Folding Pattern of a Succinyl and a Glutaric Glycine Derivative in Chloroform

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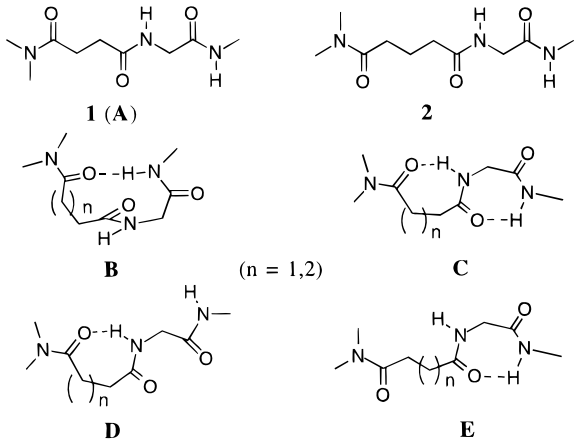
The side chain of an Asn residue at position 1 of a *β-turn* can readily hydrogen bond with the NH group of residue 3.[1] Four interactions of this type were identified in carboxypeptidase, involving Asn or Asp.[2] These side chain C═O (*i*) to NH (*i* + 2) interactions are referred to as forming *Asx turns*.[3] Marraud and co-workers studied in depth the conformational aspects of *Asx turns* using statistics, molecular mechanics, and solution structures.[4] It was found that the *Asx turn* conformation was not retained when a Glu or a Gln is substituted for an Asp or an Asn. Recently, Imperiali et al. proposed that the conformational features of the *Asx turn* is central to the N-linked glycosylation process catalyzed by the enzyme *oligosaccharyl transferase* (OT).[5,6]

We wish to report our findings of the difference in the conformational preference between a succinic glycine derivative (**1**) and a glutaric glycine derivative (**2**). The two model peptides were prepared during our study of hydrogen bonding cooperativity.[7] Each triamide can form several conformations (A−E) through intramolecular hydrogen bonding. On the basis of the IR and ^{1}H NMR data an *Asx turn* type of folding pattern (**B**) is identified to be the enthalpically favored conformation in chloroform for both triamide **1** and **2**.[8] The entropic contribution to the free energy of the folding process (from **A** to **B**) is small for triamide **1** and substantially more negative for triamide **2**.

Gellman and co-workers have carried out extensive studies on similar model peptides in CH_2Cl_2 and in $CHCl_3$ and have documented spectroscopic data on various hydrogen-bonding patterns.[9−11] Our identification of the



conformational preference of compounds **1** and **2** relies in large part on the comparison of the IR and NMR data to that in the literature. The infrared spectra (NH stretching region) for triamide **1** and **2** at high and low temperatures are shown in Figure 1. Spectra were recorded at 1 mM concentration in $CDCl_3$ on a Perkin-Elmer 1650 spectrometer.[8] The sharp absorption at ∼3452 cm^{-1} is assigned to free amide NH stretching and the broad band at ∼3350 and ∼3320 cm^{-1} to the intramolecular amide−amide hydrogen-bonded NH stretching through a 10- and an 11-membered ring, respectively.[9] The broad band at ∼3407 cm^{-1} of Figure 1c, d is due to a weak intramolecular hydrogen bond through a C_5 conformation (**A**).

The variable-temperature ^{1}H NMR data for triamide **1** and **2** are shown in Figure 2. The chemical shifts of the amide protons are plotted as a function of temperature. The ^{1}H NMR experiments were also carried out in $CDCl_3$ at 1 mM concentration. At room temperature the two NH protons of triamide **1** were distinguished by a two-dimensional ^{1}H NMR experiment (COSY). At lower temperatures, they can be identified by their coupling patterns. The internal NH proton (a triplet) of both triamide **1** and **2** exhibits relatively upfield chemical shifts (6.2−6.5 ppm) and change very little with temperature (○ and ◇ in Figure 2). In contrast, the terminal NH proton (a quartet) of triamide **1** gives substantially downfield chemical shifts (7.3−7.9 ppm) at all temperatures (● in Figure 2). The terminal NH proton of triamide **2** (◆ in Figure 2) shows a large temperature dependence ($\Delta\delta$NH/ΔT = −0.013 ppm/K) of chemical shift.

