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Quantitative Determination of Binding of ISWI to Nucleosomes and DNA Shows Allosteric Regulation of DNA Binding by Nucleotides

Gada Al-Ani,[†] Koan Briggs,[‡] Shuja Shafi Malik,[‡] Michael Conner,[†] Yoshiaki Azuma,[†] and Christopher J. Fischer*[,‡]

[†]Department of Molecular Biosciences, University of Kansas, 2034 Haworth Hall, 1200 Sunnyside Avenue, Lawrence, Kansas 66045, United States

[‡]Department of Physics and Astronomy, University of Kansas, 1082 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, Kansas 66045, United States

Supporting Information

ABSTRACT: The regulation of chromatin structure is controlled by a family of molecular motors called chromatin remodelers. The ability of these enzymes to remodel chromatin structure is dependent on their ability to couple ATP binding and hydrolysis into the mechanical work that drives nucleosome repositioning. The necessary first step in determining how these essential enzymes perform this function is to characterize both how they bind nucleosomes and how this interaction is regulated by ATP binding and hydrolysis. With this goal in mind, we monitored the interaction of the chromatin remodeler ISWI with fluorophore-labeled nucleosomes and DNA through associated changes in fluorescence anisotropy of the fluorophore upon binding of ISWI to these substrates. We determined that one ISWI molecule binds to a 20 bp double-stranded DNA substrate with an affinity of 18 ± 2 nM. In contrast, two ISWI molecules can bind to the core nucleosome with short linker DNA with stoichiometric macroscopic equilibrium constants: $1/\beta_1 = 1.3 \pm 0.6$ nM, and $1/\beta_2 = 13 \pm 7$ nM^2. Furthermore, to improve our understanding of the mechanism of DNA translocation by ISWI, and hence nucleosome repositioning, we determined the effect of nucleotide analogues on substrate binding by ISWI. While the affinity of ISWI for the nucleosome substrate with short lengths of flanking DNA was not affected by the presence of nucleotides, the affinity of ISWI for the DNA substrate is weakened in the presence of nonhydrolyzable ATP analogues but not by ADP.

$$P + D \underset{}{\overset{\beta_1}{\rightleftharpoons}} PD$$

$$P + A \overset{\beta_A}{\rightleftharpoons} PA$$

$$PA + D \overset{\beta_{1,A}}{\rightleftharpoons} PAD$$

$$PD + A \overset{\beta_{A,1}}{\rightleftharpoons} PDA$$

DNA within the nuclei of cells is packaged and organized into highly ordered structures collectively called chromatin. The basic level of chromatin is the nucleosome, which consists of the wrapping and packaging of DNA around positively charged proteins called histones.[1,2] There are four core histones (H2A, H2B, H3, and H4), and two of each of these proteins interact with each other forming a stable octamer around which ∼147 bp of DNA is wrapped.[2] In addition to protecting and organizing the DNA within the cells, the compaction of DNA into chromatin plays essential roles in DNA replication and gene expression.[3,4] The rearrangement of chromatin structure can be achieved through two known mechanisms; one mechanism involves the epigenetic modification of the nucleosomes by chromatin-modifying enzymes,[5,6] and the other mechanism involves an ATP-dependent rearrangement of the chromatin by a group of enzymes called chromatin remodelers.[7−9] On the basis of sequence and functional properties, chromatin remodelers are classified as part the SNF2 family of proteins, which in turn is part of helicase superfamily II (SF-II).[10] All chromatin remodelers share a highly conserved ATP-hydrolyzing domain and are further categorized into four subfamilies (ISWI, SWI/SNF, CHD, and INO80) on the basis of additional domains that confer specific functional properties.[4,10,11] The conserved ATP-hydrolyzing domains within the catalytic subunit of chromatin remodelers are significantly homologous to those in the helicase family of proteins.[10,12,13] The ability of helicases to translocate along DNA is necessary, but not sufficient, for their double-stranded DNA unwinding activity.[14−17] Similarly, chromatin remodelers have been shown to lack helicase activity[18] but to retain the ability to translocate along free or nucleosomal DNA in an ATP-dependent manner, a property essential for their nucleosome repositioning activity.[19−22]

The 135 kDa ISWI (imitation switch) ATPase from *Xenopus laevis* is a member of the ISWI subfamily of chromatin remodeling enzymes;[23] ISWI homologues have also been identified in humans,[24] *Drosophila melanogaster*,[25] and *Saccharomyces cerevisiae*.[26] *Xenopus* ISWI interacts with other protein subunits to form three additional chromatin remodeling complexes (ACF, CHRAC, and WICH[27]), and its nucleosome repositioning strategy has been shown to vary depending upon the complex with which it is associated.[4,28−32] ISWI has also been shown to be a conditional ATPase; DNA substrates cause only low levels of activation, while nucleosomes allow for maximal stimulation of activity.[25,33,34] This suggests that ISWI recognizes specific motifs presented by the nucleosomes. Indeed, several studies have demonstrated that interactions between the remodeler and histone tails, the H4 tail in

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particular, play an essential role in this regulation of ISWI ATPase activity.[33,35−38] Nevertheless, ISWI is capable of translocating along both single- and double-stranded DNA.[20] The ability of chromatin remodelers to translocate along DNA is fundamental to their nucleosome repositioning activity.[9,20,22,39] During these processes of DNA translocation and nucleosome repositioning, the remodeler continually experiences repeated cycles of ATP binding, ATP hydrolysis, release of ADP and inorganic phosphate, and possibly additional conformational changes.[19,22,40−42] However, processive translocation and efficient movement along the DNA, and hence effective repositioning of nucleosomes, require that the remodeler alternate between a DNA-bound state and a DNA-unbound state while simultaneously maintaining contact with the histone octamer. Identifying how the binding of ISWI to both DNA and nucleosomes is allosterically regulated by ATP binding and hydrolysis is essential not only for determining the catalytic cycle associated with nucleosome repositioning but also for understanding how DNA translocation and nucleosome repositioning are regulated.

Furthermore, a quantitative characterization of the equilibrium binding of ISWI to both DNA and nucleosomes under conditions of known stoichiometry is required to determine the kinetic mechanism of nucleosome repositioning by ISWI (i.e., the associated microscopic and macroscopic rate constants, the stoichiometry of the active oligomeric state, etc.). Indeed, currently accepted models for nucleosome repositioning by ISWI were based on the results of experiments performed with nucleosome substrates with increasing lengths of flanking DNA[20,43,44] and under conditions that had been shown to increase the stoichiometry with which ISWI complexes bind to these substrates.[45−47] Variations in the stoichiometry of the remodeler−nucleosome interaction in these experiments may have contributed to the apparent sensitivity of the repositioning rate of these enzymes to the length of the flanking DNA. In addition, and perhaps because of this, studies aimed at obtaining quantitative descriptions of remodeler−nucleosome binding or allosteric regulation of these interactions unfortunately yielded conflicting reports.[41,44,46,47]

Here we report our determination of the equilibrium constants associated with binding of ISWI to DNA, mononucleosomes, and nucleotides. Utilizing a fluorescence anisotropy-based assay, we quantitatively investigated the equilibrium binding of ISWI to fluorophore-labeled DNA and to nucleosomal substrates with short flanking DNA and found that ISWI can bind to these substrates with high affinity. Furthermore, to improve our understanding of the mechanisms of DNA translocation and nucleosome repositioning by ISWI, we determined the effect of nucleotide analogues on substrate binding by ISWI. Interestingly, we found that while the affinity of ISWI for nucleosome substrates with short flanking DNA is not affected by the presence of nucleotides, the binding of ISWI to DNA is weakened in the presence of nonhydrolyzable ATP analogues but not by ADP. These results suggest that high-affinity, non-nucleotide-regulated contacts between ISWI and histones form an anchor about which DNA translocation by ISWI results in nucleosome repositioning. Furthermore, we demonstrate that the affinity with which ISWI interacts with nucleosomes is independent of the length of the flanking DNA. These findings are further discussed in context of the current knowledge of nucleosome binding and repositioning by ISWI and ISWI-containing complexes.

