Structural Characterization of the DNA-binding Domain of the Xenopus NO38 Chaperone Complexed with its Duplex DNA Target

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Introduction

Molecular chaperones are vital for the precise assembly of nucleosomes and chromatin. Chaperone NO38 [*1*] is a Xenopus analog of human chaperone nucleophosmin (63% sequence identity) which may participate in histone assembly, regulation of DNA integrity, cell proliferation, regulation of tumor suppressors p53 and ARF and other key processes [*2*]. Both overexpression and functional loss of human nucleophosmin can lead to tumors [*3*]. The NO38/nucleophosmin polypeptide generally consists of three functional parts: the N-terminal "core" of ~120 residues proposed to bind histones [*4*], the C-terminal, DNA-binding 90-residue "tail" referred to as CTL ("C-terminal, long", preliminary data) and a long acidic linker bearing a basic NLS motif. The crystal structures of the "core" NO38/nucleophosmin fragments from several species revealed a decamer complex comprised of two pentamer rings [*1, 5*]. Each "core" monomer forms a Nucleoplasmin-like beta-barrel structural motif that is conserved in all the organisms studied [*1, 5*].

Our goal here is to determine for the first time the solution structure of the DNA-binding motif of NO38 in complex with its strongly preferred target double-stranded DNA (5'-CAGAGGCCCTGAC-3'). In particular, we are interested in identifying the NO38–DNA interface.

Experimental

Previously we collected all the data needed to perform the backbone assignment of the isolated CTL protein. These data allowed us perform the backbone resonance assignment of CTL. For the backbone NMR resonance assignment and subsequent structural characterization of the CTL fragment complexed with its DNA target, we collected the following heteronuclear solution NMR data: ^{15}N-HSQC, ^{13}C-HSQC, HNCACB, CBCA(CO)NH, HNHA, NH(CA)CO, NHCO and others. All the spectra were recorded on the 720 MHz Varian Inova spectrometers at the NHMFL/ FSU site.

Results, Discussion and Conclusion

The preliminary backbone assignment of ^{13}C/^{15}N-CTL+DNA based on the ^{15}N-HSQC only indicates that complexation with DNA results in chemical shift changes for only few CTL residues with the bulk of the resonances remaining the same as in the isolated CTL. This observation is in agreement with our prior structure determination of the core of the CTL (preliminary data) which folds independently but is not sufficient to bind DNA. Unfortunately, many 3D heteronuclear datasets of the CTL+DNA complex- e.g. HN(CA)CO, HNCO, HNHA etc- display paucity of peaks which hinders our attempts to assign the backbone and side chains of CTL complexed with its DNA partner. Repeated attempts to recollect these spectra in the best possible ways did not help. This is an unexpected development as the CTL and its shorter form CTS in isolation showed great NMR data. At the moment, we are re-recording a number of 3D NMR spectra at another facility on an 800 MHz instrument equipped with a cryoprobe, which delivers significant improvements in the number of observed resonance lines.

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References

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