

Aromatic Rings Act as Hydrogen Bond Acceptors

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Simple energy calculations show that there is a significant interaction between a hydrogen bond donor (like the $> \text{NH}$ group) and the centre of a benzene ring, which acts as a hydrogen bond acceptor. This interaction, which is about half as strong as a normal hydrogen bond, contributes approximately 3 kcal/mol (1 cal = 4184 J) of stabilizing enthalpy and is expected to play a significant role in molecular associations. It is of interest that the aromatic hydrogen bond arises from small partial charges centred on the ring carbon and hydrogen atoms: there is no need to consider delocalized electrons. Although some energy calculations have included such partial charges, their role in forming such a strong interaction was not appreciated until after aromatic hydrogen bonds had been observed in protein-drug complexes.

1. Introduction

Hydrogen bonding interactions define the precise structure of biological macromolecules and their complexes. The energy of these interactions, which occur between a donor group ($-\text{O}-\text{H}$ or $> \text{N}-\text{H}$) and an acceptor group ($\text{O}-\text{C} <$, $-\text{O}-\text{H}$ or $\text{N} <$), depends on the separation and relative orientation of the groups of atoms. Because hydrogen bonds are primarily electrostatic in nature, arising as they do from the balance of attractive and repulsive forces between partial charges on the atoms of the groups involved (Coulson, 1952; Pauling, 1939; Hagler et al., 1974), groups other than those listed above could possibly act in hydrogen bonds.

A crystallographic study of the interactions of drugs with human haemoglobin showed the amino group of an asparagine residue pointing to the centre of the benzene ring of one of the drugs, suggestive of a hydrogen bond (Perutz et al., 1986). News of this discovery led Burley & Petsko (1986) to search for similar interactions between amino and aromatic groups in 33 highly refined protein structures. They concluded that they occur more frequently than random and may make a significant enthalpic contribution to protein stability. Later Tüchsen & Woodward (1987) found the distance between an asparagine amino group and the benzene ring of a phenylalanine in pancreatic

trypsin inhibitor, a structure refined at 1.2 Å (1 Å = 0.1 nm) resolution (Wlodawer et al., 1984), to be significantly shorter than the sum of their van der Waals' radii, thus providing *prima facie* evidence in favour of a hydrogen bond. We have investigated whether the energy of conventional electrostatic interactions between amino and benzene groups is strong enough to speak of a hydrogen bond.

Partial charges on the atoms in a molecule arise from differences in electron affinity. Hydrogen atoms lose electrons and consequently carry positive partial charges, while oxygen and nitrogen atoms gain electrons and have negative partial charges. Because charge is transferred from one atom to the next through the covalent bond joining them, groups of tightly bonded atoms are often electrically neutral, with the positive partial charge on one atom of the group being balanced by the negative partial charge on the other atom. Because of this balancing, carbon atoms are negatively charged when bonded to hydrogen atoms and positively charged when bonded to oxygen or nitrogen atoms.

Consistent partial charges can be chosen to reproduce the sublimation energies and packing geometries of hydrocarbon, amide and carboxylic acid crystals (Hagler et al., 1974; Lifson et al., 1979). In these parameter sets, oxygen and nitrogen atoms carry negative partial charges of -0.4 and -0.33 electron, respectively. Hydrogen atoms bonded to carbon atoms in both aliphatic and aromatic

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hydrocarbon molecules carry positive partial charges of +0.12 electron (Williams & Starr, 1977; Warshel & Lifson, 1970). Without these partial charges on C-H groups it is not possible simultaneously to reproduce the energies and packing geometries of hydrocarbon crystals.

