Molecular recognition of the nucleosomal “supergroove”

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Chromatin is the physiological substrate in all processes involving eukaryotic DNA. By organizing 147 base pairs of DNA into two tight superhelical coils, the nucleosome generates an architecture where DNA regions that are 80 base pairs apart on linear DNA are brought into close proximity, resulting in the formation of DNA “supergrooves.” Here, we report the design of a hairpin polyamide dimer that targets one such supergroove. The 2-A crystal structure of the nucleosome–polyamide complex shows that the bivalent “clamp” effectively crosslinks the two gyres of the DNA superhelix, improves positioning of the DNA on the histone octamer, and stabilizes the nucleosome against dissociation. Our findings identify nucleosomal supergrooves as platforms for molecular recognition of condensed eukaryotic DNA. In vivo, supergrooves may foster synergistic protein–protein interactions by bringing two regulatory elements into juxtaposition. Because supergroove formation is independent of the translational position of the DNA within the histone octamer, accurate nucleosome positioning over regulatory elements is not required for supergroove participation in eukaryotic gene regulation.

Materials and Methods

Cocrystallization of NCP–PA Complexes. Previously established protocols were used to reconstitute NCP146 from recombinant Xenopus laevis histones and a 146-bp DNA fragment derived from human α-satellite DNA (1, 9, 10). PAs were synthesized as described (11, 12). The purity and identity of the clamps were established by analytical HPLC and matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy. PAs were purified by preparative HPLC, lyophilized, and stored at 4°C. Each PA was freshly dissolved in 20 mM potassium cacodylate (pH 6.0) and 1 mM EDTA, and its concentration was determined by measuring UV absorbance by using empirically determined extinction coefficients (ε280 = 68,800 for PA1; ε280 = 139,000 for PW12, PW13, and PW14). NCP146 (at 40–50 μM) was incubated with a 10-fold molar excess of clamp in solution at ambient temperature for 45 min. The integrity of the nucleosome preparation after incubation with the ligand was checked by electrophoretic mobility-shift assay (9). Crystals of NCP146–clamp complexes were grown 1–2 weeks in 40–45 mM MnCl2, 35–38 mM KCl, and 20 mM potassium cacodylate (pH 6.0) containing ≈20 μM (≈4 mg/ml−1) NCP146 at 19°C by using vapor diffusion. Crystals were harvested and flash cooled as described (2).

Data Collection, Structure Refinement, and Validation. X-ray data were collected at beamline 5.0.2 at the Advanced Light Source in Berkeley, CA. Data for each of the individual structures were collected on single crystals and processed with DENZO and SCALEPACK (13). Molecular replacement (using Protein Data Bank ID code 1AOI as the original search model) and refinement were done with CNS (14), and model building with o (15). Each model was checked by using simulated annealing omit maps during early stages of model building. PAs were clearly visible in the original difference density maps calculated after molecular replacement. An initial model for the PA was gener-

Abbreviations: NCP, nucleosome core particle; PA, polyamide.

Data deposition: The atomic coordinates and structure factors for NCP146–PW12 have been deposited in the Protein Data Bank, www.pdb.org (PDB ID code 1532).

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ated by using (i) fragments of previously published PA structures (8), (ii) information from the Uppsala Software Factory HIC-Up server (16), and (iii) Engh and Huber stereochemical parameters (17). The PA was refined along with the rest of the model. The geometry of the final model is excellent (see Table 1), with 93.7% of the residues in the most-favored regions, 5.6% in additional allowed regions, 0.7% in the generously allowed regions, and no residues in the disallowed regions of the Ramachandran plot. The refined structures were compared with unliganded nucleosome structures by using the LSQMAN program from the Uppsala Software Factory (18). Several figures in the paper were made by using the molecular graphics program PYMOL [W. L. DeLano, PYMOL Molecular Graphics System (2002), www.pymol.org].

