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Histone−DNA Binding Free Energy Cannot Be Measured in Dilution-Driven Dissociation Experiments†

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ABSTRACT: Despite decades of study on nucleosomes, there has been no experimental determination of the free energy of association between histones and DNA. Instead, only the relative free energy of association of the histone octamer for differing DNA sequences has been available. Recently, a method was developed based on quantitative analysis of nucleosome dissociation in dilution experiments that provides a simple practical measure of nucleosome stability. Solution conditions were found in which nucleosome dissociation driven by dilution fit well to a simple model involving a noncooperative nucleosome assembly/disassembly equilibrium, suggesting that this approach might allow absolute equilibrium affinity of the histone octamer for DNA to be measured. Here, we show that the nucleosome assembly/disassembly process is not strictly reversible in these solution conditions, implying that equilibrium affinities cannot be obtained from these measurements. Increases in [NaCl] or temperature, commonly employed to suppress kinetic bottlenecks in nucleosome assembly, lead to cooperative behavior that cannot be interpreted with the simple assembly/disassembly equilibrium model. We conclude that the dilution experiments provide useful measures of kinetic but not equilibrium stability. Kinetic stability is of practical importance: it may govern nucleosome function in vivo, and it may (but need not) parallel absolute thermodynamic stability.

The free energy of protein−DNA[1] interactions governs many aspects of the assembly and function of protein−DNA complexes and therefore is often among the first properties to be measured when work begins on a new experimental system. Paradoxically, despite decades of intensive study of nucleosomes, such fundamental information remains unavailable for histone−DNA interactions. Instead, methods have been developed that provide only the relative free energy of histone−DNA interactions (*1*−*6*). These methods have proven useful for revealing differences in the behavior of differing DNA sequences, thereby also revealing remarkable sequence-dependent differences in the inherent bendability of DNA itself (*7*−*9*). However, they do not replace the need for absolute measurements of free energies (equilibrium constants) for histone−DNA interactions.

Existing methods for determining relative histone−DNA interaction free energies are based on competition: differing tracer DNA molecules compete with an excess of unlabeled competitor DNA for binding to a limiting pool of histone octamer. The competition is established in elevated [NaCl] (sometimes also elevated T), such that histone−DNA interaction affinities are greatly reduced and the system is able to equilibrate freely. After an initial equilibration, the [NaCl] is slowly reduced, allowing nucleosomes to form in a reversible process; further reduction in [NaCl] to physiological or lower then freezes-in the resulting equilibrium, allowing subsequent analysis by native gel electrophoresis of the partitioning of each tracer between free DNA and nucleosomes. The distribution of a given tracer between free DNA and nucleosomes defines an equilibrium constant and a corresponding free energy valid for that competitive environment. Comparison of the results for a given pair of tracer DNAs (in the identical competitive environment) eliminates the dependence on the details of the competitive environment, yielding the difference free energy ($\Delta\Delta G$) (i.e., relative affinities) of histone interaction between the two DNAs. This method has been shown to allow equilibrium results to be obtained (*1*, *2*, *9*, *10*). The various species present are demonstrably in free exchange, and the assembly process is strictly reversible: the same apparent equilibrium is reached regardless of from which direction it is approached. Yet this method also has many shortcomings. Absolute affinities are not available; the conditions in which the equilibrium is finally established before being frozen-in are not well-defined; and how the resulting measured $\Delta\Delta G$ values relate to those that would obtain in physiological conditions is not known.

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[1] Abbreviations: DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; TE, 10 mM Tris, pH 8.0, 1 mM EDTA; PMSF, phenylmethyl sulfonyl fluoride; BZA, benzamidine hydrochloride; HO, histone octamer.

