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CONFORMATIONAL ANALYSIS OF FRAGMENT OF HUMAN PIN1 WW DOMAIN: INFLUENCE OF CHARGED AMINO-ACID RESIDUES ON β-HAIRPIN STRUCTURE

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Abstract: We examined the effect of like-charged residues on the conformation of an original nine amino-acid-residue fragment of the human Pin1 WW domain (hPin1) with the following sequence: Ac-Arg-Met-Ser-Arg-Ser-Ser-Gly-Arg-Val-NH_2 (U9). This was facilitated by CD and NMR spectroscopic measurements, and molecular dynamics calculations. Our earlier studies suggested that the presence of like-charged residues at the end of a short polypeptide chain composed of nonpolar residues could induce a chain reversal. For the U9 peptide, canonical MD simulations with NMR-derived restraints demonstrated the presence of ensembles of structures with a tendency to form a β-chain reversal. Additionally, thermal stabilities of the peptide under study were measured using differential scanning calorimetry (DSC). The estimated well defined phase transition point showed that conformational equilibria in the U9 peptide were strongly dependent on temperature.

Keywords: peptide conformations; β-hairpin; hPin1 protein; NMR

1. Introduction

Peptides, in particular those excised from sequences of native proteins that are likely to be folding-initiation sites, often serve as models of early stages of protein folding [1, 2]. By reason of its simple topology and large amount of experimental data the WW domain of the human Pin1 protein is an ideal candidate to assess theoretical approaches to protein folding. Our earlier

studies [3–5] suggested that the presence of like-charged residues at the end of a short polypeptide chain composed of nonpolar residues could induce a chain reversal. In this study we continued our investigation of the possibility of induction of a chain reversal by the presence of charged residues at the end of a loop, which screen the loop's nonpolar residues from the solvent. For this purpose, we studied the solution conformations of the hPin1 fragment with the following sequence: Ac-Arg-Met-Ser-Arg-Ser-Ser-Gly-Arg-Val-NH_2 (U9) using molecular dynamics simulation with the NMR-derived restraints.

2. Materials and methods

2.1. Peptide synthesis

The hPin1 fragment with the following sequence: Ac-Arg-Met-Ser-Arg-Ser-Ser-Gly-Arg-Val-NH_2 (U9) was synthesized using the procedure described in our earlier work [6]. The purities of the peptides were 99.8% as assessed by the analytical HPLC and MALDI-TOF analyses.

2.2. Circular Dichroism (CD)

Circular dichroism (CD) spectra were recorded in water on a Jasco-715 automatic recording spectropolarimeter in the range of temperatures: 10°C–90°C, every 10 degrees. The spectra were recorded in the 185 – 260 nm wavelength range, using a sensitivity of five milidegrees and a scanning speed of 50 nm/min. The CD measurements were made at the peptide concentration of 0.15 mM in water.

2.3. Differential Scanning Calorimetry (DSC)

Calorimetric measurements were carried out with a VP-DSC microcalorimeter (MicroCal) at a scanning rate of 90°C/1 h. Scans were obtained at the peptide concentration of ~ 1 mM. The cell volume was 0.5 ml. The results from the DSC measurements were analyzed with the Origin 7.0 software from MicroCal using the routines of the software provided with the instrument [7].

2.4. Molecular dynamic simulation (MD) with NMR-derived restrains

The NMR experiments of the U9 peptide were made on a Bruker AVANCE 700 MHz spectrometer in the NMR Laboratory at the Faculty of Chemistry (University of Gdansk). The following 2D 1H-1H NMR spectra were recorded: DQF-COSY, TOCSY (80 ms) and NOESY (250 ms) at 283 K. The samples were dissolved in $H_2O/2H_2O$ (9:1 by vol.). The concentration of the sample was about 5 mM. The spectra were processed using the Sparky program. Molecular dynamics calculations (MD) were carried out using the AMBER 11 program with the AMBER ff99SB force field at a constant volume and temperature (the NVT scheme). All simulations were performed in a periodic box of TIP3P water with the particle-mesh Ewald procedure for long-range electrostatic interactions at $T = 283$ K. In simulations, both the distance restraints resulting from the NOEs

and restraints on dihedral angles resulting from the coupling constants were included. Additionally, "anti-NOE" restraints that prevented the protons of the pairs with no NOEs observed from being close to each other were included. This approach should minimize any bias from the AMBER ff99SB force field, which seems to favor the β-turn and α-helical conformations. The test simulations of U9 were performed without using any restraints, otherwise using the same simulation protocol as in the production simulations reported in the paper. It was found that these conformations were completely different from those obtained with restraints (Figure 6 a, b). It can, therefore, be stated that it was the experimental information and not the force field used that determined the obtained conformations.

