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VOLUME 72

Peptide Solvation and H-Bonds

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Peptide Solvation and H-Bonds

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The Importance of Cooperative Interactions and a Solid-State Paradigm to Proteins: What Peptide Chemists Can Learn from Molecular Crystals

J. J. DANNENBERG

NEW DIRECTIONS IN THE STUDY OF PEPTIDE H-BONDS AND PEPTIDE SOLVATION

There are two main reasons for the rapid growth of research into the energetics of peptide H-bonds and peptide solvation: to help solve the problem of protein structure prediction and to complete the quantitation of the energetics of folding. For a long time protein chemists have accepted that the hydrophobic interaction (burial of nonpolar surface area through folding) is the major source of free energy driving folding, as proposed by Kauzmann (1959), but there has always been a nagging doubt about the role of peptide H-bonds. Even if each H-bond contributes only 0 ± 1 kcal/mol, which is sometimes used as a guesstimate, approximately 70% of the peptide groups in globular proteins make H-bonds (Stickle *et al.*, 1992) and a contribution of ± 70 kcal/mol to ΔG for folding of a 100 residue protein would make an enormous difference to its stability. The ΔH for forming an alanine peptide helix (-0.9 ± 0.1 kcal/mol per residue) has been measured accurately by titration calorimetry and it must arise from the peptide H-bond, not from burial of nonpolar surface, because of its very small ΔCp (see chapter by Makhatadze). Thus, peptide H-bonds may make a substantial favorable contribution to the enthalpy of protein folding. Moreover, every H-bonding group must make a H-bond, either within the folded protein or else to water, because the penalty for burying a free H-bonding group is large ($\sim$6 kcal/mol; Fleming and Rose, 2005). Thus, the drive for continued rapid progress in protein structure prediction (Kuhlman *et al.*, 2003), which requires a fuller understanding of protein-folding energetics, brings peptide H-bonds and peptide solvation into central focus.

Three chapters of this volume, by Dannenberg, Morozov and Kortemme, and Weinhold, deal with the problem of using quantum mechanics to represent H-bonds. Dannenberg reviews the lessons learned from analyzing chains of H-bonds in molecular crystals of small molecules such as formamide and urea. Morozov and Kortemme discuss the properties of H-bonds seen in protein structures and compare them to properties predicted by quantum mechanics. Weinhold tackles the structure of water by performing quantum mechanics on defined clusters of water molecules. These three chapters reach a common conclusion, namely that the long-standing electrostatic model of H-bond formation, which predicts linear H-bonds that are formed noncooperatively, is too simple and the H-bonds found in proteins, as well as in water clusters, are partly bent. The molecular mechanics force fields used commonly to simulate protein structures and dynamic behavior have fixed partial atomic charges

consistent with the older electrostatic model of H-bonds. Friesner tackles the problem of making a force field that can adjust to the newer view of H-bonds by allowing the partial charges on atoms to vary through induced polarization. He reports that the first results of using this force field to represent protein docking reactions are promising as regards the H-bonds formed. Hermans comments on the recent development of a method for performing quantum mechanics on an entire protein and discusses issues raised in the three other chapters, such as whether Weinhold's ''new view'' of water structure can be reconciled with the older view of water–water H-bonds resembling those found in ice. Im, Chen, and Brooks provide a tool for rapid calculation of peptide solvation in proteins as they review computation of protein electrostatics by use of generalized Born methods. Solvation of the polar peptide group is electrostatic in character and may be predicted by electrostatic algorithms, provided the protein or peptide structure is known accurately.

Each of three experimental chapters deals with a recent experimental method of investigating peptide H-bonds and peptide solvation. Powers, Deechongkit, and Kelly review the first energetic results of making mutations (amide to ester) that eliminate peptide H-bonds. Makhatadze summarizes recent calorimetric studies of peptide helix formation and considers their implications for the energetics of protein folding. White discusses the energetics of shaping membrane proteins based on the results of inserting peptides into lipid bilayers. The twin problems of making H-bonds and accounting for any free H-bonding groups take on a new character when the newly formed protein resides in a nonaqueous environment. Valuable lessons can be learned about the nature of folding energetics in aqueous solution.