■ EXPERIMENTAL PROCEDURES

Recombinant ISWI Expression and Purification. cDNA encoding *X. laevis* ISWI (A kind gift from P. Wade) was amplified using polymerase chain reaction (PCR) with primers containing a BglII restriction site at the 5′ end and an EagI restriction site at the 3′ end into the pCR4-TOPO vector (Invitrogen). The insert was then further subcloned into the BamHI- and NotI-digested pPIC3.5-CBP-Xpress-zz yeast expression vector. All recombinant constructs were confirmed by sequencing. The ISWI-containing recombinant construct was then transformed into *Pichia pastoris* strain GS115 through electroporation followed by recombinant ISWI expression according to the manufacturer's protocol (Invitrogen). Briefly, cells were grown in buffered glycerol complex medium (Invitrogen) until OD_{600} reached 10, and the cells were then resuspended in buffered methanol medium and allowed to shake for 6 h at 30 °C to induce protein expression. Cells were harvested by centrifugation at 3000*g* for 5 min at 4 °C. Cell paste was loaded into a syringe, dispensed into liquid nitrogen, and stored at −80 °C until ready for use. A mixture of dry ice and frozen yeast cells was mechanically lysed followed by addition of lysis buffer [50 mM Tris (pH 8.0), 300 mM NaCl, 0.1% Triton X-100, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10% glycerol, 10 mM PMSF, and 1.3 mM β-mercaptoethanol] and centrifugation at 15000*g* for 30 min at 4 °C. The supernatant containing 1 mM $CaCl_2$ was then incubated with Calmodulin Sepharose 4b resin (GE Healthcare) for 4 h. CBP-tagged ISWI was then eluted using 10 mM EGTA-containing buffer. Collected elutions were examined using 8% sodium dodecyl sulfate−polyacrylamide gel electrophoresis analysis, and ISWI-containing fractions were further purified using a heparin column (GE Healthcare) followed by buffer exchange [20 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM $MgCl_2$, 5% glycerol, and 0.5 mM DTT] and stored at −80 °C. The protein concentration was determined through measurements of A_{280} and the extinction coefficient and further confirmed using a Bradford assay. Subsequent analysis using dynamic light scattering confirmed that ISWI was monomeric under these solution conditions. The purity of our recombinant ISWI is demonstrated in Figure 5A of the Supporting Information. As shown in Figure 5A of the Supporting Information, the recombinant ISWI is larger than the endogenous 135 kDa ISWI because of the presence of necessary affinity tags for the purification process. ISWI activity and properties were not affected by the presence of affinity tags as demonstrated by our control experiments in Figure 5B of the Supporting Information and as reported by others.[48]

Nucleosome Reconstitution Reactions. pET28 plasmids containing untagged H2A, H2B, H3, and H4 (a kind gift from B. Cairns) were transformed into BL21(DE3)pLys cells. Recombinant yeast histone expression, purification, and octamer assembly were performed as described previously.[49,50] DNA fragments containing the 148 bp 601 high-affinity nucleosome positioning sequence[51] (the sequence-containing plasmid was a kind gift from T. J. Richmond) and an additional length of flanking DNA were amplified using large scale PCR followed by purification of the amplified fragment. Either nonlabeled primers or Alexa488 end-labeled primers (IDT) were used to reconstitute the mononucleosome substrates with the desired fluorophore label and flanking DNA length. Samples containing a mixture of DNA fragments and histone octamer in high-salt buffer [10 mM Tris (pH 7.5), 2 M KCl, 1

mM EDTA, 0.05% Tween-20, and 10 mM β-ME] were subjected to slow gradient dialysis against low-salt buffer [10 mM Tris (pH 7.5), 50 mM KCl, 1 mM EDTA, 0.05% Tween-20, and 10 mM β-ME] using peristaltic pumps as described previously.[49,50] Reconstituted mononucleosomes were evaluated using a 5% native polyacrylamide−bisacrylamide gel (60:1) run at 100 V in 0.25× Tris-Borate-EDTA (TBE) buffer followed by staining using SYBR gold or exposed for fluorescence and imaging using a Typhoon imager (GE Healthcare).

Nucleosome and DNA Binding Studies. A 20 bp 5′-FITC or Alexa488-labeled double-stranded DNA substrate (5′ CCATGTCCATGGATACGTGG 3′) (IDT) was titrated with increasing concentrations of ISWI in reaction buffer [10 mM HEPES (pH 7.0), 20 mM KCl, 10 mM $MgCl_2$, 4% glycerol, 0.1 mg/mL BSA, and 0.5 mM DTT] at 25 °C. Binding of ISWI to this DNA substrate was measured by monitoring changes in the anisotropy of the fluorophore using a Syergy2 fluorescence spectrophotometer (BioTek) set at 485 nm excitation and monitoring emission at 520 nm. To test the effect of nucleotide analogues on ISWI−DNA interactions, similar experiments were performed in the presence of varying total concentrations of ADP (Sigma-Aldrich), ATP-γ-S (Roche), or AMP-PNP (Roche) already present in solution prior to ISWI titration. Binding of ISWI to 5′ Alexa488-labeled nucleosomal substrates was performed under the same conditions. All concentrations are indicated in the figures and figure legends.

Electrophoretic Mobility Shift Assay. Reaction mixtures containing 50 nM nucleosomes were incubated with increasing concentrations of ISWI (12−300 nM) in reaction buffer [10 mM HEPES (pH 7.0), 20 mM KCl, 10 mM $MgCl_2$, 4% glycerol, 0.1 mg/mL BSA, and 0.5 mM DTT] for 30 min at 25 °C. The reaction mixtures were then analyzed using a 5% native polyacrylamide−bisacrylamide gel (60:1) run at 100 V in 0.25× TBE buffer followed by staining using SYBR gold or detecting Alexa488 fluorescence using a Typhoon imager depending on the utilized nucleosomal substrate.

ISWI ATPase Activity Assay. To screen for effective nonhydrolyzable ATP analogues, reaction mixtures containing 250 nM 50 bp DNA substrate were incubated with 500 nM ISWI in reaction buffer [10 mM HEPES (pH 7.0), 20 mM KCl, 10 mM $MgCl_2$, 4% glycerol, 0.1 mg/mL BSA, and 0.5 mM DTT] at 25 °C. Additional sets of reaction mixtures contained 1 mM ADP, AMP-PNP, or ATP-γ-S. The reactions were initiated by addition of 1 mM ATP containing 7.5 μCi of [α-^{32}P]ATP. Aliquots were withdrawn at specific time points and mixed with an equal volume of 0.5 M EDTA to stop the reaction. To separate ADP from ATP species, reaction mixtures were analyzed using thin liquid chromatography PEI-cellulose plates (EMD chemicals) in 0.6 M potassium phosphate (pH 3.4) buffer, quantified using a Typhoon Phosphor imager. Mixtures used to assess the nucleotide concentration-dependent inhibition of nucleosome-stimulated ATPase activity contained 50 nM ISWI along with 250 nM 10NF5 nucleosomes and increasing concentrations of nucleotide ranging from 50 to 500 μM. Reactions were initiated by adding 200 μM ATP containing [α-^{32}P]ATP. Reactions were stopped and mixtures analyzed as described above.