The temperature dependence of the amide NH proton chemical shifts has become a useful tool in the study of peptide conformations.[12] However, one must be careful with the interpretation because the implication changes with the solvents of the NMR experiments and the structure of the peptides.[13,14] For similar model peptides in CH_2Cl_2 or in $CHCl_3$, an intramolecularly hydrogen-bonded NH proton exhibits chemical shifts of approximately 7−8 ppm while a free NH proton is around 6 ppm.[9−11]

(1) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167−339. Abbreviations: Asn = asparagine, Asp = aspartate, Gln = glutamine, Glu = glutamate, Gly = glycine.

(2) Rees, D. C.; Lewis, M.; Lipscomb, W. N. *J. Mol. Biol.* **1983**, *168*, 367−387.

(3) Baker, E. N.; Hubbard, R. E. *Prog. Biophys. Mol. Biol.* **1984**, *44*, 97.

(4) Abbadi, A.; Mcharfi, M.; Aubry, A.; Premilat, S.; Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1991**, *113*, 2729.

(5) Imperiali, B.; Shannon, K. L.; Unno, M.; Rickert, K. W. *J. Am. Chem. Soc.* **1992**, *114*, 7944.

(6) Imperiali, B.; Spencer, J. R.; Struthers, M. D. *J. Am. Chem. Soc.* **1994**, *116*, 8424.

(7) Gung, B. W.; Zhu, Z. *Tetrahedron Lett.* **1996**, *37*, 2189. Experimental details and spectroscopic data for compounds **1** and **2** can be found in the supporting information.

(8) These IR spectra are recorded at 1 mM concentration in $CDCl_3$. Gellman has shown that the amide−amide hydrogen bonding pattern is similar in chloroform and in methylene chloride for several di- and triamides.[11] We consider the solvent-exposed NH proton to be free since chloroform is a nonpolar solvent. Deuterated chloroform is IR transparent from 3000 to 3600 cm^{-1}, which is the NH stretching region, crucial for our investigation. Methylene chloride, however, has a strong absorption at 3090 cm^{-1}. We have found that the base-line noise can be greatly reduced using $CDCl_3$ as the solvent. This is important because highly dilute solutions are required to study intramolecular hydrogen bonding. At 1 mM concentration, even low level of noise from the base line can make a big difference. For example, in order to obtain an IR spectrum with reasonable quality, 128 scans are required when CH_2Cl_2 is used as the solvent. However, with $CDCl_3$ only 32 scans are needed for a good spectrum.

(9) Gellman, S. H.; Dado, G. P.; Liang, G.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164.

(10) Gallo, E. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 9774−9788.

(11) (a) Gellman, S. H.; Adams, B. R.; Dado, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 460. (b) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228−4245.

(12) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, 512.

(13) Stevens, E. S.; Sugawara, N.; Bonara, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048.

(14) Winningham, M. J.; Sogah, D. Y. *J. Am. Chem. Soc.* **1994**, *116*, 11173−11174.

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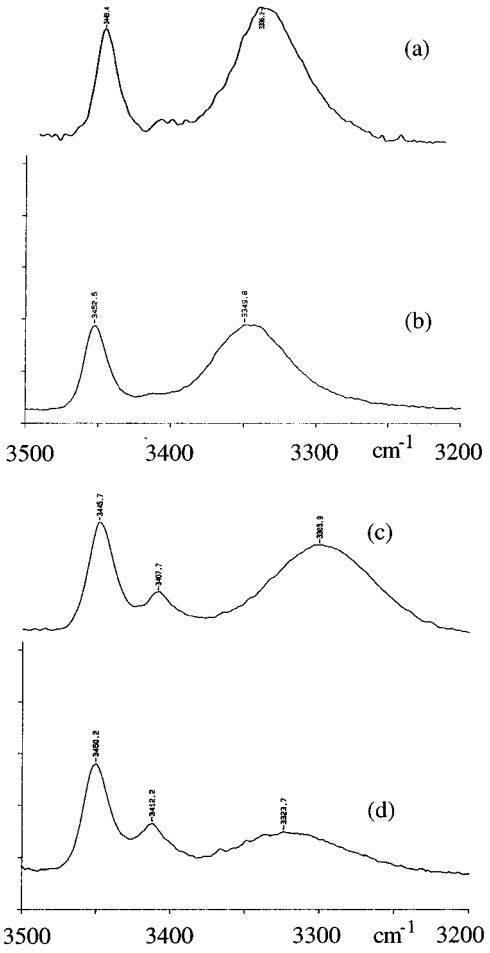