2. Methods

The potential functions found to work well for simulations on liquid water (Berendsen *et al.*, 1981; Jorgensen, 1981; R. Sharon & M. Levitt, unpublished results), organic crystals (Warshel & Lifson, 1970; Hagler *et al.*, 1974) and macromolecules (Levitt, 1983) treat interactions between non-bonded atoms as a simple sum of 2 terms. The van der Waals' interaction (in kcal/mol; 1 cal = 4.184 J) between atoms i and j is a Lennard-Jones 12-6 term given by:

$$U_{\text{vdW}} = \epsilon \{ (r_0/r)^{12} - 2(r_0/r)^6 \},$$

where r (in Å) is the separation of the 2 atoms; ϵ (in kcal/mol) is the depth of the minimum of the energy function; and r_0 (in Å) the separation at which the minimum occurs. Both ϵ and r_0 are energy parameters which depend on the type of interacting atom. The electrostatic interaction (in kcal/mol) between atoms i and j is a Coulombic term given by:

$$U_{\text{els}} = 332 \times q_i q_j / r,$$

where q_i and q_j are the partial charges on the atoms (in fractions of the electronic charge).

The simplicity of these 2 energy terms means that it is very easy to evaluate the interaction energy of an amino

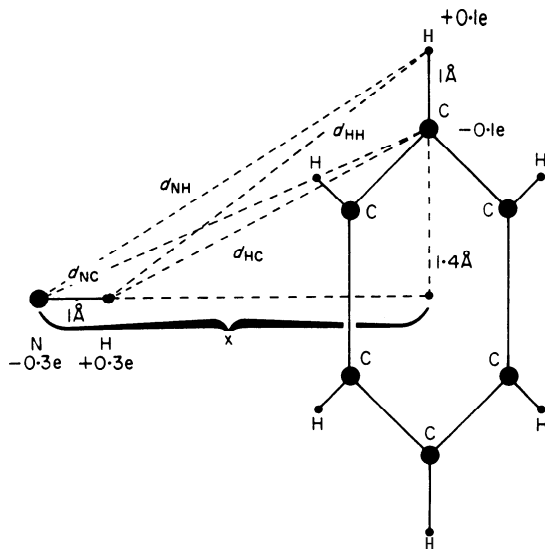


Figure 1. The idealized geometry of interaction of a >N-H group with a benzene ring. The >N-H group lies on the 6-fold symmetry axis of the ring so that the interaction with each of the 6 >C-H groups is equivalent. As the C-C bond length is 1.4 Å, each carbon atom is 1.4 Å from the ring centre; as the C-H bond length is 1 Å, each hydrogen atom is 2.4 Å from the ring centre. The 4 distances involved in the energy calculation can each be expressed in terms of x , the distance from the centre of the ring to the nitrogen atom as follows: $d_{\text{NH}} = (x^2 + 2.4^2)^{1/2}$; $d_{\text{NC}} = (x^2 + 1.4^2)^{1/2}$; $d_{\text{HC}} = ((x-1)^2 + 1.4^2)^{1/2}$ and $d_{\text{HH}} = ((x-1)^2 + 2.4^2)^{1/2}$.

>N-H group and a benzene ring using idealized geometry where the >N-H bonds lie on the symmetry axis of the ring (see Fig. 1). The van der Waals' interaction between the amino >N-H group and the ring >C-H group consists of only one significant term, that between the nitrogen and the carbon: the van der Waals' interactions with the hydrogen atoms are negligible (Hagler *et al.*, 1974):

$$U_{\text{vdW}} = \epsilon \{ (r_0/d_{\text{NC}})^{12} - 2(r_0/d_{\text{NC}})^6 \},$$

where d_{NC} is the separation of the N and C atoms. The electrostatic interaction between the amino >N-H group and the ring >C-H group, consists of 4 significant terms, 2 repulsive between charges of the same sign and 2 attractive between charges of opposite sign:

$$U_{\text{els}} = 332 \times q_{\text{N}} q_{\text{C}} (1/d_{\text{NC}} + 1/d_{\text{HH}} - 1/d_{\text{NH}} - 1/d_{\text{HC}}),$$

where d_{HH} , d_{NH} and d_{HC} are the separations of atom pairs H...H, N...H and H...C atoms, respectively.