Data Analysis. The simplest model consistent with our measured equilibrium ISWI−DNA binding isotherms is a 1:1 binding model; analysis of these isotherms with respect to alternative models is shown in Table 1 of the Supporting Information. The simplest model consistent with the equilibrium binding of ISWI, DNA, and nucleotides is shown in Scheme 1. In Scheme 1, a single ISWI (P) can bind to a single DNA molecule (D) with a stoichiometric macroscopic equilibrium constant β_1, ISWI can bind to nucleotide with a stoichiometric macroscopic equilibrium constant β_A, and a complex of ISWI and nucleotide (PA) can bind to DNA with a stoichiometric macroscopic equilibrium association constant $\beta_{1,A}$. This model is in agreement with previous mutagenesis studies showing that ISWI contains only one nucleotide binding site.[52,53]

Scheme 1. ISWI (P) Binding to DNA (D) and the Nucleotide Analogue (A)[a]

$$P + D \overset{\beta_1}{\rightleftharpoons} PD$$

$$P + A \overset{\beta_A}{\rightleftharpoons} PA$$

$$PA + D \overset{\beta_{1,A}}{\rightleftharpoons} PAD$$

$$PD + A \overset{\beta_{A,1}}{\rightleftharpoons} PDA$$

[a] β_1, β_A, $\beta_{1,A}$, and $\beta_{A,1}$ represent the stoichiometric macroscopic equilibrium constants.

Initial EMSA experiments demonstrated a stoichiometry of two ISWIs bound to each nucleosome, which thus provided an initial estimate of the stoichiometry in the analysis of equilibrium ISWI−nucleosome binding isotherms monitored using the fluorescence anisotropy-based assay; analysis of these isotherms with respect to alternative models is shown in Table 2 of the Supporting Information. The simplest model consistent with these data and this stoichiometry is shown in Scheme 2. In this model, a single ISWI (P) can bind a single nucleosome (N), to form a singly bound complex (PN) with an associated stoichiometric macroscopic equilibrium constant β_1, and two ISWIs can bind a single nucleosome to form a doubly bound complex (P_2N) with an associated stoichiometric macroscopic equilibrium constant β_2. Simultaneous global analyses of equilibrium binding isotherms with respect to the analytical expressions associated with Schemes 1 and 2 were performed using Mathematica (Wolfram Research) to determine the equilibrium constants and anisotropy signal changes associated with the PD (for DNA binding) or PN and P_2N (for nucleosome binding) species; these analytical expressions were also determined using Mathematica and are too burdensome to reproduce here. Separate independent analysis of these equilibrium binding isotherms through simultaneous implicit analysis using Conlin[54] produced

Scheme 2. ISWI (P) Binding to Nucleosomes (N)[a]

$$P + N \overset{\beta_1}{\rightleftharpoons} PN$$

$$P + PN \overset{\beta_2}{\rightleftharpoons} P_2N$$

[a] β_1 and β_2 represent the stoichiometric macroscopic equilibrium constants.

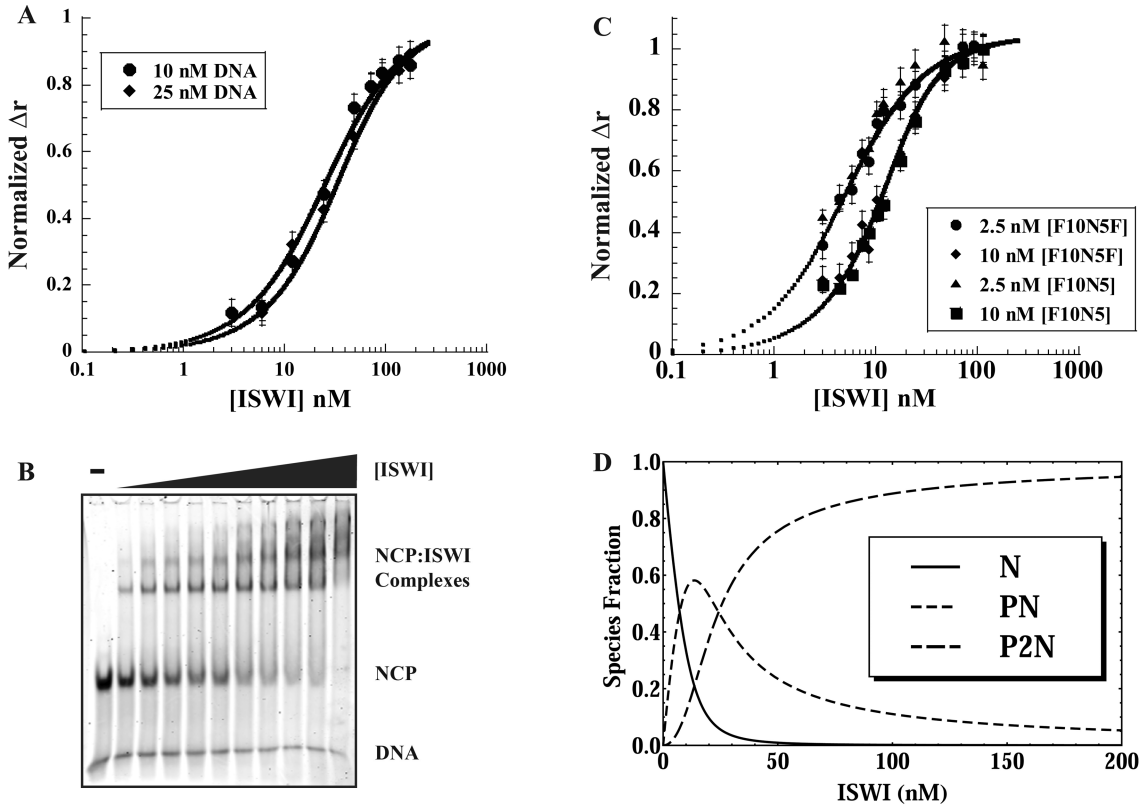

Figure 1. Fluorescence anisotropy measurements (Δr) of binding of ISWI to DNA and nucleosome substrates. (A) A 20 bp FITC-labeled DNA substrate [(●) 10 and (◆) 25 nM] was titrated with ISWI concentrations ranging from 6 to 183 nM, and changes in fluorescence anisotropy were monitored. Isotherms were analyzed using Scheme 1 as described in Experimental Procedures. The solid line represents the fit of the data to this scheme, which returned a $1/\beta_1$ value of 18 ± 2 nM. (B) Electrophoretic mobility shift assay performed by titrating a nonlabeled 10N5 nucleosome substrate (50 nM) with increasing ISWI concentrations ranging from 12 to 200 nM. Samples were analyzed using a 5% TBE−acrylamide native gel. Gels were stained using a DNA staining dye and imaged using a Typhoon imager. Independent experiments showed that high-molecular weight smearing is caused by interaction of ISWI with free DNA present (<2%) in the reconstituted nucleosome sample. (C) Fluorescence anisotropy measurements of binding of ISWI to doubly labeled Alexa488 (F10N5F) and singly labeled Alexa488 (F10N5) nucleosomal substrates. Nucleosomes at 2.5 nM (● and ▲) and 10 nM (◆ and ■) were titrated with increasing concentrations of ISWI ranging from 3 to115 nM. Equilibrium binding isotherms were analyzed using Scheme 2 as described in Experimental Procedures. The solid line represents the fit of the data to this scheme, which returned a $1/\beta_1$ value of 1.3 ± 0.6 nM and a $1/\beta_2$ value of 13 ± 7 nM^2. (D) Computer simulations according to Scheme 2 of the fraction of free nucleosome (N), singly bound nucleosome (PN), and doubly bound nucleosome (P2N) species present as a function of the concentration of ISWI. In these simulations, the total nucleosome concentration was 10 nM, and a $1/\beta_1$ value of 1.3 ± 0.6 nM and a $1/\beta_2$ value of 13 ± 7 nM^2 were taken from the analysis of the data in panel C.

