

# Effects of HU Binding on the Equilibrium Cyclization of Mismatched, Curved, and Normal DNA

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**ABSTRACT** The effects of HU, the histone-like protein from *Escherichia coli*, on the equilibrium cyclization of duplex DNAs have been observed as a function of protein concentration and DNA sequence. The results indicate that the presence of HU significantly enhances the extent of cyclization and increases the melting temperature,  $T_m$ , of the cyclized form of the DNA by >10 K. The stabilization of equilibrium cyclization by HU binding is at least  $-1.2$  kcal/mol. The results are consistent with two HU homotypic dimers binding to each of the three 29-mer duplexes studied. One of the 29-mer duplexes contains a central dA tract, one contains mismatched sites, and one a conventional sequence. Stepwise or microscopic association constants, determined from the fluorescence data, range from  $1.5$  to  $0.6 \mu\text{M}^{-1}$ . The binding affinity of the HU dimer is strongest for the mismatched duplex and lowest for the dA tract, consistent with HU dimers having a preference for flexible DNA substrates. These results demonstrate the utility of the equilibrium cyclization approach to monitor DNA-protein interactions. These results have been considered along with those previously obtained to refine a model for the interaction of HU with duplex DNA.

## INTRODUCTION

Multiprotein-DNA complexes mediate important events in the cell, such as transcription, recombination, and replication by the formation of complexes, which loop or bend DNA. The resulting curvature or bending can result in the activation or repression of transcription, replication, or recombination and may also be important in the packaging of DNA (Chinenov and Kerppola, 2001; Koopman, 1999; McGill and Fisher, 1998; Thomas, 2001; Werner et al., 1996; Zlatanova and van Holde, 1998).

HU protein is involved in a number of different genomic events including repression and replication. Originally identified as a histone-like protein (Rouviere-Yaniv and Gros, 1975), more recent studies implicate HU as a DNA chaperone or architectural protein, in which the binding and bending of DNA by HU facilitates the formation of functional protein-DNA complexes (Aki and Adhya, 1997; Goodman et al., 1992; Travers, 1993). For example, HU is required for the formation of the Mu transpososome and the Gal repressosome (Geanakopoulou et al., 2001; Lavoie et al., 1996). In both of these complexes, loop formation, putatively induced by HU, facilitates the interaction of other proteins, which then leads to the associated function. In this study a novel method, equilibrium cyclization (EC), has been developed to examine the effect of HU binding on DNA cyclization.

The DNAs used in this study have a duplex region of 29 basepairs (bp), dT<sub>18</sub> as the flexible tails, and sticky ends that can form seven basepairs as depicted in Fig. 1. DNA

cyclization is achieved when the sticky ends come close enough together to form a duplex. The equilibrium between the open and closed forms is monitored by the fluorescence of 2-aminopurine (2AP), which is present in one of the sticky ends. In the open or uncyclized form, 2AP is highly fluorescent. Upon cyclization, the region containing 2AP forms a duplex and the fluorescence is quenched. The observation of the equilibrium as a function of temperature allows the  $T_m$  of cyclization to be determined.

The ability of EC to monitor protein-DNA interactions is tested using HU, which is a DNA-binding protein with low sequence specificity. DNA footprinting (Kamashev et al., 1999), enhancement of circularization (Hodges-Garcia et al., 1989), and fluorescence studies (Wojtuszewski et al., 2001; Wojtuszewski and Mukerji, 2003) have all implied that the presence of HU induces DNA bending. The EC melting temperature,  $T_m$ , was monitored as a function of HU concentration and the results were used to determine the binding affinity of HU to the different DNA molecules. A slightly higher binding affinity was observed for the duplex containing mismatches. For all of the DNAs studied two HU dimers bind to the 29-bp duplex section and the presence of HU elevates the  $T_m$  of the cyclized form by  $\sim 10$  K, which corresponds to a stabilization free energy of  $-1.2$  kcal/mol. These findings demonstrate the capability of the equilibrium cyclization method to monitor protein-DNA interactions and further confirm the role of HU as an architectural protein.

