Conformational Analysis

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# Inherent Conformational Preferences of Ac-Gln-Gln-NHBn: Sidechain Hydrogen Bonding Supports a β-Turn in the Gas Phase

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***Abstract:*** *Gas-phase single-conformation spectroscopy is used to study Ac-Gln-Gln-NHBn in order to probe the interplay between sidechain hydrogen bonding and backbone conformational preferences. This small, amide-rich peptide offers many possibilities for backbone–backbone, sidechain–backbone, and sidechain–sidechain interactions. The major conformer observed experimentally features a type-I β-turn with a canonical 10-membered ring C=O—H–N hydrogen bond between backbone amide groups. In addition, the C=O group of each Gln sidechain participates in a seven-membered ring hydrogen bond with the backbone NH of the same residue. Thus, sidechain hydrogen-bonding potential is satisfied in a manner that is consistent with and stabilizes the β-turn secondary structure. This turn-forming propensity may be relevant to pathogenic amyloid formation by polyglutamine segments in human proteins.*

Neurodegenerative disorders such as Huntington's disease (HD) seem to arise from extension of CAG codon repeats, which produce polyglutamine (poly-Gln) segments upon translation. Long poly-Gln segments are highly prone to aggregation into β-sheet-rich fibrils. The fundamental studies reported here are motivated by the hypothesis that elucidating the intrinsic conformational propensities of glutamine-rich peptides will provide a physicochemical foundation for understanding of poly-Gln-driven disease pathogenesis.[1–4]

Recent studies have identified the formation of β-turns and β-hairpins in poly-Gln segments as critical to fibril formation. Wetzel and co-workers showed that placement of β-hairpin-promoting moieties at the center of a polyglutamine tract accelerates aggregation and reduces the number of molecules required to nucleate aggregation.[5,6] These studies, however, involved the use of glutamine-free turn-forming units; therefore, the contribution of local β-turn formation to fibril formation by pure poly-Gln segments remains uncertain. The potential for β-turn/β-hairpin formation in glutamine-rich segments is relevant not only to fibrillar assembly but also to the formation of oligomeric species that may be critical to poly-Gln-associated neurotoxicity.[7–13]

Here we describe an unusual experimental strategy for assessing intrinsic folding preferences in the short peptide Ac-Gln-Gln-NHBn (Figure 1 a). Using single-conformation laser spectroscopy, we have examined the inherent conformational preferences of this capped, two-residue peptide, which is the smallest glutamine-rich molecule whose backbone can form a β-turn. These studies have been conducted in the gas phase, in which only intramolecular noncovalent interactions are possible.[14–17] Our results demonstrate that, in the absence of intermolecular interactions with water or other peptides, the dominant observed conformer of Ac-Gln-Gln-NHBn contains a type-I β-turn, with each glutamine sidechain carbonyl forming a hydrogen bond to the nearest NH in the backbone (Figure 1 b). If β-turn formation within a poly-Gln segment is a rare event that initiates the misfolding pathway,[5] our work raises the possibility that a nonpolar environment, such as

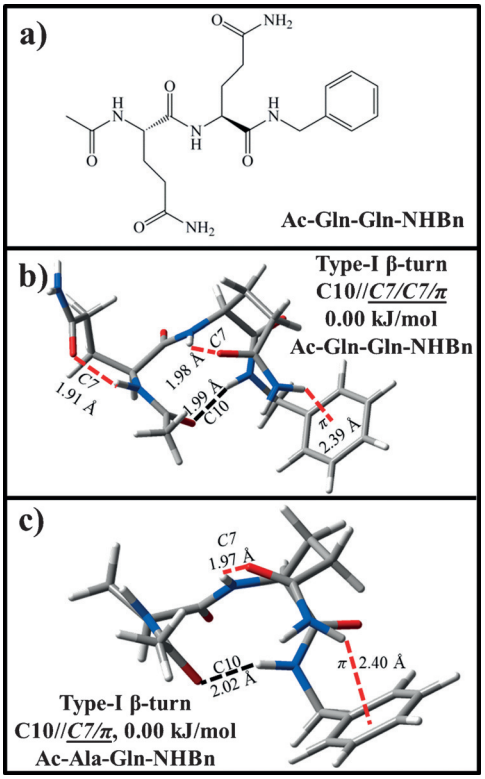


***Figure 1.*** a) The chemical structure and b) experimentally assigned type-I β-turn (C10//C7/C7/π) structure of Ac-Gln-Gln-NHBn. c) For comparison, the type-I β-turn (C10//C7/π) assigned to the sole experimentally identified structure of Ac-Ala-Gln-NHBn. Structures were calculated at the DFT M05-2x/6-31 + g(d) level of theory.[18]

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that provided by a membrane interior, may encourage misfolding.