This list of chapters raises some questions, the most evident one being: why are peptide H-bonds treated here by quantum mechanics or by introducing a new force field while peptide solvation is handled as a problem in electrostatics? Why is not peptide solvation treated as the problem of making H-bonds between water molecules and free peptide —NH and —C═O groups? There are various answers to this question, the first being that probably quantum mechanics will soon be used to study H-bonds between water and peptide —NH and —C═O groups. Weinhold looks ahead to possible methods of tackling this problem. A second answer is that if liquid water is considered to be a giant network of H-bonded water molecules, then it is just too big for present methods of performing quantum mechanics. Weinhold suggests one approach for breaking this problem into parts. A third answer is that backbone electrostatics, which result from the large partial charges on the atoms of the peptide —NH and —C═O groups, are important in other problems besides peptide

solvation, notably in the docking reactions of proteins with small ligands, other proteins, and nucleic acids. Note that amide solvation is a directly measurable quantity for small amides, as demonstrated by the pioneering work of Wolfenden (1978), and the solvation of polar groups in small molecules has been treated successfully as an electrostatic problem. The parameters of the electrostatic algorithm DelPhi are calibrated specifically to reproduce the solvation free energies of polar small molecules (Sitkoff *et al.*, 1994). When DelPhi is used to investigate how peptide solvation depends on backbone conformation and on the presence of specific neighboring residues in short peptides, surprising and interesting results are found (Avbelj and Baldwin, 2004).

As noted earlier, the theoretical chapters on representing H-bonds in proteins, as well as in water clusters, agree that a paradigm shift is under way. Morozov and Kortemme trace the evolution of the change in viewpoint. Linear electrostatic H-bonds, formed by atoms with fixed partial charges, are very convenient for rapid computation using standard force fields and the paradigm shift will not take place without a struggle. That peptide H-bonds are linear was assumed by Pauling and co-workers (1951) in their landmark paper predicting the structure of the α-helix. It is sometimes said that the business of a scientist is to introduce new ideas into the field. Readers of this volume will find that long-standing and basic assumptions of structural biology are being challenged.

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Robert L. Baldwin

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POTENTIAL FUNCTIONS FOR HYDROGEN BONDS IN PROTEIN STRUCTURE PREDICTION AND DESIGN

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Abstract

Hydrogen bonds are an important contributor to free energies of biological macromolecules and macromolecular complexes, and hence an accurate description of these interactions is important for progress in biomolecular modeling. A simple description of the hydrogen bond is based on an electrostatic dipole–dipole interaction involving hydrogen-donor and acceptor–acceptor base dipoles, but the physical nature of hydrogen bond formation is more complex. At the most fundamental level, hydrogen bonding is a quantum mechanical phenomenon with contributions from covalent effects, polarization, and charge transfer. Recent experiments and theoretical calculations suggest that both electrostatic and covalent components determine the properties of hydrogen bonds. Likely, the level of rigor required to describe hydrogen bonding will depend on the problem posed. Current approaches to modeling hydrogen bonds include knowledge-based descriptions based on surveys of hydrogen bond geometries in structural databases of proteins and small

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molecules, empirical molecular mechanics models, and quantum mechanics-based electronic structure calculations. *Ab initio* calculations of hydrogen bonding energies and geometries accurately reproduce energy landscapes obtained from the distributions of hydrogen bond geometries observed in protein structures. Orientation-dependent hydrogen bonding potentials were found to improve the quality of protein structure prediction and refinement, protein–protein docking, and protein design.