3. Results and Discussion

The estimated temperature range of the phase transition with the well-defined maximum which is noticeable on the DSC curve (Figure 2) shows that conformational equilibria in U9 are strongly dependent on temperature. As shown in Figure 3, the molar ellipticity varies with temperature. The ellipticity at 210 and 230 nm becomes more negative, whereas that at 196 nm is less negative, with increasing temperature. A negative band at $\sim$200 nm is characteristic of a statistical coil and regular β-sheet conformation, respectively [8, 14]. However, it was observed from the changes in ellipticity at selected diagnostic wavelengths that the overall changes were very small. Additionally, the CD results (Figure 3) showed a well-defined isodichroical point. This suggests that a temperature-dependent equilibrium is established between the folded and unfolded conformations. In the TOCSY spectra (Figure 4 a) we found major and minor sets of signals for almost each amino acid residue, what indicated large conformational dynamics of the U9 peptide. Both major and minor sets of signals did not give any long-range connectivities. It was only $(i, i+1)$ NOE signals that were found in the NOESY spectrum (Figure 4 b) for the U9 peptide at 283 K. Figure 5 presents NOE effects corresponding to the interproton contacts and the $^3J_{HN/H\alpha}$ coupling constants of the U9 peptide measured in H_2O at 283 K. In the NOESY spectra we found

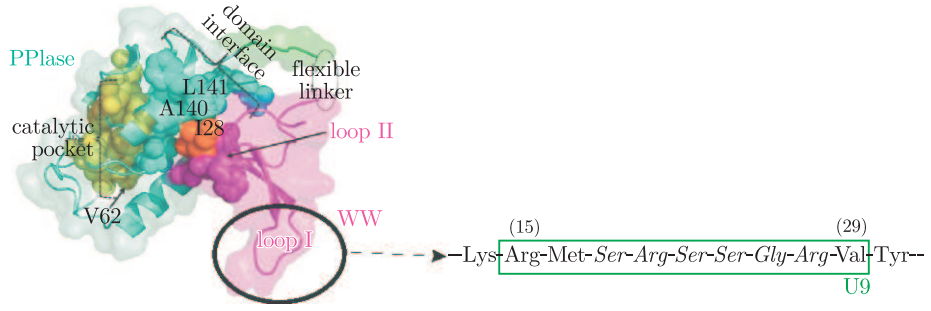

Figure 1. Structure of human Pin1 (PDB id code 1PIN) [8]; the boxed fragment of the 1PIN WW domain was synthesized and examined

