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Hairpin conformation of an 11-mer peptide

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ABSTRACT

An 11-mer peptide taken from a subsequence of the human protein ubiquitin was synthesized. The peptide has been fully characterized by NMR spectroscopy using chemical shift analysis and by NOE measurements. The conformation was calculated using state of the art MD methods of protein chemistry. A hairpin conformation was found which is to a large part identical with the structure of this peptide fragment within the human ubiquitin. The surprising result that already an 11-mer peptide adopts a hairpin conformation in aqueous solution is discussed in terms of initials sites for protein folding.

1. Introduction

The study of peptide conformation of an isolated structure derived from a protein has made an important step to bridge the understanding of stable folding and protein folding mechanism. The evidences of a stable isolated conformation are an important indication of their role in directing the subsequent folding pathway.[1] Such studies are of fundamental interests in order to decode how the primary amino acid sequence causes the folding into the native protein structure via initiation sites.

The process of identifying secondary structures in peptides fragments is challenging. To carry out structural studies of peptide fragments, the first step is to select appropriate peptides for investigation. A prior prediction of the peptides that are likely to contain protein folding initiation sites on the native protein is usually not straightforward. Secondly, the populations of folded conformers are rather low in water.[2] Nevertheless, the availability of high resolution NMR has made the structure determination of small peptides more accessible.[3]

The β-hairpin motifs are widespread in globular proteins, and often serve as initiation sites that cause the folding of the ensemble.[4,5] Considerable high interest has been focused by different groups to investigate the folding of peptides derived from the β-hairpin motif of small globular proteins such as ubiquitin and the stability of short amino acids sequences of the β-hairpin. The stability of the hairpin motiv in ubiquitin itself was pointed out in several papers.[5–7] Earlier work has demonstrated the structure of the β-hairpin motif of ubiquitin in a series of hairpin sequences (⩾15 amino acids).[8,9] It has been shown that some specific design turn sequences can strongly promote β hairpins formation.[10–12]

Recent studies have revealed evidence of small population of folded structures in water. There is, however, a lack of structure information of ubiquitin β-hairpin motif in water.[13] It has been shown previously that a peptide corresponding to residues 1–17 of ubiquitin autonomously fold into a native β-hairpin structure.[14] In the effort to investigate the propensities of turn sequence to drive the formation of a β-hairpin motif,[15,16] a much smaller peptide is studied in the present work. Here, we report the first structure of an 11-mer peptide (corresponding to residues 4–14 of ubiquitin) of the ubiquitin β-hairpin motif and focus on our NOE results and $^3J_{NH\alpha}$ spin coupling constants, although other experimental data such as correlation times and diffusion rates would also be very helpful.

2. Results and discussion

2.1. Synthesis and characterization

To investigate the importance of β-hairpin stability on strand sequences, the 11-mer peptide (Scheme 1) was synthesized and characterized by NMR. Structure evidence can first derived from deviation of chemical shifts from random coil values. The deviation of HN and Hα shifts from random coil values provides an indication of α-helix, β-sheet and loop regions formation.[17] Figure 1 indicates that some populations of β-hairpin structure are present in the 11-mer peptide. In the 11-mer peptide, the positive differences of the shift regions match with the β-strands and the negative