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Recently, we developed an alternative method for assessing nucleosome stability (*11*), based on quantitative analysis of nucleosome dissociation as a function of nucleosome concentration in a dilution series (*12*−*14*). Solution conditions were identified in which the nucleosome dissociation curves are reproducible, allowing the dissociation midpoints to serve as a useful quantitative measure of effective (kinetic) nucleosome stability. Intriguingly, the dissociation data were found to fit with the predictions of a simple equilibrium.

$$\text{nucleosome core particle} \rightleftarrows \text{free DNA} + \text{free histone octamer} \quad (1)$$

Moreover, the relative midpoints of the resulting dissociation curves (i.e., the apparent dissociation constants for eq 1) for two differing DNA sequences reproduced the relative histone−DNA interaction free energies for those DNAs obtained using the previous competition-based measurements. These observations suggested that not onlywould the dilution experiment provide a useful practical measure of nucleosome stability, but it might also provide the long-sought method for measurement of absolute histone−DNA interaction affinities.

In this new study, we critically examine this question. We confirm the earlier findings that nucleosome dissociation is driven by dilution and that consistent dilution series can be obtained when Igepal detergent is included in the buffer; but we find that the dilution-dependent dissociation is not strictly reversible, implying that true equilibrium results may not be obtained. In addition, we show examples where (with differing solution conditions) the dilution experiments fit to processes with cooperative Hill coefficients, which are inconsistent with eq 1. We conclude that the dilution experiment provides a simple and reproducible measure of relative kinetic stability—which is of practical importance and moreover may dominate nucleosome function in vivo—but that the results cannot in general be interpreted with reference to eq 1 to provide absolute equilibrium free energies. The earlier thermodynamic interpretations of the data from dilution-driven nucleosome dissociation (*11*) are incorrect.

MATERIALS AND METHODS

DNAs were 5′ end-labeled with ^{32}P using T4 polynucleotide kinase and γ-^{32}P-ATP. Nucleosome core particles (henceforth referred to as nucleosomes) were prepared from purified DNA and either recombinant *Xenopus laevis* or native chicken erythrocyte histone octamer, using stepwise dilution or dialysis from 2 M NaCl. The stepwise dilution method was carried out as described (*11*). For the dialysis method, each nucleosome reconstitution reaction contained 200 ng of ^{32}P-labeled 5S′ DNA (*15*), 10 μg of cold 5S′, and 7 μg of chicken erythrocyte histone octamer in a 50 μL volume of 2.0 M NaCl, 0.5x TE (TE is 10 mM Tris pH 8.0, 1 mM EDTA), supplemented with 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mM benzamidine (BZA). Nucleosome core particles were separated from unincorporated DNA and histones on 5−30% (w/v) sucrose gradients (in 0.5x TE) at 41 000 rpm in a Beckman SW41 rotor for 24 h at 4 °C. Gradients were fractionated into 0.5 mL fractions and quantified by a liquid scintillation counter (Beckman). Fractions containing nucleosome core particles were pooled and exchanged into 0.5x TE on Centricon-30 concentrators. The quality of the resulting nucleosome core particle preparations was assessed by native polyacrylamide gel electrophoresis before use. Nucleosome concentrations were determined by absorbance at 260 nm.

Nucleosome core particles at stock concentrations of 51 or 115 nM were diluted into 1x TE + 200 mM NaCl or into a buffer containing 10 mM Tris-HCl pH 7.6, 10% glycerol, 0.1% Igepal CA-630, 0.5 mg/mL glycogen, plus varying [NaCl] (*11*), referred to henceforth as d-buffer. Dilution reactions were incubated at ambient temperature (~23 °C) (or in certain cases at other temperatures, as indicated) for 2 h and subsequently analyzed by native polyacrylamide gel electrophoresis.

For analysis of the reversibility of nucleosome dilution, concentrated nucleosome stock samples were diluted into 1x TE + 200 mM NaCl or d-buffer + 200 mM NaCl to final concentrations of 500 or 50 pM, then reconcentrated using Centricon-3 concentrators, and run overnight at 1000 rpm (120*g* in a Beckman JA-20 rotor) slowly yielding ~7-fold concentration, and then the speed was increased to 7500 rpm (6800*g*) for an additional 4−5 h, yielding an additional ~2-fold concentration, for an overall concentration of 15-fold. In other experiments, reconcentration was achieved by dialysis against Pierce concentrating solution (catalog # 66527) using Pierce Slide-A-Lyzer concentrators with a 10 000 MW cutoff, as described (*11*).

