Solvent Influence on the Stability of the Peptide Hydrogen Bond: A Supramolecular Cooperative Effect

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Ab initio calculations at the Hartree–Fock 6-31G and 6-31G* levels are used to determine the effect of additional hydrogen-bonding groups on the strength of the *N*-methylacetamide dimer hydrogen bond. Results are given for one, two, or three hydrogen bonds involving water, ethanol, ethylene glycol, and trifluoroethanol. The cooperative effect due to multiple hydrogen bonds can be as large as 5.8 kcal/mol; the magnitude of the effect depends on the nature and the number of ligands. Ethylene glycol and trifluoroethanol are found to lead to larger stabilization than does water, which is of interest in view of experimental observations of the effect of these solvents on peptides and proteins. The observed cooperativity also has implications for the stability of helices, multiply stranded β-pleated sheets, and supramolecular structures involving hydrogen bonds.

Hydrogen bonds make important contributions to supramolecular recognition. Protein hydrogen bonds involving the peptide backbone are essential for the existence of the native structure,[1-3] but there is considerable uncertainty concerning their contribution to stability.[4-9] Most analyses,[6,9-11] including simulation studies,[12,13] have focused on the ability of water molecules to compete with peptide hydrogen bonds. In this Letter, we report *ab initio* results that show that a variety of solvents used for peptides and proteins (e.g., water, ethanol, ethylene glycol, and trifluoroethanol) strengthen peptide hydrogen bonds. The cooperative stabilizing effects are sufficiently large that they must be considered in any analysis of solvent denaturation or the enhancement of helix stability in peptides and proteins.[1,5,14-17] Moreover, they suggest that it may sometimes be necessary to introduce corrections to the pairwise additive potentials used in most simulations.[18] Although the existence of a cooperative effect in peptide hydrogen bonding has been discussed in earlier work,[2] only limited calculations have been made.[19] More extensive studies with improved quantum mechanical methods are needed to verify and extend the available results. This is particularly important because of recent papers that question the role of peptide hydrogen bonds in stabilizing secondary structure.[8,20,21]

As a model system for the peptide bond, we use two antiparallel *N*-methylacetamide (NMA) molecules (Figure 1a). We compare their hydrogen bond energy with that obtained when the exposed donor and/or acceptor group(s) are interacting with one or more solvent molecules (Figure 1b). The difference between the energy required to break the hydrogen bond between the two NMA molecules in the presence of additional ligands and in the absence of ligands is referred to as the cooperative effect, ΔE_{coop}. As donors (Y, Y') we use water (W), ethyl alcohol (EA), ethylene glycol (EG), and trifluoroethanol (TFE); as an acceptor (X) we use water (W). In addition, we consider formamide (FA) as a donor and/or acceptor and *N*-methylacetamide as an acceptor to mimic α-helical cooperativity effects.

The results are given in Table 1. Most of the complexes were studied at the HF/6-31G level. For some cases, HF/6-31G* calculations were made, and the importance of basis set superposition corrections was examined. Table 1 also lists values for HF/6-31G*(est) which were obtained by scaling the HF/6-31G energies by 0.78. From test calculations given in footnotes *a* and *b* of Table 1 and elsewhere,[22] the differences between the estimated and true HF/6-31G* energies are expected to be very small (≤0.1 kcal/mol). The HF/6-31G*(est) energies are of particular interest because they provide results for polar interactions of the type considered here, which are in good agreement with larger basis set, correlated *ab initio* calculations.[23,24] The HF/6-31G* method is now widely used for estimating water interactions with polar groups of biological interest.[25,26] The calculations with corrections for the basis set superposition error are given in footnotes *a* and *c* of Table 1; the correction reduces ΔE_{coop} by about 6% for the cases studied.

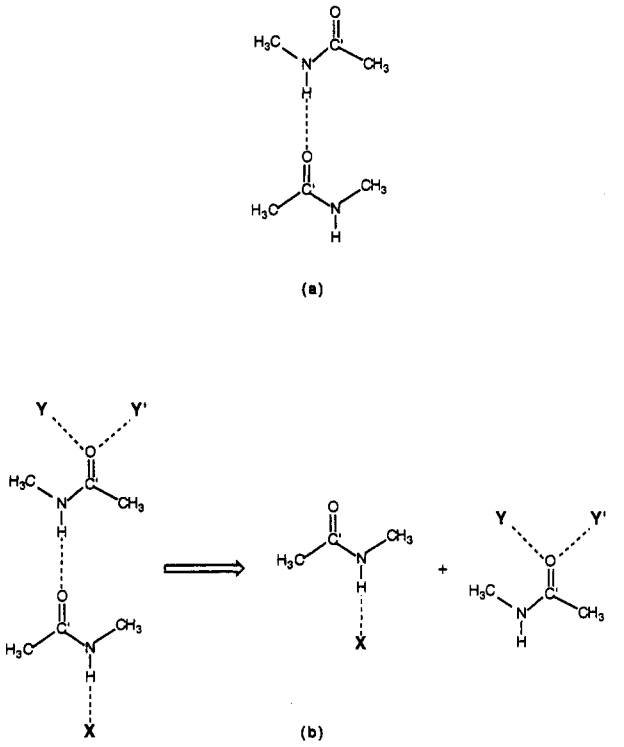

Figure 1. (a) Hydrogen bonding two antiparallel NMA molecules. (b) Breaking the hydrogen bonding between two NMA molecules in the presence of ligands.

