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Intramolecular Hydrogen Bonding in Simple Diamides

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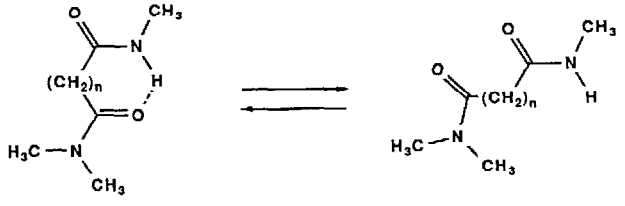
Abstract: Intramolecular hydrogen bonding is detected in a homologous series of diamides by NMR and IR spectroscopy. Variable temperature NMR experiments reveal a particularly favorable interaction when the hydrogen bond involves a nine-membered ring.

We are studying equilibria in solution between hydrogen bonded and non-hydrogen bonded conformations of the simple diamides indicated below. This system has been chosen as a starting point for exploring the effects of incremental changes in structure and environment on the intramolecular amide-amide hydrogen bond, an interaction that helps to determine three-dimensional protein structure.



1, n = 1
2, n = 2
3, n = 3
4, n = 4
5, n = 5
6, n = 6

In the early 1960s, Klotz and Franzen[2] showed that there was no tendency for intermolecular hydrogen bonding between simple secondary amides in aqueous solution. Extrapolating from this model study, these workers argued that amide-amide hydrogen bonds could not contribute to the enthalpic driving force for protein folding, because the folding process simply replaces water-amide hydrogen bonds with hydrogen bonds between amide groups on the protein and water-water hydrogen bonds. Indeed, it is now widely accepted that the driving force for protein folding is largely a hydrophobic effect.[3]

A more likely role for intramolecular hydrogen bonding is to enforce the *specificity* of the protein folding pattern. Once amide moieties are buried in the relatively non-polar core of the folded structure, conformations in which potential hydrogen bond donors and acceptors are isolated are disfavored relative to conformations in which donors and acceptors are paired. Hydrogen bond strength will be affected by the geometry with which bonding partners are juxtaposed; therefore, the structure-specifying role of amide-amide hydrogen bonding in protein folding cannot be fully understood without a knowledge of how the interaction energy varies as a function of donor-acceptor orientation. In its most essential form, the biostructural role of amide-amide hydrogen bonds reduces to a question of physical organic chemistry.

One approach to deducing the optimum geometric requirements for amide-amide hydrogen bonding involves statistical analysis of the copious crystallographic data available for proteins. The results of an extensive survey were recently reported,[4] showing a range of possible values for such parameters as N-H--O and C=O--H angles. The trends manifested in these data provide some insight on preferred donor-acceptor orientation, but conclusions based on these observed preferences must be tentative. Any hydrogen bond observed in a protein core

is affected by the energetic demands of non-covalent interactions (attractive and repulsive) involving neighboring groups. When hydrogen bond geometric parameters observed in crystal structures vary from predicted optimum values, one cannot know the energetic cost of the deviation or how that cost is paid (i.e., what competing non-covalent interactions have achieved a more optimal geometry).

In order to gain further insight into the effects of donor-acceptor orientation on intramolecular amide-amide hydrogen bonding, we are studying flexible molecules under conditions in which hydrogen bonding exerts an influence on conformation (non-polar solvent). Flexibility is a key feature here, because thermodynamic information must be derived by monitoring an equilibrium between bonded and non-bonded forms. The effects of incremental structure variation on internal hydrogen bonding should provide insight into how bond strength responds to changes in spatial juxtaposition and environment of the donor and acceptor amide groups.

Before using proton NMR to probe our diamides for intramolecular hydrogen bonding, we needed to determine the concentration range in which intermolecular interactions would be minimal. Shown in figure 1 is the concentration dependence of the amide NH chemical shift of N-methylacetamide (NMA) in 9:1 carbon tetrachloride/benzene-d_6 and methylene chloride-d_2 (data obtained at room temperature, ca. 293 K). Because exchange between bonded and non-bonded forms is rapid on the NMR time scale, only a single, averaged signal is observed for the proton bound to nitrogen. The behavior we observe by NMR in 9:1 carbon tetrachloride/benzene-d_6 correlates well with near infrared data reported by Klotz and Franzen for NMA in pure carbon tetrachloride.[2] Our control studies show that measurements made at ambient temperatures on solutions of diamides **1-6** at concentrations of 10 mM or less in methylene chloride-d_2 should be free of the effects of intermolecular association.

