Specific Interaction between H1 Histone and High Mobility Protein HMG1

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ABSTRACT: High mobility group proteins HMG1 and -2 and histone H1 are structural components of chromatin. Previously, we reported that HMG1 interacts with H1 histone in a way that modulates the ability of H1 to condense DNA *in vitro*, suggesting that these proteins may act together *in vivo* to regulate locally the condensation state of chromatin, possibly affecting replication and/or transcription. Here we show that reduced (native) HMG1 binds to H1 cooperatively at pH 6.0 as a tetramer with a dissociation constant of 3.4×10^{-8} M, and at pH 7.5 as a monomer with a dissociation constant less than 10^{-9} M. Denaturation through oxidation of sulfhydryl groups has a strong effect on the interaction of HMG1 with H1 histone, suggesting that the reduced state of HMG1 is critical to its function. Oxidized HMG1 failed to bind H1 at pH 7.5, and its binding at pH 6 was biphasic; the first three (or two) molecules of H1 were bound with a dissociation constant of 2×10^{-8} M with negative cooperativity, and the last one (or two) H1's were bound cooperatively with $K_D = 1.8 \times 10^{-7}$ M. Regulation of the pH or the concentration of some other ion may be used *in vivo* to alter the interactions between HMG1 and -2, H1 histone, and DNA.

H1 histone may serve as a generalized regulator of gene expression (Croston et al., 1991) by varying the condensation state of localized regions of chromatin (Huang & Cole, 1984; Rocha et al., 1984). Such a role is supported by the ability of H1 to condense DNA (Fasman et al., 1970; Welch & Cole, 1980; Liao & Cole, 1981a), dinucleosomes (Liao & Cole, 1981b), or chromatin fragments (Biard-Roche et al., 1982) *in vitro*. Control of condensation may be through local variation in the amount of H1 histone (Huang & Cole, 1984; Kamakak & Thomas 1990; Davie & Delcuve, 1991; Bresnick et al., 1992) or the proportion of each H1 subfraction (Jin & Cole, 1985, 1986) or through posttranslational modification of histones (Adler et al., 1972; Gurley et al., 1974; Ajiro et al., 1981). The interaction of H1 with chromatin may also be modulated by the binding of non-histone chromosomal proteins to H1 histone [Sharp, 1991; Felsenfeld, 1992; see also Giese et al. (1992)]. High mobility group (HMG) proteins 1 and 2 are good candidates for such a role. HMG1 and HMG2 are two closely related, abundant non-histone chromosomal proteins. Although the functions of HMG1 and -2 are not completely understood, some studies have implicated HMG1 and -2 in the regulation of transcription (Vidali et al., 1977; Kleinschmidt et al., 1983; Singh & Dixon, 1990; Thanos & Maniatis, 1992) or replication (Alexandrova et al., 1984), and homology between HMG1 and -2 and one or more types of transcriptional activators has been observed in a number of cases [reviewed by Ner (1992)]. Singh and Dixon (1990) reported that HMG1 and -2 serve as class II transcription factors, and it needs to be recognized that this does not rule out their interaction with histone H1, whether or not chromatin decondensation is involved [see Sharp (1991)].

There are reports that HMG1 interacts with histone H1. Smerdon and Isenberg (1976) postulated a specific interaction between certain subfractions of H1 and HMG1 which does not occur with HMG2. These workers found that HMG1 and some subfractions of H1 could form heterodimers at pH 7.6 which could be detected by sedimentation equilibrium. Yu and Spring (1977) also observed an interaction between HMG1 and H1 in a nonquantitative assay. They observed that HMG1 bound at pH 7.0 to a particular variant of H1 immobilized on agarose, but that HMG2 did not. The bound HMG1 was eluted in 50–150 mM NaCl. Cary et al. (1979) claimed that the interaction between HMG1 and histone H1 is merely a nonspecific electrostatic one, caused entirely by the electrostatic attraction between the highly cationic H1 and the anionic domain of HMG1. Their conclusion was based on the lack of perturbation of the histidine NMR spectrum when HMG1 and H1 were mixed at pH 7.0 in 100 mM NaCl. The lack of a shift was taken to indicate that no conformation change occurred upon binding and that the binding was therefore totally electrostatic in nature. We showed, however, that H1, HMG1, and DNA form a specific complex that does not form when HMG1 is denatured by oxidation of its sulfhydryl groups (Kohlstaedt et al., 1987). In the present work we explored more fully the binding of HMG1 to H1, determining the binding constant and stoichiometries for this interaction. Previously, it was reported that HMG1 and -2 are very labile to sulfhydryl oxidation (Elton & Reeves, 1985; Kohlstaedt et al., 1986). Since the oxidation caused a change in the conformation of the protein (Kohlstaedt et al., 1987), we wondered if such partial denaturation of the proteins might have escaped notice in some earlier experiments, causing inconsistencies in the reports of the properties of HMG1 and -2. Here we show that oxidation of sulfhydryls to which HMG1 is subject does affect its binding to H1 histone.