identical results. For this implicit fitting, the following equations were used for Scheme 1:

$$[P_{total}] = [P] + \beta_1[P][D] + \beta_A[P][A] + \beta_{1,A}[P][A][D] \quad (1)$$

$$[D_{total}] = [P] + \beta_1[P][D] + \beta_{1,A}[P][A][D] \quad (2)$$

$$[A_{total}] = [P] + \beta_A[P][A] + \beta_{1,A}[P][A][D] \quad (3)$$

where $[P_{total}]$, $[D_{total}]$, and $[A_{total}]$ are the total concentrations of ISWI, DNA, and nucleotide, respectively, in solution, β_1, β_A, $\beta_{1,A}$, and $\beta_{A,1}$ are the stoichiometric macroscopic equilibrium constants as defined in Scheme 1, [P] is the concentration of free ISWI, [D] is the concentration of free DNA, and [A] is the concentration of free nucleotide. The observed change in anisotropy can be determined from free concentrations of ISWI, DNA, and nucleotide using the following equation

$$f = s\left(\frac{[P][D]}{[D_{total}]}\right)(\beta_1 + C\beta_{1,A}\beta_A[A]) \quad (4)$$

where s is the signal change associated with the formation of the PD complex and C is the ratio of the signal change associated with the formation of the PDA complex to that associated with the formation of the PD complex.

For equilibrium nucleosome binding, the implicit fitting equations are

$$[P_{total}] = [P] + \beta_1[P][N] + 2\beta_2[P][P][N] \quad (5)$$

$$[N_{total}] = [N] + \beta_1[P][N] + \beta_2[P][P][N] \quad (6)$$

where $[P_{total}]$ and $[N_{total}]$ are the total concentrations of ISWI and nucleosomes, respectively, in solution, β_1 and β_2 are the stoichiometric macroscopic equilibrium binding constants as defined in Scheme 2, [P] is the concentration of free ISWI, and [N] is the concentration of free nucleosomes. The observed change in anisotropy can be determined from free concentrations of ISWI and nucleosomes using the following equation

$$f = s\left(\frac{[P][N]}{[N_{total}]}\right)(\beta_1 + C\beta_2[P]) \quad (7)$$

where s is the signal change associated with the formation of the PN complex and C is the ratio of the signal change associated with the formation of the P_2N complex to that associated with the formation of the PN complex.

Unless otherwise noted, all traces presented in the figures have been normalized to the final asymptotic value of the anisotropy change as determined from this analysis. Finally, unless otherwise noted, all uncertainties represent 68% confidence intervals (±1 standard deviation) as determined by Monte Carlo analysis.

RESULTS

DNA Binding Studies of ISWI. ISWI is able to translocate along both single- and double-stranded DNA, a trait necessary for its nucleosome repositioning activity.[20,39] However, a quantitative description of ISWI's ability to bind to and translocate along DNA is required for further delineation of the role of DNA translocation in the mechanism of nucleosome repositioning by ISWI. Perhaps more importantly, because ISWI has been demonstrated to bind to DNA flanking the nucleosome core particle,[20,47] identifying the affinity for ISWI–DNA interactions, and how these interactions are affected by the presence of nucleotides, is critical for the interpretation of data obtained in nucleosome repositioning experiments with ISWI.

We monitored the binding of ISWI to DNA using a fluorescence anisotropy-based assay.[54,55] Previous studies have shown that ISWI is unable to bind a 15 or 18 bp DNA substrate but is able to bind to a 23 bp DNA substrate.[20,55] Furthermore, both 32 and 35 bp DNA substrates have been shown to accommodate more than one bound ISWI.[20,55] To avoid the possibility of multiple ISWIs being bound to the DNA, we therefore used a fluorophore-labeled 20 bp double-stranded DNA. The titration of this DNA substrate with increasing concentrations of ISWI resulted in an increase in the fluorescence anisotropy of the fluorophore (Figure 1A);[54,55] similar increases were detected regardless of whether the DNA was labeled with FITC or Alexa488 (data not shown). This increase in the fluorescence anisotropy is consistent with the formation of an ISWI–DNA complex. Through simultaneous global least-squares analysis of equilibrium binding isotherms conducted at two different total DNA concentrations (10 and 25 nM) using Scheme 1, we determined that the simplest model consistent with the data was a 1:1 stoichiometry with an affinity ($1/\beta_1$) of 18 ± 2 nM (Figure 1A and Table 1 of the Supporting Information). This result is also in agreement with previous reports of contact and occluded site sizes of 15–23 bp for ISWI[20] and with an affinity of approximately 15 nM for ISWI binding cooperatively to a 35 bp DNA substrate.[55] It is worth mentioning that we and others have previously shown that the presence of fluorophore labels can have an effect on the affinity of the protein for the substrate.[54,56] We performed competition experiments and found that the presence of the fluorophore increased the affinity of the interaction by a factor of ∼3 (Figure 6 of the Supporting Information).

Nucleosome Binding Studies of ISWI. Previous native gel-based binding studies demonstrated that only low levels of binding were reported for ISWI[20,47] and ISWI-containing complexes ACF[43,45,46] and ISW2[52,57,58] interacting with nucleosomes containing no flanking DNA. On the other hand, multiple ISWI–nucleosome complexes were detected upon interaction of ISWI with nucleosomal substrates with longer stretches of flanking DNA.[20,47,59] Taken together, these results indicate both that flanking DNA affects the affinity of ISWI for nucleosome binding and that the presence of long flanking DNA on the nucleosome might provide an additional ISWI binding site that may not be in direct contact with the histones. Because any mechanistic study of the nucleosome repositioning activity of a remodeler requires the determination of the oligomeric state associated with the remodeler–nucleosome interaction, we sought to quantify the stoichiometry and affinity of the ISWI–nucleosome interaction.

To accomplish such analysis, we reconstituted mononucleosomal substrates using the high-affinity Widom 601 nucleosome positioning sequence.[51] This sequence contains 146 bp, which allows for the positioning of the histone octamer to one major site on the DNA fragment.[51,60] The positioning of the nucleosomes reconstituted with yeast histones and the 601 sequence or the significantly weaker 5S sequence has been demonstrated by several groups through nuclease digestion assays.[61−67]

To minimize the possibility of ISWI binding to only the flanking DNA, and not contacting the histones, we restricted the length of the flanking DNA to <23 bp, the approximate occluded site size for ISWI–DNA binding.[20,55] In our initial experiments, we monitored the binding of ISWI to a nucleosome substrate with 10 bp DNA flanking one side of the nucleosome core particle and 5 bp flanking the other side; we refer to this substrate as 10N5. The binding of ISWI to a non-fluorophore-labeled 10N5 was monitored using a native gel electrophoretic mobility shift assay (EMSA). As shown in Figure 1B, upon ISWI titration, we detect the formation of two major slow mobility bands consistent with ISWI bound to each 10N5 in a 2:1 stoichiometry at saturation. It is worth noting that EMSA experiments that we performed using fluorophore-labeled nucleosomes yielded the same stoichiometry of ISWI–nucleosome binding, eliminating the possibility of effects of the fluorophore on stoichiometry (data not shown).