The species present after dilution (or reconcentration) of the nucleosomes were resolved by native polyacrylamide gel electrophoresis as described (*6*). 18% Ficoll was added to each sample to a final concentration of 3% immediately prior to electrophoresis to facilitate the gel loading. The distribution of DNA between free DNA and nucleosomes in the native gels was quantified by phosphorimager. The concentration of free histone octamer ($[HO]_{free}$) was determined from the total concentration in the reaction ($[HO]_{total} = [nucleosome]_{total}$ and the relative fraction of free DNA as measured by phosphorimager.

$$[HO]_{free} = [HO]_{total}(1 - \text{fraction nucleosomes remaining}) \quad (2)$$

The resulting data were represented as fraction of DNA bound by histone octamer (i.e., fraction of nucleosomes remaining) versus $[HO]_{free}$ and were fit using a nonlinear least squares approach (with KaleidaGraph software) to the equation appropriate for association of histone octamer and DNA according to eq 1

$$\text{fraction bound} = \frac{1}{\left(1 + \left(\frac{K_d}{[HO]_{free}}\right)^m\right)} \quad (3)$$

where K_d is an apparent dissociation constant, and m is the cooperativity or Hill coefficient. In separate calculations, we could fix the Hill coefficient $m = 1$ (as required by eq 1) or allow it to float as a second parameter to be obtained by the curve fitting procedure.

RESULTS

Reproducible Dilution-Driven Nucleosome Dissociation Requires Additives Present in d-Buffer, Most Likely the

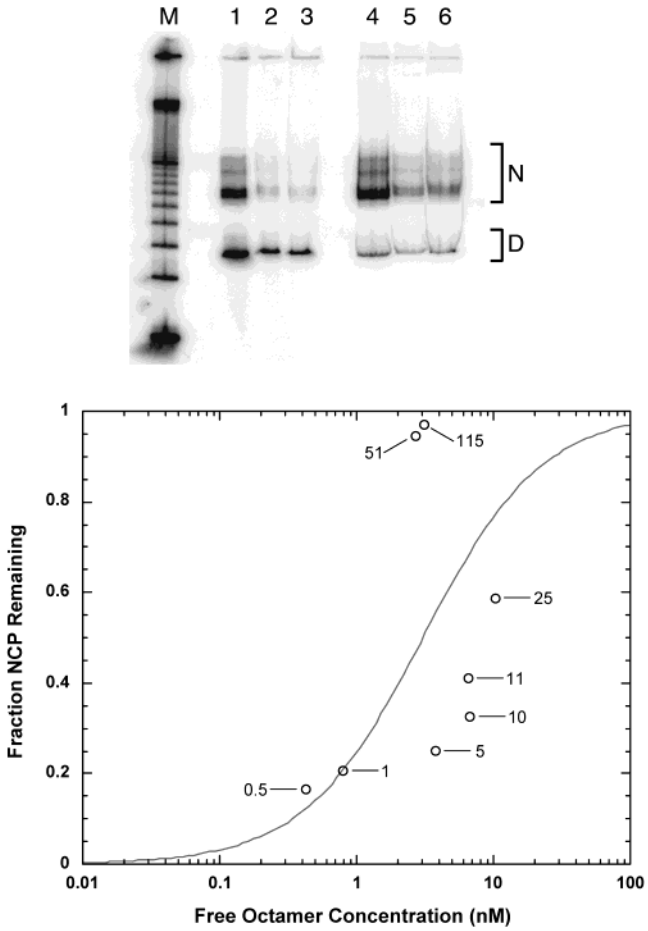

FIGURE 1: (A) Nucleosome dilution experiment in TE buffer or d-buffer. A concentrated nucleosome stock solution was diluted into TE buffer + 200 mM NaCl (lanes 1−3) or into d-buffer + 200 mM NaCl (lanes 4−6), equilibrated at 23 °C, and the resulting products were resolved by native polyacrylamide gel electrophoresis. A phosphorimage of the ^{32}P-label is shown. D and N denote the mobilities of free DNA and nucleosomes, respectively. The final nucleosome concentrations were 11 nM (lanes 1 and 4), 1 nM (lanes 2 and 5), and 0.5 nM (lanes 3 and 6). Lane M, 100 bp ladder used as a marker. (B) Quantitative analysis of dilution-driven nucleosome dissociation in TE buffer. Total nucleosome concentrations (in nM), from which $[HO]_{free}$ are calculated, are indicated for each datapoint. Nucleosome dissociation in TE buffer is erratic within a given dataset (and irreproducible between datasets—data not shown) and plainly not in accord with mass action. The curve drawn through the data represents a least-squares fit for noncooperative binding (eq 1, Hill coefficient $m = 1$).