Related previous studies revealed sidechain-to-backbone and backbone-to-backbone hydrogen bonding patterns that are most prevalent for an isolated glutamine residue.[18] The current study of Ac-Gln-Gln-NHBn was aimed at probing the more complex interplay among internal interactions when sidechain-to-sidechain hydrogen bonding is possible. The naming scheme used in this work is retained from the earlier study on Ac-Ala-Gln-NHBn, by listing the hydrogen bonds by type ("Cn" = an *n*-membered H-bonded ring), ordered from *N*- to C-terminus as follows: backbone–backbone// *sidechain–backbone*// **sidechain–sidechain**.[18]

Figure 2a shows the R2PI (resonant two-photon ionization) spectrum of Ac-Gln-Gln-NHBn recorded in the $S_0$–$S_1$ origin region of the -NHBn UV chromophore (ca. 37450–37800 $cm^{-1}$). The spectrum shows several sharp transitions that appear on a background that likely arises due to

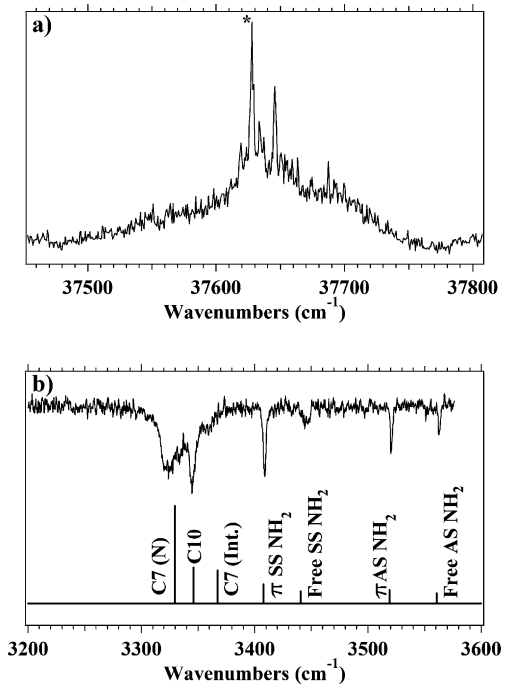


***Figure 2.*** a) R2PI spectrum of Ac-Gln-Gln-NHBn recorded in the monomer mass channel ($m/z = 405$) in the -NHBn $S_0$–$S_1$ origin region. b) RIDIR spectrum recorded in the NH-stretch region for the major conformer, with labeled stick spectra displaying the scaled harmonic frequencies for the assigned C10//*C7/C7/π* type-I β-turn conformer (0.9406). The starred transition (*) was used to record the RIDIR spectrum.

incomplete cooling of a fraction of the laser-desorbed molecules in the molecular beam, as deduced from changing desorption conditions. The transition at 37628 $cm^{-1}$ is the $S_0$–$S_1$ origin transition for the single identified conformer for Ac-Gln-Gln-NHBn. Figure 2b shows the experimental RIDIR (resonant ion-dip infrared) spectrum in the NH-stretch region. The other sharp peaks in the R2PI spectrum possess the same RIDIR spectrum, indicating that only one conformer is responsible for them. Five well-resolved IR transitions are evident at 3562, 3522, 3445, 3404, and 3345 $cm^{-1}$, while a sixth asymmetrically broadened transition at 3324 $cm^{-1}$ shows a shoulder centered around 3334 $cm^{-1}$ (see Table S1 in the Supporting Information). Comparison of the experimental spectrum with the calculated frequencies and infrared intensities for the global minimum structure—a type-I β-turn C10//*C7/C7/π* conformation—shows excellent agreement in both absolute frequency and intensity pattern (Table S1 and Figure 2b).