EXPERIMENTAL PROCEDURES

Protein Purification. HMG1 in the reduced form was purified from steer thymus as described before (Kohlstaedt et al., 1986). Purification was by perchloric acid extraction of the tissue followed by acetone fractionation and purification by reversed-phase HPLC.[1] H1 histone was prepared from

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[1] Abbreviations: HPLC, high-pressure liquid chromatography; SDS, sodium dodecyl sulfate; MES, 2-(*N*-morpholino)ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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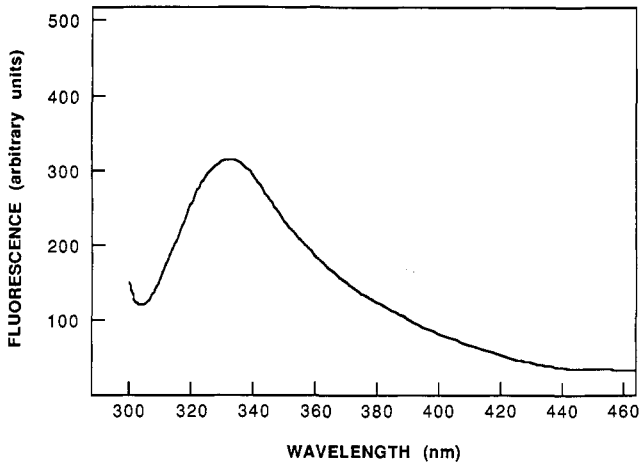

FIGURE 1: Tryptophan emission spectrum of HMG1. HMG1 was dissolved in and dialyzed against 10 mM MES, 150 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol, pH 6.0. The sample was diluted to about 1 mM in the same buffer just prior to analysis. The sample was excited at 295 nm, and the emission spectrum was recorded while a buffer blank was subtracted. Fluorescence units are arbitrary.

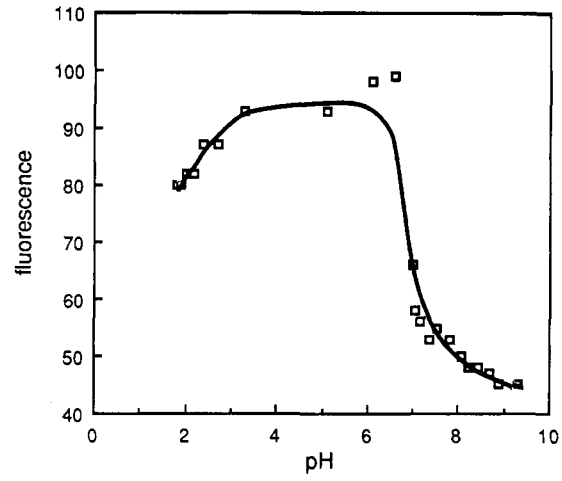

FIGURE 2: Fluorescence of HMG1 as a function of pH. HMG1 was dissolved in and dialyzed against 10 mM Tris, 150 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol, pH 7.0. The sample was diluted to about 2 μM in the same buffer just prior to analysis. The pH was varied by adding measured quantities of NaOH or HCl. The pH of the sample at any point was determined by comparison to a titration curve that related the molar ratio of added acid or base to the pH of this buffer. The sample was excited at 295 nm, and the emission spectrum was recorded while a buffer blank was subtracted. Fluorescence units are an arbitrary expression of the area under the emission curve.

steer thymus by perchloric acid extraction and precipitation from 78% acidified acetone. Purification was completed by passage over a Bio-gel P-100 column in 0.01 N HCl as described previously (Pehrson & Cole, 1980). H1 histone used in these experiments was an unfractionated mixture of the H1 variants found in calf thymus.

Determination of Protein Concentration. Protein concentration was determined by UV absorbance. The extinction coefficient for HMG1 at 280 nm was taken to be 33 000 M^{-1} cm^{-1}, a value determined by comparison to an amino acid analysis. The extinction coefficient for H1 histone at 230 nm was taken to be 4.1×10^4 M^{-1} cm^{-1}.

Polyacrylamide Gel Electrophoresis. SDS gel electrophoresis was run according to the method of Laemmli (1970) with 12.5% acrylamide and 0.8% bis(acrylamide).