## MATERIALS AND METHODS

### DNA sample preparation

The DNA samples were obtained from IDT (Coralville, IA) as polyacrylamide-purified samples. The concentrations of the DNAs were determined using the extinction coefficients given below.

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79-mer:

d(GCCCA{2AP}GTTTTTTTTTTTTTTTTTCCGGCGCGGCGCAAAATC  
GCGCGCCCGGTTTTTTTTTTTTTTTTTCTTGGGC);  $\epsilon_{260} = 672, 400$ .

79-mer-scrambled:

d(GCCCA{2AP}GTTTTTTTTTTTTTTTTTCCGGCGCGGCGCATATATC  
GCGCGCCCGGTTTTTTTTTTTTTTTTTCTTGGGC);  $\epsilon_{260} = 668, 600$ .

dA-tract-29-mer:

d(CCGGGCGCGCATTTTTGCGCCGCGCCGG);  $\epsilon_{260} = 250, 600$ .

Scrambled-29-mer:

d(CCGGGCGCGCATATATGCGCCGCGCCGG);  $\epsilon_{260} = 260, 100$ .

The scrambled sequence duplex was formed by the combination of the 79-mer-scrambled and the scrambled 29-mer, the dA-tract duplex was formed by the combination of the 79-mer with the dA-tract 29-mer and the mismatched sample was formed by the combination of the scrambled 29-mer with the 79-mer.

## HU protein

HU protein was isolated from *Escherichia coli* strain RLM1078 and purified according to the procedure previously described (Wojtuszewski et al., 2001). 20 mg of purified HU was obtained from a liter growth and the purity was >95% as determined by SDS-PAGE and by amino acid analysis (see Supplementary Material). Dialyzed cell lysate was loaded onto a SP-Sepharose (Amersham-Pharmacia, Piscataway, NJ) cation exchange column. HU-containing fractions were pooled, dialyzed, and loaded onto a DNA-cellulose column, which was prepared by UV cross-linking calf thymus DNA to Whatman (Clifton, NJ) CF-11 cellulose (Alberts and Herrick, 1971). To eliminate a contaminating nuclease that copurifies with HU, an FPLC MonoS 5/5 cation exchange column (Amersham-Pharmacia) was used and the nuclease-free HU eluted at ~0.35 M NaCl. The lack of nuclease activity was verified by the absence of any digested products after

incubating the purified protein with plasmid DNA. HU protein concentration was determined by amino acid analysis (Keck Facility, Yale University, New Haven, CT). The extinction coefficient at 230 nm was determined to be 37,500 based on seven independent analyses.

## Equilibrium cyclization experiments

All experiments were done in 20 mM Tris, 140 mM NaCl, and 0.1 mM EDTA at pH 7.6. All DNA samples were at a duplex concentration of  $1 \times 10^{-6}$  M. The complementary 29-mer single-strand DNA, which does not contain the fluorescence probe 2-aminopurine, was added at a ratio of 1:1.2 so that there was an excess of the nonfluorescent strand. DNA samples were annealed by heating to 358 K followed by slow cooling to room temperature over 6 h. Individual samples were made for each molar ratio of DNA:HU. HU was added to the annealed samples and equilibrated for 20 min. The maximum volume increase upon addition of HU was <8%. The melting curves were found to be fully reversible as illustrated by the results in the Supplementary Material, indicating that denaturation of HU does not occur under these conditions.

The fluorescence experiments were carried out using a Fluoromax-2 fluorimeter (Instruments SA, Metuchen, NJ). The excitation wavelength was 309 nm and the emission was monitored at 371 nm with a slit setting of 0.59 nm corresponding to a 2.5-nm spectral bandpass. The sample was stirred to ensure uniform temperature. The temperature was controlled using a Neslab RTE-111 temperature bath (Thermo Electron, Beverly, MA). Samples were equilibrated for 4 min at each temperature.