The total energy for all 6 interactions is then given by:

$$U_{\text{total}} = 6 \times U_{\text{els}} + 6 \times U_{\text{vdW}}.$$

3. Results and Discussion

Figure 2(a) shows the variation of the energy of interaction with distance of the nitrogen atom from the centre of the ring. The interaction has a clearly

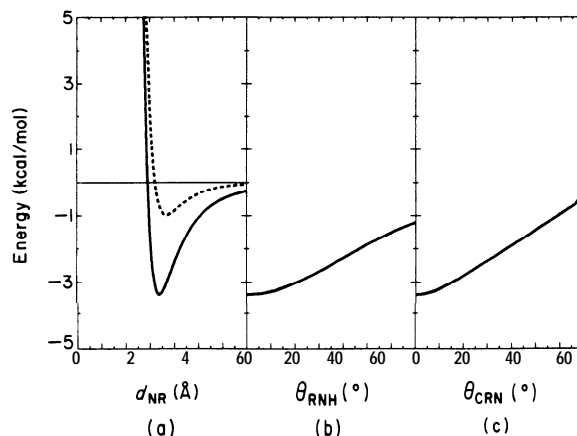


Figure 2. (a) The variation of the amino-benzene interaction energy with distance of the >N-H group from the centre of the benzene ring; the distance to the H atom is 1 Å closer. The continuous line shows the total energy, while the dotted line shows the van der Waals' energy. The energy minimum of -3.35 kcal/mol occurs at a N to ring-centre separation of 3.40 Å. (b) The variation of energy as the H atom of the >N-H group is rotated off the ring axis changing the angle R...N-H, where R is a point at the centre of the ring. (c) The variation of energy as the entire >N-H group is rotated off the ring axis changing the angle C...R...N, while keeping the >N-H group pointed towards R.

The energy parameters used here have the following values: $q_{\text{H}} = 0.119e$; $q_{\text{C}} = -0.199e$; $q_{\text{N}} = -0.333e$; $q_{\text{H}} = 0.333e$; $r_{\text{NC}}^0 = 4.013 \text{ Å}$; $\epsilon_{\text{NC}} = 0.125 \text{ kcal/mol}$; $r_{\text{NH}}^0 = 3.469 \text{ Å}$; $\epsilon_{\text{NH}} = 0.038 \text{ kcal/mol}$ (values of r_0 and ϵ for a pair of different atom types are taken as the geometric mean of the values for pairs of the same type, using $r_{\text{HH}}^0 = 2.852 \text{ Å}$; $\epsilon_{\text{HH}} = 0.038 \text{ kcal/mol}$; $r_{\text{NN}}^0 = 3.8171 \text{ Å}$; $\epsilon_{\text{NN}} = 0.41315 \text{ kcal/mol}$; $r_{\text{CC}}^0 = 4.2202 \text{ Å}$ and $\epsilon_{\text{CC}} = 0.03763 \text{ kcal/mol}$.

Table 1
How the values of the energy parameters affect the strength of the aromatic hydrogen bond

q_N	Energy parameter			R_{\min} (Å)	Strength?		(kcal/mol) <i>Els</i>
	q_H (electrons)	r_0 (Å)	ϵ (kcal/mol)		U_{\min} Total	VdW	
0.333†	0.119	4.01	0.125	3.40	-3.4	-0.7	-2.7
0.33	0.12	3.6	0.1	2.90	-4.4	-0.3	-4.1
0.33	0.12	3.8	0.1	3.12	-3.7	-0.3	-3.4
0.33	0.12	4.0	0.1	3.35	-3.2	-0.4	-2.8
0.33	0.12	4.2	0.1	3.58	-2.7	-0.5	-2.2
0.33	0.12	3.8	0.2	3.26	-4.1	-1.2	-2.9
0.33	0.12	4.0	0.2	3.49	-3.6	-1.2	-2.4
0.33	0.10	3.8	0.1	3.16	-3.2	-0.5	-2.7
0.33	0.14	3.8	0.1	3.08	-4.3	-0.2	-4.1
0.33	0.16	3.8	0.1	3.05	-4.9	-0.1	-4.8

† The strength of the aromatic hydrogen bond is measured by U_{\min} , the minimum energy, and R_{\min} , the $>NH \dots$ ring centre separation at which the minimum occurs. VdW is the van der Waals' energy, Els is the electrostatic energy and Total is the total energy.

