Effect of pH on Interactions between DNA and High-Mobility Group Protein HMG1†

Lori A. Kohlstaedt‡ and R. David Cole*

Department of Molecular and Cell Biology, Stanley/Donner Administrative Services Unit, University of California, Berkeley, California 94720

Received April 19, 1994; Revised Manuscript Received August 8, 1994®

ABSTRACT: Fluorescence quenching was used to test the effect of pH changes on the binding of high-mobility group protein 1 (HMG1) to double-stranded and single-stranded DNA. At pH 7.5, the binding constant K for double-stranded DNA was 3×10^6 M^{-1}, the binding site size n was 13, and the cooperativity factor q was 78, while at pH 6 the corresponding values were $K = 12 \times 10^6$ M^{-1}, $n = 54$, and $q = 770$. For the binding of HMG1 to single-stranded DNA at pH 7.5, the values were $K = 2 \times 10^6$ M^{-1}, $n = 7$, and $q = 60$, whereas at pH 6 they were $K = 3 \times 10^6$ M^{-1}, $n = 14$, and $q = 440$. Denaturation of HMG1 by oxidation of its sulfhydryl groups substantially affected the binding parameters. At pH 6, double-stranded DNA bound oxidized HMG1 with $K = 6 \times 10^6$ M^{-1}, $n = 16$, and $q = 200$, and single-stranded DNA bound with $K = 3 \times 10^6$ M^{-1}, $n = 7$, and $q = 180$. The sensitivity of the double-stranded DNA–HMG1 interaction to pH, along with an earlier report of a sharp optimum of binding at 140 mM NaCl, reveals a potential for *in vivo* regulation of the strength and mode of HMG1 binding by DNA through the action of analogous factors in the cellular milieu.

High-mobility group proteins 1 and 2 (HMG1 and 2[1]) are chromosomal proteins found in relatively high abundance in the nucleus (Johns, 1982). HMG1 and -2 were first described by Goodwin et al. (1973) as proteins extractable from the nucleus in 5% perchloric acid, running faster than histone H1 in SDS–polyacrylamide gel electrophoresis. Since these proteins are very abundant [$\sim 10^6$ molecules per nucleus or about 1 HMG1 or -2 for every 10 nucleosomes (Goodwin et al., 1978)], they were postulated to be structural components of chromatin. These nonhistone chromosomal proteins appear to be enriched in transcriptionally active chromatin (Singh & Dixon, 1990; Thanos & Maniatis, 1992; Vidali et al., 1977; Kleinschmidt et al., 1983), and the levels of HMG1 and -2 decrease when cells become committed to differentiation (Seyedin & Kistler, 1979; Seyedin et al., 1981). HMG1 is the prototype of a class of proteins containing an HMG box (Jantzen et al., 1990), which all appear to bind DNA but with varying degrees of specificity.

The proposals for the structural role of HMG1 and -2, whether dynamic or static, led to many studies of their interactions with components of chromatin, notably histone H1 and DNA. There are reports on complexes formed between H1 and HMG1 and -2 (Smerdon & Isenberg 1976; Yu & Spring, 1977; Cary et al., 1979), and we observed a ternary interaction between H1, DNA, and HMG1 (Kohlstaedt et al., 1987) that might provide an additional basis for the suggestion of Paull et al. (1993) that HMG1 facilitates cooperative interactions between cis-acting proteins by promoting DNA bending.

In spite of numerous studies, the interaction of HMG1 and -2 with DNA is not thoroughly understood. There is evidence that HMG1 and -2 binding can change the linking number during the formation of circular DNA (Javaherian et al., 1978, 1979), and a recent report shows that it is negatively supercoiled DNA that HMG1 and -2 preferentially bind rather than linear or positively supercoiled forms (Sheflin et al., 1993). That HMG1 and -2 discriminate among various conformations of DNA is further indicated by their strong binding to cruciform structures (Bianchi, 1988, 1991; Bianchi et al., 1989). It has been reported several times that HMG1 and -2 bind to single-stranded DNA in preference over double-stranded DNA [Isackson et al., 1979; for a review Einck and Bustin (1985)]. Both raising and lowering of the melting temperature of DNA by HMG1 and -2 have been reported (Yu et al., 1977; Javaherian et al., 1979; Makiguchi et al., 1984; Yoshida & Shimura, 1985), the direction of the effect depending on salt concentration and the ratio of HMG to DNA. Complexity in the HMG1 and -2 interaction with DNA was further suggested by the report of a "beaded" complex of HMG1 and -2 with double-stranded DNA (Mathis et al., 1980) and the report of monomer and tetramer forms of HMG1 that differ from each other in their binding to DNA (Duguet & de Recondo, 1978; Bonne et al., 1982). The basis for the variety in these observations is not clear, but a possible factor is the variation in solution conditions from one experiment to another.