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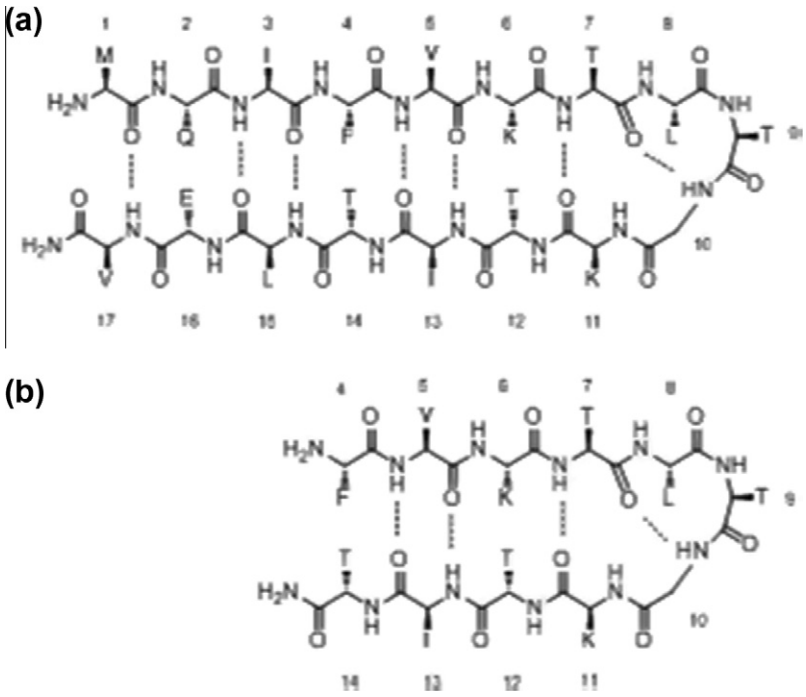

Scheme 1. Schematic diagrams of the β-hairpin structure formed by (a) the native 17-mer, and (b) the 11-mer peptide in which residues 1, 2, 3, 15, 16 and 17 have been deleted.

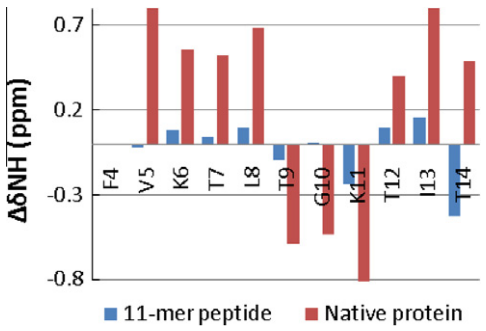

Figure 1. HN chemical shift differences of the 11-mer peptide and native ubiquitin from random coil values.

differences shift regions match with the hairpin turn regions of native ubiquitin. Fraying of terminal residues is common in β-hairpin peptides.[18]

There are also numbers of long range NOEs involving main chain to side chain, main chain to side chain and side chain—side chain interactions observed along the sequence. The majority of residues exhibit strong dαN(i, i+1) sequential NOEs and the dNN(i, i+1) NOE is absent, therefore indicating the involvement of β-sheet conformation.[2] The network of NOEs is represented in the diagonal plot in Figure 2. The NOE connectivity indicates that in the 11-mer peptide T4 is close to F14 and therefore a hairpin conformation is likely even when measured at 298 K. The NOE's include a number of cross-strand NOE's, mainly involving side chains, but also including main-chain NOEs. These NOE patterns indicate a roughly native like folded structure which consists of two strands of β-sheet and a turn region located near the center of the sequence. A summary of the NOE values observed is given in Table 1.

The substantially greater coupling constant $^{3}J_{NH\alpha}$ is also an evidence of the folded conformation of the beta sheet structure. The coupling constant $^{3}J_{NH\alpha}$ for the 11-mer peptide are listed in