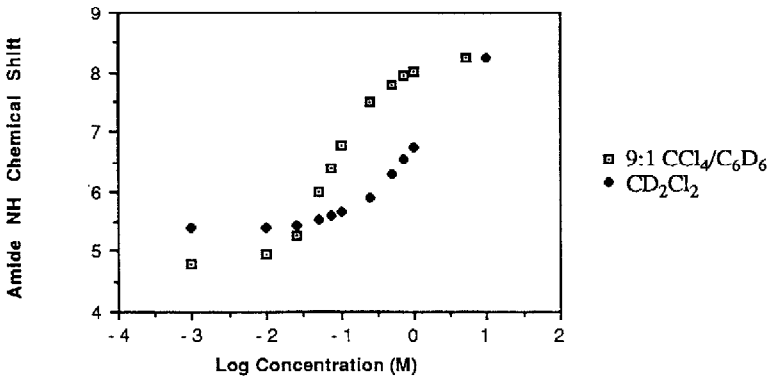


Figure 1. Concentration dependence of the amide NH chemical shift of N-methylacetamide in CD_2Cl_2 and 9:1 CCl_4/C_6D_6.

Figure 2 shows the temperature dependence of the amide proton chemical shifts measured with 1 mM solutions of compounds **1-6** in methylene chloride-d_2 over the range 193-273 K. Also shown are the data for a 1:1 mixture of N-methylacetamide and N,N-dimethylacetamide (NNDMA) (1 mM in each component). At a constant temperature, any downfield shift in the amide proton resonance of a diamide, relative to the amide proton signal from the 1:1 mixture of monoamides, should be due to intramolecular hydrogen bonding. Our data suggest

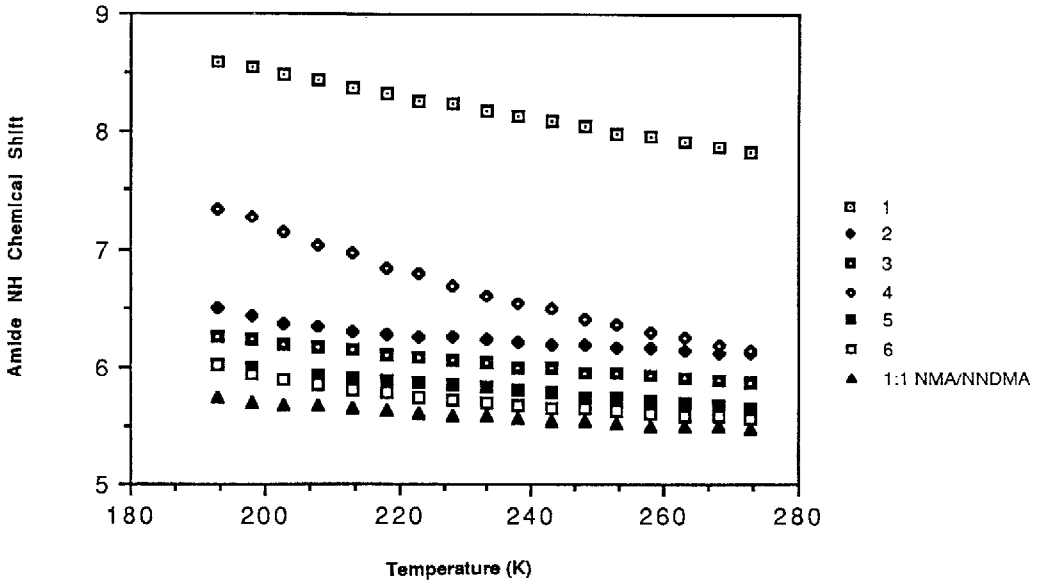


Figure 2. Temperature dependence of diamide NH chemical shift (samples 1 mM in CD_2Cl_2).

that all six diamides are engaged in internal hydrogen bonding, although the interaction is minimal for **6**. This qualitative picture is corroborated by the IR spectra of these diamides (10 mM solutions; spectra obtained at ambient temperature). Because IR measurements have a shorter time scale, amide protons equilibrating between hydrogen bonded and non-hydrogen bonded forms show characteristic N-H stretch bands for each form. Compound **1** shows only a signal for a hydrogen bonded N-H, and compounds **2-5** show bands for free and hydrogen bonded N-H. The IR spectrum of **6** is ambiguous; it is impossible to rule out a small amount of the hydrogen bonded form.