We discovered that the conformation of HMG1 changes substantially between pH 6.5 and 7.2 and that the change alters the mode of interaction between HMG1 and H1 histone (Kohlstaedt & Cole, 1994; L. A. Kohlstaedt and R. D. Cole, unpublished data). Therefore, it seemed reasonable to ask whether pH also affects the interaction between HMG1 and DNA. Since essentially all of the previous studies on HMG1 binding to DNA were done at pH's higher than 7, we undertook this present investigation of the effect of pH on the interactions of HMG1 with both double-stranded and single-stranded DNA. Binding was monitored by fluorescence quenching to determine site size, cooperativity, and binding constants.

† This work was supported in part by a research grant (GM 20338) from the National Institutes of Health and by the Experimental Research Station.

‡ Present address: Department of Chemistry, University of California, Santa Barbara, CA 93106.

® Abstract published in *Advance ACS Abstracts,* September 15, 1994.

[1] Abbreviations: HPLC, high-pressure liquid chromatography; MES, 2-morpholinoethanesulfonic acid; HMG1 and HMG2, high-mobility group proteins 1 and 2.

0006-2960/94/0433-12702$04.50/0 © 1994 American Chemical Society

MATERIALS AND METHODS

Protein Purification and DNA Preparation. HMG1 and -2 were purified from steer thymus as described previously (Kohlstaedt et al., 1986). Purification was by perchloric acid extraction of the tissue, followed by acetone fractionation, and finally by reversed phase HPLC. The DNA used in binding experiments was highly polymerized calf thymus DNA from Sigma. [If double-stranded regions of this DNA were cross-linked, or if protein contaminants were present in our DNA samples, the differences between pure single- and double-stranded DNA would be even greater than those shown in Table 1 (see below)]. Single-stranded DNA was made by boiling calf thymus DNA for 10 min followed by rapid cooling under cold tap water. Protein and DNA concentrations for studies of the interactions of HMG1 were determined by UV absorbance. The extinction coefficient for HMG1 at 280 nm was taken to be 33 000 M^{-1} cm^{-1}. The absorbance of DNA was assumed to be such that a 1 mg/mL solution would give an absorbance of 20.0 at 260 nm.

Fluorescence Spectrophotometry. Fluorescence spectra were measured on a Perkin-Elmer Model MFP-44B fluorescence spectrophotometer equipped with a DSU-2 differential corrected spectra unit. Spectra were recorded on a Hitachi strip chart recorder. Excitation was at 295 nm, and emission spectra were recorded between 300 and 500 nm. A blank spectrum was recorded and automatically subtracted from each emission spectrum, eliminating any effect on the emission spectrum by light scattering from the added DNA solution. Quenching was measured by determining the area under the curve of the emission spectrum.

Samples were prepared for fluorescence spectrophotometry by dissolving the lyophilized protein in the chosen buffer followed by overnight dialysis against the same buffer. Dialysis ensured a controlled salt concentration in binding experiments. For experiments at pH 6.0, the buffer was 10 mM MES, 150 mM NaCl, and 1 mM EDTA; 1 mM dithiothreitol was added to reduced HMG1 but not to oxidized HMG1. For experiments at pH 7.5, Tris was substituted for MES.

RESULTS

Interaction of HMG1 with Double-Stranded DNA. The interaction of HMG1 with DNA was studied by fluorescence quenching. HMG1 was added to long, double-stranded DNA, and the quenching of the tryptophan fluorescence of HMG1 that accompanied its binding to DNA was measured by calculating the total emission as the area under the curve of the fluorescence emission spectrum between 300 and 500 nm. In earlier studies over the range pH 2–9, the conformation of HMG1 was found to change sharply from one state to another between pH 6.0 and 7.5 (Kohlstaedt & Cole, 1994). Therefore, binding was measured at pH 6.0 and 7.5. No shift in the wavelength of the emission maximum was observed when HMG1 bound to DNA.

Since the binding of large ligands to DNA is complex (McGee & von Hippel, 1974), we chose to interpret the binding of HMG1 to DNA by the method of Schwarz and Watanabe rather than by a more conventional Scatchard analysis (Schwarz & Watanabe, 1983; Watanabe & Schwarz, 1983; Watanabe, 1986). Figure 1 shows the fluorescence of mixtures of HMG1 and DNA as a function of total HMG1 concentration at pH 6.0. DNA concentration was constant at about 10.8 μM base pairs (the concentration of DNA was calculated independently after each addition of HMG1). Fluorescence increased initially with a moderate slope until saturation, where

