1,2,3-triazole bridge as conformational constrain in β-hairpin peptides: Analysis of hydrogen-bonded positions

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Abstract

Conformational constrained β-hairpin peptides are useful tool to modulate protein-protein interactions. A triazole bridge in hydrogen bonded positions between two antiparallel strands induces a conformational stabilization of β-hairpin peptide. The entity of the stability of the β-hairpin peptide depends on the length of the bridge.

Secondary structure elements present on protein surface are very often involved in protein-protein interactions governing biomolecules activity and function. Small peptides able to reproduce these secondary structure elements are useful tools to modulate protein-protein interaction with potential application, for example, in chemical biology and medicine. It is well known that amino acid segments excised from the protein environment seldom reproduce the native conformation as they are unfolded in solution.[1] In this context, the design of conformational constrained peptides, that mimic secondary structure motifs, is key to obtain bioactive molecules[2] or robust scaffolds onto graft the interacting residues keeping the correct spatial orientation.[3]

The molecular factors governing β-hairpin folding and stability have been deeply studied and molecular tools to reproduce stable β-hairpin peptides have been reported.[4] Among the covalent tools to conformational stabilize a β-hairpin the closure of intramolecular triazole ring has been explored. A 1-4 disubstituted 1,2,3 triazole is obtained from side chains cyclization of alkyne and azide unnatural amino acids by means of Cu-catalyzed alkyne-azide cycloaddition (CuAAC),[5] forming a triazole ring linking the two antiparallel strands. This reaction is easy to perform, furnishing an enzymatic and chemical stable product in high yields, and does not need additional deprotection steps as the alkyne and azide functional groups are compatible with solid phase peptide synthesis protocol. In the last years, the CuAAC reaction gained much popularity and it has been widely employed for bioconjugation and biomolecules immobilization.[6] Recently, CuAAC has been also used to modify β-hairpin peptides. Holland-Nell and Meldal have demonstrated that the triazole can replace a disulfide bridge within β-hairpin structures,[7] Waters and co-workers reported a study on the conformational stability and function of the β-hairpin peptide modified by an interstrand triazole linkage,[8] and a triazolyl-bridged peptide mimicking the EGFR dimerization arm was developed, showing the inhibition of the allosteric activation of EGFR and an increased proteolytic stability.[9]

Recently, we reported the use of interstrand 1-4 disubstituted 1,2,3 triazole as chemical tools to increase the conformational and proteolytic stability in β-hairpin peptide.[10] In particular, we analyzed the conformational stability of a series of β-hairpin peptides presenting the triazole bridge, with variable lengths, in a non-hydrogen bonded position and determined the structural

requirements of the triazole bridge to achieve the greatest stabilization. The present study aims to evaluate the stabilizing effect of the triazole bridge in hydrogen-bonded (HB) positions of the β-hairpin and the dependence, if any, on the length of the bridge.

We analyzed a set of peptides which correspond to the previously described NHB peptides[10], based on Trpzip2 peptide[11], except for the position of the triazole bridge which was inserted in a hydrogen-bonded site (position 3 and 10) (Figure 1 and Figure S1).

The peptides were referred to as HB x.y, where x and y represent the number of methylene groups on the side chain of the alkyne and azido amino acids; they also correspond to the methylene unit in position 4 and 1 of the triazole ring, respectively. The full peptide sequences are reported in table S1. HB peptides were synthesized by solid phase peptide synthesis using Fmoc chemistry. The purified linear peptides were cyclized through a Cu-catalyzed azide-alkyne cycloaddition reaction. HPLC and LC-MS analyses are reported in figure S2 and S3.

The linear peptides and the corresponding cyclic peptides have the same molecular mass. As proof of intramolecular cyclization by CuAAC and the formation of 1,4 -disubstituted 1,2,3-triazole bridge we observed that: a) the HPLC retention times of cyclic peptides is shorter than the linear ones[12] (Table S2 and S3); b) the absence of odd multicharged peaks in the ESI-MS spectra characteristic of covalent dimer (Figure S3); c) cyclic peptides are stable to treatment with TCEP while linear peptide loss a mass of 26 Da as a consequence of the reduction of azide to amine[10,13] (see in Experimental section in supplementary information).