Fluorescence Spectrophotometry. Fluorescence spectra were measured on a Perkin-Elmer Model MFP-44B fluorescence spectrophotometer equipped with a DSU-2 differential corrected spectra unit and a temperature controlled cell set at 23 °C. 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 subtracted automatically from each 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 controlled salt concentrations 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

Fluorescence Properties of HMG1. The quantum yield of tryptophan fluorescence for HMG1 is unusually low. HMG1 in its native, reduced form was dissolved in buffer at pH 6.0 and dialyzed against the same buffer. The HMG1 solution was diluted to a known concentration near 1 μM with the same buffer, and the fluorescence emission spectrum with excitation of the HMG1 at 295 nm was recorded (Figure 1). It showed a maximum emission at 330 nm. The area of this spectrum was compared to the area of a spectrum of tryptophan in water under the same conditions. The quantum yield for tryptophan was assumed to be $f = 0.2$ (Lakowicz, 1983). The quantum yield for HMG1, the average of five determinations, was found to be $f = 0.030$. Quantum yield was independent of concentration between 0.025 and 2.0 μM HMG1; higher concentrations of HMG1 were not investigated.

Most proteins have quantum yields that exceed that of tryptophan in water because their tryptophan residues are buried inside the protein away from the quenching effects of water (Lakowicz, 1983). Apparently, the fluorescence of tryptophan in HMG1 is quenched by some aspect of protein structure. This confirms the earlier report by Baker et al. (1976) that the structure of HMG1 quenches its tryptophan as much as water does. The emission spectrum of HMG1 in 4.2 M guanidinium chloride was determined. The quantum yield for the denatured HMG1, which was expected to be nearly the same as that for tryptophan in water since tryptophans in the fully denatured protein should be completely exposed to solvent, was not substantially different. That the tryptophan was actually exposed to water was evident because the peak of fluorescence emission was shifted to 350 nm, the same as the peak found for tryptophan in water. Therefore, there may be some residues close to the tryptophans of HMG1 in the primary sequence that cause quenching. Butler et al. (1985) suggested that the tryptophan fluorophore was relatively exposed to solvent, but constrained in a rigid, positively charged environment, and they suggested that an arginine and a lysine flanking Trp56 (Walker et al., 1980) were quenchers.

The fluorescence of tryptophan in HMG1 was highly dependent on pH. The total relative fluorescence (area under the emission curve) of HMG1 was determined as a function of pH (Figure 2). The fluorescence yield dropped dramatically as the pH was raised, and it showed an inflection at pH 7.0. Either the protein experienced a dramatic conformational change at this point or a nearby quenching group titrated at pH 7.0. The amino acid that commonly has a pK near 7.0 is histidine, but an α-amino group could also have a pK near 7.0. It was reported that two histidines in HMG1 titrate with

p*K*'s near 6.7 (Cary et al., 1979), but neither histidine nor an amino group is likely to exert the primary quenching effect on tryptophan, because it would be expected that such a mechanism would lead to decreased quenching at high pH when in fact the opposite was observed. Of course it is possible that titration of histidine caused a conformation change that moved a quencher into proximity to a tryptophan.

To obtain the observed quenching as pH is increased probably requires an increase in the charge of a negative group, if a conformational change is not the mechanism. Sulfhydryl groups can have p*K*'s near 7.0 if they are constrained in a positively charged environment, and carboxyl groups may have such a p*K* if clustered with other negatively charged residues. If the observed quenching above pH 6.5–7.0 was caused by the titration of a carboxyl group surrounded by other carboxyl groups, the titration curve would be expected to have a broad inflection. The observed titration curve was sharp, however, so increased quenching at high pH is not likely to be due to the titration of carboxyl groups. A more likely possibility is that the observed change in fluorescence with pH was due to the titration of a sulfhydryl group constrained in a positively charged environment. This possibility was supported by the fact that after oxidation the fluorescence of HMG1 was the same between pH 6.0 and 7.5.

Interaction of HMG1 with H1 Histone. The interaction of HMG1 with H1 histone was measured by quenching of the fluorescence of tryptophans in HMG1. H1 histone has no tryptophan residues. Small increments of H1 were added to a solution of HMG1, which was then excited at 295 nm; the fluorescence spectrum was recorded between 300 and 500 nm. A blank containing the same concentration of H1 (but without HMG1) was subtracted from each spectrum. Blank subtraction eliminated the Raman spectrum from the emission spectrum and eliminated emission from some component of the H1 solution that had an emission peak around 300 nm. The total fluorescence of each spectrum was calculated as the area under the curve of the emission spectrum. It was found that addition of H1 to a solution of HMG1 could quench about one-third of the fluorescence from the tryptophans of HMG1. The H1 may interact with the HMG1 in such a way as to quench the fluorescence of only one of the two tryptophans of HMG1. Alternatively, the H1 may cause partial quenching of both tryptophans either directly or by causing a conformational change in the HMG1. No shift in the emission maximum was found. Maximum quenching of fluorescence was taken to represent complete binding, and intermediate degrees of quenching were assumed to represent intermediate degrees of saturation by HMG1. Binding was assumed to be a linear function of fluorescence quenching.