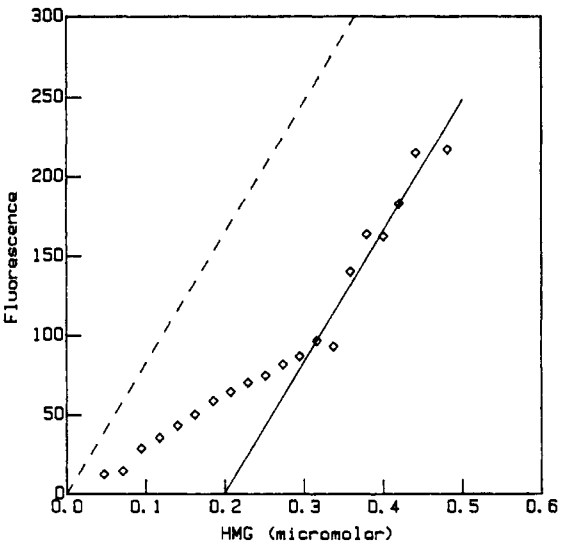

FIGURE 1: Fluorescence of tryptophans of HMG1 during the titration of double-stranded DNA by HMG1 at pH 6.0, 23 °C. The DNA concentration was 10.8 μM base pairs. The dashed line represents the amount of fluorescence expected from the same concentrations of HMG1 in the absence of DNA. The solid line is drawn through the points representing the fluorescence of the HMG1 after saturation of the DNA. Excitation was at 295 nm. Fluorescence is expressed in arbitrary units.

the slope abruptly became steeper. This steeper postsaturation line has a slope equal to that found for the addition of HMG1 to a solution containing no DNA (dashed line in Figure 1). The intercept of the steeper postsaturation line with the abscissa is equal to the amount of HMG1 needed to saturate the DNA; therefore, the stoichiometric number (n), the number of base pairs covered by a molecule of HMG1, can be estimated to be about 54.

Following Schwarz and Watanabe, the degree of saturation at each point, θ, and the stoichiometric number, n, were calculated as illustrated in Figure 2. The amount of free HMG1 could be calculated this way because binding to DNA caused essentially complete quenching of fluorescence. In the general case, the less quenching that occurs, the steeper the slope of the presaturation experimental curve, with the experimental curve approaching the line (e.g., the dashed line in Figure 1) for no ligand binding. Since binding must occur to produce quenching, the practical limit of the experimental slope is well under the slope of the curve for zero binding. The slope of the experimental curve in Figure 1 is 70% of the slope of the dashed line, and since binding occurred, the quenching must have been substantially greater than 70%. For the purposes of our calculations, we assumed 100% quenching. The calculations were then tested for the effect of assuming 80% quenching, and the latter assumption would change the binding constants by less than a factor of 2.

The concentrations of free HMG1 and θ were calculated as indicated in Figure 2 using the best smooth curve through the data points. Errors in this analysis stem from selection of this curve and placement of the straight line passing through the postsaturation data points. A test of the extremes in judging these lines showed that the calculated values for binding constants, stoichiometric number, and other parameters would not vary by more than 2-fold. Therefore, the calculations about to be presented have a precision of ±50% or better.

The degree of saturation as a function of free HMG1 concentration is shown in Figure 3. The sigmoidal shape of this plot is an indication of positive cooperativity. Half-

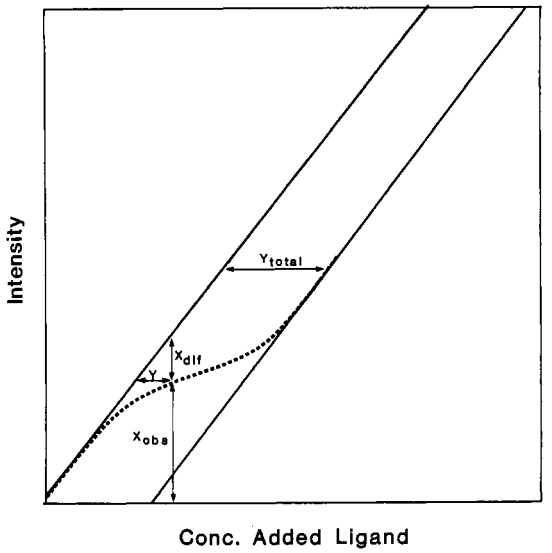

FIGURE 2: Fictitious example of the determination of the degree of DNA saturation and the concentration of free HMG1 (Schwarz & Watanabe, 1983; Watanabe & Schwarz, 1983; Watanabe, 1986). Y is the difference along the abscissa between a point on the observed curve and a point on the line that would be observed in the absence of substrate (DNA). The degree of binding, θ, is determined as $\theta = Y/Y_{total}$ for each point on the curve. The concentration of free ligand (free HMG1 here) is calculated from the difference between the observed magnitude of the property being measured (fluorescence here) and the magnitude expected if no substrate were present: [free ligand] = $(X_{obs}/X_{dif} + X_{obs})$[total ligand]. The stoichiometric number, n (in this case, the number of base pairs covered by a single HMG1 molecule), is determined as the intercept of the postsaturation line with the abscissa divided by the concentration of substrate present (DNA here).

