NMR assignment of the intrinsically disordered C-terminal region of *Homo sapiens* FCP1 in the unbound state

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Received: 18 May 2009 / Accepted: 3 June 2009 / Published online: 19 June 2009 © Springer Science+Business Media B.V. 2009

**Abstract** The phosphorylation state of the RNA polymerase II C-terminal repeat domain (CTD) regulates progression through the mRNA biogenesis cycle. Termination of transcription and recycling of RNA polymerase II is promoted by an interaction between the general transcription factor IIF (TFIIF) and the TFIIF-associating CTD phosphatase (FCP1). The acidic C-terminal region of FCP1 is disordered in the free state, but adopts an α-helical conformation upon binding to the heavy chain of TFIIF. Here we report 1H, 13C, and 15N resonance assignments for the intrinsically disordered unbound form of human C-terminal FCP1 (residues 879–961). The use of recently developed 13C direct detected “protonless” NMR experiments allowed the nearly complete assignment of FCP1 reported here and is likely to be a generally effective strategy for the chemical shift assignment of disordered proteins.

**Keywords** TFIIF-associating CTD phosphatase · NMR resonance assignments · 13C Direct detection

**Biological context**

The C-terminal repeat domain (CTD) of RNA polymerase II undergoes a cycle of phosphorylation and dephosphorylation as the polymerase progresses through the messenger RNA biogenesis cycle (Meinhart et al. 2005). The transcription factor IIF-associating CTD phosphatase (FCP1) is the only known phosphatase to act on RNA polymerase II CTD. FCP1 phosphatase activity is promoted through interaction of its C-terminal acidic domain (residues 879–961) with the C-terminal domain of the RAP74 chain of transcription factor IIF (Archambault et al. 1998). The structure of the FCP1 acidic region bound to RAP74 has been determined by crystallography (Kamada et al. 2003) and NMR spectroscopy (Nguyen et al. 2003a) and reveals an α-helical conformation for residues 944–960 of FCP1. The remainder of the C-terminal acidic region of FCP1 (879–943) is disordered in the RAP74 bound complex and the entire region is disordered under non-denaturing conditions in the absence of RAP74 (Nguyen et al. 2003a). Partial NMR resonance assignments have been reported for RAP74 bound FCP1 (Nguyen et al. 2003b), but no assignments have been reported for the unbound state. Here we report the 1H, 13C, and 15N resonance assignment of unbound *Homo sapiens* FCP1 (879–961).

**Methods and experiments**

A synthetic gene coding for the acidic region of FCP1 was purchased from GENEART (Regensburg, Germany), cloned into the expression vector pET-47b, and transformed into *Escherichia coli* BL21(DE3) competent cells. Cells were grown in M9 media containing 15NH4Cl and u-13C glucose at 37°C to an optical density (OD600) of 0.6, induced with 0.5 mM IPTG, and grown for an additional 3 h prior to harvesting by centrifugation. Following cell lysis in a French pressure device, the recombinant His-tagged FCP1 was purified by Ni-NTA chromatography. Removal of the His-tag was accomplished through dialysis overnight at 4°C in the presence of His-tagged 3Cprotease. The only non-native sequence remaining after cleavage
was a Pro–Gly dipeptide at the N-terminus of the 9.0 kDa FCP1(879–961) construct. Following dialysis, the 3Cpro-
tease was removed by passage over a Ni-NTA column
resulting in highly pure FCP1 protein. An NMR sample of
$^{15}$N/$^{13}$C FCP1 was prepared by concentration in a Viva-
spin-20 3 K centrifugal concentrator (Sartorius-Stedim)
and buffer exchanged to produce a final sample of 1 mM
FCP1, 20 mM sodium phosphate, pH 7.0, 100 mM NaCl,
0.02% (w/v) NaN$_3$, 10% (v/v) D$_2$O.