Nonionic Detergent. We first asked whether consistently reproducible nucleosome dissociation data could be obtained in simple solutions containing only TE buffer (see Materials Methods) and NaCl or whether additional surface active compounds present in d-buffer (glycerol, Igepal detergent, and glycogen; see Materials and Methods) were required. Samples of concentrated nucleosome stock solutions were diluted in parallel into TE buffer + 200 mM NaCl or into d-buffer + 200 mM NaCl. Samples at varying final nucleosome concentrations were incubated at 23 °C for 2 h and were then analyzed by native polyacrylamide gel electrophoresis. Typical results from such an experiment are illustrated in Figure 1A. The raw phosphorimager data of Figure 1A reveal that nucleosomes in TE buffer are much less stable against dilution than those in d-buffer. Quantitative results from this experiment and additional such studies (in TE buffer) are represented in Figure 1B. The fraction of nucleosomes remaining undissociated in the TE buffer decreases monotonically as [nucleosomes] is decreased, as expected for a process involving mass action, but the process appears strongly cooperative; moreover, the data themselves are highly erratic, both within and between datasets, and cannot be fit to eq 1.

In contrast, as will be seen below, we reconfirm our earlier observations that dilution experiments carried out in d-buffer are smooth functions of [nucleosomes] within a given dataset and are reproducible between datasets. Evidently, in the absence of glycerol, Igepal detergent, and/or glycogen, nucleosome stability is governed in part by properties other than mass action, perhaps involving nonspecific adsorption of one or more components to tubes or pipet tips. We have not systematically characterized the separate roles of the three additives, although in concurrent studies we observed that a related nonionic detergent (Triton X-100) reduced loss or inactivation of a fraction of histone H2A/H2B heterodimers in dilute histone octamer solutions (V. A. T. Huynh and J. Widom, unpublished), while no such protective effects of glycerol alone have been observed in our studies. We further note that Nonidet NP-40 (now known as Igepal CA-630) is routinely included in some nucleosome assembly procedures (*3*) and that more generally nonionic detergents such as Igepal or Triton are frequently included in protein purifications and enzyme reactions to suppress losses due to adsorption and surface denaturation.

Dilution-Driven Nucleosome Dissociation Is Incompletely Reversible Even in d-Buffer. A critical test for a potential equilibrium measurement is whether the process is reversible. Irreversible processes cannot in general be analyzed to yield equilibrium properties. To test the reversibility of dilution-driven nucleosome dissociation, stock solutions of nucleosomes were diluted to final concentrations of 0.5 or 0.05 nM (50 pM) in TE + 200 mM NaCl or in d-buffer + 200 mM NaCl and then reconcentrated 15-fold using centricon microconcentrators to final concentrations of 7.5 or 0.75 nM, respectively, and the resulting products were analyzed by gel electrophoresis. An example of such an experiment is shown in Figure 2.

Nucleosomes in TE buffer + 200 mM NaCl diluted to 0.5 nM and then concentrated back up 15-fold to 7.5 nM failed to yield any detectable reassembly into nucleosomes (Figure 2, lanes 1 and 3 show results from two identical but separate experiments). Evidently, dilution-driven nucleosome dissociation is largely irreversible in this buffer. Equivalent experiments carried out in d-buffer (again in separate but duplicate experiments) reveal that significant nucleosome reassembly occurs in this buffer (e.g., Figure 2, lanes 5 and 6), but the process is not quantitatively reversible. In a typical experiment, nucleosomes were diluted in d-buffer to 0.5 nM, yielding ~12 ± 4% dissociation (e.g., see Figure 1, lane 6 and Figure 3D, lane 2); after reconcentration of these samples 15-fold, to 7.5 nM, the resulting distribution of naked DNA and nucleosomes remained unchanged: 13 and 12% dissociated nucleosomes, respectively (Figure 2, lanes 2 and 4). That is, the apparent equilibrium did not respond at all to mass action. However, when the samples were diluted in d-buffer to a lower starting concentration, 0.05 nM (50 pM; 30 ± 8% dissociation), the reassembly process was better reversible: 15-fold reconcentration, to