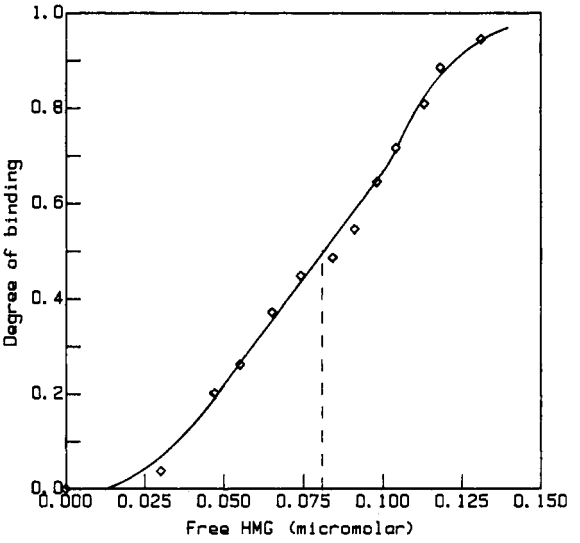

FIGURE 3: Degree of binding as a function of free HMG1 concentration for double-stranded DNA, pH 6.0. The midpoint gives the dissociation constant, 8×10^{-8} M^{-1}. The degree of binding and concentration of free HMG1 were calculated from data in Figure 1.

saturation occurred at about 8×10^{-8} M HMG1; therefore, $K_D = 8 \times 10^{-8}$ M.

The degree of binding is plotted as a function of the molar ratio of total (input) HMG1 to DNA (molar concentration of nucleotide pairs) in Figure 4. The values for each point on this curve minus the molar ratio of free HMG1 to DNA for each point define a straight line that would be followed at infinite DNA concentration (Schwarz & Watanabe, 1983; Watanabe & Schwarz, 1983). Extrapolation of this line to complete saturation ($\theta = 1.0$) yielded an estimation of the

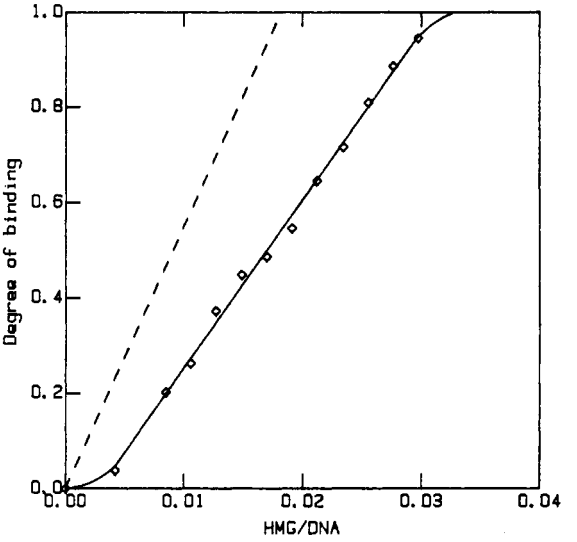

FIGURE 4: Degree of binding as a function of the molar ratio of added HMG1 to base pairs of DNA at pH 6.0. The dashed line represents the curve that would be obtained at infinite DNA concentration. This line is obtained by subtracting the molar ratio of free HMG to DNA (as base pairs) from the value for each point on the experimental curve. The dashed line reaches 1.0, complete saturation, at the reciprocal of the site size, indicating that each HMG1 molecule binds 54 base pairs of DNA.

reciprocal of the stoichiometric number. The site size determined by this method was 55 base pairs per HMG1 polypeptide, essentially the same as that determined in Figure 1.

The large ligand model of Schwarz and Watanabe (1983) was used to interpret the binding data further. The binding constant, K, the nucleation constant, K', and the cooperativity parameter, q, were calculated using the following relations:

$$(2\theta - 1)/\sqrt{\theta(1-\theta)} = \sqrt{q/n}(Kc_A - 1)$$

when $(q/n)^{1/2} > 2$, and

$$K' = K/q$$

where c_A is the concentration of free HMG1 and n is the stoichiometric number. Figure 5 is a graph of $(2\theta - 1)/(\theta(1 - \theta))^{1/2}$ as a function of free HMG1 concentration. One advantage of such a plot is its relative insensitivity to errors in the determination of θ. From such a plot, K and q may be estimated with fair accuracy, even when $(q/n)^{1/2} \leq 2$ (Schwarz & Watanabe, 1983). The intercept on the abscissa is equivalent to the reciprocal of the binding constant, and the intercept on the ordinate is equal to $-(q/n)^{1/2}$ (a line tangent to the curve at $y = 0$ is used to estimate q when $(q/n)^{1/2} \leq 2$). Using the value of n determined here, it was calculated that $K = 12 \times 10^6$ M^{-1}, $q = 770$, and $K' = 2 \times 10^4$ M^{-1}. This corresponds to $1/K = K_D = 8 \times 10^{-8}$ M, in agreement with the result determined in Figure 3.