To assess the conformational properties of HB peptides a conformational analysis in aqueous solution was performed by NMR spectroscopy. In particular, linear and cyclic peptides were analyzed by using a combination of 1D ^{1}H, 2D [^{1}H, ^{1}H] TOCSY, 2D [^{1}H, ^{1}H] NOESY and 2D [^{1}H, ^{1}H] COSY spectra. The 1D ^{1}H NMR spectra of linear peptides display poor backbone amide protons (HN) chemical shift dispersion consistent with mostly unstructured conformations; instead, the ^{1}H NMR spectra of cyclic peptides showed a good degree of dispersion, as occurs in a folded peptide (Figure 2 and Figure S4). The conformational state of linear and cyclic peptides was confirmed by the analysis of the splitting of the Gly7 diastereotopic Hα proton in the 2D TOCSY

spectra. Particularly, linear peptides did not show the splitting of Gly7 Hα protons, confirming that linear peptides lack of turn structure, whereas cyclic peptides showed a significant Gly7 Hα splitting (Figure 3 and Table 1).

The conformational properties of linear and cyclic peptides were determined qualitatively by comparing Hα chemical shifts relative to random coil values.[14] The plots of Hα chemical shift difference (ΔδHα) values for each cyclized peptide in aqueous solution at room temperature have the characteristic signature of a β-hairpin, indicating the presence of two β-strands (from residue Trp2 to Glu5 and from Thr9 to Val11) separated by a turn region centered on residues Asn6 and Gly7 (Figure 4). In contrast, the closeness of Hα chemical shifts to their random coil values (ΔδHα < 0.1 ppm) indicate that linear peptides in aqueous solution behave as mainly random coil conformation (Figure S5).

Next, we investigated the influence of the number and position of substituent methylene units of the triazole ring on β-hairpin fold. The folded population content of each β-hairpin peptide was assessed with two methods. The first method estimates the extent of the diastereotopic Hα splitting of the turn residue Gly7 (ΔδGly).[15] The second method is based on the root mean square deviation (RMSD) values of Hα protons taken over all residues (RMSΔδHα).[16] These two methods examine different structural elements: the ΔδGly reflects the change in population of the turn residues, while RMSΔδHα values mainly reflect the contribution of residues in the β-strands.

The folded populations were determined relative to the ΔδGly (0.50 ppm) and the RMSΔδHα values of trpzip2. The list of the observed Gly7 Hα splitting and RMSΔδHα values, together with the calculated fraction of folded population for all cyclic peptides are reported in table 1, figure 5 and S6. The two methods resulted in good agreement; comparison of fraction-folded values derived from both Gly7 Hα splitting and the RMSΔδHα reveals that the cyclic peptides retain a folded β-hairpin in the range of 20−68% with respect to Trpzip2 population. The ΔδGly and the RMSΔδHα differ significantly for each cyclic peptide suggesting that β-hairpin content depends on the length of the triazole bridge with peptide HB3.3 showing the highest values between the HB peptide series (Figure 5).

To gain a more detailed depiction of HB 3.3 folded structure, examination of long-range NOEs between residues in anti-parallel strands of the β-hairpin was carried out. Several intense inter-strand H_N-H_N and H_α-H_N interactions (Table S4) are readily detected, constituting a strong evidence for HB 3.3 β-strand conformation.

Overall, the formation of the triazole bridge in a hydrogen-bonded position induces a substantial conformational stabilization of a β-hairpin peptide with respect to the linear peptide. This result is interesting considering that a covalent tool, as disulfide bridge, failed to induce a conformational stabilization in β-hairpin peptides when inserted in a HB position.[17] The length of the bridge affects the peptide stability. In particular, a short bridge (HB 1.1, HB 2.1, HB 1.2) is weakly β-hairpin stabilizer, while the peptide folding content rise increasing the number of methylene units, being HB 3.3 the most folded peptide.

Comparing the HB series with the previous reported NHB series[10] is evident that the triazole tool induces a similar energetic stabilization in both β-hairpin positions, being the β-hairpin content of the most folded peptide (HB 3.3 vs NHB 2.1) comparable in the two series (0.68 vs 0.60 respectively). However, the highest stabilization is achieved with two different triazole bridges (HB3.3 vs NHB2.1). In particular, it seems that the most constrained position (HB) requires a more flexible bridge to achieve the maximal β-hairpin stabilization.