To confirm this stoichiometry and to more readily determine the equilibrium constants associated with the binding of ISWI to nucleosomes, we monitored the binding interaction using a fluorescence anisotropy-based assay[55] and a nucleosome substrate in which the 5′ end of the flanking DNA and both 5′ ends of the flanking DNA were labeled with Alexa488; we refer to these substrates as F10N5 and F10N5F, respectively. In these experiments, the titration of the nucleosome substrates with increasing concentrations of ISWI resulted in an increase in the fluorescence anisotropy of the fluorophore, consistent with the formation of ISWI–nucleosome complexes (Figure 1C).[54,55] The simplest model consistent with the observed 2:1 stoichiometry of binding of ISWI to these substrates is shown in Scheme 2. Through the global least-squares analysis using Scheme 2 of equilibrium binding isotherms collected with two different total F10N5F concentrations, we determined the following associated overall equilibrium constants: $1/\beta_1 = 1.3 \pm 0.6$ nM, and $1/\beta_2 = 13 \pm 7$ nM^2. Using these parameters, we simulated the species distribution for the equilibrium binding of ISWI to F10N5F nucleosomes (Figure 1D). As indicated in Figure 1D, cooperativity in the binding of ISWI to this substrate, if it exists, is weak. Additional models, including one postulating that ISWI can exist as a dimer in solution and thus bind the substrate as either a monomer or a dimer, were also tested but were not consistent with the binding isotherms (see Table 2 of the Supporting Information).

ISWI Binding to DNA and Nucleosomes in the Presence of ADP and ATP Analogues. We repeated our

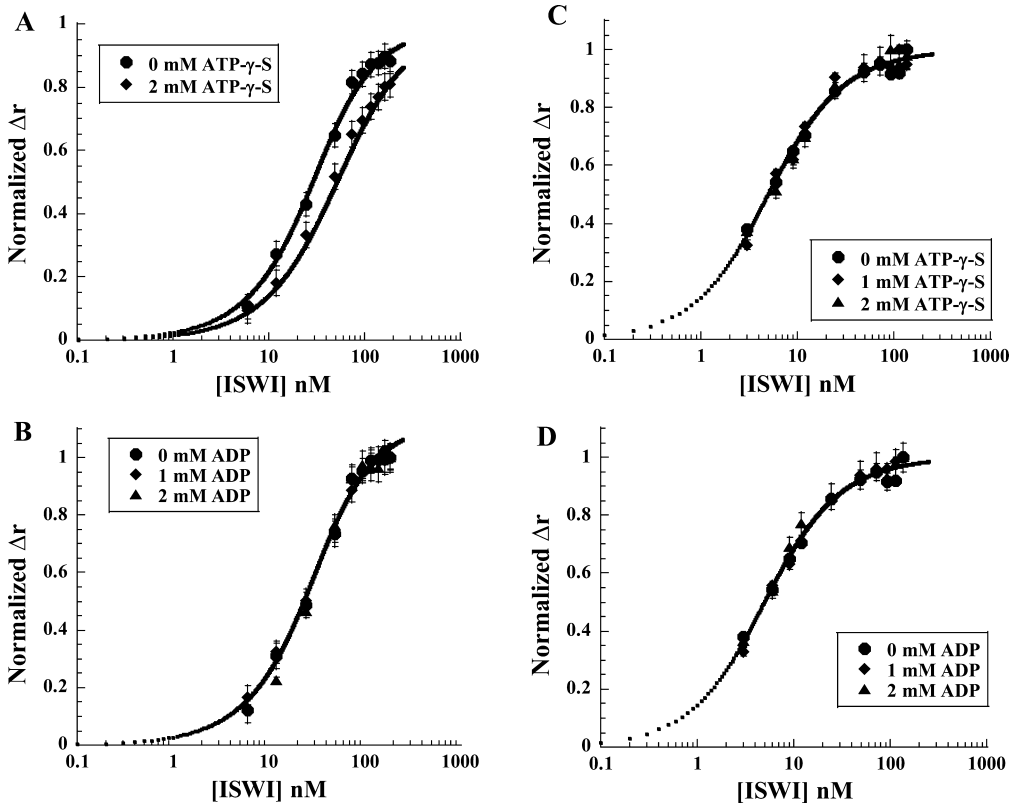

Figure 2. Fluorescence anisotropy measurements (Δr) of equilibrium binding of ISWI to DNA and nucleosomes in the presence of nucleotides. (A) Equilibrium binding to a 20 bp FITC-labeled DNA substrate (25 nM) in the presence of ATP-γ-S. These data were analyzed using Scheme 1 as described in Experimental Procedures. The solid lines in the figure represent the fits of the data to this scheme, which returned the following values: $1/\beta_A = 140 \pm 30$ μM, $1/\beta_{A,1} = 390 \pm 70$ μM, and $1/\beta_{1,A} = 42 \pm 8$ nM. (B) Equilibrium binding to a 20 bp FITC-labeled DNA substrate (25 nM) in the presence of ADP. The solid line in this figure represents the fit of equilibrium DNA binding data collected in the absence of nucleotide (Figure 1A). (C) Equilibrium binding to an Alexa488-labeled 10N5 nucleosome substrate in the presence of ATP-γ-S. (D) Equilibrium binding to an Alexa488-labeled 10N5 nucleosome substrate in the presence of ADP. The solid lines in panels C and D are the fits of the equilibrium nucleosome binding data collected in the absence of nucleotides (Figure 1C).

equilibrium binding studies in the presence of ADP and nonhydrolyzable ATP analogues to investigate the effect of the ATP hydrolysis cycle on DNA and nucleosome binding. Such information is critical for the proper modeling of the nucleosome repositioning activity of ISWI [the following paper (DOI: 10.1021/bi500226b)]. To determine the proper analogue for these experiments, we measured the DNA-stimulated ATPase activity of ISWI in the presence of ADP and the nonhydrolyzable ATP analogues, ATP-γ-S and AMP-PNP; in these experiments, the concentration of the ADP or ATP analogue was equal to the concentration of the ATP in solution. We found that ATP-γ-S was the most effective in competing with ATP for binding to ISWI as demonstrated by its ability to inhibit the ATPase activity of ISWI (Figure 1 of the Supporting Information). Similarly, we found that at 1:1 equimolar concentrations ADP was effectively competing with ATP for ISWI binding and consequently inhibiting ISWI ATPase activity. Next, we performed equilibrium DNA binding studies in the presence of concentrations of ADP and ATP-γ-S ranging from 0.5 to 2 mM. The presence of ADP had no effect on ISWI–DNA interactions (Figure 2B). In contrast, in the presence of ATP-γ-S, the affinity of ISWI for DNA was reduced significantly (Figure 2A). We globally fit the equilibrium DNA binding isotherms in the presence of this analogue using Scheme 1. This analysis returned the following values: $1/\beta_A = 140 \pm 30$ μM, $1/\beta_{A,1} = 390 \pm 70$ μM, and $1/\beta_{1,A} = 42 \pm 8$ nM (indicating that the affinity of ISWI for binding DNA is reduced by a factor of 3 in the presence of ATP-γ-S). It is worth noting that our estimate of $1/\beta_A$ (140 ± 30 μM) is consistent with a recent report of a K_m of 150 ± 50 μM for the steady-state ATPase activity of *Drosophila* ISWI in the presence of DNA.[53] We observed a similar decrease in the affinity of DNA binding in the presence of ATP-γ-S in additional experiments conducted with a 60 bp DNA substrate (data not shown), confirming that this effect is not a DNA length effect (i.e., resulting from the partial contact of ISWI with the DNA). Interestingly, as shown in panels C and D of Figure 2, the affinity of nucleosome binding by ISWI was independent of the presence of ADP and ATP-γ-S. To confirm that nucleosome bound ISWI can still bind ATP-γ-S and ADP, we performed nucleosome-stimulated ATPase assays in the presence of increasing concentrations of ATP-γ-S or ADP. We found that both nucleotides inhibit the ATPase activity of ISWI in a concentration-dependent manner, demonstrating the ability of nucleosome-bound ISWI to bind to these nucleotides (Figure 2A,B of the Supporting Information).