The 5' nucleotide of 2-aminopurine (2AP) was a gift from Glen Research (Sterling, VA); its concentration was determined using the extinction coefficient of 6800. 2AP samples were at  $1 \times 10^{-6}$  M. A slit setting of 0.23 nm corresponding to a 1-nm bandpass was used for the 2AP nucleotide experiments.

## Data analysis

The fluorescence was measured over the temperature range from 278 K to 328 K. At 328 K the cyclized form is completely melted. The fluorescence data was corrected for the intrinsic, temperature dependence of 2AP (see Supplementary Material). The  $T_m$  values were determined from fits to the Boltzmann function using the nonlinear curve fitting program of Origin version 6.0 (Microcal Software, Northampton, MA). Representative fit and error values are reported in the Supplementary Material. The fluorescence melting curves were also fit using  $I = I_o + (I_{\max} - I_o)/(K + K)$ , by nonlinear regression using the program NLREG, version 5.4 (NLREG, Brentwood, TN). From these fits  $\Delta H$  and  $\Delta S$  were determined and the ratio of  $\Delta H/\Delta S$  was used to determine  $T_m$  values. Essentially identical  $T_m$  values were obtained by the two methods as shown in the Supplementary Material.

The reproducibility of the experimental results was investigated for samples in the presence and in the absence of HU protein. In all cases the reproducibility of the melting curves was very high and essentially identical  $T_m$  values were obtained from the data obtained in independent runs or on independent samples. Data showing the reproducibility is included in the Supplementary Material.

## Determination of equilibrium binding constants

Equilibrium binding curves were fit assuming identical, noninteracting binding sites,

$$i = i_0 + \frac{nk_a[L](i_1 - i_0)}{1 + k_a[L]}, \quad (1)$$

where  $i$ ,  $i_0$ , and  $i_1$  represent the fluorescence intensity of the titrated sample, free DNA, and with one HU protein bound, respectively.  $k_a$  is the

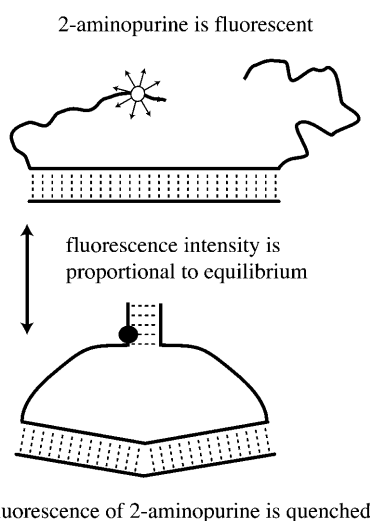


FIGURE 1 The equilibrium cyclization of DNA is depicted. In the open state, shown at the top, the 2-aminopurine, 2AP, is fluorescent and the sticky ends are not basepaired. In the closed state the fluorescence of the 2AP is quenched as it is in a duplex region formed by the sticky ends. The equilibrium between the open and closed states depends on the curvature and flexibility of the DNA, which can be altered by protein binding.

microscopic association or stepwise binding constant and  $n$  represents the number of ligands bound (Eftink, 1997). The fluorescence intensities ( $i_0, i_1, i$ ) at one temperature were extracted from the melting curves obtained at HU:DNA ratios ranging from 0.0 to 3.0. The number of HU dimers bound was determined by equilibrium stoichiometry to be 2 and was held constant during the fitting. Binding to either site is considered equivalent and independent, such that the equilibrium binding constants for binding of the first and second HU dimer are determined as  $K_1 = 2k_a$  and  $K_2 = k_a/2$  and the overall binding constant is  $K_1 \times K_2$ . In this formulation, the fractional change in fluorescence intensity was considered equal for each binding event.  $[L]$  corresponds to the concentration of free ligand, in this case HU protein and was assumed to be equal to total HU.