In conclusion, the formation of a triazole bridge between two antiparallel strands induces a conformational stabilization of β-hairpin peptide when the tools is inserted in hydrogen bonded positions. The entity of β-hairpin stabilization depends on the length of the bridge, being maximum for longer bridge. This work completes our study on the determination of the optimal “triazole bridge” tool for the conformational stabilization of a β-hairpin peptide.

Experimental Section

Experimental details are reported as supplementary material.

Acknowledgements

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Legends

Figure 1. Generic molecular structure of HB peptides. The alkyne (Xaa) and azide (Yaa) amino acids are indicated in bold. Propargylglycine (Pra), homopropargylglycine (Hpg), bishomopropargylglycine (Bpg), L-β-azidoalanine (Dap(N3)), L-γ-azidohomoalanine (Dab (N3)), L-δ-azidoornitine (Orn(N3)).

Figure 2. ^{1}H NMR spectra of A) HB 3.3 peptide and B) HB 3.3 linear peptide in aqueous solution. Spectra were recorded at 400 MHz and at 298 K.

Figure 3. Section of the low field 2D [^{1}H-^{1}H]TOCSY spectra of cyclic and linear HB 3.3 peptides in water. Spectra were recorded at 400 MHz and 298 K.

Figure 4. Bar plot of the deviation of Hα chemical shift from random-coil values (ΔδHα) for Trpzip2 and cyclized HB peptides.

Figure 5. Fraction folded of peptides as determined by RMSΔδHα and ΔδGly Hα splitting values at 298 K, using limiting shift values derived from trpzip2.

TABLE

Table 1. Fraction folded values for the HB clicked peptides

Peptide	ΔδGly	Fraction folded[a]	RMSΔδHα	Fraction folded[b]
HB 1.1	0.10 (±0.02)	0.20 (±0.02)	0.11 (±0.03)	0.25 (±0.03)
HB 1.2	0.17 (±0.02)	0.34 (±0.01)	0.13 (±0.03)	0.30 (±0.01)
HB 1.3	0.18 (±0.04)	0.36 (±0.02)	0.19 (±0.02)	0.43 (±0.03)
HB 2.1	0.18 (±0.02)	0.36 (±0.03)	0.14 (±0.02)	0.32 (±0.02)
HB 2.2	0.28 (±0.02)	0.56 (±0.02)	0.23 (±0.03)	0.52 (±0.02)
HB 2.3	0.19 (±0.01)	0.38 (±0.02)	0.20 (±0.02)	0.45 (±0.02)
HB 3.1	0.22 (±0.02)	0.44 (±0.01)	0.18 (±0.04)	0.41 (±0.02)
HB 3.2	0.20 (±0.01)	0.40 (±0.01)	0.19 (±0.02)	0.43 (±0.02)
HB 3.3	0.34 (±0.03)	0.68 (±0.03)	0.27 (±0.03)	0.61 (±0.03)

[a] Fraction folded determined from ΔδGly Hα splitting values; [b] Fraction folded determined from the RMSΔδHα data.

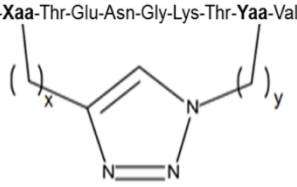
Text for Table of Contents

Click chemistry to constrain β-hairpin peptides. Inserting the appropriate azide and alkyne component on a peptide chain can brings to the conformational stabilization of a β-hairpin peptide.

Keywords: β-hairpin • click chemistry • NMR • constrain • peptide

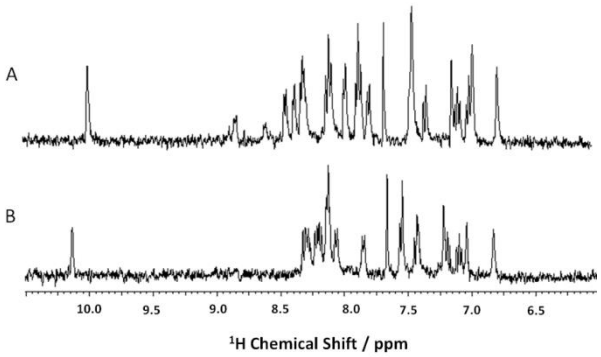
Trpzip2 Ser-Trp-Thr-Trp-Glu-Asn-Gly-Lys-Trp-Thr-Trp-Lys-NH_2