NMR data were recorded on a 500 MHz Bruker Avance
3 spectrometer equipped with a TCI cryoprobe. Standard
triple resonance assignment based on amide detected
spectra was attempted using $^1$H/$^15$N-HSQC, $^1$H/$^13$C-HSQC,
$^1$H/$^15$N-TOCSY-HSQC, HNCO, HN(CA)CO, CBCA
(CO)NH, HNCACB, H(CC(CO)NH, CC(CO)NH), and
HCCH-TOCSY. These data proved insufficient for com-
plete resonance assignment and were augmented by the
following $^{13}$C$^0$ direct detected experiments: $^{13}$C/$^15$N CON,
CACO, CBCANCO, CCCO, and CCCON (Bermel et al.
2006a, b), resulting in sufficient spectral dispersion for
resonance assignment. The $^1$H chemical shifts were refer-
cenced to external 2,2-dimethyl-2-silapentanesulfonic acid
(DSS) and the $^{13}$C and $^{15}$N chemical shifts referenced
indirectly.

All $^1$H-detected NMR spectra were processed in
NMRPipe (Delaglio et al. 1995) and the $^{13}$C-detected
spectra in Topspin 2.0 (Bruker). Peak picking and reso-
nance assignment was done in Sparky (Goddard and
Kneller 2006).

Assignments and data deposition

$^{13}$C-direct detected experiments have recently been utilised
in a study of the partially disordered protein securin
(Csizmok et al. 2008), which led us to investigate whether
they would facilitate resonance assignment of FCP1. As
seen in the $^{13}$C/$^{15}$N CON (Fig. 1a) and $^1$H/$^{15}$N-HSQC
(Fig. 1b) spectra, the chemical shift dispersion obtained
through $^{13}$C-carbonyl detection is preferable to that of $^1$H-
amide detected experiments. Furthermore, resonances for
the six proline residues in the N-terminal half of the protein
are all present and well resolved in the $^{13}$C/$^{15}$N CON
(circled in Fig. 1a), yielding straightforward backbone
connectivity in the 3D spectra.

Complete backbone resonance assignments were
attainable for 80 of the 83 native residues in the construct
with a total of >97% backbone assignment. Nguyen et al.
(2003b) have reported partial chemical shift assignments
for RAP74 bound FCP1 (BMRB accession number 5685).
As residues 944–961 undergo a conformational change to
the $\alpha$-helical state upon binding and 25 of the remaining 65
residues in FCP1 were unassigned, no reference was made
to the bound shifts in assigning the unbound state. Compar-
ing the RAP74 bound shifts for the backbone heavy
atoms to the new unbound shifts reveals the predicted
result of extensive chemical shift changes in the RAP74
binding region (Fig. 2). The only other residue with
significantly changed chemical shifts, excepting the final two residues assigned by Nguyen et al. (2003b) at the N-terminus, is His 918. No resonance is found in the unbound FCP1 \(^{1}H,^{15}N\)-HSQC in the region of the bound His 918 assignment, excepting Met 961 which is clearly established as adjacent to Leu 960 in the 3D spectra.

The overall assignment of the routinely observable \(^{13}C\) resonances was 89\% with the majority of the missing resonances being the C\(_{\gamma}\) of Asp and C\(_{\delta}\) of Glu. His 918 is the only aromatic residue, and the aromatic carbons of its side chain were not assigned. Use of the CBCACO allowed assignment of the Asn C\(_{\gamma}\) and Gln C\(_{\delta}\) resonances. The proline \(^{15}N\) and \(^{13}C\) resonances were all readily assigned through the \(^{13}C,^{15}N\) CON and CACO based 3D experiments. The overall \(^{1}H\) assignment rate was 86\% despite the poor dispersion in the \(^{1}H\) detected experiments. Almost all of the proline \(^{1}H\) resonances were unassigned owing to the absence of a \(^{1}H\)-amide and extreme overlap in the HCCH-TOCSY. The complete resonance list has been deposited in the BMRB (accession number 16296).

**Acknowledgments**  This work was supported by start-up funds from the Pennsylvania State University to SAS. We are grateful to Alan Benesi for assistance with the NMR spectrometers.

**Ethical Compliance**  The experiments performed comply with the current laws of the United States.

**References**


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