The binding of HMG1 to double-stranded DNA was also measured at pH 7.5. Binding was measured at a constant DNA concentration of 5.8 μM base pairs, and binding data were analyzed as described earlier (data not shown). The binding constant was $K = 3 \times 10^6$ M^{-1}, which is a factor of 4 weaker than that measured for HMG1 at pH 6.0. Cooperativity was also much reduced at pH 7.5; the cooperativity parameter was determined to be $q = 78$. Perhaps most striking was the change in the stoichiometric number, which was only 13 at pH 7.5 compared to 54 at pH 6.0. The nucleation constant was $K' = 4 \times 10^5$ M^{-1}.

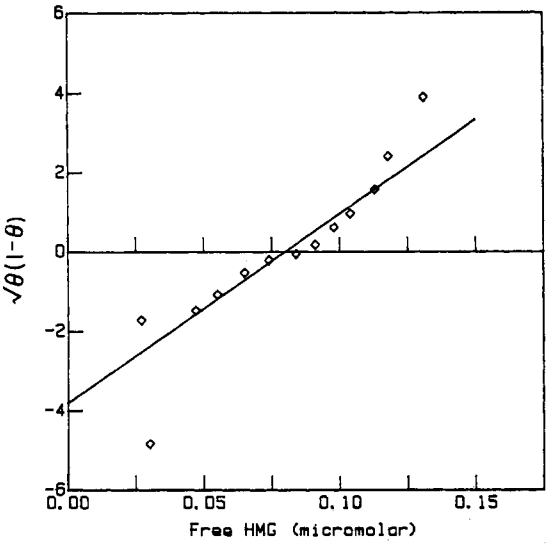

FIGURE 5: Binding of HMG1 to double-stranded DNA at pH 6.0—determination of dissociation constant. $2\theta - 1/(\theta(1-\theta))^{1/2}$ is plotted as a function of free HMG1 concentration. The intercept with the abscissa is equal to the dissociation constant, and the intercept on the ordinate is equal to $-(q/n)^{1/2}$, $1/K = K_D = 8 \times 10^{-8}$ M, in perfect agreement with the result determined in Figure 3.

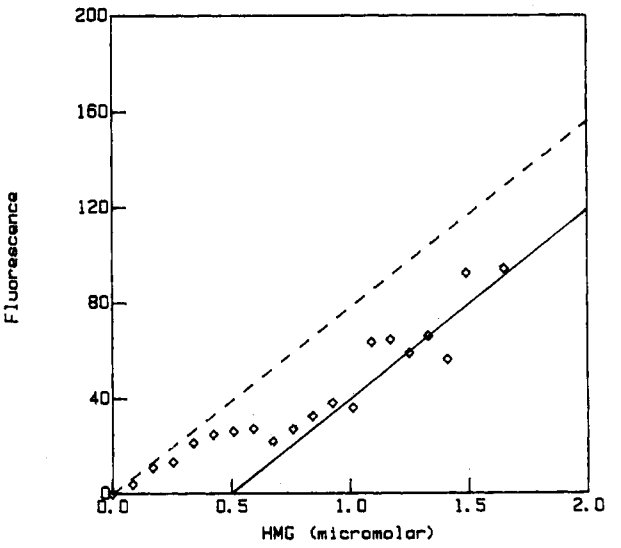

FIGURE 6: Fluorescence of tryptophans of HMG1 during titration of single-stranded DNA by HMG1 at pH 6.0, 23 °C. The DNA concentration was 9.4 μM bases. The dashed line represents the amount of fluorescence expected from the same concentrations of HMG1 in the absence of DNA. The solid line is drawn through the points representing the fluorescence of the HMG1 after saturation of the DNA. Excitation was at 295 nm. Fluorescence is expressed in arbitrary units.

Denaturation of HMG1 by the oxidation of sulfhydryls has been shown to prevent the conformation characteristic of reduced HMG1 at pH 6.0 when oxidized HMG1 was brought to pH 6.0 (Kohlstaedt & Cole, 1994). The importance of free sulfhydryl groups for the structure and function of HMG1 was also revealed when the HMG–DNA interaction was altered by covalently blocking sulfhydryl groups (Billings et al., 1992; Sheflin et al., 1993). When we denatured HMG1 by oxidation, it interacted with double-stranded DNA at pH 6.0, with a binding constant of $K = 6 \times 10^6$ M^{-1}. Cooperativity was greatly reduced; the cooperativity parameter for oxidized protein was found to be $q = 200$. When oxidized HMG1 was added to DNA at pH 7.5, erratic fluorescence measurements were obtained, perhaps due to light scattering. DNA seemed to induce a shift in the fluorescence maximum to 350 nm, a wavelength characteristic of total exposure of tryptophan to water. The DNA may have induced a further denaturing conformational change. DNA-HMG1 interaction may have occurred, but could not be quantified by fluorescence.