HB Ser-Trp-**Xaa**-Thr-Glu-Asn-Gly-Lys-Thr-**Yaa**-Val-Lys-NH_2



Peptide	Xaa	Yaa	x	y
HB 1.1	Pra	Dap (N3)	1	1
HB 1.2	Pra	Dab (N3)	1	2
HB 1.3	Pra	Orn (N3)	1	3
HB 2.1	Hpg	Dap (N3)	2	1
HB 2.2	Hpg	Dab (N3)	2	2
HB 2.3	Hpg	Orn (N3)	2	3
HB 3.1	Bpg	Dap (N3)	3	1
HB 3.2	Bpg	Dab (N3)	3	2
HB 3.3	Bpg	Orn (N3)	3	3

FIGURE 1



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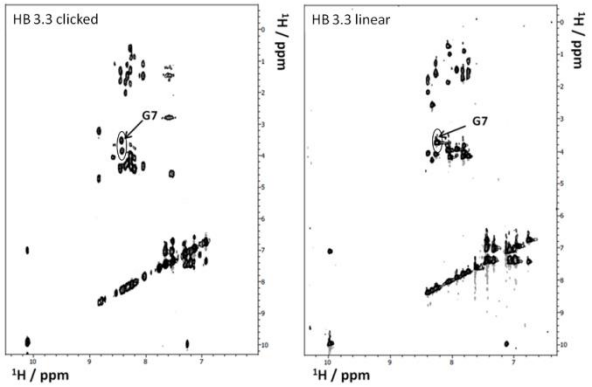

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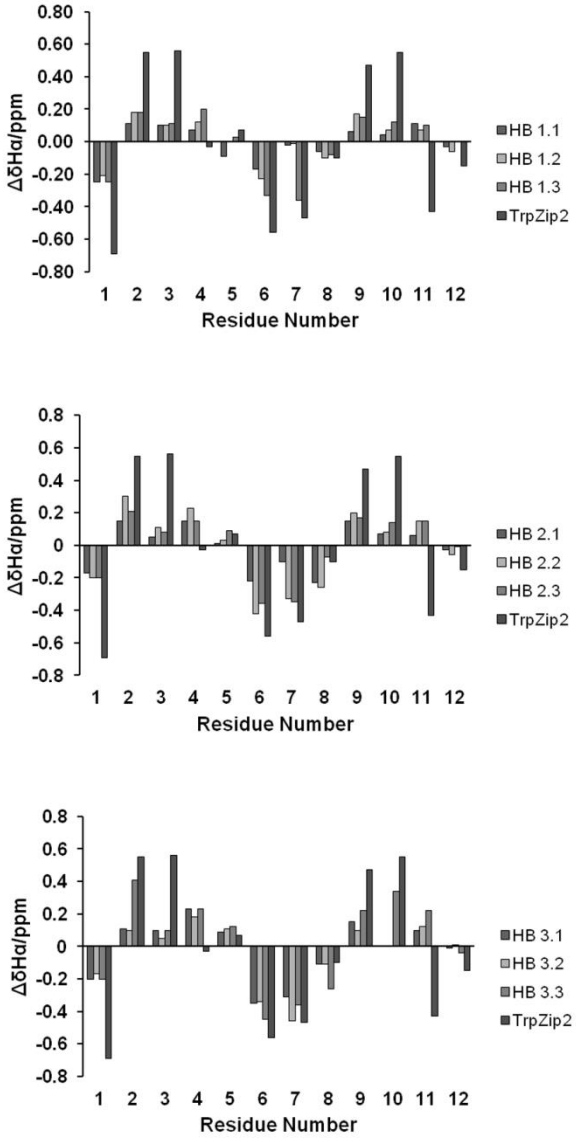
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0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
ΔδHα/ppm
1 2 3 4 5 6 7 8 9 10 11 12
Residue Number
HB 1.1
HB 1.2
HB 1.3
TrpZip2
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
ΔδHα/ppm
1 2 3 4 5 6 7 8 9 10 11 12
Residue Number
HB 2.1
HB 2.2
HB 2.3
TrpZip2
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
ΔδHα/ppm
1 2 3 4 5 6 7 8 9 10 11 12
Residue Number
HB 3.1
HB 3.2
HB 3.3
TrpZip2