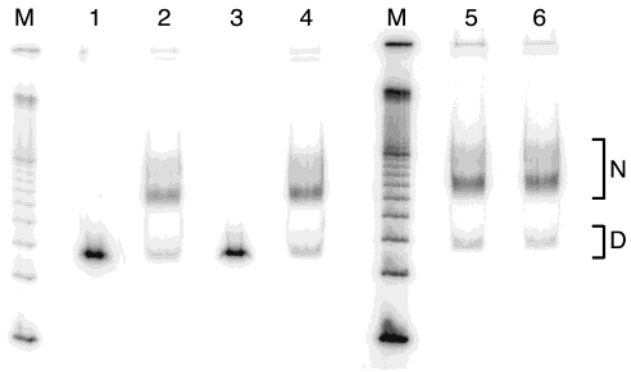

FIGURE 2: Test for reversibility of dilution-driven nucleosome dissociation. Nucleosomes at a stock concentration of 51 nM were diluted in duplicate to 0.5 nM in TE buffer + 200 mM NaCl or to 0.5 and 0.05 nM (50 pM) in d-buffer + 200 mM NaCl. The starting fractions of dissociated nucleosomes, determined from phosphorimager analysis, were ~100, 12.5 ± 4, and 30 ± 8% for these three samples, respectively. The samples were then concentrated 15-fold and analyzed by native gel electrophoresis (shown) and phosphorimaging. Lanes 1 and 3: samples from two identical but separate experiments diluted in TE buffer + 200 mM NaCl to 0.5 nM and then reconcentrated 15-fold. After reconcentration, the fraction of dissociated nucleosomes remained at ~100%: dissociation was effectively irreversible. Lanes 2 and 4: nucleosomes diluted in d-buffer + 200 mM NaCl to 0.5 nM and then reconcentrated 15-fold to 7.5 nM (two identical but separate experiments). After reconcentration, the fraction of dissociated nucleosomes was 13 and 12%, respectively. Lanes 5 and 6: nucleosomes diluted in d-buffer + 200 mM NaCl to 0.05 nM (50 pM) and then reconcentrated 15-fold to 0.75 nM (two identical but separate experiments). After reconcentration, the fraction of dissociated nucleosomes was 8 and 7%, respectively. M, 100 bp ladder used as a marker.

Table 1: NCP Dissociation Parameters[a]

dissociation conditions				
temperature (°C)	[NaCl] (mM)	[free octamer] (nM) at 50% dissociation	cooperativity coefficient	*R* value
32	50	0.12 ± 0.02	1.06 ± 0.15	0.985
37	50	0.13 ± 0.01	1.14 ± 0.14	0.990
42	50	0.19 ± 0.01	1.51 ± 0.14	0.996
23	400	2.53 ± 0.10	2.98 ± 0.34	0.996

[a] From a least-squares analysis of the data shown in Figure 3A−D, as described in Materials and Methods. NCP, nucleosome core particle.

0.75 nM, yielded 8 and 7% dissociated nucleosomes, respectively (Figure 2, lanes 5 and 6). This represents less dissociation than was obtained for the samples at 10-fold higher nucleosome concentration—another clear contradiction of the law of mass action. We conclude from these studies that dilution-driven nucleosome dissociation is significantly but incompletely reversible in d-buffer. The lower fraction of reassociated nucleosomes at the higher histone concentration suggests that reassembly may be hindered by kinetic traps that become increasingly significant at higher concentrations.

Dilution Curves Reveal Apparent Cooperativity in Nucleosome Assembly/Disassembly Processes under Certain Conditions. We explored further the situations in which nucleosome dissociation curves follow the form required for eq 1. We confirm that there is a range of conditions for which the dissociation curves can be fit to eq 1 (that is, with a Hill coefficient of 1, implying dissociative behavior that is neither cooperative nor anticooperative).