Equilibrium binding affinities and stepwise binding constants were also determined from the  $\Delta T_m$  as a function of HU concentration using a similar formulation. The number of binding sites was constrained to 2, based on the stoichiometry experiments and the binding sites were assumed to be identical and noninteracting. The fractional change in  $T_m$  was assumed to be the same for each binding event:

$$T_m = T_{m0} + \frac{nk_a[L](T_{m1} - T_{m0})}{1 + k_a[L]}.$$

## RESULTS AND DISCUSSION

The cyclization of the DNAs containing the 2AP was examined in the presence and in the absence of the complementary 29-mer with selected results shown in Fig. 2. The temperature dependence of the 2AP fluorescence was analyzed to determine the melting temperature,  $T_m$ , of the transition by the methods described above. The  $T_m$  of the single-stranded DNA was found to be 312 K in the absence of the complementary 29-mer and 294 K in the presence of the complementary strand. This indicates that the presence of the 29-mer duplex region decreases the extent of cyclization as

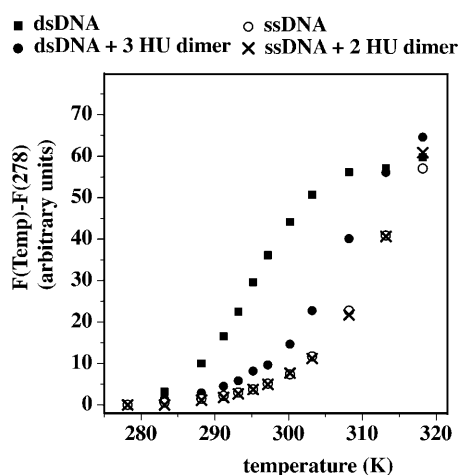


FIGURE 2 The temperature dependence of the fluorescence of 2AP in the scrambled sequence DNA, dsDNA, in the presence and absence of HU dimer is shown. Also shown is the temperature dependence of the fluorescence of 2AP in the single-stranded DNA, ssDNA, in the presence and absence of HU dimers. The DNA concentration in both cases was  $1 \times 10^{-6}$  M. The values shown are the difference between the fluorescence at the indicated temperature and that obtained at 278 K. The sequences of the central six basepairs of each of the three DNAs are also included.

expected. The  $\sim 18$ -K difference in  $T_m$  between the single-stranded and duplex DNAs indicates the effective range of difference in  $T_m$  values that can be observed in an EC experiment. An increase in the flexibility of the duplex region will increase the  $T_m$  by a fraction of this 18-K difference. The  $T_m$  values were found to be reproducible from sample to sample and from experiment to experiment as described in Materials and Methods. The melting curves do not exhibit hysteresis as shown in the Supplementary Material. The optical  $T_m$  values of all of the 29-mer duplex regions is  $>353$  K. The melting transition of the 29-mer duplex is a normal, cooperative transition. There is no evidence of any premelting or other transition of the 29-mer duplex region below 340 K.

The  $T_m$  values of DNAs containing a 29-mer duplex region, a 29-mer duplex region containing two, nonadjacent TT mismatches, and a 29-mer duplex region containing a central d(AAAAAT) sequence were found to be 294, 294, and 295 K, respectively. These results indicate that for this duplex length, under the conditions used here, the extent of cyclization is not strongly dependent on the sequence of the duplex region of the DNA. The absence of a strong dependence of the  $T_m$  on DNA sequence is consistent with previous findings, which indicated that the most pronounced effect on DNA properties is observed with the phasing of dA tracts (Hagerman, 1990).

The effect of HU dimer binding on the EC of the DNAs was examined. The addition of HU dimer to the single-stranded DNA was found to have little, if any, effect on the  $T_m$  of the EC as shown in Fig. 2. The change in  $T_m$  in the single-stranded DNA was on the order of 0.1 K, and this small change is well within the experimental error (see Supplementary Material).