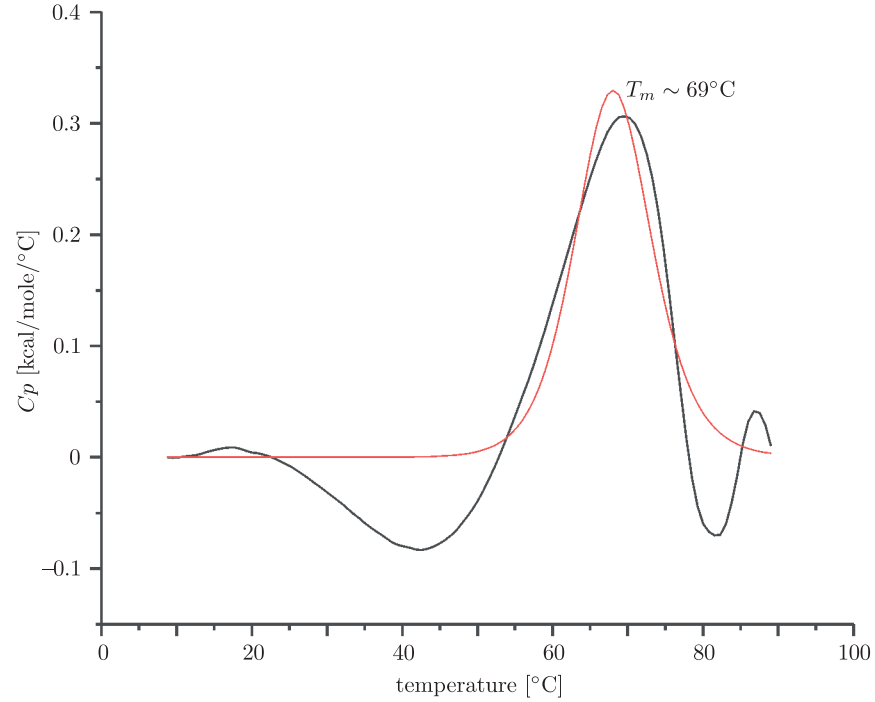

Figure 2. Heat-capacity curve of U9 peptide determined by DSC (black line) and the fit to the two-state model (red line)