Interaction of HMG1 with Single-Stranded DNA. The interaction of HMG1 with single-stranded calf thymus DNA was measured by the same technique that was used for double-stranded DNA. No shift in emission maximum was observed when HMG1 bound to single-stranded DNA. The single-stranded DNA concentration was expressed in moles of nucleotides rather than nucleotide pairs. The differences between single- and double-stranded DNA in their characteristics for binding HMG1 at pH 6.0 were conspicuous even in the raw data, as can be observed by comparing Figure 6 to Figure 1; note the difference in scale along the abscissa. The additional calculations based on these data showed that, at pH 6.0, HMG1 bound to single-stranded DNA with a binding constant of $K = 3 \times 10^6$ M^{-1} and a site size of 14 bases. Cooperativity and the nucleation constant, calculated by the method of Schwarz and Watanabe, were $q = 440$, and $K' = 1 \times 10^4$ M^{-1}. The differences between single- and double-stranded DNA were seen to be more modest when the binding of HMG1 to single-stranded DNA was measured at pH 7.5. Both the site size and the degree of cooperativity were reduced; the parameters determined were $n = 7$, $q = 60$, and $K' = 3 \times 10^4$ M^{-1}. The binding constant at pH 7.5 was $K = 2 \times 10^6$ M^{-1}.

Table 1: Parameters for Binding of HMG1 to DNA[a]

HMG1	DNA	pH	K	n	q	K'
reduced	ds	6.0	12	54	770	2
	ds	7.5	3	13	78	4
	ss	6.0	3	14	440	1
	ss	7.5	2	7	60	3
oxidized	ds	6.0	6	16	200	3
	ss	6.0	3	7	180	2

[a] DNA is referred to as ds for double-stranded or ss for single-stranded; K is the binding coefficient multiplied by 10^{-6} M; K' is the nucleation constant multiplied by 10^{-4} M; n is the stoichiometric number; and q is the cooperativity parameter.

The characteristics of binding of HMG1 to both double- and single-stranded DNA are summarized in Table 1. It should be noted that the interaction of HMG1 with double-stranded DNA was stronger than that with single-stranded DNA at pH 6.0. At pH 7.5, the interactions were of about equal strength for the two forms of DNA. Thus, under the conditions of our experiments, HMG1 does not preferentially bind to single-stranded DNA. This conclusion is supported by the fact that circular dichroism experiments at pH 6.0 and 7.5 show no detectable distortion of the structure of double-stranded DNA upon the binding of HMG1 [data not shown; see also Kohlstaedt et al. (1987)].

DISCUSSION

The present measurements of binding constant and binding sites may be compared to those of Butler et al. (1985). Butler et al. (1985) measured the binding of HMG1 to DNA by monitoring the quenching of fluorescence of HMG1 when DNA was added to a solution of HMG1 at pH 8.0. These authors reported binding constants of $K = 3 \times 10^5$ and 4×10^5 M^{-1}, respectively, for double-stranded and single-stranded DNA in 140 mM NaCl and a site size of 14 for both. This site size is in reasonable agreement with ours (at pH 7.5 and 150 mM NaCl), but their binding constants are about 10-fold

lower than ours. Moreover, Butler et al. observed only 30% quenching, while we observed quenching greater than 80%. The modest amount of quenching observed by Butler et al. may be an indication that they failed to saturate all of the HMG1 with DNA. The method they used, the addition of DNA to a solution of binding ligand, has been shown to underestimate the binding constant where there is cooperativity (Watanabe & Schwarz, 1983; Schwarz & Watanabe, 1983). In any case, our binding constant at pH 7.5 (3×10^6 M^{-1}) is in better agreement with that of Shooter et al. (1974), who reported $K = 2 \times 10^6$ M^{-1} (and a site size of 13–16) for double-stranded DNA in 100 mM Tris (pH 8.5). Sheflin et al. (1993) did not measure binding constants, but they did show HMG1 and -2 binding more strongly to negatively supercoiled DNA than to linear DNA at pH 7.5, with binding sites estimated at 20 base pairs, similar to the size measured in our studies with linear DNA.