Figure 3A,B shows the results of native gels and the corresponding quantitative analyses of dilution experiments carried out in d-buffer + 50 mM NaCl at 32 and 37 °C, respectively. The data are fit well by eq 3, and when the cooperativity parameter is allowed to float in the fits, cooperativity parameter values of 1.1 are obtained for both datasets (Table 1), close to the theoretical value of 1 for simple noncooperative binding. Nucleosome dissociation in these conditions is clearly dependent on concentration and is highly repeatable. Moreover, as we have shown before (*11*), the midpoints of these dissociation curves serve as measures of relative nucleosome stability. However, because the dissociation process is not fully reversible, one cannot equate these values with absolute equilibrium free energies of histone octamer−DNA association.

This noncooperative behavior is only observed for a restricted range of solution conditions. When otherwise equivalent experiments are carried out at moderately elevated temperature (42 °C, Figure 3C) or moderately elevated [NaCl] (0.4 M, Figure 3D), the resulting quantitative data shows clear evidence of apparent positive cooperativity (Table 1). The data in Figure 3C fit to a cooperativity parameter of 1.5, which implies that the dissociation/reassociation process occurring differs from, and cannot be interpreted in the context of, eq 1. Even more striking, when dilution is carried out in 400 mM NaCl, a cooperativity parameter of ~3 was obtained (Figure 3D), again inconsistent with eq 1.

DISCUSSION

Multiple States and Kinetic Traps in Nucleosome Dissociation and Reassembly. The possible use of nucleosome dilution experiments for measurement of equilibrium thermodynamic quantities is complicated by the existence of competing structures and processes that have been documented in other studies. These include the following (*10*).

In solution conditions analogous to those used here (except lacking Igepal, glycerol, and glycogen), histone octamers dissociate into three subunits: two H2A−H2B heterodimers and an $H3_2H4_2$ tetramer (*16*−*18*). This process occurs rapidly (*19*). Consequently, rather than eq 1, one expects the process occurring in solution to be

$$\text{nucleosome core particle} \rightleftarrows \text{free DNA} + 2\,(\text{H2A}-\text{H2B}) + (\text{H3}_2\text{H4}_2) \quad (4)$$

In addition, as expected on general physicochemical grounds, the isolated histone subunits themselves are known to bind with high affinity to DNA, forming various nonnucleosomal structures (*20*)

$$\text{DNA} + (\text{H2A}-\text{H2B}) \rightleftarrows \text{DNA} - (\text{H2A}-\text{H2B}) \quad (5)$$

and

$$\text{DNA} + (\text{H3}_2\text{H4}_2) \rightleftarrows \text{DNA} - (\text{H3}_2\text{H4}_2) \quad (6)$$

Finally, Eisenberg and colleagues (*12*, *21*) have shown that intact nucleosomes themselves can bind extra histones (most likely in the form of oligomers such as H2A−H2B heterodimers and $H3_2H4_2$ tetramers, although this