ISWI Binding to Nucleosome Substrates with Long Flanking DNA. Recent studies of the nucleosome repositioning activity of the ISWI-containing remodeling complex ACF have suggested that the dependence of the affinity of ACF for nucleosomes on the length of the DNA flanking the core particle results in ACF generating evenly spaced nucleosome

arrays.[43−45] Similarly, the affinity of the ISWI-containing complex ISW2 for nucleosome binding has been shown to increase with an increasing length of the DNA flanking the nucleosome core particle, with a minimal length of 20 bp required for any binding and optimal binding requiring at least 60 bp of DNA.[44] Because of these results, we sought to determine whether ISWI has a different affinity for binding to nucleosomal substrates with longer flanking DNA and whether the binding to these substrates is regulated by nucleotides in a manner that is similar to the regulation observed for our free DNA substrate (Figure 2).

To address this question, we redesigned our previous nucleosomal substrate by increasing the length of the flanking DNA on one side from 5 to 18 bp; this new substrate is denoted F10N18F. We chose this length of flanking DNA to minimize the possibility of an additional ISWI binding to the flanking DNA alone (i.e., not in contact with the histones); furthermore, a similar length of flanking DNA was shown by photochemical cross-linking assays to be contacted by the catalytic subunit (Isw2) of the ISW2 complex[57] and is a length that is below the ISWI−DNA occluded site size.[20] We monitored binding of ISWI to this substrate using the same native gel analysis and fluorescence anisotropy assay; the resulting data suggest that ISWI binds to this substrate with the same affinity and stoichiometry as the F10N5F substrate (Figure 3A,B). Binding studies performed with 10N24 and with symmetrical substrates (18N18F or 24N24) yielded similar outcomes (Figure 4A,B of the Supporting Information). Similar to the F10N5 substrate, the presence of nucleotides had no effect on the affinity of ISWI for the F10N18 substrate (Figure 3B) or the F10N24 substrate (Figure 4A of the Supporting Information). In contrast, when using a substrate with very long flanking DNA, 5N71, we found additional ISWI can be accommodated as demonstrated in our EMSA experiment (Figure 3C). Furthermore, the affinity of ISWI for this substrate was reduced in the presence of ATP-γ-S (Figure 7A,B of the Supporting Information). Overall, these findings suggest that the presence of additional flanking DNA is not required for stable ISWI binding or the nucleotide regulation of the binding to the nucleosome core, which is evident upon comparison of the binding to the 10N5 and 10N18 substrates. Furthermore, the presence of very long flanking DNA can provide an additional binding site for an ISWI molecule that may be regulated by nucleotides.

■ DISCUSSION

The ability of ISWI to translocate along DNA in an ATP-dependent manner is necessary for its nucleosome repositioning activity.[20,22] During these processes, the enzyme undergoes continual rounds of ATP binding, hydrolysis, and product release. For further delineation of the role of DNA translocation in the mechanism of nucleosome repositioning by ISWI, we quantitatively characterized the DNA and nucleosome binding properties of ISWI. Furthermore, to understand the role of the ATP binding and hydrolysis cycle in regulating translocation, we quantified binding of ISWI to DNA and nucleosome substrates in the presence of nucleotide analogues.

ISWI Binding to DNA Substrates. The simplest model consistent with our studies of the equilibrium binding of ISWI with a 20 bp double-stranded DNA substrate is a 1:1 interaction with an equilibrium constant $1/\beta_1$ of 18 ± 2 nM (Scheme 1). This result agrees with previous studies showing that while *Drosophila* ISWI is unable to bind a 15 or 18 bp

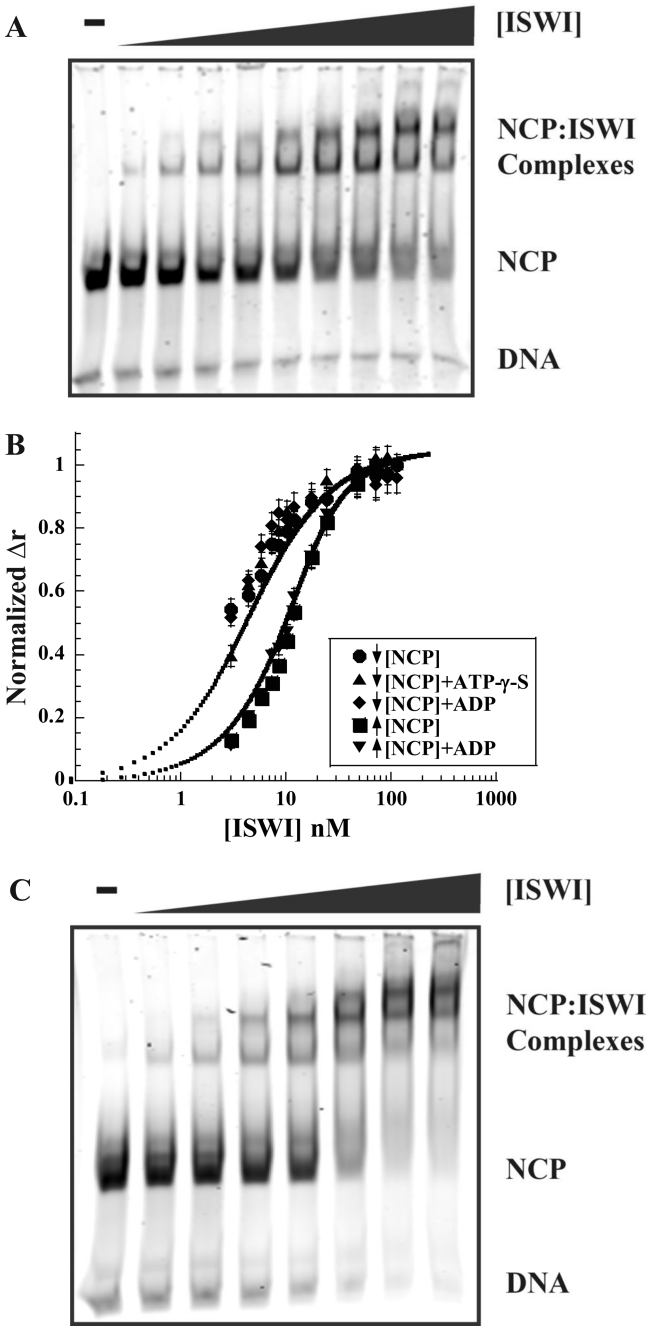

Figure 3. ISWI binding to nucleosome substrate with long flanking DNA. (A) EMSA performed by titrating a 10N18 nucleosome substrate (50 nM) with increasing concentrations of ISWI ranging from 12 to 300 nM. Samples were analyzed using a 5% TBE−acrylamide native gel. Gels were stained using a DNA staining dye and imaged using a Typhoon imager. (B) Fluorescence anisotropy measurements (Δr) of equilibrium binding of ISWI to Alexa488-labeled 10N18 nucleosomes in the presence of 2 mM nucleotides. To more readily determine the effect of ADP on ISWI binding, two different concentrations [2.5 nM (●, ▲, and ◆) and 10 nM (■ and ▼)] of the 10N18 substrates were used in the associated binding experiments. The solid line in this panel represents the fit of the equilibrium nucleosome binding data collected in the absence of nucleotides. (C) Electrophoretic mobility shift assay performed by titrating a nonlabeled 5N71 nucleosome substrate (50 nM) with increasing concentrations of ISWI ranging from 12 to 300 nM. Samples were analyzed using a 5% TBE−acrylamide native gel. Gels

