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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and the secondary structures of human Coactosin like protein (hCLP) D123N

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Biological context

Nucleotide sequence of human Coactosin Like Protein (hCLP) was first found as a sequence flanking a deletion on chromosome 17 characterizing the Smith-Magenis syndrome (Chen et al., 1997). It has 142 amino acid residues, and was so named for its similarity to coactosin (33% identity and 75% similarity), which can bind F-actin (filamentous actin) *in vitro* (de Hostos et al., 1993). In addition, coactosin showed no effect on actin polymerization, but could interfere with the capping of actin filaments (Hohring et al., 1995). HCLP is similar to coactosin in the F-actin binging ability, but does not form a stable complex with globular actin and Lysine-75 plays an important role in its binding (Provost et al., 2001b). Besides, hCLP can bind to 5LO (5-Lipoxygenase) in which lysine 131 plays an important role (Provost et al., 2001a). 5LO is of central importance in cellular leukotriene (LT) synthesis; it converts arachidonic acid released from the membranes by the cytosolic phospholipase A_2 into 5(S)-hydroperoxy-6,8,11,14-eicosatertraenoic acid (5-HPETE) and subsequently into the epoxide intermediate LTA_4(Samuelsson, 1983). Therefore, 5LO plays an important role in inflammatory disorders, including arthritis, asthma, and allergic reactions. Both the binding of hCLP to F-actin and 5LO are in Ca^{2+} independent manner. Furthermore, the binding of hCLP to 5LO might inhibit its binding to F-actin (Provost et al., 2001a). Up to now, the function of hCLP *in vivo* was not clear.

The study of proteins that bind to F-actin is become one of the most attractive research field and many structures are determined (McGough, 1998). In PDB, there is a crystal structure of the actin-binding protein actophorin from *Acanthamoeba* having about 21% identity and 37% similarity to hCLP, which has the highest identity and similarity (Leonard et al., 1997). Here we report the backbone and side chain assignments of hCLP, as well as the secondary structures, as a first step in the study of its full 3D structure and the relativity between the structure and function.

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Methods and experiments

The hCLP cDNA was obtained by PCR from a human brain cDNA library using the two primers 5′-CATATGGCCACCAAGATCGACAAAGAGG-3′ and 5′-CTCGAGCTCCGTCTGGGCGTCGTAA TTG-3′ and cloned in frame into the NdeI/XhoI sites of pET-22b (+) (Novagen). The recombinant vector was then transformed to the *Escherichia coli* bacterial strain BL21 (DE3). Recombinant hCLP has 150 amino acids, the last 8 amino acids attribute to His-tag. We found D123 was replaced by N through DNA sequencing. Uniformly labeled recombinant hCLP D123N 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 hCLP D123N was then purified by Ni-chelating column according to the manufacturer's instructions.

The NMR samples were prepared with 50 mM phosphate buffer at pH 5.0 in 90% H_2O/10% D_2O and contained 0.45 ml of about 1.5 mM protein. The following experiments were carried out: 2D ^{1}H,^{15}N-HSQC,3D ^{15}N-edited TOCSY-HSQC(67 ms mixing time), 3D ^{15}N-edited NOESY-HSQC(130 ms mixing time), 3D HNCO, 3D HNCACO, 3D HNCA, 3D HNCOCA, 3D CBCA(CO)NH, 3D CBCANH, 3D H(C)(CO)NH-TOCSY, 3D (H)C(CO)NH-TOCSY, 3D HBHA(CBCACO)NH, 3D HCCH-TOCSY, 3D HCCH-COSY, and 3D ^{13}C-edited NOESY-HSQC

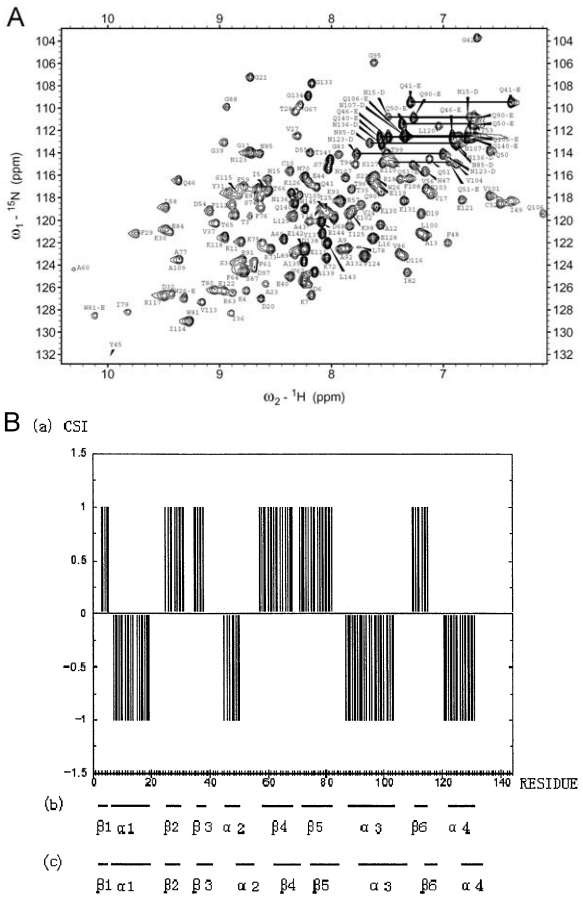

Figure 1. (A) ^{1}H,^{15}N-HSQC spectrum of recombinant hCLP obtained at 296 K. The NMR sample contained about 1.5 mM hCLP in 50 mM phosphate buffer, pH 5.0. The resonance assignments are indicated with the one-letter amino acid code and residue number. The side NH_2 groups of Asn and Gln are indicated by horizontal lines and marked with D or E, respectively; the NH side-chain of Trp is marked with E. (B) CSI consensus plot for recombinant hCLP generated using $^1H^\alpha$, $^{13}C^\alpha$ and $^{13}C^\beta$ and ^{13}CO chemical shifts (a). For comparison, the secondary structures of hCLP (b) and actophorin from *Acanthamoeba* (c) are shown here with thick horizontal lines respectively.

(130 ms mixing time). All NMR experiments were recorded at 296 K on a Bruker DMX 500 spectrometer equipped with cryoprobe except ^{15}N-NOESY was recorded by Bruker DMX 600. NMR data processing was completed using NMRPipe and NMRDraw software (Delaglio et al., 1995) and analyzed with SPARKY. The chemical shift indices (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 hCLP (D123N) was shown in Figure 1A, all the backbone amide resonances A2-E144 (including the first 2 amino acids of His-tag) and the side NH_2 groups of Asn and Gln was marked. Complete backbone assignments of residues from A2-E144 were made for $^1H^N$, ^{15}N, $^1H^\alpha$, $^1H^\beta$, $^{13}C^\alpha$, $^{13}C^\beta$ and ^{13}CO. Assignments of side-chain resonances (A2-E144) were mostly completed, excluding the ^{1}H and ^{13}C resonances in the aromatic rings and some of residue R63, K75, K102. Some resonances of the side-chain ^{1}H and ^{13}C of P38, K93 and K131 need to be further confirmed.

The secondary structure prediction based on CSI and short-range NOEs analysis show the existence of four α-helices and six β-strands characteristic of hCLP D123N. The secondary structure unit distribution of hCLP is very similar to actophorin from *Acanthamoeba* (Leonard et al., 1997) (Figure 1B). The chemical shift values of the proton, nitrogen and carbon resonances have been deposited in the BioMagResBank (accession number: 6071).

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