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Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments of an 18.2 kDa protein, *E. coli* peptidyl-prolyl *cis-trans* isomerase b (EPPIb)

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Biological context

Peptidyl-prolyl *cis-trans* isomerases (PPIs), which are ubiquitous and phylogenetically well-conserved proteins, catalyze the *cis-trans* isomerization of peptidyl-prolyl bonds (Xaa-Pro) in proteins and are believed to assist in protein folding *in vivo*, by accelerating the rate-limiting step in folding pathways (Galat, 1993). There are three PPI superfamilies, which are sequentially unrelated: cyclophilin, FK506-binding protein (FKBP), and parvulin.

In *E. coli* cells, two distinct PPIs, designated as EPPIa and EPPIb, are separately expressed in the periplasmic and cytoplasmic compartments, respectively (Hayano et al., 1991), indicating that the substrate proteins of EPPIa are different from those of EPPIb. In spite of their physiological significance, little is known about their enzymatic properties, such as their substrate specificities. Information about their solution structures obtained by NMR studies will be helpful for further investigations of their enzymatic properties. Here we report the backbone chemical shifts of EPPIb, which will be useful for such investigations.

Methods and results

The EPPIb expression vector was constructed by introducing the PCR-amplified DNA fragment encoding EPPIb into the *Nde*I/*Bam*HI site of pET-3a (Novagen). Uniformly ^{15}N-labeled or ^{15}N,^{13}C-labeled EPPIb was synthesized by a cell-free reaction using an S30 extract (Pratt, 1984; Kramer et al., 1999), which was prepared from the *E. coli* A 19 (obtained from the Genetic Stock Center of Yale University), using the dialysis method (Davis et al., 1996; Kigawa et al., 1999). A 1 mM mixture of ^{15}N-labeled or ^{15}N, ^{13}C-doubly labeled amino acids was used for the reaction mixture and also for the dialysis solution. From 8 ml of the cell-free reaction mixture with 40 ml of the dialysis solution, 25 mg of the ^{15}N-labeled and 21 mg of the ^{15}N,^{15}C-labeled EPPIb were obtained by chromatography on a DEAE Sepharose FF (Amersham Pharmacia Biotech) column and a HiLoad 16/60 Sephadex 75 prep grade column (Amersham Pharmacia Biotech). For NMR measurements, protein concentrations were adjusted to 1.6 mM by Centricon ultrafiltration (MWCO: 10 kDa, Millipore).

The sample conditions were optimized by monitoring a series of 2D-CBCA(CO)NH spectra at various protein concentrations, pHs, and temperatures. We found that the NMR spectrum of EPPIb became broad at higher protein concentrations, or at lower temperatures, possibly due to protein aggregation. The optimum sample conditions were: [protein] = 1.6 mM, [sodium phosphate] = 50 mM, [DTT] = 1 mM, pH = 6.2, and 35 °C, respectively. To establish the full backbone assignments, we performed the following set of NMR experiments on a Bruker DRX-600 spectrometer: HNCA, HNCO, CBCANH, CBCA(CO)NH, HBHA(CO)NH, ^{1}H-^{15}N HSQC, ^{15}N-edited NOESY, and ^{15}N-edited TOCSY. The obtained NMR data were

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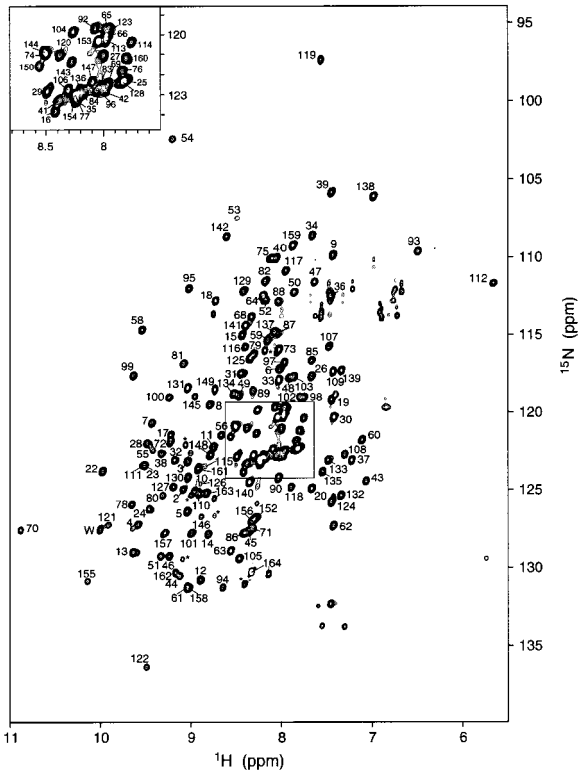

Figure 1. 600 MHz ^{1}H-^{15}N HSQC spectrum of [*ul*-^{15}N]-EPPIb with full assignments. The sample conditions: 1.6 mM, 5% D_2O, 50 mM sodium phosphate, pH 6.2, 35 °C. Note that the side-chain amide cross peaks for Gln and Asn were weak in this particular spectrum, since the ^{15}N-enrichments for these amides were low in this preparation.

processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and were analyzed by the use of PIPP (Garrett et al., 1991). All of the chemical shifts were indirectly referenced to that of DSS.

Extent of assignments and data deposition

All of the $^{1}H^{N}$-, ^{15}N-, $^{13}C^{\alpha}$-, ^{13}CO-, and $^{13}C^{\beta}$-assignments were completed. Most of the backbone (^{1}H, ^{15}N, and $^{13}C^{\alpha}$) and side-chain $^{13}C^{\beta}$ resonances were assigned with a combination of CBCANH and CBCA(CO)NH, by correlating the i and (i−1) residue pairs. For the broader peaks in these two spectra, HNCA was found to be helpful to find the connectivity. Since the molecular mass (18.2 kDa) is relatively large, the NMR spectra were complicated and some of the peaks had ambiguity. The established assignments were double-checked by various amino acid-selectively labeled proteins, which cover more than 40% of all amino acid residues. An example of an NMR spectrum of EPPIb is shown in Figure 1. The $^{1}H^{N}$-, ^{15}N-, and ^{13}C-chemical shifts for EPPIb have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4765.

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