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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments and secondary structure of human pancreatitis-associated protein (hPAP)

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Biological context

Pancreatitis associated protein (PAP) is a secretory stress protein first identified in rat pancreas during the acute phase of pancreatitis (Iovanna et al., 1991). Human pancreatitis associated protein (hPAP), also known as HIP (hepatocarcinoma-intestine-pancreas), is composed of 149 amino-acid residues (16 kDa) cross-linked with three disulfide bridges (Christa et al., 1994). The biological functions of hPAP remain elusive, although several potential functions have been reported (Christa et al., 1999). hPAP showed a 43% identity with human lithostathine (hLITA), another major protein expressed in the pancreas. Both hPAP and hLITA contain a trypsin cleavage site between residues Arg and Ile at N-terminus and were found to form highly organized fibrillar structures at physiological pH upon tryptic activation that converts 16 kDa proteins into 14 kDa isoforms. Furthermore, hPAP and hLITA were found to be highly expressed at the very early stages of Alzheimer disease (AD) and the level remained elevated during the course of AD, showing that they might be involved with AD (Duplan et al., 2001). The molecular characterization of hPAP reveals that it is a lactose-binding protein and belongs to the group VII of C-type lectin family. It was recently identified that hLITA binds with Ca^{2+} with 1:1 stoichiometry, and a cluster of acidic residues on the molecular surface are critical for Ca^{2+} binding (Lee et al., 2003). Interestingly, this acidic-residue cluster is not seen in hPAP, revealing that the binding characteristics might be different between hLITA and hPAP. To date, X-ray crystal structure of hLITA at a resolution of 1.55 Å (Bertrand et al., 1996) and a preliminary X-ray study of hPAP (Abergel et al., 1999) have been reported, and there is no NMR study reported for hPAP. Knowing hPAP structure may provide new insight into its functional properties as well as the conformational change from globule to fibril. Here we report the resonance assignments and secondary structure of the recombinant hPAP (138 residues, 14 kDa) that lacks the N-terminal 11 residues.

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Methods and experiments

The gene encoding hPAP with an additional 9 amino acids, MKHHHHHHQ, at N-terminus was expressed in *E. coli* M15 containing the constructed vector [pQE1]. The cells were grown in LB broth or in M9 minimum medium at 37 °C with 100 μg/ml ampicillin and 25 μg/ml kanamycin until OD_{600} reading reaches 0.6. Protein was then induced for another 3 h with 1 mM IPTG. After refolding, the unlabeled and isotopically labeled ($^{13}C/^{15}N$-labeled, $^{13}C/^{15}N/^{2}H$-labeled, and ^{15}N-specifically labeled) hPAP were purified by Ni-NTA and Mono S column chromatographies. To simplify NMR data and to confirm the resonance locations of the extra residues, the 6xHis tag was removed by TAGZyme kit (Qiagen) with approximately 50% recovery. The authenticity of hPAP was verified by SDS-PAGE and ES/

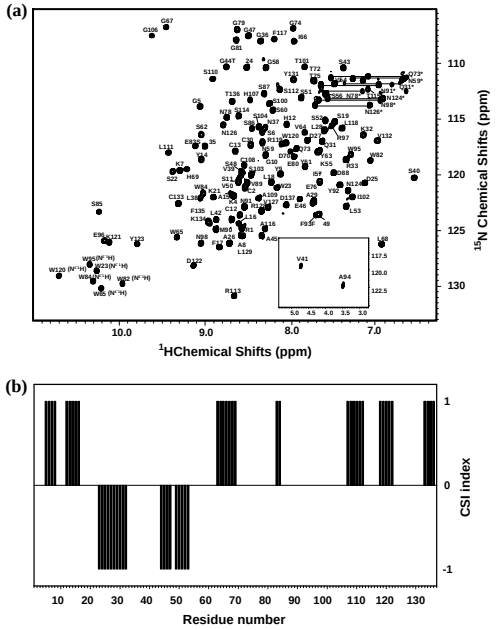

Figure 1. (a) 2D ^{1}H-^{15}N HSQC spectrum shows correlations for hPAP recorded at 298 K and pH 4.0. The assignments are indicated with the one-letter amino acid code and residue number. Side-chain amide protons of Asn and Gln are indicated by horizontal lines, and the unusual H^N shifts of Val^{41} and Ala^{94} are shown in the small square. (b) The CSI consensus plot generated on the basis of $^{1}H^{\alpha}$, $^{13}C^{\beta}$, $^{13}C^{\beta}$ and $^{13}C'$ chemical shifts clearly displays that hPAP contains 2 α-helices and 7 β-strands secondary structures.

MS analysis. All NMR experiments were performed on a Bruker AVANCE 600 spectrometer equipped with a triple (^{1}H, ^{13}C and ^{15}N) resonance probe including shielded z-gradient. NMR samples were prepared in 20 mM phosphate buffer in 90% H_2O/10% D_2O at pH 4.0 and contained 0.3 ml of 1.0 mM protein in a Shigemi NMR tube (Allison Park. PA, USA). All heteronuclear NMR experiments were carried out as described in review articles (Kay, 1995). Sequence-specific assignment of the backbone atoms was achieved by the independent connectivity analysis of CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO and C(CO)NH. The ^{1}H resonances were assigned using TOCSY-HSQC, HAHB(CO)NH, HCCH-TOCSY and HC(CO)NH. A combined information from 2D ^{1}H-^{15}N HSQC and 3D NOESY-HSQC experiments yielded assignments for side-chain amide resonances of the Asn and Gln residues. Aromatic resonances were assigned based on 2D TOCSY, NOESY, (Hβ)Cβ(CγCδ)Hδ and (Hβ)Cβ(Cγ CδCε)Hε data.

Extent of assignments and data deposition

We have assigned all resonances of the backbone nuclei and over 96% of the side-chain resonances based on a variety of triple-resonance experiments. Most of unassigned side-chain resonances, due to severe overlapping, come from the aromatic rings of Phe and Trp residues. A large number of unusual chemical shifts were observed for backbone as well as side chain atoms. For example, the amide protons of Val^{41} and Ala^{94} were assigned at very upfield region of 4.72 and 3.53 ppm, respectively, as shown in Figure 1a. We suggested that the unusual shifts are primarily due to the ring-current effects because hPAP contains 7 Tyr, 5 Phe and 6 Trp residues. The consensus chemical shift index (CSI) plot shown in Figure 1b (Wishart and Sykes, 1994) reveals that hPAP consists of 2 α-helices and 7 β-strands. The ^{1}H, ^{13}C and ^{15}N chemical shifts at 298 K and pH 4.0 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 6231.

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