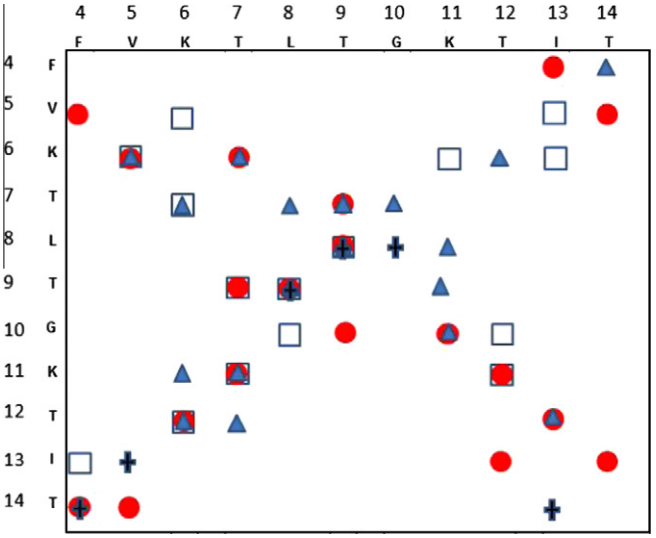

Figure 2. The diagonal plot shows the NOE's network of 11-mer peptide where 'red dot' represents main chain to main chain NOEs, 'blue square' represents main chain to side chain, 'blue filled triangle represents side chain to main chain and '+' represents side chain to side chain NOE's. Due to signal overlap, some signals have been observed only once.

Table 1
Classification of NOESY restraints used for structural calculation

NOE distance restraints	Number
Sequential	32
Intra-residual	24
Medium range	17
Long range	17
Ambigous	4
Sum	90

Table 2
$^{3}J_{NH\alpha}$ values in random coil structures,[19] in the 11-mer peptide and in native ubiquitin[20]

Residue	Random coil structure	11-Mer peptide	Native ubiquitin
F4	7.5	—	9.9
V5	7.7	6.7	9.6
K6	7.1	5.1	8.9
T7	7.6	7.2	8.2
L8	7.1	6.2	5.3
T9	7.6	7.5	—
G10	—	5.6	—
K11	7.1	7.0	—
T12	7.6	7.9	9.4
I13	7.6	8.8	9.6
T14	7.6	8.6	9.5

Table 2. The coupling constants observed in native ubiquitin were adapted from Zerella et al.[19] and were used as estimates of the folded state, while the values of unfolded state were adapted from by Smith et al.[20] The coupling constant $^{3}J_{NH\alpha}$ for the investigated 11-mer peptide is apparently not greater for residue 5–7 but substantially greater for residue 12–14. Again, this might indicate that the 11-mer peptide being partially folded.

Structural calculation of the 11-mer peptide was performed via dynamic simulated annealing. A total of 90 distances restraints as given in Table 1 were used for the structure calculation. Both the distances restraints obtained from NOESY experiments and the dihedral angle restraints obtained from TALOS+ evaluation were used to calculate structure of the 11-mer. The 10 lowest energy NMR structures are shown in Figure 3. All of the 10 models in the ensemble have ϕ/ψ angles of the residues at position i+1 and i+2 in the turn (L8 and T9) matches with the native like type I turn. However, ψi+2, which is residue T9, has a value of around 32° Compared to the ideal value of 0°.[21] The side chain conformations are well defined for several residues, mainly V5, T7, L8, K11 and I13. These side chains have a higher number of restraints and thus contributed to local packing interaction. F4 and T14 are packed together while V5 exhibit contact with I13. Interestingly, T7, L8 and K11 appear to form a hydrophobic cluster that stabilize the peptide.

The NMR evidence presented might seem to have some contradicting results. From the chemical shifts deviation analysis, not all residues display chemical shift deviations of more than 0.1 ppm whereas the NOE based data strongly suggest that a native like β-hairpin structure is present. Chemical shifts and NOEs are NMR parameters that detect characteristics for structural conformation.

However, these parameters detect the structural conformation at different time scales. The chemical shift can be considered to detect population-weight average over all conformers while the NOE spectrum will contain all cross peaks representation of conformers that have sufficiently high populations of near distance residues.

Hence, different NMR parameters could possibly show different characteristics of a specific structure. Perhaps the 11-mer peptide could be folded in fast exchange between random coil and β-hairpin and therefore gives raise to weak chemical shift deviation but relatively stronger NOE effects.