Note that both Gln sidechains engage in C7 hydrogen bonds with the peptide backbone, thereby forming a tightly held and highly stable β-turn. The close agreement between the experimental and calculated IR spectra allows the transitions to be readily assigned. The transitions at 3562 and 3445 $cm^{-1}$ are the free antisymmetric and symmetric stretches of the $i+1$ Gln-$NH_2$ moiety, respectively. The π-bound antisymmetric and symmetric -$NH_2$ stretch fundamentals of the $i+2$ Gln are shifted slightly to lower frequency from their nominally free positions, to 3521 and 3409 $cm^{-1}$, respectively due to formation of a π H-bond. The final closely spaced set of broadened transitions arise from the strongly hydrogen-bonded backbone amide NH groups. The transitions at 3345, 3334, and 3324 $cm^{-1}$ are assigned to the *C7* ring formed between the interior NH and the $i+2$ Gln sidechain, the backbone–backbone C10, and the *C7* formed by the $i+1$ Gln sidechain and the N-terminal NH, respectively (Table S1 and Figure 2b).

These three hydrogen bonds work in concert to stabilize the structure. Their NH stretch fundamentals are shifted down in frequency by about 20–40 $cm^{-1}$ relative to the corresponding C10/C7 pair in Ac-Ala-Gln-NHBn (Figure S1).

The type-I β-turns formed by Ac-Ala-Gln-NHBn and Ac-Gln-Gln-NHBn are structurally similar (Figure 1b and c, Figure S1, Table S2), but there is both spectroscopic and computational evidence for a synergistic stabilization induced by the pair of C7 H-bonds between the two Gln sidechains and the peptide backbone. The *C7* H-bond formed by the $i+1$ Gln C=O group to the N-terminal NH is calculated to be unusually short (1.91 Å), and this is reflected in a lowering in the frequency of its NH stretch fundamental by about 30 $cm^{-1}$ relative to the corresponding C7 H-bond in Ac-Ala-Gln-NHBn (Figure S1, Tables S2 and S3).[18] A similar shift is observed in the C10 H-bond that characterizes the β-turn, with calculated reduction in length from 2.02 to 1.99 Å.[18] These transitions are also broadened significantly compared to non-hydrogen-bonded NH stretch fundamentals as a result of the strong hydrogen-bonding interaction.

The observed gas-phase structure can be directly compared with naturally occurring type-I β-turns of sequence Xaa-Gln-Gln-Yaa from crystal and NMR structures of proteins.[19] Table S3 provides the dihedral angles for the $i+1$ and $i+2$ residues for eight such structures. It is immediately clear from this comparison that the gas phase and condensed phase structures share the same backbone arrangement, confirming the gas phase structure as an accurate representation of folding patterns common to Gln-Gln segments in condensed phases. The crystal and NMR structures collec-

  

tively manifest a degree of inhomogeneity in the β-turns that is not observed in the Ac-Ala-Gln-NHBn or Ac-Gln-Gln-NHBn gas phase structures, due to interactions of Gln sidechains with their surroundings in crystals or solution, a factor that is irrelevant in the gas phase. Our observation of a specific pattern of Gln sidechain-to-backbone hydrogen bonding in the gas phase, and the evidence that such peripheral hydrogen bonds stabilize the β-turn, raises the possibility that nonpolar environments promote turn formation and ultimately fibril formation by glutamine-rich segments of proteins.

In order to better understand the role of β-turn structures in the larger context of other possible structures for the isolated molecule, the energy level diagram shown in Figure 3 was constructed that summarizes the relative energies of all structures within 37 kJ mol$^{-1}$ of the global minimum calculated at the DFT M05-2X/6-31 + g(d) level of theory. This diagram is surprisingly sparse in its low energy region, with only 8 structures observed in the first 15 kJ mol$^{-1}$. Even more striking, of these eight, four are type-I β-turns, as are three of the lowest four, including the global minimum, which is the single observed conformation. It is clear on this basis that the β-turn is preferred over other structures for Ac-Gln-Gln-NHBn in non-polar environments. The -NHBn cap used in this molecule contributes modestly to stabilizing the observed conformation through the NH···π hydrogen bond. As is shown in Figure 3 b, when the low energy structures are re-optimized with the -NHBn cap replaced by -NHMe, the assigned conformation is not the global minimum, but remains amongst the lowest energy structures.

