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ARTICLE

^{1}H, ^{13}C and ^{15}N resonance assignments of human parvulin 17

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Abstract A 25-residue elongation at the N-terminus endows parvulin 17 (Par17) with altered functional properties compared to parvulin 14 (Par14), such as an enhanced influence on microtubule assembly. Therefore the three-dimensional structure of this N-terminal elongation is of particular interest. Here, we report the nearly complete ^{1}H, ^{13}C and ^{15}N chemical shift assignments of Par17. Subsequent chemical shift index analysis indicated that Par17 features a parvulin-type PPIase domain at the C-terminus, analogous to Par14, and an unstructured N-terminus encompassing the first 60 residues. Hence the N-terminus of Par17 apparently adopts a functionally-relevant structure only in presence of the respective interaction partner(s).

Keywords PPIase · Par14 · Par17 · DNA binding · Microtubule assembly

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Biological context

Peptidyl prolyl *cis/trans* isomerases (PPIases) play a role in protein folding and function by accelerating the slow interconversion between the *cis* and the *trans* conformational state of peptide bonds preceding proline residues in polypeptide chains (Fanghänel and Fischer 2004). Parvulins represent one family of the PPIases, which are conserved from bacteria to the human species. Recently, parvulin 17 (Par17) was identified as the third member of the human parvulin family of PPIases, which include Pin1, parvulin 14 (Par14) and Par17 (Mueller et al. 2006).

Human Pin1 comprises an N-terminal WW domain and a C-terminal PPIase domain, whereas Par14 and Par17 both lack the WW domain. Moreover, in contrast to Pin1, which specifically accelerates *cis/trans* isomerization of (pSer/pThr)-Pro moieties (Yaffe et al. 1997; Lu et al. 1999), Par14 and Par17 do not show any preference for phosphorylated substrates due to a lack of certain basic residues that are responsible for the interaction with phosphorylated substrates in Pin1, such as Lys63, Arg68 and Arg69 (Uchida et al. 1999). This positively-charged interaction site is replaced in the Par14 structure by a more negatively-charged surface made up of Glu46 and Asp74 for example (Sekerina et al. 2000; Terada et al. 2001; Mueller et al. 2011), resulting in a substrate specificity for basic amino acids preceding proline (Zoldák et al. 2009).

Par14, which has been associated with cell cycle progression and chromatin remodeling, is localized mainly in the nucleus, whereby the N-terminal lysine- and glycine-rich region apparently is important for nuclear localization and DNA binding (Surmacz et al. 2002). NMR structure analysis of Par14 revealed that the 35 N-terminal residues

preceding the PPIase domain adopt a random-coil structure (Sekerina et al. 2000). Similar to Par14, Par17 has been reported to also bind to double-stranded DNA at physiological salt concentrations (Kessler et al. 2007). However, in contrast to Par14, which is found mainly in the nucleus, Par17 is encountered predominantly in the cytosol, associated to the mitochondrial matrix or colocalized with microtubules (Kessler et al. 2007; Thiele et al. 2011), suggesting that the additional residues in Par17 may mask the nuclear localization signal (Reimer et al. 2003).

Compared to Par14, Par17 contains 25 additional residues at the N-terminus, which are uniquely present in Hominidae (Mueller et al. 2006; Kessler et al. 2007). This N-terminal elongation supposedly functions as a novel mitochondrial targeting signal (Kessler et al. 2007). Furthermore it was shown recently (Thiele et al. 2011) that Par17 binds tubulin with a 1:2 stoichiometric ratio and catalyzes tubulin polymerization in vitro 2.5-fold more efficiently than Par14. However, as the N-terminal elongation of Par17 is not sufficient to promote microtubule assembly by itself, it is assumed that this effect results from a combined action of both the N-terminal elongation and at least part of the Par14-like C-terminal domain of Par17. Hence, due to the above-described novel functional properties of Par17 compared to Par14, the structure adopted by the 25 N-terminal residues of Par17 is of particular interest.

Methods and experiments

Protein preparation

GST-Par17 was expressed and purified as previously described (Thiele et al. 2011), except that isotope-labelled protein was produced using M9 minimal medium. Par17 was finally obtained via thrombin cleavage. Mass spectrometric analysis revealed that a Gly–Ser residual from the thrombin cleavage site precedes Met1 at the N-terminus.