The NMR structure of the 11-mer derived peptide resembles the native ubiquitin structure (Fig. 4). The comparison between the two structures reveals some interesting features. Firstly, the main variation from the native backbone conformation is the ψ angle of T9, which adopts a value of approximately 32° rather than approximately 0°. This is perhaps not surprising, as the T9 has been previously identified as the residue that most likely determine the stability of native ubiquitin by having extraordinary high NH-exchange rate.[22] In the case of the 11-mer peptide, the peptide has lost all tertiary network connectivity. T9 forms different interactions with neighbouring residues and thus a different ψ angle to compensate for the loss. A residue specific NH-exchange rate study by a NMR diffusion experiment[23] also reveals that T9 in our 11-mer peptide has with 14 Hz the tendency of being more than two times

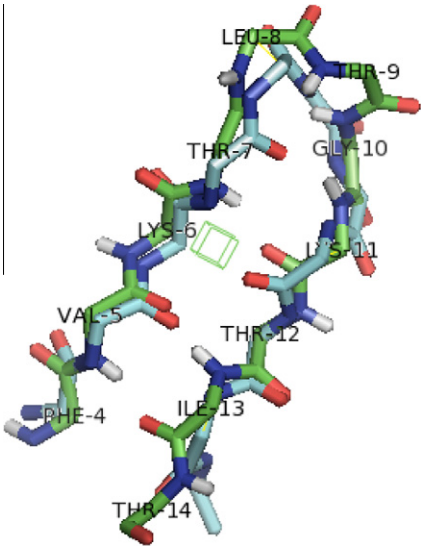

Figure 4. Superposition of the main chain of the 11-mer peptide with the native ubiquitin structure. Blue = 11-mer peptide, green = native protein with RMSD value of 2.709 Å.

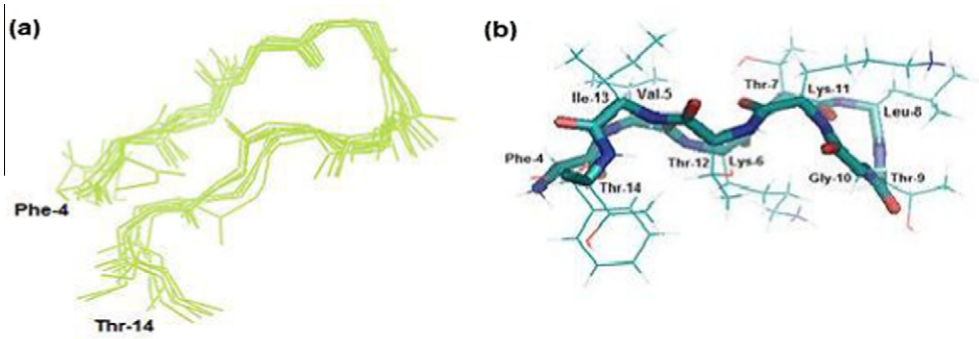

Figure 3. (a) Superposition of the backbone atoms of 10 calculated structures for the 11-mer peptide. (b) Molecular representation of the orientation of the side chain residues.

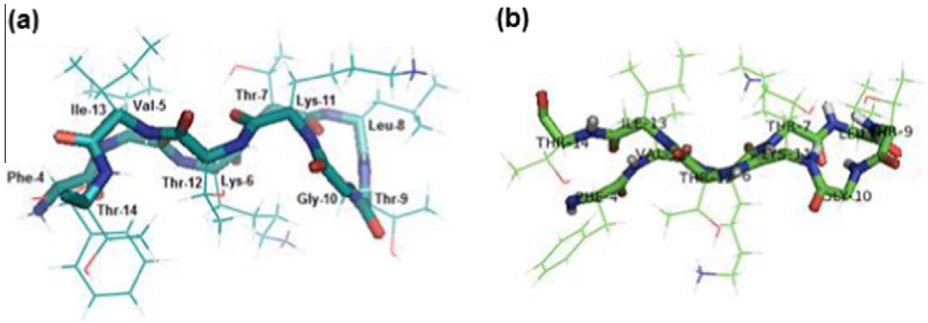

Figure 5. Molecular representation showing the side chain interaction of (a) the 11-mer peptide and (b) the native ubiquitin.