The preference for β-turn formation in Ac-Gln-Gln-NHBn is all the more notable when compared with the preferences observed in other capped dipeptides studied to date that do not contain Gln residues.[20,21] In these studies similar β-turn motifs (C10) are observed experimentally in some cases, but the C10 structures are often minor conformers that are in competition with C5/C5 and C5/C7 hydrogen-bonded families. The presence of β-turn motifs is not surprising in small peptides because of the limited number of hydrogen bonding patterns available to peptides this size. However, the studies of Ac-Ala-Gln-NHBn and Ac-Gln-Gln-NHBn mark the first study on naturally occurring amino acids in which only a β-turn motif is observed.[20,21] We surmise on this basis that the glutamine sidechains are "locking-in" the type-I β-turn.

Although the assigned global minimum structure possesses no sidechain-to-sidechain hydrogen bonds, Ac-Gln-Gln-NHBn is the smallest molecule in which such interactions are possible. Indeed, such sidechain-to-sidechain hydrogen bonds are prevalent among the higher energy structures (Figures 3, S2, and S3). The most common calculated sidechain–sidechain interaction is the **C13** hydrogen bond formed

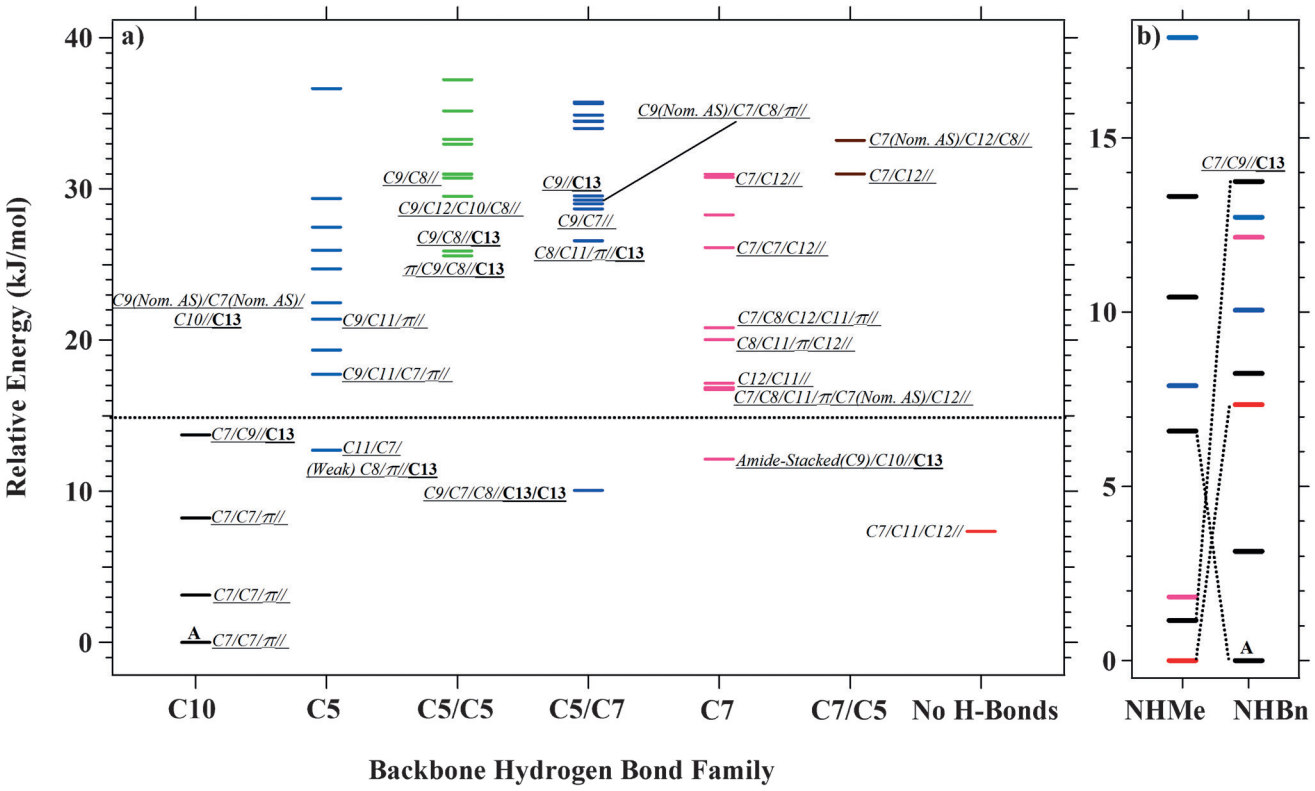


***Figure 3.*** a) Energy-level diagram containing all calculated structures within 37 kJ mol$^{-1}$ of the calculated global minimum at the DFT M05-2x/6-31 + g(d) level of theory. Structures are grouped by their backbone hydrogen-bonding architecture. Labels on individual structures list the sidechain–backbone (first, in italics) and sidechain–sidechain (second, in bold) hydrogen bonds, separated by a double slash (//). b) Comparison of the energies of low-energy (below dashed line in (a)) conformations calculated with the -NHBn or -NHMe caps. Backbone–backbone hydrogen-bond families are color-coded as in part (a).

between the -$NH_2$ and C=O groups of the two adjacent Gln sidechains. Close inspection of Figure 3 reveals that this type of interaction is possible in nearly all families of backbone geometries (C10, C5, C5/C5, C7, and C5/C7). In this sense, it appears that these sidechain-sidechain interactions are typically hydrogen bonds of convenience rather than interactions that uniquely stabilize a particular backbone conformation.

Although sidechain-to-sidechain interactions are important features of the aggregated fibril structures, the gas phase results point towards a diminished role for such interactions in non-aggregated poly-Gln segments. The low-energy region ($< 10\,kJ\,mol^{-1}$) of the calculated potential energy diagram (Figure 3) shows that the four lowest-energy structures ($< 10\,kJ\,mol^{-1}$) are devoid of any sidechain-to-sidechain interactions and instead contain exclusively backbone-backbone and sidechain-backbone H-bonds. The first calculated structure that contains a sidechain-sidechain hydrogen bond is a C5/C7//*C9/C7/C8*//**C13/C13** (Figure S2) structure calculated to be about $10\,kJ\,mol^{-1}$ above the global minimum.

