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Letter to the Editor: Backbone and side chain assignments of human Peptidylprolyl Isomerase Like 1 (hPPIL1)

Chao Xu[a], Yingqi Xu[a], Yajun Tang[a], Jihui Wu[a,*], Yunyu Shi[a,*], Qiuhua Huang[b] & Qinghua Zhang[b]

[a]*School of Life Science and Hefei National Laboratory for Physical Sciences at Microscale, University of Science and Technology of China, Hefei, Anhui 230026, P.R. China;* [b]*Shanghai Institute of Hematology, Ruijin Hospital affiliated to Shanghai Second Medical University, Shanghai, P.R. China*

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Biological context

PPIL1 was identified as a member of cyclophilin family several years ago (Ozaki et al., 1998; Mann et al., 1998). PPIL1 is similar to cyclophilins in the PPIase ability and could be inhibited by cyclosporin.

In the past few years, PPIL1 has been found as a member of spliceosome in the activated form (Makarov et al., 2002) coexisting with SNW/SKIP. Pre-mRNA splicing, the excision of introns from mRNA precursors, is a prerequisite for the expression of most eukaryotic genes. Major structural changes occur in the spliceosome during its activation just before catalyzing the splicing of hnRNAs. SNW/SKIP may aid in conformational transition of the gene expression machine through its recruiting appropriate effector molecules, such as the prolyl isomerase PPILs (Folk et al., 2004). So PPIL1 may play an important role in the processing of transition of spliceosome. It was found that the PPIL1–SKIP/SNW interaction was not affected by cyclosporin, the potent inhibitor of PPIase. Thus, PPIL1–SKIP/SNW interaction seems not to occupy the prolyl isomerase catalytic site. But the mode of interaction is still unknown (Skruzny et al., 2001).

*To whom correspondence should be addressed. E-mails: {yyshi, wujihui} @ ustc.edu.cn

In PDB, there is a crystal structure of the cyclophilin B from *Homo sapiens* having about 47% identity and 67% similarity to hPPIL1, which is with the highest identity and similarity in publications (Mikol et al., 1994). Here we report the backbone and side chain assignments of hPPIL1 as the first step in the study of its full 3D structure, which could provide the platform for the interaction of hPPIL1 with its substrates.

Methods and experiments

The hPPIL1 cDNA was obtained from human CD34+ hematopoietic stem/progenitor cell cDNA library, cloned in frame into pET-22b (+) in the *Nde*I/*Xho*I sites (Novagen). The recombinant vector was then transformed to the *Escherichia coli* bacterial strain in BL21 (DE3). Recombinant hPPIL1 has 174 amino acids, the last 8 amino acids attribute to His-tag. Uniformly labeled recombinant hPPIL1 was overproduced using minimal medium containing 0.5 g/l 99% ^{15}N-ammonium sulfate and 2.5 g/l 99% ^{13}C-glucose as the sole nitrogen and carbon source, respectively. Labeled hPPIL1 was then purified by Ni-chelating column according to the manufacturer's instructions.

The NMR samples were prepared with 20 mM phosphate buffer (containing 50 mM NaCl) at pH 6.8 in 90% H_2O/10% D_2O and contained

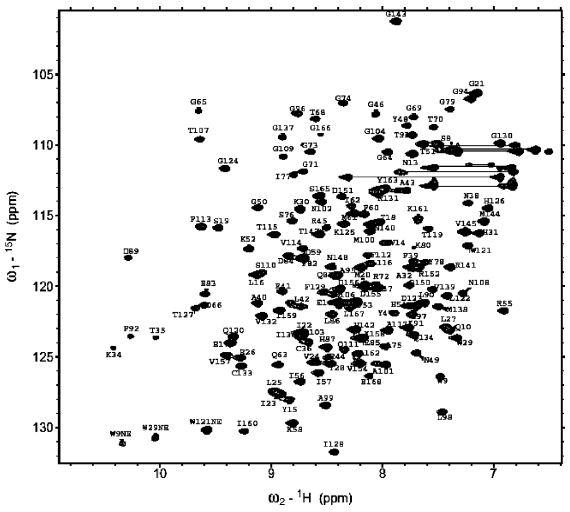

Figure 1. (a) ^{1}H,^{15}N-HSQC spectrum of recombinant hPPIL1 obtained at 295 K. The NMR sample contained about 0.5 mM hPPIL1 in 20 mM phosphate buffer, 50 mM NaCl at pH 6.8. The resonance assignments are indicated with the one–letter amino acid code and residue number. The NH side-chain of Trp is marked with NE.

0.45 ml of about 0.5 mM protein. The following experiments were carried out : 2D ^{1}H, ^{15}N-HSQC, 3D, 3D ^{15}N-edited NOESY-HSQC(130 ms mixing time), 3D HNCO, 3D HNCACO, 3D HNCA, 3D HNCOCA, 3D CBCA(CO)NH, 3D HNCACB. All NMR experiments were recorded at 295 K on a Bruker DMX 500 MHz spectrometer equipped with cryoprobe. 3D H(C)(CO)NH-TOCSY, 3D (H)C(CO)NH-TOCSY, 3D HBHA(CBCAC-O)NH, 3D HCCH-TOCSY, 3D HCCH-COSY, and 3D ^{13}C-edited NOESY-HSQC (130 ms mixing time) was recorded by Bruker DMX 600 MHz. NMR data processing was accomplished using NMRPipe and NMRDraw software (Delaglio et al., 1995) and analyzed with SPARKY. The chemical shift index (CSI) were obtained using the CSI software (Wishart and Sykes, 1994).

Extent of assignment and data deposition

The 2D ^{1}H, ^{15}N-HSQC spectrum of hPPIL1 was shown in Figure 1, all the backbone amide resonances S8-L167 (including the first amino acid of His-tag). Because the resonance peaks of the first seven amino acids could not be found in any spectrums recorded. Complete backbone assignments of residues from S8–L167 were made for $^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO except the N and NH of G135 and S149, which could not be found in ^{1}H, ^{15}N-HSQC. Their side chain resonances were assigned on the aid of HCCH-COSY and were confirmed by 3D ^{15}N-edited NOESY-HSQC and 3D ^{13}C-edited NOESY-HSQC furtherly. Assignments of side-chain resonances (S8–L167) of non-proline residues were mostly completed, excluding some side chain of K37, R45, R152 and prolines P11, P12, P67, P88, P105, P118, P153 due to overlapping resonance peaks.

The secondary structure prediction based on CSI and short-range NOEs analysis shows the existence of three helices and eight β-strands characteristic of human cyclophilin family. The secondary structure unit distribution of hPPIL1 is very similar to other members of cyclophiln family. The chemical shift values of the proton, nitrogen and carbon resonances have been deposited in the BioMagResBank (accession number: 6310).

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