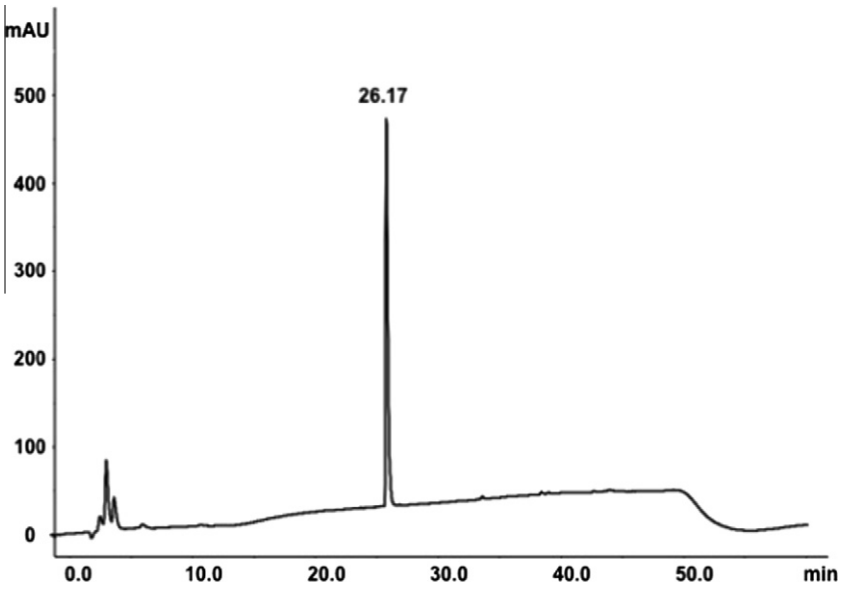

Figure 6. HPLC chromatogram of the 11-mer peptide on a RP18 column using an increasing MeCN gradient with added TFA.

higher flexible than in the native protein form (5.7 Hz). The NH-exchange study again reinforces the existence of 11-mer peptide as a quite mobile structure appearing between the random coil amino acid and the native protein.

To further check the similarity between the derived peptide versus the native ubiquitin, we compared their side chain conformations. From Figure 5, it is found that the β-strands of the derived peptide are being stabilized by some quite different side-chain interactions compared to the native ubiquitin. For example, we see partial burial of the side chain of F4 between T14 and T12 in native ubiquitin but side chain of F4 and T14 packs against each other in the derived peptide. In the absence of tertiary interaction within the derived peptide, the hydrophobic interaction between cross strands residues seems to be of importance.

The formation of a native like structure of the 11-mer peptide shows that the sequences of both arms of the hairpin do stabilize the β-hairpin structure, but their role is less critical compared to the turn sequences. The observed specific conformation of the shortened arm of β-hairpin peptide compared to the native β-hairpin indicates that the terminal arms of the ubiquitin β-hairpin peptide should not be the initiation site. In contrast, the turn region should be the initiation site for protein folding which determines the stabilization of the protein. A similar conclusion has been drawn for a few other hairpin peptide systems. These previous studies have shown that the stability of the derived structure can be drastically increased by mutation of important residues in the turn region.[19,24]

The observation of a native like β-hairpin for the 11-mer peptide is unexpected. It is generally accepted that the correlation time for peptides less than 20 residues in aqueous solution is very short. The populations of the folded forms are frequently very small, which also limits the inter proton distances for which a NOE can be detected. The fact that such a short peptide also forms a native like β-hairpin structure reveals several interesting features and helps understanding the protein folding of ubiquitin. The observation of transiently stable folding of a 11-mer peptide suggests the important role of the initiation of protein folding. The conformation of this part of ubiquitin is encoded in its own sequence rather than being imposed by the requirement of the overall secondary and tertiary interactions of the protein. Formation of such a local structure, which mainly depends on short range interaction of neighboring amino acids, is again supporting the framework model of protein folding.[25]

3. Conclusion

We have presented evidences that the 11-mer peptide resembles the native like β-hairpin structure in aqueous solution. The native like structural formation of 11-mer peptide supports the hypothesis that folding of the N terminal 4–14 residues could play

a role in the early folding of ubiquitin. Comparing with some earlier studies, the structural formation of this shorter peptide implicates the important role of the turns sequence in the nucleation of protein folding rather than the terminal end residues. Thus, the structure conformation of this relatively shorter peptide comparing to previous studies provide an aspect of reference during peptide design.