The addition of HU to the DNA with the scrambled duplex sequence was found to have a pronounced effect on the  $T_m$  of the EC. The temperature dependence of the fluorescence is shown in Fig. 3 over the range of 0 to 3 HU dimers per DNA. The addition of a single HU dimer to the DNA increases the  $T_m$  of the EC by almost 8 K. This can be compared with the 18-K increase in  $T_m$  when going from 29 basepairs to a 29-mer single-stranded region. The addition of two HU dimers per DNA gives rise to an additional increase in the  $T_m$  of EC. Thus, HU dimers give rise to an 11-K increase in  $T_m$ , which is more than one-half of the difference in  $T_m$  that is observed between single-stranded and duplex DNA.

The change in  $T_m$  as a function of the ratio of the concentrations of HU dimer to DNA is also shown in Fig. 3. The results indicate that the addition of more than two HU dimers per DNA has little additional effect on the  $T_m$  of cyclization. The qualitative analysis of the curve of  $T_m$  versus the ratio of HU dimer to DNA indicates that two dimers interact with each DNA, that the two dimers bind independently, and that the magnitude of the effect of each of the dimers on the  $T_m$  of EC is similar.

Analogous experiments were carried out on the DNA that contained two, nonadjacent TT mismatches in the duplex

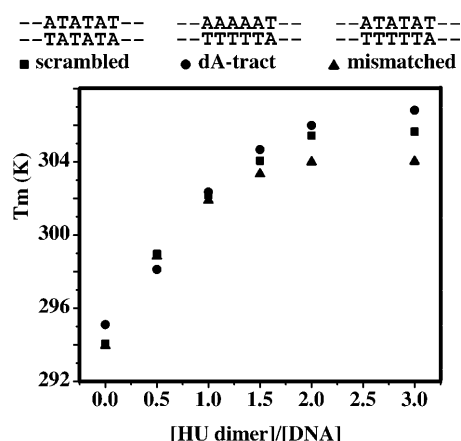


FIGURE 3 The melting temperatures,  $T_m$ , of the cyclization of three duplex DNAs are shown as a function of  $[HU \text{ dimer}]/[DNA]$ . The  $T_m$  values were determined as described in the text for the scrambled, dA-tract, and mismatched sequence DNAs. The  $T_m$  values are listed in the Supplementary Material along with the errors.

region. The examination of the results in Fig. 3 indicates that the effects of HU dimer on the  $T_m$  for cyclization of the mismatch containing DNA is qualitatively the same as for the DNA without the mismatches. In both cases the addition of HU dimer dramatically increases the  $T_m$  of EC and the effect is mostly complete at approximately two dimers per DNA. The total change in the  $T_m$  of EC for the mismatch containing DNA is somewhat smaller than that observed for the fully duplex DNA—10 K and 12 K, respectively. However, the change in  $T_m$  that is observed at one HU dimer per DNA is the same in both of these cases.

The effect of HU dimer on the  $T_m$  of the EC of the DNA containing a central dA tract was also determined. The results are shown in Fig. 3 and these are qualitatively similar to that observed for the mismatch containing and fully duplex DNA. Again, the change in  $T_m$  is nearly complete at two HU dimers per DNA and the maximal change, 12 K, is the same as that observed for fully duplex DNA.

These experiments were carried out in the presence of 140 mM sodium. Additional experiments were carried out in the presence of 200 mM sodium. Over this relatively narrow range of salt concentration, no significant dependence of the EC results on salt concentration was observed. At these salt concentrations binding of HU to linear DNA is relatively weak,  $K_d = 2500$  nM as measured by gel shift experiments (Pinson et al., 1999). Results obtained in the presence and in the absence of HU dimers are shown in the Supplementary Material.