Figure 3. continued

were stained using a DNA staining dye and imaged using a Typhoon imager.

double-stranded DNA with detectable affinity,[20,55] binding to a 23 bp DNA substrate was observed.[20] *Drosophila* ISWI has also been shown to bind a 35 bp DNA in a cooperative manner, indicating that more than one ISWI molecule binds to this substrate, with a reported $K_{1/2}$ of 15 nM.[55] Consistent with our model, these data suggest that the contact and occluded site sizes for DNA binding are between 18 and 23 bp. Interestingly, an apparent weaker DNA binding affinity ($K_{1/2}$) for SNF2h was determined from analysis of its DNA-stimulated ATPase activity.[44] While these results suggest that ISWI and SNF2h have different intrinsic affinities for DNA binding, the weaker affinity for DNA binding by SNF2h might also result in part from it being determined indirectly through DNA-stimulated ATPase assays.[44] Furthermore, this affinity was found to vary with DNA length from >1400 nM for 10 bp DNA to 4 nM for 100 bp DNA.[44] It is not surprising that the apparent affinity determined from these experiments would increase with an increasing DNA length because the rate of DNA binding will scale with the number of DNA binding sites, and hence with the length of the DNA.[19,68] It is worth noting that although ISWI binds double-stranded DNA more tightly than the SWI/SNF chromatin remodeler RSC ($K_d \sim 140$ nM[54]), the k_{cat} for ISWI is much lower than for RSC[54] and may suggest a constraint related to the catalytic domain common to both ISWI and RSC.

ISWI Binding to Nucleosomal Substrates. Our EMSA studies of the equilibrium binding of ISWI to nucleosomal substrates with very short flanking DNA, 10N5, demonstrated that ISWI binds to this substrate with a 2:1 stoichiometry. Through subsequent global analysis of anisotropy-based equilibrium binding studies of ISWI binding to fluorophore-labeled nucleosomal substrates, we determined the following associated overall equilibrium constants: $1/\beta_1 = 1.3 \pm 0.6$ nM, and $1/\beta_2 = 13 \pm 7$ nM^2. Increasing the length of the flanking DNA to 18 bp from one side did not affect the affinity or the stoichiometry of ISWI binding, while further increasing the length to 71 bp provides an additional binding site leading to the binding of an additional ISWI.

Previous studies of equilibrium nucleosome binding by ISWI have presented conflicting results regarding the ability of ISWI to bind nucleosome core particles that lack flanking DNA.[20,47,69] One possibility for these differences is in the sequence used to reconstitute the nucleosomes; indeed, it is known that different positioning sequences give rise to different dynamic nucleosome states.[70] Similarly, measurements of affinity for nucleosome binding determined indirectly through ATPase assays showed that SNF2h binds to nucleosomes with short (<20 bp) flanking DNA with affinities ranging from 25 to >250 nM.[44,71] These results form the basis of a model in which the affinity of SNF2h for nucleosomes is a function of the length of the flanking DNA and that the presence of additional noncatalytic subunits is required for efficient binding to nucleosomes with very short flanking DNA.[44] In comparison to our observed stoichiometry, a negative stain electron microscopy study showed that two SNF2h molecules are bound to a nucleosome substrate with 60 bp of flanking DNA. It is worth noting that, unfortunately, in these images the flanking DNA was not visible. Although it was suggested that this might be a result of flanking DNA flexibility or the fact that DNA is occupied by one of the bound SNF2h molecules,[46] other native gel studies have shown that multiple ISWI molecules can bind to a nucleosome substrate with 36−64 bp of flanking DNA.[32,47]

Studies conducted with other ISWI-containing complexes, such as the yeast ISW2, have shown that the affinity of nucleosome binding is dependent upon the length of the flanking DNA with a minimum of 20 bp required for stable binding.[57] Affinities of ISW2 for nucleosome binding similar to what we report here for ISWI required more than 70 bp of flanking DNA,[57] suggesting that interactions mediated by the noncatalytic protein subunits in the ISW2 complex to the flanking DNA might be contributing to the observed affinity. Unfortunately, more quantitative comparisons between these results are complicated by the fact that conflicting estimates of the stoichiometries for binding of ISW2 to various nucleosome substrates (0N20, 0N67, 0N70, and 0N109) have been reported.[57,72,73] Naturally, any ambiguity in the stoichiometry of the interaction of ISW2 with these substrates complicates estimates of the associated affinity.

Nucleotide Regulation of Binding of ISWI to DNA. We investigated the effect of the ATP hydrolysis cycle on DNA binding and found that while ADP has no effect on the binding affinity of ISWI for DNA, ATP analogues weakened the interaction between ISWI and DNA. The binding of other members of the SF-II superfamily to single- and double-stranded DNA was shown to be modulated by the ATP hydrolysis cycle, and this allosteric effect is central to the processive DNA translocation activity of these enzymes.[74−77] Our observation that binding of ADP by ISWI has no regulatory effects on binding of DNA by ISWI is also consistent with a previous study of *Drosophila* ISWI.[55] However, studies that characterized how nucleotides allosterically regulate the DNA binding affinity of ISW2 have yielded conflicting results: while one study showed that ADP reduced the DNA binding affinity of ISW2,[52] another study showed that ADP had no effect on DNA binding affinity.[72] It was suggested that this discrepancy results from differences between recombinant and native preparations of ISW2.[72] Furthermore, our observations are different from the regulation that was reported for the SWI/SNF subfamily chromatin remodeler RSC.[54] This suggests a difference in the mechanisms of DNA translocation by ISWI and RSC that might contribute to the differences in the proposed models of their nucleosome repositioning activities.[21,22,39]

Nucleotide Regulation of Binding of ISWI to Nucleosomes. We also characterized the effect of nucleotide binding on nucleosome binding by ISWI and found that the binding of ISWI to nucleosomes with flanking DNA ranging from 5 to 18 bp in length was unaltered by ADP or ATP analogues. In agreement with our observations for ISWI, neither ATP-γ-S nor ADP affects the nucleosome binding affinity of ISW2;[52,72] however, the results of restriction mapping experiments suggest that slight changes in contacts with the nucleosomes occur in the presence of ATP analogues.[52] Interestingly, a recent study has demonstrated that only a small percent (1−3%) of the nucleosome-bound SNF2h *in vivo* was affected by ATP levels.[48] More striking are differences between the nucleotide-mediated regulation of nucleosome binding by ISWI and SNF2h. The affinity of SNF2h for a 0N40 nucleosome has been shown to increase in the presence of an ATP analogue and decrease in the presence

of ADP.[46] It is worth mentioning that the length of the flanking DNA used in these experiments is beyond both the contact and occluded site sizes of DNA binding by ISWI[20,47,55] and that additional ISWI complexes have been observed for nucleosomes with comparable lengths (36−64 bp) of flanking DNA.[47] Thus, the presence of an additional SNF2h binding site on the flanking DNA was possible in these experiments. Because SNF2h is known to bind DNA with affinities comparable to those for core nucleosome binding[71] and if the binding of SNF2h to DNA is regulated by nucleotides similar to ISWI, the presence of an additional SNF2h binding site on flanking DNA would lead to the overall observation of nucleotide-mediated regulation of nucleosome binding by ISWI or SNF2h. Consistent with this hypothesis was our observation that the affinity of ISWI for a 5N71 nucleosome substrate was reduced in the presence of ATP-γ-S. This substrate can accommodate an additional ISWI bound on the flanking DNA that is not in contact with the nucleosome core.

