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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C NMR backbone assignments and secondary structure of the C-terminal recombinant fragment of auxilin including the J-domain

Chae J. Han[a], James M. Gruschus[b], Tsvika Greener[a], Lois E. Greene[a], James Ferretti[b] & Evan Eisenberg[a,*]
[a]*Laboratory of Cell Biology and* [b]*Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-0301, U.S.A.*

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Biological context

Auxilin is a 100 kDa brain-specific J-domain protein that is required for uncoating of clathrin-coated vesicles by Hsc70 in vitro (Ungewickell et al., 1995). Auxilin has three domains; an N-terminal tensin-like domain, a clathrin-binding domain that polymerizes clathrin and binds to the resulting baskets, and a C-terminal J-domain containing the canonical HPDK motif that interacts with Hsc70 (Schroder et al., 1995; Ungewickell et al., 1995). Auxilin is also one of the few J-domain proteins that have a C-terminal rather than an N-terminal J-domain. Auxilin shares many of the functional properties of other J-domain proteins; it presents a substrate to Hsc70 and stimulates Hsc70 ATPase activity. However, auxilin specifically presents clathrin baskets and not other substrates to Hsc70, and conversely other J-domain proteins cannot substitute for auxilin in inducing Hsc70 to bind to clathrin baskets, thus suggesting that the clathrin-binding domain of auxilin plays a unique functional role.

Surprisingly, we found that, after deleting much of the clathrin binding domain, the residual 20 kDa (182 amino acid residues) recombinant C-terminal fragment of auxilin (Aux20) acts like intact auxilin, catalytically supporting uncoating by Hsc70 at pH 7 and inducing Hsc70 to bind to clathrin-coated vesicles at pH 6 (Greener et al., 1998). Therefore, the 20 kDa C-terminal portion of auxilin not only contains the J-domain but also a functional portion of the clathrin-binding domain of auxilin. The NMR structures of the N-terminal J-domains of *E. coli* DnaJ and human Hsp40 have been determined (Pellecchia et al., 1996; Qian et al., 1996). The auxilin J-domain is only 19% homologous to these J-domains. Furthermore, there have not yet been studies on either the structure of any C-terminal J-domains, or even more importantly, the structure of J-domains linked with active substrate binding sites. Therefore, we have undertaken a multi-dimensional heteronuclear NMR study of the 20 kDa recombinant C-terminal fragment of auxilin, Aux20, which consists of the last 182 residues of bovine auxilin. We report here the assignment of backbone ^{1}H, ^{15}N, and ^{13}C resonances and secondary structure of Aux20.

Methods and results

Bovine auxilin was truncated to give the 20 kDa C-terminal recombinant protein Aux20. Aux20 was expressed as a glutathione S-transferase (GST) fusion protein. The ^{15}N and ^{15}N/^{13}C labeled fusion proteins were purified by glutathione affinity chromatography. The Aux20 was cleaved from the fusion protein using PreScission protease (Amersham Pharmacia) and then concentrated. The final NMR sample contained Aux20 at a concentration of ca. 1.5 mM in 5 mM ^{16}D-EDTA, 25 mM phosphate buffer (pH 7.0) of 90% H_2O/10% D_2O.

All NMR spectral data were obtained at 298 K on a Bruker DRX 600 spectrometer. The NMR experiments performed included 3D ^{15}N-HSQC

*To whom correspondence should be addressed. E-mail: eisenbee@nhlbi.nih.gov

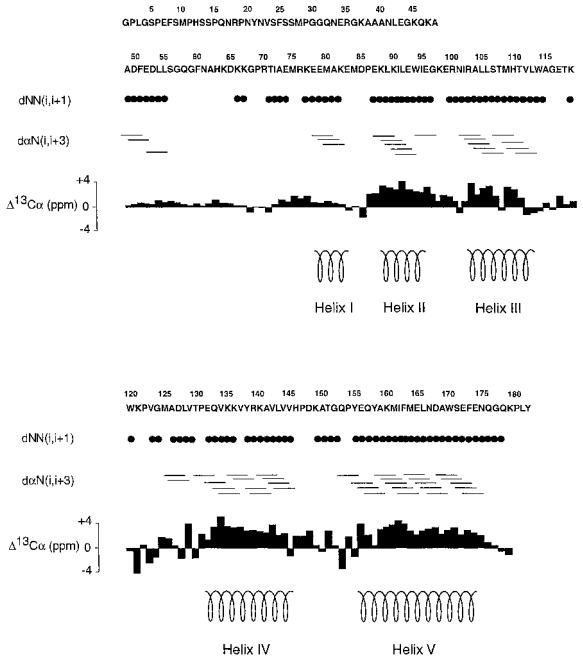

Figure 1. The amino acid sequence of Aux 20 with dNN(i, $i+1$) and dαN(i, $i+3$) connectivities and summary of the secondary structure elements based on connectivities and ^{13}Cα chemical shifts.

NOESY (Gruschus and Ferretti, 1999), CBCACONH (Muhandiram and Kay, 1994), and CBCANH (Grzesiek and Bax, 1992). All the NMR spectra were processed and analyzed with the NMRPipe software package (Delaglio et al., 1995). Figure 1 shows the chemical shift of ^{13}Cα and the NOE connectivity profiles of dNN(i, $i+1$) and dαN(i, $i+3$). Figure 1 also shows the summary of the secondary structure elements identified in Aux20. We found that, except for the first 49 N-terminal residues, Aux20 contains well-defined structural elements. This structure spans at least part of the clathrin-binding domain as well as the entire core C-terminal J-domain (residues from 120 to 182). The secondary structural analysis yielded at least five helices. The J-domain contains a loop composed of residues 144 to 155, which encompasses the highly conserved HPDK region (residues 146 to 149) that connects, probably anti-parallel, helices IV and V (see Figure 1); this is similar to the NMR structures of J-domains determined from recombinant fragments of *E. coli* DnaJ and human Hsp40 (Pellecchia et al., 1996; Qian et al., 1996). Although most of the chemical shifts of the auxilin J-domain, with only 19% sequence homology, were clearly different from those of the DnaJ J-domain (Szyperski et al., 1994), the chemical shift of the amide proton resonance of the aspartate residue in the functionally important HPDK motif in both proteins showed a similar, unusually large downfield shift. The putative clathrin-binding domain showed three helices composed of residues 77 to 82 (helix I), 87 to 94 (helix II) and 101 to 114 (helix III). The chemical shifts of the ^{13}Cα resonances indicate that the rest of the N-terminal residues are random coil.

Extent of assignments and data deposition

Sequential assignments of amide proton, carbon and nitrogen chemical shifts of most of the C-terminal residues of Aux20 were made. Many of the signals for the residues in the N-terminal region of Aux20 (residues from 1 to 49) were not observable because of the fast exchange rates of the unstructured backbone amide protons, due, in turn, to the relatively high pH (pH 7) necessary for solution stability of Aux20. The chemical shift values of proton, carbon and nitrogen resonances have been deposited in the BioMagResBank database under accession number 4716.

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