FIGURE 4

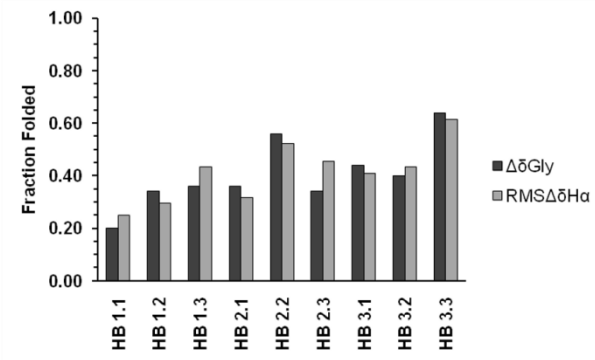
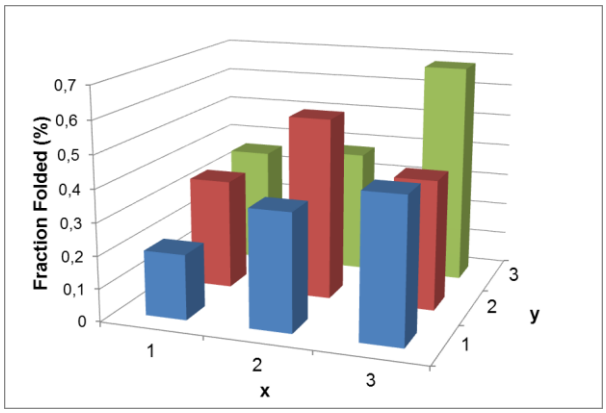

1.00
0.80
0.60
0.40
0.20
0.00
Fraction Folded
HB 1.1
HB 1.2
HB 1.3
HB 2.1
HB 2.2
HB 2.3
HB 3.1
HB 3.2
HB 3.3
ΔδGly
RMSΔδHα

FIGURE 5



GRAPHICAL ABSTRACT

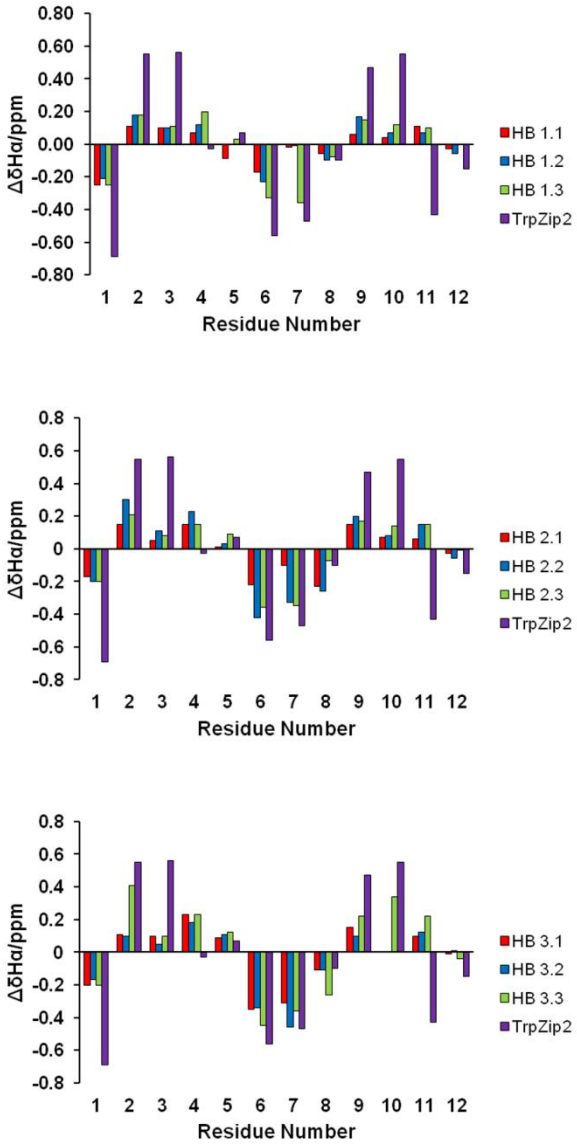


FIGURE 4 COLOR for online version