The stabilization of the cyclized form as a consequence of HU dimer binding can be estimated from the increase in the  $T_m$ . A  $\Delta\Delta G$  value can be determined from the difference in equilibrium populations of the DNAs at the  $T_m$  values obtained with and without HU dimers bound. These analyses indicate that binding of HU dimers stabilizes the EC of the dA-tract-containing sequence by  $-1.5$  kcal/mol, whereas

HU binding leads to only a  $-1.2$  kcal/mol stabilization for the mismatch sequence. HU binding stabilizes the EC of the scrambled sequence by  $-1.4$  kcal/mol. Thus, a comparison of all three DNAs reveals small, but significant differences that can potentially be related to the relative flexibility of the DNA sequences.

### Use of EC to determine the stoichiometry of the complex

The estimation of the stoichiometry of the HU dimer-DNA complex as discussed above was carried out at a DNA concentration that is similar to the  $K_d$  for the complex. To more accurately determine the stoichiometry, EC has been used to monitor the complex formation at a DNA concentration,  $7.5 \times 10^{-6}$  M, that is significantly greater than the  $K_d$  for the complex. In this experiment the DNA concentration was held constant and the effect of the addition of HU dimer on EC was monitored with results shown in Fig. 4. At this relatively high concentration of DNA, all the added HU dimer binds until the substrate is saturated. The intersection of the linear region with the plateau region is indicative of the number of HU dimers bound. For the current results the intersection occurs at an HU dimer/DNA ratio of 1.8. From this we infer that the most likely binding stoichiometry is 2:1, which is in agreement with the discussion above and with what visual inspection of the data suggests.

### Use of the EC data for the determination of stepwise association constants

Although the maximum change in  $T_m$  may be the same for all three DNAs this need not imply that the binding affinities are the same. The binding of two HU dimers can have the same effect on the  $T_m$  for the cyclization of all three DNAs

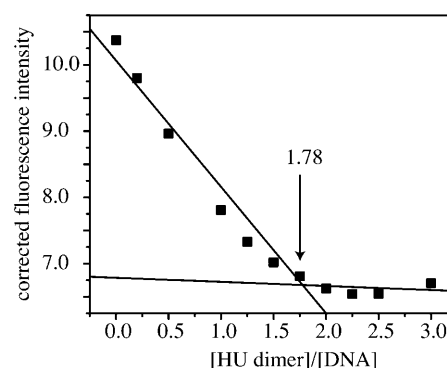


FIGURE 4 The plot of the fluorescence of the 2AP in the scrambled DNA as a function of  $[HU \text{ dimer}]/[DNA]$  is shown. The data was obtained with a DNA concentration of  $7.5 \times 10^{-6}$  M at 297 K, which is significantly greater than that of the  $K_d$ . The analysis was carried out as described in the text. The arrow indicates the  $[HU \text{ dimer}]/[DNA]$  where the linear and plateau regions intersect and indicates a binding stoichiometry of two HU dimers to one DNA.

whereas HU dimers could have different affinity for each of the DNAs. Therefore, the association constants of HU dimers for the three DNAs were examined. The results were analyzed using a model of identical, noninteracting binding sites and a binding stoichiometry of 2. From this analysis a microscopic association or stepwise binding constant,  $k_a$ , is determined for which  $K_1 = 2k_a$  and  $K_2 = k_a/2$ . Binding to the two sites is equivalent, and the factor-of-4 difference between  $K_1$  and  $K_2$  arises because of the differences in site availability caused by the binding of the first ligand. Fig. 5 contains a determination of the stepwise binding constant for the scrambled DNA,  $1.2 \pm 0.08 \mu\text{M}^{-1}$ , at a temperature of 297 K. Similar plots were obtained at other temperatures and for the other DNAs.