Although there have been many reports that HMG1 prefers to bind single-stranded rather than double-stranded DNA (Bidney & Reeck, 1978; Isackson et al., 1979; Javaherian et al., 1979; Bustin & Soares, 1985; Hamada & Bustin, 1985; Brown & Anderson, 1986), our measurements showed approximately equal binding constants for the two kinds of DNA at pH 7.5 (essentially all of the earlier studies were done near pH 7.5). Similarly, Butler et al. (1985) found nearly equal binding constants for double-stranded DNA and poly(dA), and Shooter et al. (1974) found approximately equal constants for double-stranded DNA and denatured DNA. All of those experiments reporting a preference of HMG1 for single-stranded DNA were done at salt concentrations of less than 50 mM or at 200 mM. Butler et al. showed that the preference of HMG1 for single-stranded DNA went down from 10-fold at 2.5 mM to 1.3-fold at 140 mM and then back up to 7-fold at 200 mM. Thus, the results of Butler et al. seem to reconcile all of the reports regarding the preference of HMG1 for single-stranded DNA near pH 7.5.

The principal purpose of the present study was to learn whether the binding of HMG1 by DNA was significantly affected by changes in pH. The interaction of HMG1 with DNA at pH 6.0 was strikingly different from that at pH 7.5. For double-stranded DNA, the binding constant decreased 4-fold when the pH was raised from 6.0 to 7.5. The binding constant for single-stranded DNA, however, was substantially unchanged by the same shift in pH, becoming about equal to that for double-stranded DNA at the higher pH. Cooperativity was dramatically decreased by the increase in pH; q dropped by an order of magnitude for either double- or single-stranded DNA.

The site size decreased for both single- and double-stranded DNA when the pH was raised. For single-stranded DNA, it changed from about 14 bases to about 7 bases per HMG1 monomer. For double-stranded DNA, the change was from about 54 base pairs to about 13 base pairs. The surprisingly large site size for double-stranded DNA at pH 6 might be due to a winding of DNA around an oligomer of HMG1 molecules. Such winding would involve bending the DNA, and in fact, HMG1 has been shown to bend DNA (Read et al., 1993; Paull et al., 1993; Pil et al., 1993). Under some conditions, complexes of superhelical DNA and HMG1 can form beadlike structures (Mathis et al., 1980); perhaps something analogous happened with our HMG1–DNA complexes at pH 6. If so, the conformation of HMG1 at pH 7.5 would not allow the formation of the oligomer, and the denaturation of HMG1 by oxidation would block oligomer formation.

The pH sensitivity of HMG1 binding to DNA parallels the pH dependence of the interaction of HMG1 with H1 histone (Kohlstaedt & Cole, 1994). Both are probably a reflection of the conformational change that was observed by fluorescence quenching when the pH was shifted from 6.0 to 7.5 (Kohlstaedt & Cole, 1994). The fluorescence quenching was probably caused by changes in the structure surrounding a tryptophan that is central in the B-domain HMG box (Weir et al., 1993; Read et al., 1993). Weir et al. noted the presence of up to eight potential salt bridges that could stabilize the structure of that domain, and it may be that it is the titration of one or more of these bridges that caused the conformational change, fluorescence quenching, and decrease in the binding of HMG1 to double-stranded DNA. Whether or not this is the mechanism underlying the pH-induced changes in conformation and binding properties of HMG1, it is clear that caution must be exercised for DNA binding at pH's near 7.5 to the structure, on the basis of NMR studies at pH 5 (Weir et al., 1993; Read et al., 1993).

Our results show that pH changes in the physiological pH range dramatically affect the tightness of HMG1 binding by DNA and the mode of binding, and this is especially true for double-stranded DNA. This effect, combined with the sharp optimum for HMG1 binding to double-stranded DNA at physiological levels of salt concentration (Butler et al., 1985), reveals that the structure of these HMG proteins is poised for manipulation by factors analogous to protons and salts, for example, polyamines or Ca^{2+}. The potential exists for such factors to regulate HMG1–DNA interactions *in vivo* as a way of modulating DNA functions.

ACKNOWLEDGMENT

For the use of his fluorescence spectrophotometer at the University of California at San Francisco, we thank Dr. Thomas Boyer, now at Emory University.

