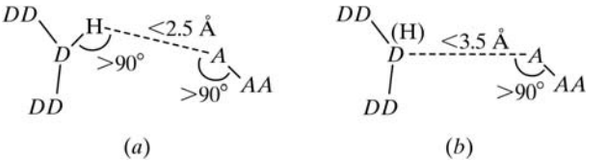

Fig. 22.2.4.1. Suggested criteria for identifying likely hydrogen bonds. *DD* and *AA* represent atoms covalently bonded to the donor atom, *D*, and acceptor atom, *A*, respectively. Here, (*a*) represents the criteria when the donor H atom can be placed, and (*b*) when it cannot be placed. Additional criteria based on the angle *DD*—*D*···*A* could be incorporated with (*b*). Adapted from Baker & Hubbard (1984) and McDonald & Thornton (1994*a*).

22.2.4. Identification of hydrogen bonds: geometrical considerations

Because hydrogen bonds are electrostatic interactions for which the attractive energy falls off rather slowly (Hagler *et al.*, 1974), it is not possible to choose an exact cutoff for hydrogen-bonding distances. Rather, both distances and angles must be considered together; the latter are particularly important because of the directionality of hydrogen bonding. Inferences drawn from distances alone can be highly misleading. An approach with an N—H···O angle of 90° and an H···O distance of 2.5 Å would be very unfavourable for hydrogen bonding, yet it translates to a N···O distance of 2.7 Å. This could (wrongly) be taken as evidence of a strong hydrogen bond.

For macromolecular structures determined by X-ray crystallography, problems also arise from the imprecision of atomic positions and the fact that H atoms cannot usually be seen. Thus, the geometric criteria must be relatively liberal. H atoms should also be added in calculated positions where this is possible; this can be done reliably for most NH groups (peptide NH, side chains of Trp, Asn, Gln, Arg, His, and all >NH and NH_2 groups in nucleic acid bases).

The hydrogen-bond criteria used by Baker & Hubbard (1984) are shown in Fig. 22.2.4.1. Very similar criteria are used in the program *HBPLUS* (McDonald & Thornton, 1994*a*), which also adds H atoms in their calculated positions if they are not already present in the coordinate file. In general, hydrogen bonds may be inferred if an interatomic contact obeys *all* of the following criteria:

(1) The distance H···*A* is less than 2.5 Å (or *D*···*A* less than 3.5 Å if the donor is an —OH or —NH_3^+ group or a water molecule).

(2) The angle at the H atom, *D*—H···*A*, is greater than 90°.

(3) The angle at the acceptor, *AA*—*A*···H (or *AA*—*A*···*D* if the H-atom position is unreliable), is greater than 90°.

Other criteria can be applied, for example taking into account the hybridization state of the atoms involved and the degree to which any approach lies in the plane of the lone pair(s). In all analyses of hydrogen bonding, however, it is clear that a combination of distance and angle criteria is effective in excluding unlikely hydrogen bonds.

22.2.5. Hydrogen bonding in proteins

22.2.5.1. *Contribution to protein folding and stability*

The net contribution of hydrogen bonding to protein folding and stability has been the subject of much debate over the years. The current view is that although the hydrophobic effect provides the driving force for protein folding (Kauzmann, 1959), many polar groups, notably peptide NH and C=O groups, inevitably become buried during this process, and failure of these groups to find hydrogen-bonding partners in the folded protein would be strongly destabilizing. This, therefore, favours the formation of secondary

references