For an amide experiencing conformational equilibrium between hydrogen bonded and non-hydrogen bonded forms, the temperature dependence of the NH chemical shift should reflect, in part, any temperature dependent variation in the equilibrium constant for internal hydrogen bonding. The most striking aspect of the data in figure 2 is the unique and relatively steep temperature dependence shown by **4**. Diamides **2**, **3**, **5** and **6** show smaller temperature dependences, similar to that displayed by the 1:1 NMA/NNDMA mixture. These data suggest that diamide **4** can adopt an internally hydrogen bonded conformation that is particularly favorable enthalpically, since the temperature dependence of the equilibrium constant derives from an enthalpy term ($\ln K_{eq} = \Delta S/R - \Delta H/RT$). Increasing enthalpic favorability of intramolecularly hydrogen bonded conformations as the ring size grows from six (diamide **1**) to nine (diamide **4**) can be rationalized as resulting from improved spatial juxtaposition of the donor (N-H) and acceptor (dimethylamide C=O) in the larger ring. In particular, molecular models indicate that the nine membered ring is the smallest that allows the N-H--O angle to approach 180^{o}, which is expected to be optimal.[4] These enthalpic differences cannot be ascribed wholly to changes in hydrogen bond donor-acceptor orientation, however, since internal steric interactions will also vary with ring size.

The observation of a particularly favorable hydrogen bonded conformation in nine-membered rings may be biologically relevant, since the side chain amide moieties of asparagine and glutamine residues in polypeptides can engage in nine-membered ring internal hydrogen bonds with proximal main chain amide groups. The internal hydrogen bonds observed in many peptide γ- and β-turns occur in seven- and ten-membered rings, respectively. There has been considerable debate over whether these amide-amide hydrogen bonds are gratuitous or contribute to the stability of the turn structures.[5] Our results suggest that hydrogen bonds in seven- and ten-membered rings may not contribute significantly to the stability of peptide turns. Stronger conclusions about the roles of seven-, nine- and ten-membered ring amide-amide hydrogen bonds in determining protein structure require experiments with appropriate oligopeptides; these studies are currently underway in our laboratory.

In principle, we should be able to deduce an equilibrium constant for formation of internally hydrogen bonded conformations from the amide proton chemical shift of any diamide experiencing partial hydrogen bonding. The temperature dependence of the equilibrium constant should then allow access to the entropy and enthalpy associated with hydrogen bonded ring formation. However, extraction of these thermodynamic parameters from the variable temperature NMR data for diamides **1-6** is complicated by several factors. The allowed orientations of hydrogen bond donor and acceptor vary across the series, and we do not know how this variation will affect the limiting chemical shift for a fully hydrogen bonded amide proton. Further, even when an amide proton is locked in a hydrogen bond, its chemical shift has a residual temperature dependence.[6] This intrinsic temperature dependence should also vary as a function of donor-acceptor orientation.

Despite these ambiguities, it is clear from our data that small changes in intramolecular juxtaposition of amide hydrogen bonding partners can have a significant impact on the driving force for hydrogen bond formation. Using the data for the 1:1 mixture of NMA and NNDMA as the limiting behavior for a non-hydrogen bonded amide proton and the data for **1** as the limiting behavior for a fully hydrogen bonded amide proton, we estimate the observed internally hydrogen bonded conformation(s) of diamide **4**, in methylene chloride-d_2, to be enthalpically favored by roughly 1.3-1.4 kcal/mol, but entropically disfavored by 7-8 e.u.

Although the analysis of chemical shift temperature dependences has become a very popular method for probing whether or not amide protons are hydrogen bonded, we are not aware of any study in which such data have been used to analyze the thermodynamics of hydrogen bond formation. Experiments aimed at an accurate dissection of the energetics of intramolecular hydrogen bond formation in diamides **1-6** are in progress.

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