NMR spectroscopy

The NMR samples contained ca. 0.9 mM Par17 (non-labelled or $^{13}C/^{15}N$-labelled) in 10 mM MES buffer (50 mM KCl, 6 mM $CaCl_2$, pH 6.8) and were acquired at 291 K using Bruker DRX 500 and Avance 800 (with cryoprobe) spectrometers. The carrier was placed in the center of the spectrum on the water resonance, which was suppressed by applying a WATERGATE sequence. Quadrature detection in the indirectly-detected dimension was obtained by the States-TPPI method. All NMR spectra were acquired and processed on Silicon Graphics computers using the programs XWINNMR 3.5 and TopSpin 2.1 (Bruker Bio-Spin, Rheinstetten, Germany). A 90° phase-shifted squared sine-bell function was used for apodization in all dimensions. Polynomial baseline correction was applied to the processed spectra in the directly-detected ^{1}H dimension. The chemical shifts were referenced to external DSS in order to ensure consistency among all spectra (Wishart et al. 1995). Spectra were analyzed with SPARKY 3 (University of California, San Francisco, USA).

Homonuclear 2D $^{1}H/^{1}H$-TOCSY and heteronuclear 2D $^{1}H/^{15}N$-TROSY, $^{1}H/^{15}N$-HSQC, and $^{1}H/^{13}C$-HSQC spectra were collected and analyzed. In addition, the following 3D triple-resonance datasets were acquired: the sequential backbone assignment was based on TROSY-type HNCACB and HN(CA)CO experiments. Side-chain ^{1}H and ^{13}C resonances were derived from CC(CO)NH experiment (17.5 ms spinlock time) as well as H(C)CH-TOCSY and (H)CCH-TOCSY experiments (15.4 ms spinlock time each).

Assignments and data deposition

The backbone amide assignment of human Par17 is shown in Fig. 1. All backbone amide groups have been assigned except for residues Gly(−1) and Ser(0) at the N-terminus, i.e. the residual of the thrombin cleavage site, as well as the segment Lys100-Gly104, which in the X-ray structure of Par14 represents a short 3_{10}-helical loop that apparently could not be fully characterized either in two independent NMR studies on human Par14 (Sekerina et al. 2000; Terada et al. 2001), possibly indicating that this loop exhibits an increased conformational variability in solution.

The resonance assignment of Par17 is nearly complete, including those 30 N-terminal residues that had not been assigned at all in case of Par14 (Sekerina et al. 2000; Terada et al. 2001). The carbon-associated ^{1}H resonances of the Par17 N-terminus up to residue Gly59, however, are missing due to a very high degree of resonance degeneracy within this apparently unstructured region. Hence, considering the usually observed protein resonances, 72 % of the ^{1}H, 93 % of the ^{13}C and 95 % of the ^{15}N assignments have been achieved for the entire protein. In case of the structured domain, i.e. residues Asn61-Lys156, 98 % of the ^{1}H, 94 % of the ^{13}C and 95 % of the ^{15}N resonances have been assigned. The thus obtained resonance assignments of parvulin 17 have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) database under accession number BMRB-18615.

Chemical shift index (CSI) analysis (Wishart and Sykes 1994) of Par17 revealed a secondary structure pattern at the C-terminal end that corresponds very closely to the