**Nucleosome Dilution Assay.** The nucleosome dilution experiments were performed as described (19). Briefly, NCP146 (reconstituted at a molar ratio of 0.6 histone octamer:DNA) was subjected to serial dilution from an initial concentration of 10 nM in steps of 3.3, 1.1, 0.34, 0.11, 0.035, 0.01, and 0.004 nM, in a

Fig. 1. Site-specific recognition of nucleosomal DNA by clamp PAs. (A) NCP146 structure (PDB ID code 1AOI, ref. 2) viewed with the superhelical axis along the z axis. The particle pseudo-two-fold dyad axis (d) is shown for orientation. DNA (blue and white) and associated histone proteins (H2A, yellow; H2B, red; H3, blue; H4, green) are shown in sphere or surface representation. (B) Supergrooves in NCP146. Shown is a different view of NCP146 with the superhelical axis along the y axis. Color scheme is the same as in A. One of the DNA supergrooves is indicated by two asterisks. (C) Chemical structures of clamp PAs, PW12 to -14. (D) Hydrogen bonding model of PW12 to its target DNA site. Circles with dots represent lone pairs of N3 of purines and O2 of pyrimidines. Circles containing H represent the N2 hydrogen of guanine. Putative hydrogen bonds are illustrated by dotted lines.
buffer containing 200 mM NaCl, 10 mM Tris·Cl (pH 7.5), 1 mM EDTA, 0.05% Nonidet P-40, and 10% (vol/vol) glycerol. After incubation at 37°C for 1 h, the samples were subjected to nondenaturing gel electrophoresis, followed by PhosphorImager (Molecular Dynamics) analysis of the gel.

**Results and Discussion**

**Bivalent Clamp Binds to a Nucleosomal Supergroove.** Three bivalent hairpin PA dimers (PW12, PW13, and PW14) were synthesized. A previously characterized eight-ring hairpin, whose binding sites were separated by 80 bp on the palindromic α-satellite DNA fragment used for previous crystallographic studies (PA1) (7, 8), was bridged in a head-to-head fashion with one, two, or three ethylene glycol units (resulting in distances of ~11–18 Å between the two hairpins; Fig. 1C) to create the bivalent clamps. The predicted hydrogen-bonding pattern of one of the clamps, PW12, to its target DNA sequence is shown in Fig. 1D. The NCP146–clamp complexes have the same electrophoretic mobility as unliganded NCP146 and do not show signs of dissociation or aggregation upon binding the clamp (data not shown). Quantitative DNase I footprint titrations (20) (data not shown) demonstrate that the clamps bind NCP146 with affinities in the low nanomolar range. Nonspecific binding of the clamp to NCP146 was not observed.

To obtain unambiguous evidence for the mode of interaction of the clamp with nucleosomes, we crystallized NCP146 in complex with each of the three clamps (NCP146–PW12, NCP146–PW13, and NCP146–PW14). Because the biophysical and structural parameters were very similar for the three co-crystal structures, only NCP146–PW12 will be further discussed. Clamp binding increased the size, order, and resolution of NCP146 crystals. High quality x-ray data to 2-Å resolution were obtained for NCP146–PW12 from a single crystal, without detectable radiation damage (Table 1). In contrast, nucleosome

![Clamp binding in the NCP146–PW12 complex. Color code for histone proteins is as in Fig. 1. DNA is shown in green and white, PW12 in green or magenta. (A) Overview of NCP146–PW12 structure, orientation same as in Fig. 1B. (B) Stereoview of PW12 bound to its target DNA site. Omit density for the clamp is shown at 2σ contour level. Chains 1 and 2 denote the two hairpin moieties. (C) The linker in the clamp is buried between the two gyres of superhelical DNA. A close-up surface representation of the NCP146–PW12 structure is shown.](image-url)
Clamp Binding to a Supergroove Prevents Nucleosome Dissociation. Nucleosome dissociation is likely to initiate by the unraveling of the DNA ends from the surface of the histone octamer, followed by the dissociation of one or both (H2A-H2B) dimers (Fig. 3). Furthermore, unwrapping of the ends of nucleosomal DNA by chromatin remodeling complexes such as SWI/SNF (22) and Swr1 (23) is thought to expose the DNA-binding surface of the (H2A-H2B) dimer and initiate dissociation and/or exchange of the (H2A-H2B) dimer. The PA clamp may counteract dissociation because it effectively cross-braces the two gyres of nucleosomal DNA and forms a closed DNA circle around the histone octamer (Fig. 3). Although partial unraveling of the DNA ends may occur in the presence of the clamp, complete dissociation of the DNA is likely to be precluded beyond the clamp binding sites (Fig. 3), which would in turn stabilize the nucleosome from dissociation.