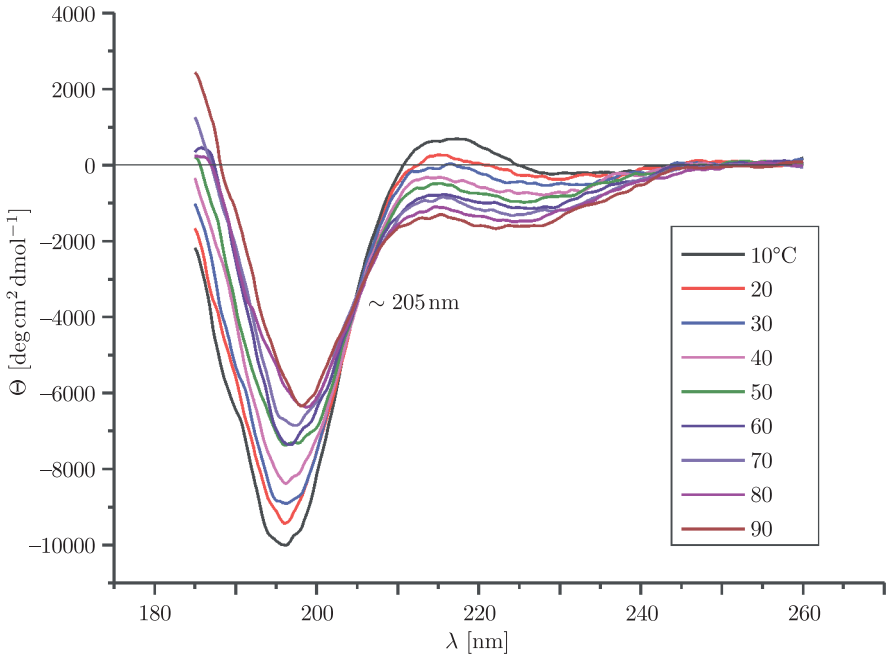

Figure 3. CD spectra of U9 in water at 9 different temperatures

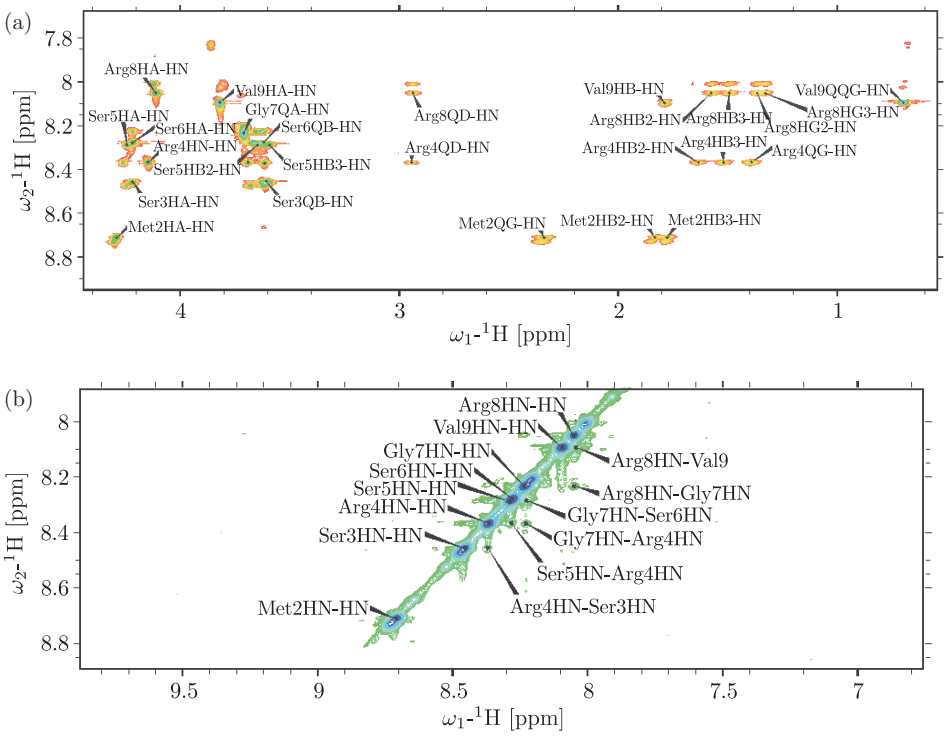

Figure 4. (a) NOESY (b) Fingerprint region of the TOCSY spectra of U9 peptide recorded at 283 K

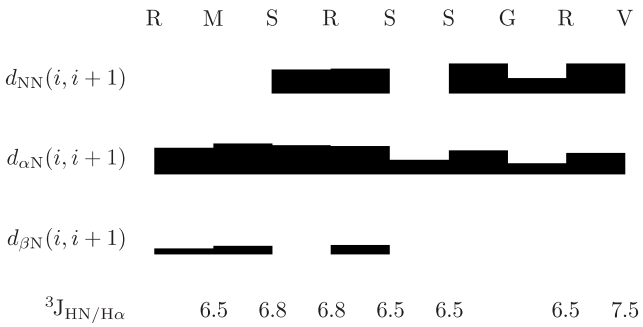

Figure 5. ROE effects for U9 peptide. The height of the bars reflects the strength of the ROE correlation

only interactions between residues, which are neighboring in the amino acid sequence. $H_{\alpha(i)}$-$HN_{(i+1)}$, $H_{\beta(i)}$-$HN_{(i+1)}$ and $HN_{(i)}$-$HN_{(i+1)}$ NOE connectivities were observed. A relatively small number of NOE interactions observed for U9 peptide entailed quite substantial conformational freedom of the investigated peptide. It should be stressed that vicinal $^3J_{HN/H\alpha}$ coupling constants (Figure 5) estimated for the analyzed peptide excluded the occurrence of an α-helical structure. 5000 conformations (the conformations were collected every 1000 steps) were obtained for the U9 peptide and the last 800 of those were analyzed. The set of the final