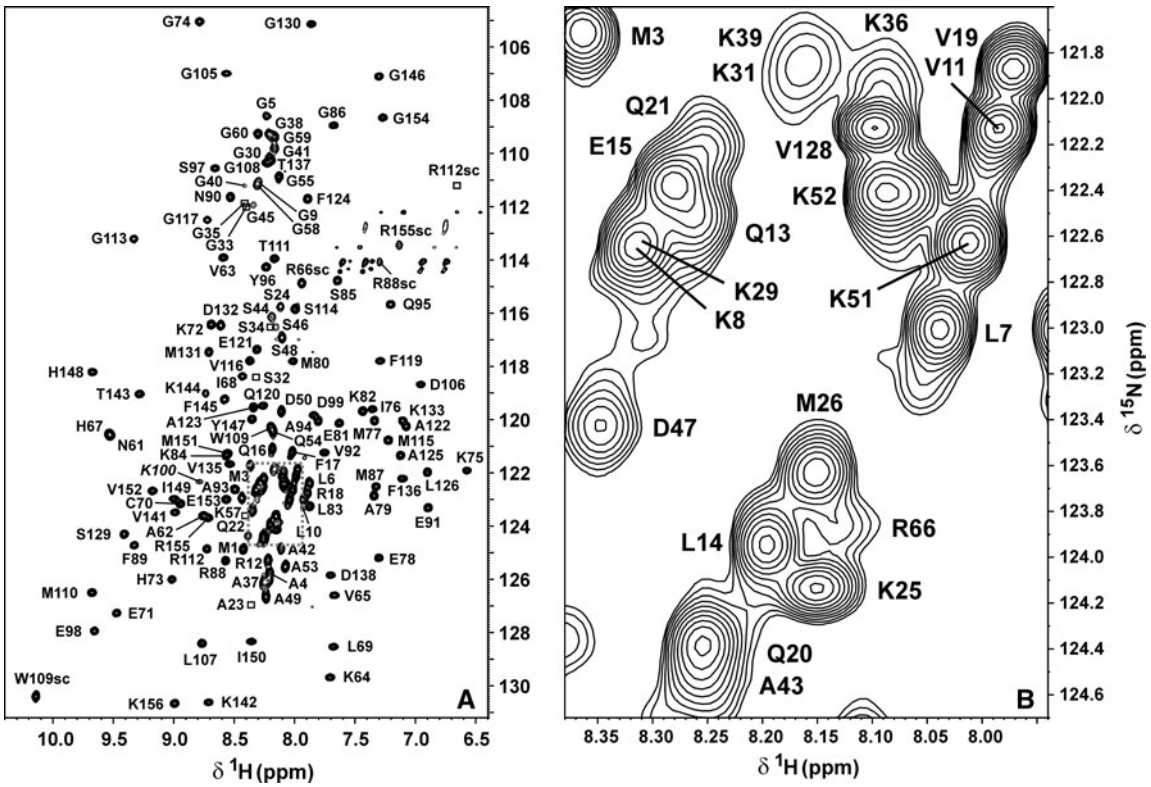

Fig. 1 **a** ^{1}H/^{15}N-TROSY spectrum collected in 10 mM MES buffer (pH 6.8) at 298 K showing the backbone amide resonance assignments of human Par17. Signals belonging to the first 60 N-terminal residues of Par17 show a low amide proton resonance dispersion (between 8.55 and 8.01 ppm), indicating that this region of the protein is largely unstructured. Side-chain signals are labelled by the notation 'sc' behind the residue name. Positions of low-intensity peaks observed below the plot level are marked with a *square box*. **b** Close-up of the central TROSY region

parvulin-type PPIase domain of Par14 (Fig. 2). Nevertheless, a few minor differences appear to exist: as mentioned above, in case of segment Lys100–Gly104, which constitutes a 3_{10}-helical loop in the X-ray structure of Par14 (Mueller et al. 2011), missing resonance assignments suggest a less well-defined conformation in solution for both Par14 and Par17. Moreover, the adjacent strand βB, which is found not only in the X-ray structure but also in the solution structures of Par14, is not identified in Par17 although the resonance assignments of the corresponding residues are quite similar in both parvulin types. On the other hand, the CSI analysis suggests that an additional β-strand element spanning residues Val135–Asp138 may exist in front of the diproline segment Pro139–Pro140, which is followed by the rather short strand βC that encompasses residues Val141–Thr143. Based on the C^{β} and C^{γ} chemical shift values (Schubert et al. 2002), Pro139 in this diproline segment features a *cis* conformation analogous to the corresponding proline in Par14. It could be envisioned that the additional β-strand element between residues Val135 and Asp138 represents an extension of strand βC, which possibly stretches from Val135 to Thr143 in Par17, interrupted by the rather unusual *cis*Pro139–*trans*Pro140 diproline segment that forms a kind of bulge in the analogous structure of Par14.

