doi:10.1016/j.jmb.2007.11.031 J. Mol. Biol. (2008) 375, 1076–1085

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

A β-Hairpin Comprising the Nuclear Localization Sequence Sustains the Self-associated States of Nucleosome Assembly Protein 1

Young-Jun Park, Steven J. McBryant and Karolin Luger*

Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523-1870, USA

Received 9 August 2007; received in revised form 6 November 2007; accepted 10 November 2007
Available online 19 November 2007

The histone chaperone nucleosome assembly protein 1 (NAP1) is implicated in histone shuttling as well as nucleosome assembly and disassembly. Under physiological conditions, NAP1 dimers exist in a mixture of various high-molecular-weight oligomers whose size may be regulated by the cell cycle-dependent concentration of NAP1. Both the functional and structural significance of the observed oligomers are unknown. We have resolved the molecular mechanism by which yeast NAP1 (yNAP1) dimers oligomerize by applying x-ray crystallographic, hydrodynamic, and functional approaches. We found that an extended β-hairpin that protrudes from the compact core of the yNAP1 dimer forms a stable β-sheet with β-hairpins of neighboring yNAP1 dimers. Disruption of the β-hairpin (whose sequence is conserved among NAP1 proteins in various species) by the replacement of one or more amino acids with proline results in complete loss of yNAP1 dimer oligomerization. The *in vitro* functions of yNAP1 remain unaffected by the mutations. We have thus identified a conserved structural feature of NAP1 whose function, in addition to presenting the nuclear localization sequence, appears to be the formation of higher-order oligomers.

Edited by J. O. Thomas

Keywords: NAP-1; NAP1; oligomerization; histone binding; nucleosome assembly

Introduction

The nucleosome core particle (NCP) is the universally repeating unit in chromatin, consisting of an approximately equal mass of protein and DNA in a complex macromolecular assembly of 210 kDa.[1] Nucleosome assembly is a stepwise process that starts with the association of histones H3 and H4 with the DNA, followed by the incorporation of H2A–H2B dimers to form the nucleosome.[2] This process is orchestrated by a set of diverse chromatin assembly factors and chaperones.[3–5]

Nucleosome assembly protein 1 (NAP-1 or NAP1) is a histone chaperone that binds H2A–H2B[6] and linker histones[7] (reviewed in Ref. 8). Chromatin assembly activity has been demonstrated *in vitro*.[9,6,10] Since H2A–H2B dimer deposition is likely reversible *in vivo*, NAP1 has been implicated in promoting chromatin fluidity through the removal of H2A–H2B dimers.[11–15] We have shown previously that yeast NAP1 (yNAP1) promotes nucleosome sliding through histone H2A–H2B dimer exchange, which under certain conditions results in histone variant incorporation into existing nucleosomes.[11] NAP1 is a member of a growing family of related proteins whose members are implicated in transcription regulation, cell cycle regulation, apoptosis, histone modification, chromatin assembly, and histone shuttling.[8]

We have previously determined the crystal structure of yNAP1 and demonstrated that the protein is an obligate dimer with an extensive dimerization interface.[16] Studies on NAP1 or NAP1/histone complexes revealed that NAP1 exists in multiple states of oligomers of varying size.[6,9,17–19] Oligomerization was shown to depend on the protein concentration and the ionic strength of the buffer using sedimentation velocity experiments.[17] Similar

*Corresponding author. E-mail address: kluger@lamar.colostate.edu.

Abbreviations used: NAP1, nucleosome assembly protein 1; yNAP1, yeast NAP1; NCP, nucleosome core particle; AUC, analytical ultracentrifugation; NLS, nuclear localization sequence; GST, glutathione *S*-transferase.

experiments suggested that yNAP1 exists in a dimer–octamer equilibrium.[20] Based on sedimentation data, a hexadecameric ring-shaped assembly was proposed for the largest observed yNAP1 complex, similar to the assemblies formed by the unrelated chaperone nucleoplasmin.[21] Since formation of higher-order assemblies is concentration dependent and the nuclear concentration of NAP1 fluctuates in a cell cycle-dependent manner, it has been suggested that the oligomerization of yNAP1 dimers could exist at different times of the cell cycle.[20,22]