REFERENCES

Bianchi, M. E. (1988) *EMBO J. 7*, 843–849.
Bianchi, M. E. (1991) *Gene 104*, 271–275.
Bianchi, M. E., Beltrame, M., & Paonessa, G. (1989) *Science 243*, 1056–1059.
Bidney, D. L., & Reeck, G. R. (1978) *Biochem. Biophys. Res. Commun. 85*, 1211–1218.
Billings, P. C., Davis, R. J., Englesberg, B. N., Skov, K. A., & Hughes, E. N. (1992) *Biochem. Biophys. Res. Commun. 188*, 1286–1294.
Bonne, C., Sutiere, P., Duguet, M., & de Recondo, A.-M. (1982) *J. Biol. Chem. 257*, 2722–2725.
Brown, J. W., & Anderson, J. A. (1986) *J. Biol. Chem. 261*, 1349–1354.
Bustin, M., & Soares, N. (1985) *Biochem. Biophys. Res. Commun. 133*, 633–640.
Butler, A. P., Mardian, J. K., & Olins, D. E. (1985) *J. Biol. Chem. 260*, 10613–10620.
Cary, P. D., Shooter, K. V., Goodwin, G. H., Johns, E. W., Olayemi, J. Y., Hartman, P. G., & Bradbury, E. M. (1979) *Biochem. J. 183*, 657–662.
Duguet, M., & de Recondo, A.-M. (1978) *J. Biol. Chem. 253*, 1660–1666.
Einck, L., & Bustin, M. (1985) *Exp. Cell Res. 156*, 295–310.
Goodwin, G. H., Sanders, C., & Johns, E. W. (1973) *Eur. J. Biochem. 38*, 14–19.
Goodwin, G. H., Walker, J. M., & Johns., E. W. (1978) in *The Cell Nucleus*, Vol. VI, pp 181–219, Academic Press, New York.
Hamada, H., & Bustin, M. (1985) *Biochemistry 24*, 1428–1433.

Isackson, P. J., Fishback, J. L., Bidney, D. L., & Reeck, G. R. (1979) *J. Biol. Chem. 254*, 5569–5572.
Isackson, P. J., Chow, L. G., & Reeck, G. R. (1981) *FEBS Lett. 125*, 30–34.
Jantzen, H.-M., Admon, A., Bell, S. P., & Tjian, R. (1990) *Nature 344*, 830–836.
Javaherian, K., Liu, L. F., & Wang, J. C. (1978) *Science 199*, 1345–1346.
Javaherian, K., Sadeghi, M., & Liu, L. F. (1979) *Nucleic Acids Res. 6*, 3569–3580.
Johns, E. W. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., Ed.) Academic Press, New York.
Kleinschmidt, T. A., Scheer, U., Dabaville, M. C., Bustin, M., & Franke, W. W. (1983) *J. Cell Biol. 97*, 838–848.
Kohlstaedt, L. A., & Cole, R. D. (1994) *Biochemistry 33*, 570–575.
Kohlstaedt, L. A., King, D. S., & Cole, R. D. (1986) *Biochemistry 25*, 4562–4565.
Kohlstaedt, L. A., Sung, E. C., Fugishige, A., & Cole, R. D. (1987) *J. Biol. Chem. 262*, 524–526.
Makiguchi, K., Chida, Y., Yoshida, M., & Shimura, K. (1984) *J. Biochem. 95*, 423–429.
Mathis, D. J., Kindelis, A., & Spadafora, D. (1980) *Nucleic Acids Res. 8*, 2577–2590.
McGee, J. D., & von Hippel, P. H. (1974) *J. Mol. Biol. 86*, 469–480.
Paull, T. T., Haykinson, M. J., & Johnson, R. C. (1993) *Genes Dev. 7*, 1521–1534.
Pil, P. M., Chow, C. S., & Lippard, S. J. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 9465–9469.
Read, C. M., Cary, P. D., Crane-Robinson, C., Driscoll, P. C., & Norman, D. G. (1993) *Nucleic Acids Res. 21*, 3427–3436.
Schwarz, G., & Watanabe, F. (1983) *J. Mol. Biol. 163*, 467–484.
Seyedin, S. M., & Kistler, W. S. (1979) *J. Biol. Chem. 254*, 11264–11271.
Seyedin, S. M., Pehrson, J. R., & Cole, R. D. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5988–5992.
Sheflin, L. G., Fucile, N. W., & Spalding, S. W. (1993) *Biochemistry 32*, 3238–3248.
Shooter, K. V., Goodwin, G. H., & Johns, E. W. (1974) *Eur. J. Biochem. 47*, 263–270.
Singh, J., & Dixon, G. H. (1990) *Biochemistry 29*, 6295–6302.
Smerdon, M. J., & Isenberg, I. (1976) *Biochemistry 15*, 4242–4247.
Thanos, D., & Maniatis, T. (1992) *Cell 71*, 777–789.
Vidali, G., Boffa, L. C., & Allfrey, V. G. (1977) *Cell 12*, 409–415.
Watanabe, F. (1986) *Nucleic Acids Res. 14*, 3573–3585.
Watanabe, F., & Schwarz, G. (1983) *J. Mol. Biol. 163*, 485–498.
Yoshida, M., & Shimura, K. (1985) *J. Biochem. 95*, 117–124.
Yu, S. S., & Spring, T. G. (1977) *Biochim. Biophys. Acta 492*, 20–28.