Figure 1. NH stretching region of the IR spectra for triamide **1** (a) at 213 K (b) at 298 K, and **2** (c) at 213 K (d) at 298 K. Spectra were recorded at 1 mM concentration in $CDCl_3$ on a Perkin-Elmer 1650 spectrometer.[8]

On the basis of the documented cases, a large proportion of the terminal NH proton of triamide **1** is intramolecularly hydrogen bonded at all temperatures. For triamide **2**, the terminal NH proton is largely free at higher temperatures, but becomes more and more hydrogen bonded as the temperature is lowered. The internal amide NH proton of triamide **1** remains predominantly free throughout the temperature range. However, the internal amide NH proton of triamide **2** is involved in a C_5 conformation that gives rise to a broad band at 3407 cm^{-1} in the IR spectra, Figure 1. This weak intramolecular hydrogen bond decreases in intensity as the temperature is lowered.

On the basis of the above analysis, two conformations cannot be enthalpically favored, the straight chain form **A** and the seven-membered ring **D.** The terminal NH proton is free in these forms, contrary to the indication of the ^1H NMR data. Conformation **C** can also be ruled out because the terminal and the internal NH protons do not have similar chemical shifts and temperature dependence. Although conformation **E** utilizes the terminal NH proton to form a hydrogen bond, a C_7 conformation (*γ-turn*) is not observed in $CHCl_3$ for nonproline residues.[15] Thus, the head-to-tail intramolecularly hydrogen bonded form **B** is consistent with the IR and ^1H NMR data, but the other conformations are not.

(15) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. P.; Scheraga, H. A. *Biopolymers* **1979**, *18*, 2507.

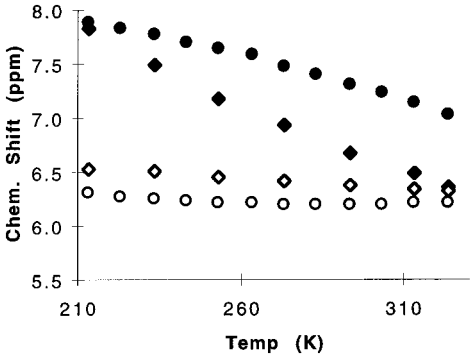

Figure 2. Internal (○ = **1**, ◇ = **2**) and terminal (● = **1**, ◆ = **2**) NH proton chemical shifts as a function of temperature for triamides **1** and **2**.

The terminal NH proton of triamide **1** exhibits substantial downfield chemical shifts throughout the temperature range. However, triamide **2** has a greater reduced temperature coefficient than triamide **1**. The infrared spectra (Figure 1) indicate that triamide **1** has more proportions of intramolecular hydrogen bonds than triamide **2** at 25 °C. Therefore, it appears that triamide **1** assumes predominantly conformation **B** at all temperatures. The corresponding glutaric glycine derivative **2**, on the other hand, experiences an equilibrium between the hydrogen-bonded and nonbonded states. Conformation **B** becomes more populated at lower temperatures when the entropic effect becomes smaller. A van't Hoff analysis of the ^1H NMR variable-temperature data using the *temperature-dependent* upper and lower limits of chemical shifts from the literature (Figure 20 in ref 11b) gives a ΔH of -1.7 ± 0.5 kcal/mol and a ΔS of -5.7 ± 2 eu for the equilibrium between the nonhydrogen-bonded states and conformation **B** for triamide **2**. Since the IR spectra indicate that the intramolecular hydrogen bond of triamide **1** is nearly complete in chloroform at all temperatures, thermodynamic parameters cannot be obtained from the temperature-dependent data. A temperature-independent intramolecular hydrogen bond is an indication of either a small entropic effect or an overwhelmingly enthalpically favored hydrogen bond or a combination of both. In the present case, both entropic and enthalpic factors appear to be at work. Thus, conformation **B**, the *Asx turn* type of folding pattern, is enthalpically favored for both triamide **1** and **2** but is entropically disfavored for triamide **2**.

We have shown that in $CDCl_3$ both triamide **1** and **2** prefer conformation **B**, an *Asx turn* type of intramolecular hydrogen bonding, enthalpically. It is interesting to note that there is little internal hydrogen bonding in diamides derived from pimelic and suberic acids,[9] which are analogs of triamides **1** and **2**, respectively. This difference between the diamides and the present triamides indicates that the 10- and 11-membered ring hydrogen bonds in the triamides must somehow be strongly promoted by the central amide group.

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Supporting Information Available: Experimental procedures and ^1H NMR spectra for compounds **1** and **2** (5 pages).

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