Due to the strong propensity of yNAP1 dimers to form oligomers under physiological conditions, it is unknown whether dimeric and oligomeric forms of yNAP1 exhibit different functions. Furthermore, the molecular details of the interactions responsible for the oligomerization of yNAP1 dimers are unknown. In this study, we investigated yNAP1 oligomerization using x-ray crystallography, gel filtration, analytical ultracentrifugation (AUC), and functional assays. We have identified an extended β-hairpin that encompasses the nuclear localization sequence (NLS) as the site of intermolecular interactions through the formation of an extended β-sheet, and we have evidence that yNAP1 assemblies become more elongated with increasing size. We have disrupted the β-hairpin using site-directed mutagenesis and found that this region is indeed responsible for the formation of oligomers, while it has no apparent effect on the various functions attributed to yNAP1 *in vitro*.

Results

The structure of yNAP1$_{(74–365)}$ reveals a highly defined β-hairpin

The central domain of yNAP1 [residues 74 to 365: yNAP1$_{(74–365)}$] retains native-like activity in histone binding and nucleosome assembly.[9,23] In contrast to wild-type yNAP1, which crystallizes at pH 4.85, crystals of NAP1$_{(74–365)}$ were obtained at neutral pH in a tetragonal space group ($P4_22_12$) (Table 1). The refined structure of this truncated form of yNAP1 is for the most part identical with the previously published structure of full-length yNAP1 (Fig. 1a). The two structures superimpose with an rmsd of ~1 Å. Like full-length yNAP1, yNAP1$_{(74–365)}$ forms a stable homodimer. However, while the two monomers are related by crystallographic symmetry in full-length yNAP1, the asymmetric unit for yNAP1$_{(74–365)}$ encompasses the two monomers of the physiological dimer. The two independently built and refined monomers are identical and superimpose with a least-squares difference of 0.79 Å.

The only striking difference between the two structures is found in β5 and β6 (Fig. 1a). This region was structurally ill-defined and partially disordered in the previously published structure.[16] In yNAP1$_{(74–365)}$, it forms a β-hairpin that extends over 11 amino acids for each β-strand and is stabilized by nine hydrogen bonds (Fig. 1b). Amino acids 295–298 form a typical β-turn. The entire hairpin is exceedingly well ordered, as signified by the highly defined electron density (Fig. 1b) and *B*-factors that are ~20 Å^2 lower than the average for the entire molecule. The β-hairpin protrudes by a distance of 3.7 nm from either side of the compact core of the NAP1 dimer.

Table 1. Data collection and refinement statistics for yNAP1$_{(74–365)}$

	Native
Data collection	
Space group	$P4_22_12$
Cell dimensions	
a, *b*, *c* (Å)	125, 125, 146.7
α, β, γ (°)	90, 90, 90
Wavelength	1.1
Resolution (Å)	50–3.2
R_{merge}[a]	0.07 (0.38)
$I/\sigma I$[a]	14 (3.5)
Completeness (%)[a]	99 (92)
Redundancy[a]	5 (5)
Refinement	
Resolution (Å)	50–3.2
No. of reflections[b]	17,067
R_{work}/R_{free}	0.26/0.30
No. of atoms	
Protein	4468
Ligand/Ion	0
Water	0
B-factors	
Protein	85
Ligand/Ion	N/A
Water	N/A
rmsd values	
Bond length (Å)	0.0091
Bond angle (°)	1.5369

[a] Values in parentheses are for the highest-resolution shell.
[b] Reflections of $|F_{obs}| > 1.0$.

The high definition of this region in the present structure can be attributed to its strong contributions to crystal contacts (Fig. 2a). Eight β-strands are arranged to form a consecutive antiparallel β-sheet that links four yNAP1 dimers. The β-hairpin interacts with the β-hairpin of a neighboring yNAP1 dimer through an extensive hydrogen bonding network over its entire length (Fig. 2b). One additional yNAP1 dimer docks on either side of this dimer of dimers. This interaction is slightly weaker due to a larger twist of the β-sheet imposed by lattice constraints. The distance of the adjacent β-strand backbones and the distances between facing oxygen and hydrogen atoms are within the canonical range.

The β-hairpin is implicated in the formation of yNAP1 oligomers

It is well established that interactions between exposed edges of β-sheets are an important mode of protein–protein interaction with potentially pathological consequences (e.g., Ref. 24). Single point mutations were designed to disrupt the β-hairpin to