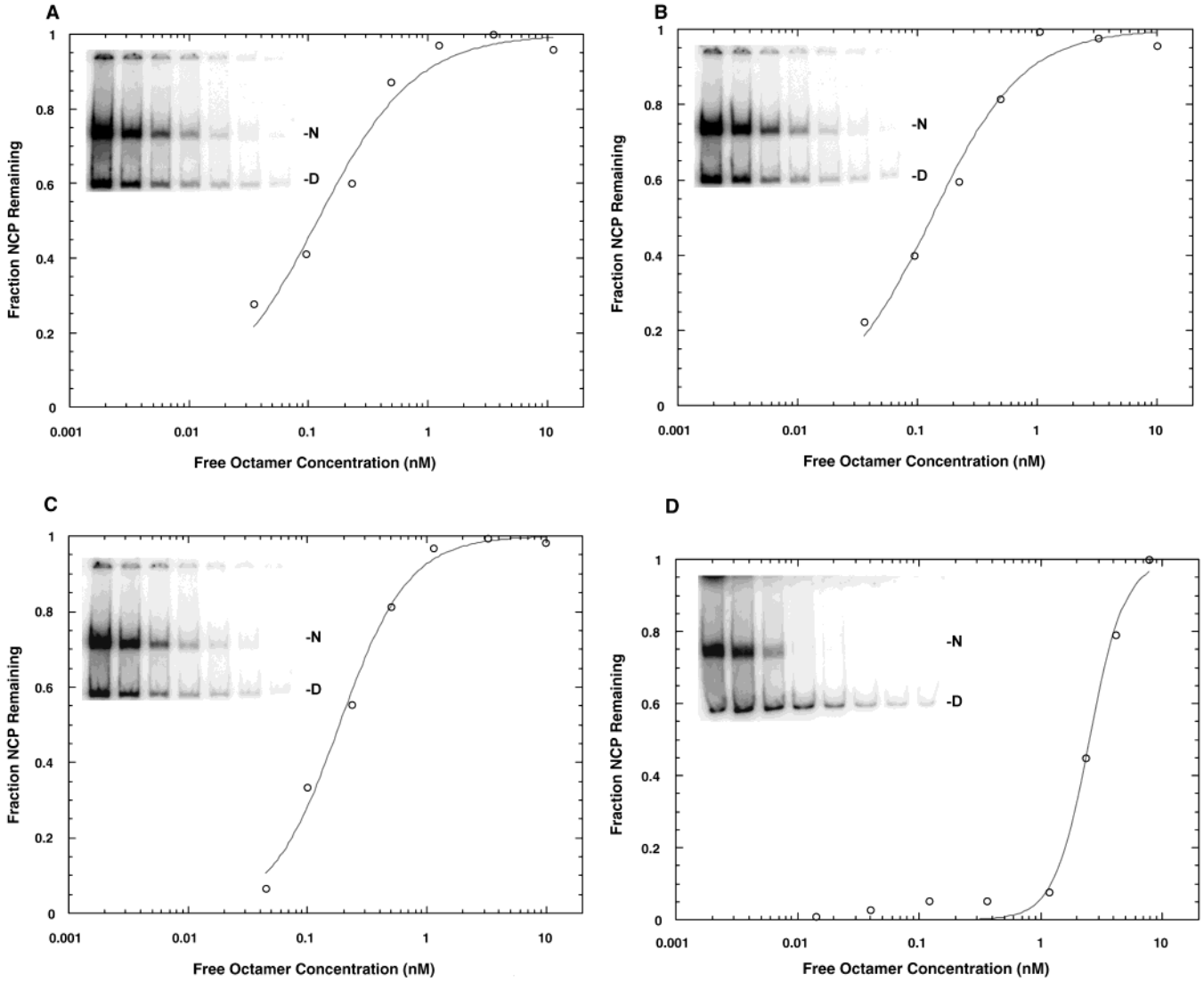

FIGURE 3: Quantitative analysis of dilution-driven nucleosome dissociation. (A−C) Nucleosomes assembled with a 146 bp 5S positioning sequence DNA were diluted in d-buffer + 50 mM NaCl at temperatures of 32, 37, and 42 °C, respectively, and then resolved by native gel electrophoresis and analyzed by phosphorimaging. Phosphorimages are shown as insets to the corresponding quantitative analyses. The curves represent least-squares fits to eq 3, with the cooperativity parameter allowed to float. The fit values for cooperativity are 1.1, 1.1, and 1.5, respectively. (D) Nucleosomes were diluted in d-buffer + 400 mM NaCl at 23 °C and then resolved by native gel electrophoresis. The phosphorimage is shown as an inset in the analysis of the binding data, as above. The cooperativity parameter was allowed to float, and the best fit yielded a cooperativity value of 3. The full set of parameters obtained from curve fitting are presented in Table 1.

has not been analyzed in detail), giving rise to a set of additional processes of the form

$$\text{nucleosome core particle} + \text{free histones} \rightleftarrows \{\text{core particle} - \text{extra histones}\} \quad (7)$$

Indeed, hints of processes such as eqs 5−7 may be present in the phosphorimages of Figures 1−3 themselves, which reveal some smearing and nonnucleosomal product bands, albeit in relatively low amounts.