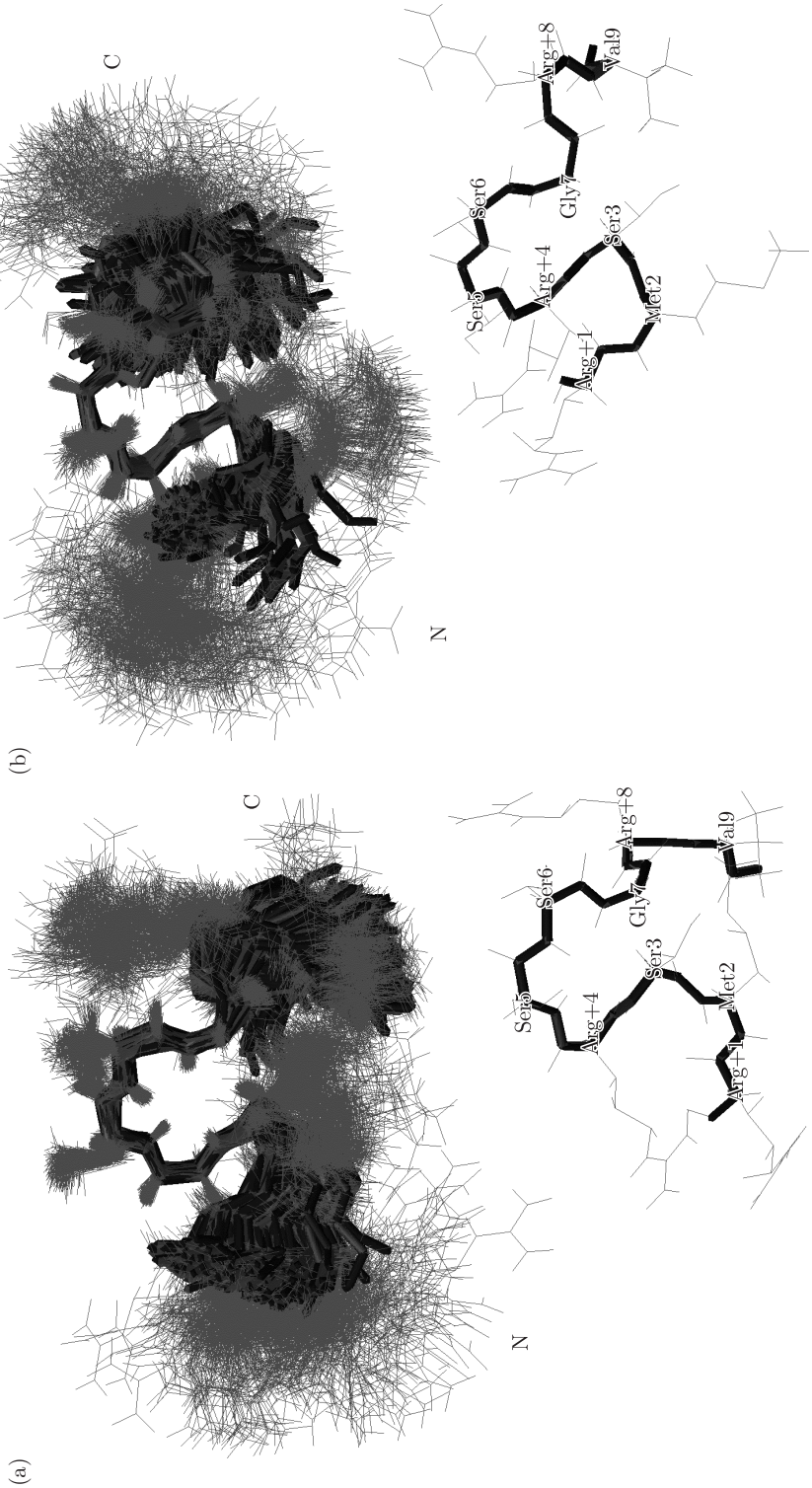

Figure 6. Main families clustered using the MOLMOL program [9] (hierarchical minimal spanning tree method) [10–13] and the most representative conformations: (a) family 1, (b) family 2, for U9 peptide

conformations was clustered using the MOLMOL program [9]. MOLMOL uses a hierarchical minimal spanning tree method [10–13]. The conformations presented in Figures 6 (a), (b) correspond to the major set of signals. The conformational analysis of the U9 peptide showed that the major shape of dominated conformations was bent and that it was only the ends of the main conformations that were mobile while the central part seemed to have a well-defined bent structure. The flanked charged arginine side-chain groups (Arg1, Arg8) were located relatively far from each other which suggested that they did not form a hydrogen bond. Two dominant families of conformations were obtained for the U9 peptide. In both cases, Arg1 and Arg4 were located close to each other and Arg1 was in parallel with Arg4. However, no hydrogen bonds between these two residues were observed. Some conformations, where Ser6 was close to the Arg8 appeared for the most populated family. In the dominant family (Family1) the side chain of Met2 was close to the Val9 but this was not observed in the second family. It appears that the presence of Arg1 and Arg4 in the N-terminal part of the sequence stabilized the fold in the middle of the U9 peptide because they rendered any easy deformation of the strand impossible. The presence of charged residues in the U9 peptide chain (especially in the ends of the tread) seemed to prevent the peptide from deforming a bent. Agreement between calculated and experimental data was observed.

Acknowledgements

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