4. Experimental section

4.1. Synthesis and purification of the 11-mer peptide

To study NH-exchange rates the partially ^{15}N labeled 11-mer peptide ubiquitin4–14[15N-Thr9] was synthesized by Fmoc-chemitry using DIC/Hobt as activation agents. The peptide from position 10–14 was synthesized automatically with the peptide synthesis robot Syro2000 in a 25 μmol scale using a polystyrene based Rinkamide resin as solid support. 12.5 μmol of the 5-mer peptide was manually elongated by ^{15}N labeled threonine to yield the corresponding peptide ubiquitin9–14[15N-Thr9]. Subsequently, amino acids 4–8 were coupled to the peptide using the peptide synthesis robot. To improve purity and yield amino acids at positions 6 and 7 were introduced as pseudoproline dipeptide.[26,27] After the synthesis, the peptide ubiquitin4–14[15N-Thr9] was liberated from the resin including simultaneous cleavage of the side chain protection groups with 85% TFA and 15% Scavanger (0.5:1:1:1, ethanditiol, m-cresol, thioanisole, water). The final product was purified using RP-(C-18) chromatography using an increasing MeCN gradient and characterized by MALDI Mass Spectrometry. In Figure 6 the result of the HPLC run is shown. The final peptide was obtained with purity above 95% and a yield of 75%. The HPLC conditions are denaturing and remove intermolecular complexes, however, give no argument with respect to dimer formation during the NMR measurements.

4.2. Aggregation test

1D ^1H NMR spectra were recorded at varying concentration of derived concentration of 0.4 mM–3 mM. The absence of any substantial change in either line widths or chemical shifts implies the absence of aggregation in peptides.

As both referees asked for more arguments to distinguish between a putative anti-parallel β-strand dimer from a monomer β-hairpin structure we want to add the results of our NMR diffusion measurements which yielded a diffusion constant $D = 2.8 \times 10^{-10}\ m^2/s$. From this value the hydrodynamic radius and the apparent molecular weight[28] can be calculated which gave a value below 1000 Daltons and renders such a dimer with 2400 Daltons very unlikely.

4.3. NMR spectroscopy

NMR samples were prepared by dissolving the peptide in 500 μL 9:1 H_2O:D_2O or D_2O. Solution pH values were measured using a pH electrode for an NMR tube and were adjusted to a final pH of 5.8. Spectra were referenced to the singlet resonance of DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) at 0 ppm. Spectra were recorded on Bruker DRX-600 with 5-mm-TBI-probe and Bruker AVANCE-700 with Cryoprobe spectrometers. All the spectra were measured at the concentration ranging from 1 mM to 3.0 mM at 298 K.

Two dimensional double quantum filtered correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY) nuclear Overhauser spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments were carried out using the pulse sequences from the Bruker software. NOESY experiments were taken with mixing times of 50–500 ms. All spectra were analyzed using shifted square sine bell window functions. Excitation sculpting sequence was used for water resonance suppression.[29] Assignments were carried out as described by Wüthrich.[30] The 3D 1H, 15N-DOSY-HSQC[31] sequence was used for determine the NH-exchange rate. A diffusion time Δ of 80 ms was used, with nine different gradient strengths.

4.4. Structure calculation

Structures were calculated using ARIA version 2.3.1.[32] Total of 90 distance restraints derived from NOE data were classified as strong, medium and weak. 20 randomly generated structures were annealed using 10000 dynamics steps followed by 4000 minimization steps. 7 of the 20 structures were randomly chosen as starting structures for simulated annealing by molecular dynamics using CNS[33] with water refinement.

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