The existence of these multiple processes that can compete with equilibrium nucleosome reassembly raises the likelihood that reassembly may be kinetically blocked. This is in fact a phenomenon that is well-known to those who assemble and study nucleosomes in vitro. Simply mixing together free histones and DNA in quasi-physiological solution conditions produces some nucleosomes but also large fractions of diverse nonnucleosomal aggregates (*22*, *23*), which do not resolve into nucleosomes over any reasonable time scale.

Dilution Studies Do Not Yield Equilibrium Free Energies of Histone−DNA Interactions. We find that the dilution experiments are not properly reversible; hence, one cannot be certain that the midpoint of the measured dissociation curves does in fact reflect the true equilibrium affinity of histone octamer for DNA even if the process were otherwise to accord with eq 1. Moreover, we show examples where the system behaves cooperatively, and this outcome cannot be interpreted in the context of eq 1.

More importantly, the actual processes in solution are expected to include those in eqs 4−7. Consequently, a quantitative analysis of nucleosome dissociation experiments would need to take these processes into account in any expression used to derive an equilibrium histone octamer−DNA interaction free energy or affinity. Indeed, given the multiple parallel processes occurring, it is unlikely that the histone−DNA interactions in nucleosomes can appropriately be described by a single affinity. In accord with this view,

in other studies we have reported that the apparent affinities for histone−DNA interactions (site exposure equilibrium constants) vary with position inside the nucleosome. Stretches of DNA just inside the nucleosome are held orders of magnitude less tightly onto the histone surface than are stretches of DNA near the middle of the nucleosome (*9*, *15*, *24*−*26*).

Kinetic Stability May Mirror Thermodynamic Stability and May Control Biological Activity. The nucleosome dilution method does not yield equilibrium nucleosome stabilities, yet the results are robustly repeatable, and moreover, they can mirror the relative equilibrium stabilities (affinities) for pairs of DNA sequences obtained using the earlier competition methods. We conclude that the nucleosome dissociation process monitored by the dilution method reports on kinetic or effective nucleosome stability. These data are valuable for three reasons.

First, and of practical significance, studies of nucleosomes in vitro need to be carried out in conditions in which the nucleosomes do in fact exist. Often such studies utilize nanomolar concentrations of nucleosomes and sometimes even lower concentrations. Our studies show that these studies are at risk for having a substantial fraction of the nucleosomes dissociated into free DNA and other nonnucleosomal histone−DNA complexes. The use of higher affinity nucleosome forming DNA sequences may somewhat alleviate if not eliminate this problem.

Second, it is plausible that nucleosome function in vivo may be under kinetic, not equilibrium, control. In that case, the effective relative stabilities measured in nucleosome dissociation experiments may be directly relevant to biological phenomena, more so even than are hypothetical true equilibrium constants. The dilute nonionic detergent and glycerol used in these experiments are routine additives in protein chemistry and do not diminish the likelihood that the kinetic stabilities measured in these conditions could pertain to physiological conditions.

Third, it is frequently the case that relative rates or processes of reactions are found to mirror relative equilibrium stabilities. For many biochemical association/dissociation processes, the forward (association) rate constants are essentially diffusion controlled; hence, relative dissociation rates accurately reflect relative equilibrium stabilities. Separately, in organic chemistry, it is common to observe linear free energy relationships between relative chemical reaction rates and relative equilibrium constants for a linked process (e.g., rate constant for a catalyzed reaction and the equilibrium dissociation constant for a proton from a general acid catalyst). Thus, for a set of comparable chemical reactions on a set of closely related compounds, one frequently finds that the relative reaction rates are proportional to relative equilibrium constants. Consequently, for either of these two classes of reasons, the relative stability of histone−DNA interactions measured by the dilution experiment may prove to mirror true thermodynamic stabilities—although one cannot be certain of such a correspondence.

General Lessons for Analysis of Protein−DNA Interactions. Finally, we note that these findings have implications for analyses of other protein−DNA interactions. It is common for the affinities (free energies) of protein−DNA interactions to be obtained by experimental measurement of association and dissociation kinetic constants or from competitive transfer experiments. Our studies highlight the importance of establishing that the apparent equilibria being studied are in fact reversible.

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