Naturally, it is also possible that, although highly conserved, ISWI and SNF2h proteins from different species display distinct behaviors. Indeed, the allosteric effect of nucleotides on DNA binding was found to vary when comparing helicases from different superfamilies with very similar structures.[74−80] Nevertheless, without an independent determination of the stoichiometry of binding of SNF2h or ISWI to these nucleosome substrates, a determination of the mechanism through which nucleotide binding allosterically regulates nucleosome binding is problematic. Indeed, as demonstrated in the following paper (DOI: 10.1021/bi500226b), such information is critical for correct modeling of the nucleosome repositioning activity of ISWI.

Implications for Nucleosome Repositioning. Our findings show that ISWI binds nucleosomes with 5 or 10 bp of flanking DNA with very high affinity, and that additional lengths of flanking DNA are not required for further enhancement of the stability of the binding. The fact that free DNA but not nucleosome binding is allosterically regulated by nucleotides might suggest a model in which ISWI stably contacts structures that are present on the nucleosome but not on free DNA. This agrees with the nucleosome repositioning model for ISWI put forward by Längst and Becker,[59] with previously published data showing that the ATPase activity of ISWI is maximally stimulated in the presence of nucleosomes but not free DNA,[25,33,34] and with previously published data demonstrating that interactions between ISWI and the H4 tail regulate ISWI's ATPase activity.[33,35−38] On the other hand, it is possible that ISWI bound to the nucleosome core still interacts weakly with the flanking DNA and those interactions are regulated by nucleotides. However, even if those weaker contacts with the flanking DNA are present and regulated by nucleotides in a manner similar to the regulation of binding to free DNA, the weak nature of those contacts in comparison to the nature of the interactions made with the nucleosome core would still result in no observed nucleotide-mediated regulation of nucleosome binding. This possibility is in agreement with our observations that increasing the length of the DNA to 24 bp has no effect on the affinity of ISWI for the nucleosome core, and that the binding to this longer substrate is also not regulated by nucleotides.

Because ISWI has two domains that are known to interact with both DNA and nucleosomes [the ATPase domain and the C-terminal HAND-SANT-SLIDE (HSS) domain[69]], determining the origin of the differences in how ISWI binds to nucleosomes and DNA requires resolution of how these two domains interact with these substrates. It was recently demonstrated that the N-terminal ATPase domain of ISWI formed the same ATPase stimulating contacts with nucleosomes as the full length ISWI and is sufficient for nucleosome sliding activity.[53] While the deletion of the entire C-terminal HSS domain decreased the affinity of ISWI for DNA and nucleosomes, compromising mutations of DNA-interacting residues of the SLIDE domain had no effect on the affinity for nucleosome binding but did alter contacts with the flanking DNA.[53,81] These results are consistent with a regulatory role for the C-terminal DNA binding domain of ISWI in the repositioning activity, such as determining directionality of translocation or remodeling efficiency and/or processivity. Furthermore, these findings suggest that regulatory mechanisms, such as nucleotide binding or flanking DNA binding, but not the stable contacts made with the nucleosome core, might play a supporting role in the regulation of nucleosome repositioning. Future mutagenesis and deletion studies aiming to dissect the details of the binding to DNA and nucleosomes, nucleotide-mediated allosteric regulation, and potential cross-talk between the different domains will be of great interest and will contribute further to the understanding of the role of each domain in regulating the nucleosome binding and repositioning activity of ISWI.

It is also possible that other noncatalytic subunits associated with ISWI in ISWI-containing complexes play a role in the allosteric regulation of nucleosome binding by those complexes. Conformational changes in ISWI upon ATP binding and hydrolysis can be also translated into these protein subunits causing pronounced changes in the interaction with flanking DNA, or changes occurring in the ISWI conformation when in complex with these subunits alter the interactions of ISWI with DNA and nucleosomes. Indeed, it has been demonstrated that noncatalytic proteins within the ISW2 complex contact the flanking DNA and that those contacts can extend as far as 53 bp[57,73] and that additional subunits within the ACF complex and the CHRAC complex appear to modulate its nucleosome binding and repositioning activities.[44,82] Future studies that directly compare the nucleosome binding and its associated allosteric regulation for ISWI-containing complexes are required to further resolve these issues.

Finally, our observation that a single nucleosome can accommodate up to two bound ISWI enzymes raises several questions regarding the nucleosome repositioning activity of such a complex: if two ISWI enzymes were bound simultaneously to the same nucleosome, would only one or both be active during repositioning? Does any cross-talk occur between the two ISWI during repositioning? In other words, do the two enzymes work independently or concertedly? In light of the difficulty in interpreting nucleosome repositioning data in the absence of information about the stoichiometry with which the remodeler binds the nucleosome substrate, we argue that resolution of these questions would require measuring the nucleosome repositioning activity of ISWI under conditions of known bound stoichiometry and information regarding nucleotide regulation.[85] Nevertheless, it is worth noting that both SNF2h and human ACF are believed to function as dimers[45,46] and that the dependency of ATP hydrolysis on the length of the flanking DNA[43] along with the allosteric regulation by nucleotides controls which subunit of the dimer is active.[46] In contrast, recent estimates of the total concentration of SNF2h and nucleosomes in human cells

would argue that the predominant bound species *in vivo* is monomeric SNF2h.[48] The fact that some remodeling complexes, such as WCRF and human CHRAC, contain multiple ISWI subunits[83,84] only further emphasizes the need to determine how multiple ISWIs bound to the same nucleosome coordinate their nucleosome repositioning activity.

■ ASSOCIATED CONTENT

Supporting Information

Analysis of equilibrium DNA binding isotherms in the absence of nucleotides using different models (Table 1), comparison of different stoichiometries of binding of ISWI to nucleosomes (Table 2), nucleotide analogue screening (Figure 1), nucleosome-stimulated ATPase−nucleotide competition assay (Figure 2), non-normalized change in fluorescence anisotropy measurements of ISWI−DNA and ISWI−nucleosome binding (Figure 3), fluorescence anisotropy measurements (Δr) of binding of ISWI to F10N24, F18N18F, and F24N24F nucleosomes (Figure 4), effect of the CBP tag on ISWI−DNA interactions (Figure 5), effect of the fluorophore on ISWI−DNA interactions (Figure 6), and ISWI binding to 5N71 nucleosomes in the presence of ATP-γ-S (Figure 7). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Department of Physics and Astronomy, University of Kansas, 1251 Wescoe Hall Dr., 1082 Malott, Lawrence, KS 66045-7582. E-mail: shark@ku.edu. Phone: (785) 864-4579. Fax: (785) 864-5262.

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Notes

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■ ABBREVIATIONS

ISWI, imitation switch; AMP-PNP, adenylyl-imidodiphosphate; ATP-γ-S, adenosine 5′-(3-trithiophosphate).

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