Table 1 shows that the value of ΔE_{coop} correlates approximately with the number of ligands. For one donor or acceptor, ΔE_{coop} can be as large as 2.1 kcal/mol; for two ligands the range of ΔE_{coop} is 1.9–4.6 kcal/mol; and for three ligands, ΔE_{coop} varies from 3.6 to 5.8 kcal/mol. Different ligands yield different ΔE_{coop} values, and there is no simple correlation between the strength of the hydrogen bond between NMA and the ligand and the magnitude of ΔE_{coop}. For example, FA as a donor generally led to larger cooperative energy than W, even though the NH group in FA is a weaker hydrogen bond donor than OH in the W. Of

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TABLE 1: Cooperativity Correction to the Hydrogen Bonding of Two NMA Molecules (kcal/mol)

ligand[a]			ΔE_{coop}	
X	Y	Y′	6-31G	6-31G*(est)[b]
	W		1.1	0.8
		W	1.0	0.8
	W	W	1.9	1.5
		FA	2.0	1.5
	W	FA	3.0	2.3
		TFE	2.0	1.5
W			1.4	1.1
W	W		2.7	2.1
W	W	W	3.6[c]	2.8
W		FA	3.9	3.0
W	W	FA	5.0	3.9
W		EA	2.7	2.1
W		EG[d]	3.6	2.8
W	EG[e]	EG[e]	4.4	3.4
FA			1.9	1.5
FA	W	W	4.2	3.3
FA		FA	4.6[c]	3.6
FA	W	FA	5.8	4.5
FA		EA	3.2	2.5
FA		EG[d]	4.2	3.3
FA	EG[e]	EG[e]	5.0	3.9
NMA			2.1	1.6

[a] The interaction energy of the isolated NMA dimer (Figure 1a) at the 6-31G level is –8.7 kcal/mol; this is reduced to –7.5 kcal/mol with a correction for the basis set superposition error, and the 6-31G* value is –6.8 kcal/mol. Information on the calculations is given in ref 33. [b] The numbers are HF/6-31G*(est) values, obtained as described in the text; calculated HF/6-31G* values were obtained for X = W, Y = W, Y′ = W (2.8 kcal/mol) and for X = FA and Y′ = FA (3.6 kcal/mol), in agreement with the 6-31G* (est). [c] Correction for the basis set superposition error (Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553) yields 3.4 kcal/mol for X = W, Y = W, Y′ = W and 4.3 kcal/mol for X = FA and Y′ = FA. [d] tGg′ conformation. See: Van Alsenoy, C.; Van Den Enden, L.; Schäfer, L. *J. Mol. Struct.* **1984**, *108*, 121. [e] g′Gg′ conformation. There is multiple hydrogen bonding between the C=O group of NMA and the two O–H groups of EG (i.e., only a single EG involved).

particular interest is the fact that the stabilizing effects induced by the OH groups of EG and TFE are significantly greater than that of the OH group of W. For example, ΔE_{coop} for Y′ = TFE is 0.9–1 kcal/mol larger than those for Y′ = H_2O or Y = H_2O. Similarly, for X = H_2O and Y′ = EG, ΔE_{coop} is 0.9 kcal/mol larger than for X = Y = H_2O. By contrast, the same ΔE_{coop} value was found for the cases involving water and ethanol.

The results of the *ab initio* calculations on a model system for peptides in the presence of certain solvent molecules suggest that there is a significant cooperative effect that can enhance the strength of peptide hydrogen bonds. This needs to be considered in estimating the stability of α-helices and β-pleated sheets in solution. Since the stability of a given structure involves the balance of the interactions in that structure with the interactions of the components with water when the structure is unfolded, solvent cooperative interactions must also be considered; this will be treated in a subsequent paper. Of particular interest is the stronger cooperative interaction with the peptide hydrogen bond of certain ligands, such as EG and TFE, relative to water. This may contribute to the ineffectiveness of EG as a denaturant[5,14] and the helix-enhancing properties of TFE,[15-17] although other factors are likely to be involved as well.[8,16,17,28,29] The cooperative effect involving TFE could play a role in its selective binding to the helical conformation of peptides; for a recent experimental study, see Jasanoff and Fersht.[29] The relatively large ΔE_{coop} induced by NMA is expected to increase the stability of helices and of multiply-stranded β-pleated sheets. It may also contribute to stabilizing higher aggregates of NMA.[10,30] Since the net enthalpy for helix formation in water has been estimated to be about 1–1.5 kcal/mol per residue,[31] the cooperative effect within a helix (1.5–2 kcal/mol) and between carbonyl groups of a helix and the solvent (0.5 kcal/mol) could too be significant. Finally, the self-organization of supramolecular systems, such as nanotubules constructed from cyclic peptides,[32] is expect to be aided by cooperative interactions.

Future work will consider interactions with denaturing solvents like urea and the competition between the bonding of structured water and peptide groups in determining the stability of biologically relevant supramolecular structures.

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(32) All calculations were done with the GAUSSIAN 90 program on a CRAY-YMP at the Illinois Supercomputing Center. Full geometry optimization was performed for NMA and NMA–NMA complexes in the presence or absence of ligands; the only approximation in the geometry optimization is the use of C_s symmetry. For the complexes containing EG or TFE, the C_s symmetry requirement was relaxed for the internal structure of EG(TFE) as well as for the H-bond geometry between NMA and EG(TFE). The NMA molecules in the complexes were assumed to have a staggered *N*-methyl group and an eclipsed *C*-methyl group with respect to the *C*′–N bond; see ref 22.