The effect of clamp binding on NCP146 stability was investigated by using a simple dilution assay (19, 24, 25). This method allows us to compare the kinetic stability of NCP146 in the presence and absence of the clamp. We find that a saturating concentration of PW12 dramatically stabilizes NCP146 against dissociation into free DNA and histones (Fig. 4A and B). In the absence of PA, we observed 50% dissociation at a NCP146 concentration of 0.33 nM. In the presence of the unlinked parent PA (PA1), 50% dissociation is observed at ~9-fold lower concentration of NCP146 (0.038 nM). In contrast, dissociation is minimal for NCP146–PW12 (compare lane 8 in the three panels in Fig. 4A, and see Fig. 4B). The off-rate for PW12 on NCP146 is increased almost by an order of magnitude relative to that of PA1 on NCP146 whereas the equilibrium affinities of both the clamp and PA1 for NCP146 are similar (Table 2). Thus, it seems that the kinetic stability ($k_{off}$) of the clamp on NCP146 is significantly higher than that of PA1 on NCP146. These results are also consistent with our observation that NCP146–clamp complexes form ordered and highly diffracting crystals. PA clamps could be potent reagents to determine whether chromatin remodelers transiently unravel DNA as a step en route to histone exchange. Furthermore, this class of molecules might be used to

<p>| Table 2. Dissociation rate constants for PA1 and PW12 with NCP146 |</p>
<table>
<thead>
<tr>
<th>Polyamide</th>
<th>PA1</th>
<th>PW12</th>
</tr>
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<tbody>
<tr>
<td>$K_s$ (M$^{-1}$)</td>
<td>$1.1 (\pm 0.1) \times 10^9$</td>
<td>$0.5 (\pm 0.05) \times 10^9$</td>
</tr>
<tr>
<td>Half-life, min$^1$</td>
<td>15</td>
<td>180</td>
</tr>
<tr>
<td>$k_{off}$ (s$^{-1}$)</td>
<td>$7.7 \times 10^{-4}$</td>
<td>$6.4 \times 10^{-5}$</td>
</tr>
<tr>
<td>$k_{on}$ (M$^{-1}$ s$^{-1}$)</td>
<td>$8.5 \times 10^5$</td>
<td>$3.2 \times 10^4$</td>
</tr>
</tbody>
</table>

$^1$Calculated according to $k_{off} = 0.693/\text{Half-life}$. Values in parentheses denote the SDs. Values reported here are averages and SDs of four independent determinations.

$^2$Calculated by competition footprinting using unlabeled 146-bp DNA as a competitor (data not shown).

$^3$Calculated according to $K_s = (K_d/k_{off})$.
explore and modulate many aspects of chromatin function: for example, to improve nucleosome positioning and stability on poorly positioned nucleosomal templates, to study the role of nucleosome dissociation and dynamics during transcription, replication, and chromatin remodeling, or to target reagents or recruit proteins specifically to particular nucleosomes.

Nucleosomal Supergrooves Are Platforms of Molecular Recognition.

Here, we show in vitro that DNA architecture embedded within the nucleosome generates interaction platforms or supergrooves that can be targeted by sequence-specific bivalent DNA binding ligands to mediate short- and medium-range interactions, and to modulate nucleosome stability. In vivo, nucleosomal supergrooves may mediate medium- and long-range DNA interactions. Indeed, there is evidence that such interactions may regulate transcription of several genes. For example, on the mouse mammary tumor virus long terminal repeat, the precise spacing between two glucocorticoid receptor recognition elements brings about nucleosome-mediated synergistic transcriptional activation (ref. 26 and references therein). In several other genes, a positioned nucleosome brings two precisely spaced regulatory elements into juxtaposition; examples of this are the X. laevis vitellogenin B1 and TRβA genes, the Drosophila Adh, hsp26, and hsp27 genes, and the human U6 gene (refs. 26 and 27 and references therein). We found numerous examples of tandem or multiple repeat regulatory sequences that are arranged with a regular spacing of ~80 bp. Such spacing may have evolved to promote crosstalk between factors bound to noncontiguous regulatory elements in the context of chromatin. Supergrooves occur all along the nucleosomal DNA (seven minor and six major supergrooves per nucleosome), and their occurrence requires only that the two DNA segments be separated by ~80 bp and be within the context of a folded nucleosome. Because they do not require precise translational nucleosome positioning, the presence of nucleosomes over such regulatory elements, and their role in transcription regulation, may often go undetected, and synergistic interactions between regulatory factors may be much more frequent than previously assumed.

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