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Letter to the Editor: Assignment of a 15 kDa protein complex formed between the p160 coactivator ACTR and CREB binding protein

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Biological context

Nuclear hormone receptors (NHRs) regulate the expression of target genes in a ligand dependent fashion (Mangelsdorf et al., 1995). To accomplish this, NHRs recruit coactivator proteins in the presence of ligand or corepressor proteins in the absence of ligand which work in concert with the receptors to generate an activation or repression response. p160 coactivator proteins bind directly to NHRs and recruit other proteins such as CBP/p300, a general transcriptional coactivator with strong histone acetyltransferase activity (Chakravarti et al., 1996), which are necessary to allow RNA polymerase II and the basal transcriptional machinery to operate. Both the p160 family of proteins and CBP/p300 are essential for normal cell cycle control, differentiation and apoptosis and are implicated in human disease (Chen et al., 1997; Goodman and Smolik, 2000).

We have expressed and purified the interacting domains from the p160 coactivator protein ACTR and from CBP (Chen et al., 1997). The first construct consists of residues 1018–1088 of ACTR and the second of residues 2059–2117 of CBP. The 2D ^{1}H-^{15}N HSQC spectra of the isolated domains of both proteins display limited resonance dispersion, an indication that they are unfolded. The two polypeptides combine with high affinity to form a cooperatively folded protein domain. Upon formation of the complex, there is a significant increase in backbone NH dispersion throughout both proteins. This result strongly suggests that folding and binding are coupled events.

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Methods and experiments

The ACTR and CBP interaction domains were subcloned into a pET22b vector containing two separate insertion regions and two separate ribosomal binding sites (provided by R.M. Evans, Salk Institute). Plasmids were transformed into *E. Coli* and the bacteria were cultured in M9 media containing $^{13}C_6$-glucose, $^{15}NH_4Cl$ and $(^{15}NH_4)_2SO_4$ as the sole carbon and nitrogen sources. Cell pellets were spun down at 4000 *g*, resuspended in 8 M urea, and lysed by sonication. Purification to > 95% purity for each protein component was achieved using reverse phase HPLC with preparative C4 columns. The mobile phase consisted of standard H_2O/acetonitrile buffers in 0.1% (v/v) TFA. ACTR, CBP and ACTR/CBP samples were prepared by direct reconstitution from lyophilized powder into a solution of 10 mM TrisHCl, 50 mM NaCl, 0.02% NaN_3 and 5% D_2O and the resulting samples were titrated to pH 6.6 with NaOH. ^{15}N/^{13}C-labeled ACTR or CBP was mixed with a 2–4 fold excess of unlabeled CBP or ACTR, respectively, for NMR studies of the complex. NMR samples ranged from 2–3 mM labeled material.

NMR spectra were recorded on Bruker DRX-600, DMX-600, and AMX-500 MHz spectrometers and referenced internally to DSS. Probe temperatures were calibrated to 304 K using a methanol standard. Two separate sets of 3D HNCA, CBCA(CO)NH, and HNCACB were collected to obtain sequence specific assignments of the backbone and βCH_n resonances for both the complexed CBP domain and the complexed ACTR domain. Subsequently, 3D (H)CCH-COSY, (H)CCH-TOCSY and H(C)CH-TOCSY experiments were collected for both protein domains to obtain sidechain ^{1}H and ^{13}C resonance assign-

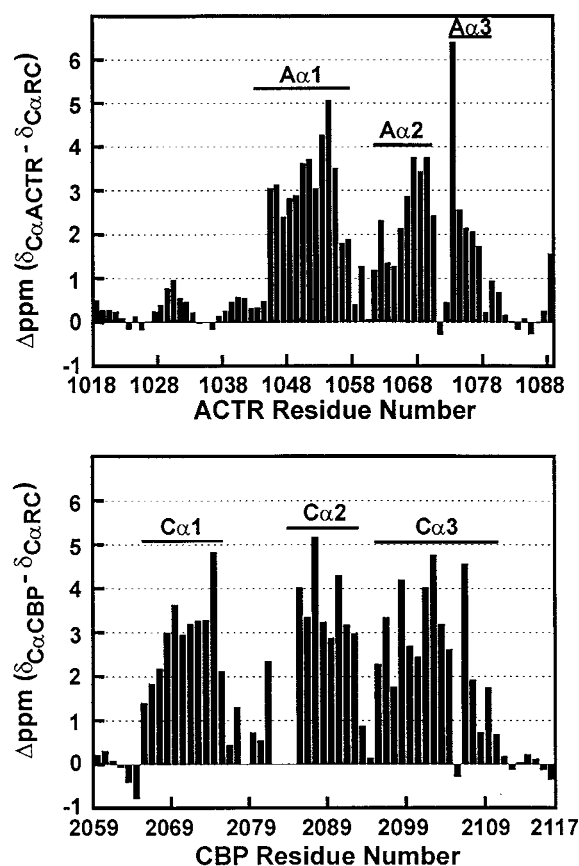

Figure 1. Plots of the deviations from random coil values for the $^{13}C_{\alpha}$ resonances of ACTR:1018–1088 (top) and CBP:2059–2117 (bottom). Sustained positive $^{13}C_{\alpha}$ deviations indicate helical backbone configurations. Both ACTR:1018–1088 and CBP:2059–2117 clearly form three helices, each of which are labeled on the diagram.

ments. The complex contains only three aromatic residues. ^{1}H and ^{13}C assignments for the single Tyr and Phe residues were obtained using 3D ^{13}C-resolved [^{1}H,^{1}H]-NOESY spectra. Side chain resonances of His1053 of ACTR could not be observed. Stereospecific assignment of Val methyl groups was achieved using 2D CGCN, CGCO experiments (Bax et al., 1994). Methionine methyl groups were assigned by analysis of the NOE patterns associated with each methyl group in the 3D ^{13}C resolved [^{1}H,^{1}H]-NOESY spectra. NMR spectra were processed using NMR-Pipe/NMRDraw (Delaglio et al., 1995) and analyzed using NMRView 3.0 (Johnson and Blevins, 1994).

Extent of assignments and data deposition

Near complete assignment of the 130 backbone resonances was achieved: 98% of $^{13}C_{\alpha}$, 96% of $^{1}H_{\alpha}$, 95% of ^{1}HN, 95% of ^{15}N and 86% of $^{13}C'$ have been assigned. Missing backbone assignments arise from the central residues of a polyglutamine stretch within CBP:2059–2117. Only HNCO experiments were performed to assign $^{13}C'$ resonances; therefore, all $^{13}C'$ resonances N-terminal to prolines are unassigned. Deviations from random coil chemical shift values for the backbone $^{13}C_{\alpha}$ resonances are displayed in Figure 1 (Wishart et al., 1995). 63% of the side chains (^{13}C, ^{1}H, and observable ^{1}H-^{15}N resonances) were completely assigned, 32% were partially assigned and 5% were completely unassigned. The ^{13}C, ^{1}H, and ^{15}N chemical shift assignments for the ACTR:1018–1088/CBP:2059–2117 complex have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers 5228 (ACTR domain) and 5229 (CBP domain).

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