The mismatched DNA has the largest stepwise binding constant,  $1.4 \pm 0.1 \mu\text{M}^{-1}$ , followed by that of the scrambled DNA,  $1.2 \pm 0.08 \mu\text{M}^{-1}$ , and the dA-tract DNA has the smallest  $k$ -value,  $0.6 \pm 0.04 \mu\text{M}^{-1}$ . The stepwise binding constant for the mismatch containing DNA is more than twice that of the dA-tract DNA. These results suggest that the binding of two HU dimers to all three of the DNAs has approximately the same effect on the  $T_m$  of the EC. However, the binding affinity of HU dimers does depend on the sequence of the DNA and on the presence of mismatched sites, where the mismatched sites lead to a more than twofold higher binding affinity in terms of the binding of each HU dimer compared to the A-tract-containing duplex.

A similar analysis of the  $T_m$  values as a function of HU concentration yields comparable results. In this case, the  $\Delta T_m$  corresponds to the increase in the population of molecules stabilized in the loop conformation in the presence of HU dimers. These analyses yield stepwise  $k_a$  values of  $1.00 \pm 0.05 \mu\text{M}^{-1}$ ,  $0.57 \pm 0.04 \mu\text{M}^{-1}$ , and  $1.42 \pm 0.1 \mu\text{M}^{-1}$  for the scrambled, dA-tract, and mismatched DNA

sequences, respectively, which are in excellent agreement with those determined from the fluorescence intensities and from the gel shift results (see below). These  $T_m$ -determined  $k_a$  values further support the finding that HU binds to the mismatched sequence with twofold higher affinity relative to the A-tract-containing sequence. All three analysis methods yield approximately the same binding constants, which further confirms that formation of the 7-bp duplex from the sticky ends is stabilized by HU binding to the 29-bp duplex of the loop.

Gel shifts of the DNA-HU dimer system were also examined to see how the results compare with those determined by EC (see Supplementary Material). The gel results indicated that the apparent  $K_d$  is  $3.9 \pm 1.1 \times 10^{-7} \text{ M}$ , which is in good agreement with previously determined values for HU binding to linear DNA (Azam and Ishihama, 1999; Pinson et al., 1999). Because of rapid dissociation of the complexes in the gel, which leads to the appearance of a smear rather than a discrete band, the apparent binding constant is determined from the concentration of free DNA rather than the concentration of bound complex. As has been observed previously with gel shifts, the apparent  $K_d$  is approximately one-half that of the value determined in solution. The lower  $K_d$  observed in the gel is attributed to its *caging effect* (Fried and Liu, 1994); nevertheless, the comparability of the  $K_d$  values determined from the EC and the gel shift data indicates that the observed changes in EC are a consequence of HU binding. Although multiple bands are observed on the gel, the stoichiometry of the complexes cannot be determined from the gel because of the anomalous migration of bent complexes (Wojtuszewski et al., 2001).

### Comparison of the EC results to those obtained previously

These results and the interpretation presented above are entirely consistent with previous studies on the interaction of HU with DNA. The consensus model is that the HU dimers bind independently, there is no indication that they bind cooperatively, and that there is one binding site for ~9–12 bp each of duplex DNA (Bonnefoy et al., 1994; Broyles and Pettijohn, 1986; Wojtuszewski et al., 2001).

The HU dimers do not appear to bind to the sticky ends of the cyclized DNA under the conditions used here. As shown in the Supplementary Material, the HU dimers have no discernable effect on the EC of a DNA with the sticky ends but without the complementary strand to form the duplex region.

The binding of HU dimers to DNA with a 3' single-stranded overhang has been reported to be ~60 times stronger than to a duplex region, whereas binding to a DNA with a 5' overhang is comparable to that of the duplex region. In the 3' case, this increase in binding affinity over that of a duplex was mainly attributed to folding of the