It is notable that structural preferences manifested by isolated Ac-Gln-Gln-NHBn are evident to some degree also in the single-glutamine analogs Ac-Ala-Gln-NHBn and Ac-Gln-NHBn (Figure 1, Table S3). In all three molecules, low-energy structures involve the Gln sidechain C=O in a C7 H-bond with the backbone NH of that residue, an interaction that induces a turn in the backbone.[18]

The present results strongly suggest that a -Gln-Gln- segment in a nonpolar environment will readily and preferentially adopt a sidechain-stabilized β-turn. This folding behavior could be important for disease pathogenesis. Experimental efforts to characterize the forms of monomeric or oligomeric poly-Gln segments that are proposed to exert toxic effects are complicated by the transience of these hypothetical species. Moreover, such species are likely to occur at low abundance in the conformational equilibria available to poly-Gln segments in solution. For segments with a Gln repeat length beyond the threshold for disease onset, the "misfolded" monomers aggregate quickly to insoluble states, so that little of the proposed toxic forms would be present.[5,9–13] Available data for poly-Gln segments in solution suggest a predominance of random coil-like structures.[9] Since recent studies have identified formation of β-turn/β-hairpin structures as the bottle neck along the pathogenic pathway,[5–13] the present results suggest that transient entry of a short Gln-rich portion into a nonpolar environment, perhaps within a lipid bilayer, might lead to local β-turn formation and thereby promote intermolecular association of poly-Gln segments. Studies of longer Gln-rich peptides or of Gln-containing peptides with different capping arrangements would be helpful in seeking further tests of this intriguing possibility.

## Experimental Section

A full description of the experimental and theoretical approaches used in this study are found in the Supporting Information, including detailed descriptions of resonant two-photon ionization (R2PI) and resonant ion-dip infrared (RIDIR) spectroscopies.[14–16] Briefly, molecules of interest are vaporized using laser desorption, entrained in a supersonic expansion (Argon backing gas), and cooled to their vibrational zero-point level. The cold, isolated molecules then enter the extraction region of a time-of-flight mass spectrometer where they are interrogated with IR and UV laser pulses. UV excitation spectra are recorded using R2PI, while single-conformation IR spectra are provided by RIDIR spectroscopy. Experimental results were compared against scaled harmonic frequencies generated from calculations on structures optimized using DFT M05-2x/6-31 + g(d) in Gaussian09. Initial starting geometries for DFT optimization were generated using molecular dynamics calculations starting from geometries with hydrogen-bonded patterns consistent with experiment.[22–24]

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