I. Introduction

Accurate modeling of hydrogen bonding interactions is critical for progress in protein structure prediction, protein–protein docking, and protein design. While the large number of hydrogen bonds in proteins and protein interfaces underlines their importance, there may be no net gain in free energy for hydrogen bond formation in protein folding and binding; the formation of hydrogen bonds between protein atoms results in the loss of hydrogen bonds made with water. Most polar groups in the protein interior form hydrogen bonds to satisfy their hydrogen bonding potential (Baker and Hubbard, 1984; McDonald and Thornton, 1994). These requirements result in considerable energetic and structural constraints and are in part responsible for the regular backbone–backbone hydrogen bonding patterns of α-helix and β-sheet regular secondary structure elements (Pauling and Corey, 1951). Similarly, hydrogen bonds, particularly side chain–side chain hydrogen bonds, are thought to play important roles in the specificity of macromolecular interactions (Lumb and Kim, 1995; Petrey and Honig, 2000) and need to be taken into account in the prediction of protein interaction preferences. Hydrogen bonds may be crucial for enabling a unique three-dimensional protein conformation or binding mode in protein design applications (Looger *et al.*, 2003; Lumb and Kim, 1995).

What is needed for an accurate description of hydrogen bonding interactions within and between proteins? The physical nature of hydrogen bonds is complex, and calculation of electrostatics, polarization, exchange repulsion, charge-transfer, and coupling contributions to hydrogen bonding energetics (Kollman, 1977; Morokuma, 1971; Singh and Kollman, 1985; Umeyana and Morokuma, 1977) from first principles is not straightforward for biological macromolecules. Likely, the level of rigor required to explain certain molecular properties in question will depend on the problem posed. Which simplifications can be made in which context? An example discussed in detail in this chapter is the orientation dependence of hydrogen bonds, which has been a subject of considerable debate. An electrostatic dipole–dipole model of a hydrogen bond would predict a

linear arrangement of the donor and acceptor dipoles. However, a ''lone pair'' concept would imply directionality of the hydrogen bond (Fig. 1a). What are the structural and energetic characteristics of hydrogen bonds in protein structures and how can a model be devised that reproduces them?

Any simplified description of hydrogen bonds in biological molecules needs to be tested by comparing its predictions against a large body of experimental data, preferably obtained from macromolecules. A direct comparison of predicted and observed hydrogen bonding energies in biological macromolecules is not straightforward because the individual components of the free energy cannot readily be measured independently in experiments. More feasible but less direct strategies rely on the vast information available on protein sequences and structures and use concepts from computational protein design, protein structure prediction, and protein–protein docking. The structure prediction and docking tests

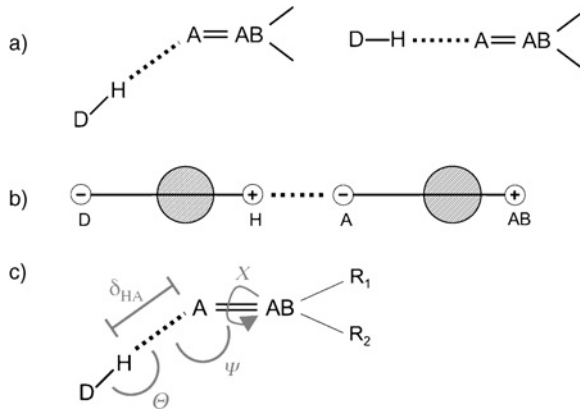

FIG. 1. Mechanism and orientation dependence of hydrogen bond formation. A, acceptor; D, donor; H, hydrogen; AB, acceptor base. (a) Orientation dependence of hydrogen bond formation. Hydrogen bond formation along lone-pair directions would predict hydrogen bonding geometries such as the one shown on the left, whereas an electrostatically dominated mechanism based on a dipole–dipole interaction (see b) would favor the linear arrangement on the right. (b) Simple description of hydrogen bonding interactions as the interaction of two dipoles with atom-centered partial point charges. Shaded spheres represent electron density at H and AB shifted along the H–D and AB–A covalent bonds toward more electronegative atoms, resulting in the appearance of partial charges on all four atoms. (c) Schematic representation of hydrogen bond geometry. D, donor atom; H, hydrogen atom; A, acceptor atom; AB, acceptor base; R_1, R_2, atoms bound to the acceptor base. Geometric parameters used here to describe hydrogen bonds are as follows: δ_{HA} (Å), distance between hydrogen and acceptor atoms; Ψ (degree), angle at the acceptor atom; θ (degree), angle at the hydrogen atom; X (degree), dihedral angle around the A–AB axis.