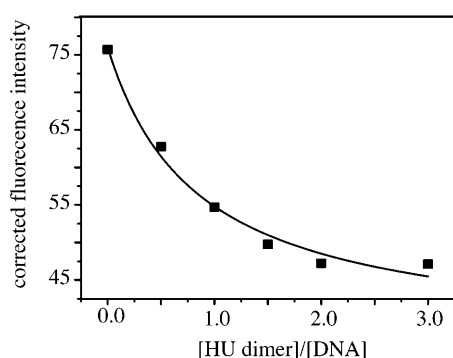


FIGURE 5 A typical binding curve is shown. The fluorescence intensity of the scrambled DNA at 297 K as a function of  $[\text{HU dimer}]/[\text{DNA}]$  is shown along with a fit. The fit was done as a function of HU concentration and gave a stepwise association constant of  $1.18 \times 10^6 \text{ M}^{-1}$ .  $I = I(0) + (n \times k \times x \times [I(1) - I(0)] / (1 + k \times x))$ , where  $I$  is the corrected fluorescence intensity and  $x$  is the concentration of HU dimer. The data shown is for the scrambled sequence DNA and the errors are listed in the Supplementary Material for all three DNAs.

terminus of the overhang such that it can interact with the positively charged regions of the HU dimer (Kamashev and Rouviere-Yaniv, 2000). The EC results and the gel results, shown in the Supplementary Material, do not indicate the presence of this stronger binding with the DNAs studied here. However, the EC results do not discriminate between the possible binding site locations of the HU dimers.

### Effects of HU on the cyclization of a DNA with a dA tract

The specificity of HU dimer binding is of interest and in particular, whether the binding affinity to curved or damaged DNA is greater than that of normal DNA. If the DNA is already curved, then this may provide a premade binding site for the HU dimers. There have been prior investigations of the binding of HU to dA-tract DNAs to investigate this question (Shimizu et al., 1995; Tanaka et al., 1993; Wojtuszewski and Mukerji, 2003) and we examined the effects of HU on the EC of a duplex DNA containing a dA tract. Our results indicate that HU dimers have a smaller apparent  $k_a$  when a dA tract is present than with a scrambled sequence, suggesting that the rigidity of the dA tract affects HU binding affinity.

### Effects of HU on the cyclization of DNA containing a mismatched site

The results indicate that once the two HU dimers are bound to DNA containing a mismatched site the effect on the  $T_m$  of EC is the same as when two HU dimers bind to DNA without a mismatched site. The stepwise  $k_a$  value for the DNA with a mismatched site is slightly larger than that of the DNA without a mismatched site. This is consistent with the notion that HU dimers prefer to bind to more flexible DNA. The difference that is observed in the stepwise  $k_a$  values between the DNAs with and without a mismatched site is <50%, which corresponds to a small difference in free energy of  $\sim 1$  kcal.

### SUMMARY

These results indicate that the stepwise binding constant of HU dimers exhibits an approximately twofold variation depending on the sequence of the DNA and whether the DNA contains a mismatched site. The binding of two HU dimers to DNA appears to have a similar effect on the  $T_m$  of the EC process for all three DNA sequences examined. These results indicate that HU dimers have somewhat different affinities for DNAs of different sequences but that the final complex has much the same structure independent of the sequence of the DNA.

Elevation of the cyclization  $T_m$  as a consequence of HU binding demonstrates that HU binding stabilizes the loop structure, consistent with the proposed function of HU in vivo. The relative flexibility of the 29-mer duplex influences

HU binding affinity and the extent of stabilization, in which the relatively rigid dA-tract-containing sequence results in lower binding affinity and the stabilization by HU for EC is greater ( $\sim 0.3$  kcal/mol) than for the scrambled or mismatch sequences. These results also show that EC can be used to monitor the interactions of proteins with DNA in a convenient and sensitive manner. To determine the extent of flexibility or curvature introduced by protein binding requires being able to directly relate the change in  $T_m$  of EC to changes in flexibility and/or curvature. EC can also be used to examine the interactions of proteins, such as the integration host factor, which bind to specific sequences of DNA. We anticipate carrying out additional studies in both of these areas.

### SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at <http://www.biophysj.org>.

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