Although the ^{13}CO chemical shift values might hint at helical propensity within segments Ala4–Leu7 and Ser32–Ser46, such a secondary structure preference was not confirmed by the corresponding ^{13}Cα and ^{13}Cβ resonances (Fig. 2). As a consequence, the N-terminus of Par17 up to Gly60 revealed no regular secondary structure in the CSI consensus. Hence, in addition to the first 35 residues of Par14, which were reported earlier as unstructured (Sekerina et al. 2000; Terada et al. 2001), the N-terminal elongation of Par17, comprising 25 additional residues, apparently also features a mostly random-coil conformation in solution. Considering the importance of the N-terminal elongation in terms of Par17 function (Kessler et al. 2007; Mueller et al. 2006; Thiele et al. 2011), it must be therefore assumed that the Par17 N-terminus adopts a functionally-relevant conformation only in the presence of a physiological ligand but remains largely unstructured in the non-liganded form.

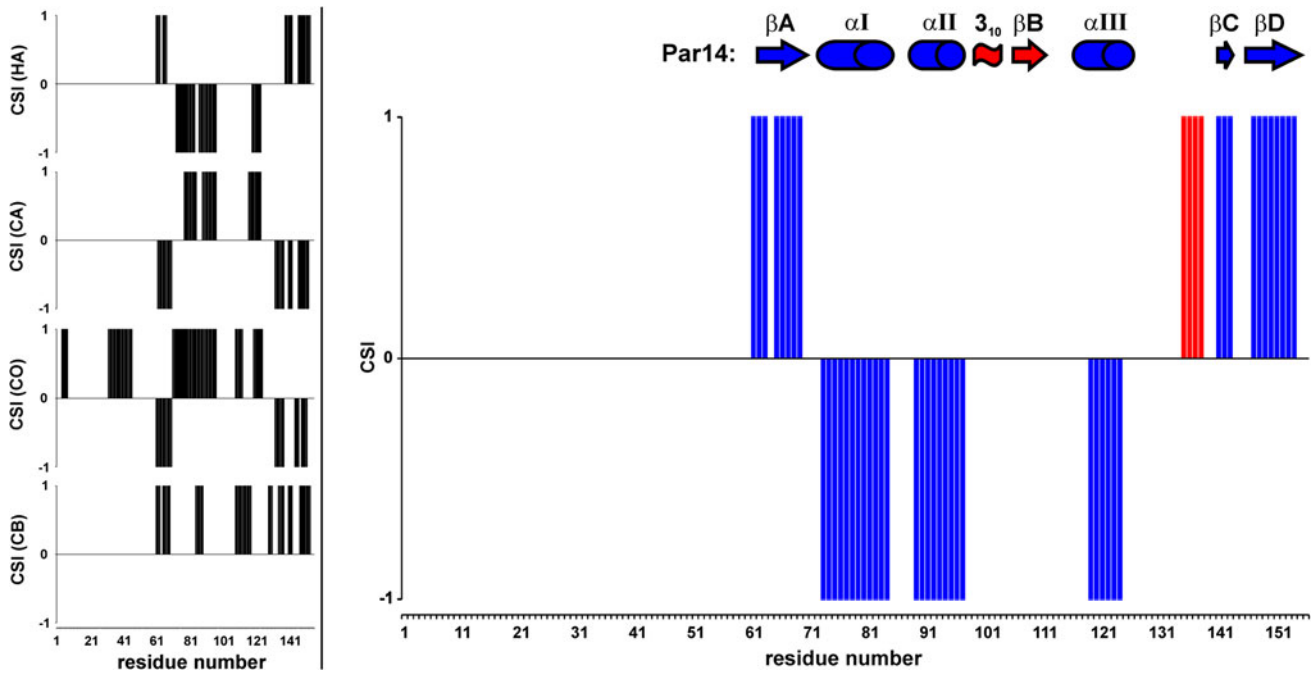

Fig. 2 The *left panel* displays from *top* to *bottom* the chemical shift index analyses of the $^{1}H\alpha$, $^{13}C\alpha$, ^{13}CO and $^{13}C\beta$ resonances throughout the Par17 sequence. The *right panel* shows a comparison of the Par17 CSI consensus (*bars*) with the secondary structure elements (*cartoon*) of the Par14 crystal structure (PDB ID code 3UI4). Positive and negative values in the CSI consensus indicate β-strand and α-helix segments in Par17, respectively. In the secondary structure cartoon at the *top*, which is based on sequence homology alignment with Par14, arrows, cylinders and waves indicate β-strands, α-helices and 3_{10}-helices, respectively. Secondary structure elements that correlate between Par17 and Par14 are colored in *blue*, whereas apparent structural disagreements are indicated by *red color*

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