‡ This entry uses the same energy parameters as used for Fig. 1.

defined minimum energy; the position and depth of which depend on the energy parameters as shown in Table 1. For values of the energy parameters that encompass all reasonable values, the optimum distance from the ring centre to the N atom varies from 2.9 to 3.6 Å, and the minimum energy varies from -2.7 to -4.9 kcal/mol. At least 75% of this interaction energy arises from the electrostatic terms. In their study of amino-aromatic contacts in highly refined globular proteins, Burley & Petsko (1986) found a preponderance of distances of 3.3 Å.

Our more complete calculations, which do not assume 6-fold symmetry and treat all 14 atoms of the system explicitly, confirm these findings and also give the angular dependence of the interaction. Rotating the N-H group so that the H atom moves off the ring axis while the N atom stays on it, makes the interaction less favourable: for a 60° rotation the interaction is -1.5 kcal/mol opposed to -3.3 kcal/mol for no rotation (Fig. 2(b)). Rotating the entire N-H group off the ring axis while keeping the N-H directed towards the ring centre has a larger effect on the interaction energy: for a 60° rotation, the interaction energy is

-1.0 kcal/mol, depending on whether the N-H group moves towards a C atom or towards the midpoint between adjacent C atoms (Fig. 2(c)). The optimum distances of the N atom from the ring plane is almost constant (to within 0.2 Å) provided that these rotation angles are less than 40°.

Is this interaction strong enough to qualify as a hydrogen bond? The aromatic acceptor group gives rise to a hydrogen bond that is about half the strength of a normal hydrogen bond (see Table 2). At room temperature, the amino-benzene interaction energy of about -3 kcal/mol is five times the room temperature thermal energy ($ET = 0.6$ kcal/mol for $T = 300K$) and can be expected to play a significant role in stabilizing molecular interactions.

In our study, the aromatic group acts as a hydrogen bond acceptor because of the partial charges on the carbon and hydrogen atoms and the 6-fold symmetry of the ring, which amplifies a weak interaction. There is no need to invoke lone-pair electrons, or a delocalized electron cloud in the centre of the ring. Because of the partial charges, the $>C-H$ group can also act as weak hydrogen bond donors; the partial charges on the $>C-H$ group are about one-third of those on the $>N-H$ group, so that the $>C-H \dots$ aromatic ring interaction will be weaker, with energies of approximately -1 kcal/mol. Such interactions also occur frequently in proteins (Burley & Petsko, 1985; Singh & Thornton, 1985). The interaction found here with a model benzene group should also occur in the amino acids phenylalanine, tyrosine and tryptophan, although the precise energy of interaction will depend on the values of the partial charges and on the symmetry of the ring system.

4. Conclusion

Benzene rings and other aromatic systems can interact with hydrogen bond donors such as NH,

Table 2

Comparison of strength of the aromatic hydrogen bond to that of other commonly occurring hydrogen bonds

Interaction	Atoms involved	Energy (kcal/mol)
Water. . . water	H-OH. . . $\begin{array}{c} O-H \\ \diagdown \\ H \end{array}$	-6.6
Peptide. . . peptide	$>N-H \dots O-C<$	-5.8
Water. . . peptide	H-O-H. . . $O-C<$	-7.0
Peptide. . . water	$>N-H \dots \begin{array}{c} O-H \\ \diagdown \\ H \end{array}$	-5.0
Amino. . . benzene	$>N-H \dots \begin{array}{c} \bigcirc \\ \bigcirc \end{array}$	-2.7 to -4.9

Values are taken from Hermans et al. (1984); calculations with our potential yield similar values.

giving rise to hydrogen bonds of about half normal strength. This interaction arises from the common van der Waals' and electrostatic interaction between atoms. There is no need to consider delocalized electron clouds, but it is important to include all the hydrogen atoms, especially those on non-polar atoms.

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