At pH 6.0 in the presence of 150 mM NaCl and 1 mM EDTA, H1 histone interacted with HMG1 in a cooperative fashion to form a complex that contained four HMG1 molecules and one molecule of H1. In the titration fluorescence dropped from 7.45 to 4.40 (arbitrary units), and the latter was assumed to represent saturation. This assumption was confirmed in finding that the raw data were well represented by the equation

$$\phi = 4.35 + 2.11 \log\left(\frac{1}{(\mathrm{H1/HMG1})}\right)$$

with a correlation coefficient $R^2 = 0.893$.

The degree of binding (θ) is plotted against the molar ratio of H1 to HMG1 in Figure 3. If binding were infinitely tight, the curve in Figure 3 would intersect a horizontal line drawn at $\theta = 1.0$ at a point equal to the reciprocal of the stoichiometric

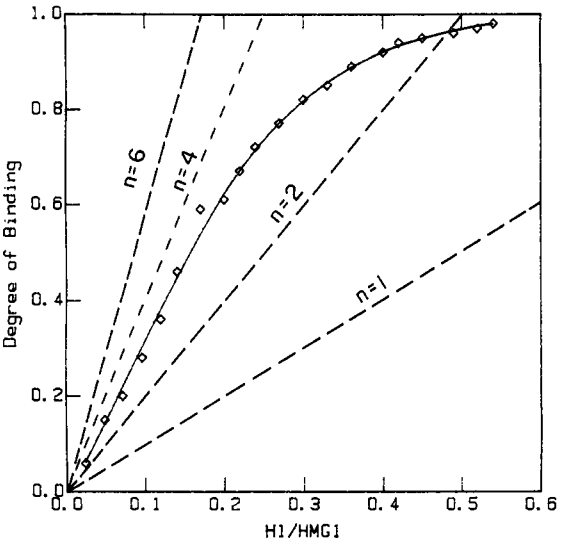

FIGURE 3: Binding of HMG1 to H1 at pH 6.0 and 23 °C as a function of H1/HMG1 molar ratio. Degree of binding was calculated by assuming that the maximum attained quenching represented saturation of HMG1 and that binding was a linear function of fluorescence quenching. Free H1 concentration was calculated from these data by assuming $n = 4$ (see text). Total HMG1 concentration was 0.8 μM. Theoretical lines (dashed lines) for the indicated values of n can be compared to the experimental data (solid line).

number (n) with respect to HMG1 (molecules of HMG1 bound to each H1 molecule). Theoretical lines (dashed) are drawn for $n = 1$, 2, 4, and 6, on the basis of infinitely tight binding; any real case will fall to the right of the corresponding theoretical curve. With this in mind, the actual data for H1 binding to HMG1 strongly suggested $n = 4$. It can be seen from Figure 3 that $n \geq 3$ (greater than or equal to three HMG1 molecules per H1 intercept of $\theta = 1$ at 0.33) is the only possible stoichiometry for the interaction. Since tetramerization is a common form of protein interaction, and trimerization is rare, we concluded that the stoichiometry of the interaction was most likely to be $n = 4$, four HMG1 molecules per H1 molecule. It is not clear whether tetramerization occurs independently or only as a result of H1 binding to HMG1. There are published results of tetrameric HMG1 (Duguet & de Recondo, 1978; Bonne et al., 1982) and conflicting ones of monomeric HMG1 (Shooter et al., 1974; Goodwin et al., 1975). We were unable to resolve this conflict, occasionally observing tetramers but usually observing monomers. A long search for conditions promoting tetramerization failed. It is most likely therefore that HMG1 tetramers form only as HMG1 is complexed with H1 histone. The molar concentration of free H1 at any point in the titration was then calculated for the difference between the observed curve and the line for complete H1 binding (infinitely tight binding) if $n = 4$.

A Scatchard plot assuming $n = 4$ for the histone H1–HMG1 interaction at pH 6.0 revealed substantial positive cooperativity (Figure 4). The dissociation constant, calculated from the slope of the linear part of the Scatchard plot, was $K_D = 3.2 \times 10^{-8}$ M. The degree of HMG1 saturation is plotted as a function of log(K[free H1]) in Figure 5. The fact that a reasonably smooth sigmoid curve was obtained with its inflection at 0 indicates that the estimation of the dissociation constant from the Scatchard plot was good. Positive cooperativity was once again evident ($\theta \approx 10^2$).

Log[$\theta/(1 - \theta)$] was plotted as a function of log[free H1] in Figure 6. In such a plot the intercept in the abscissa is equal to the dissociation constant, and the slope is an indication

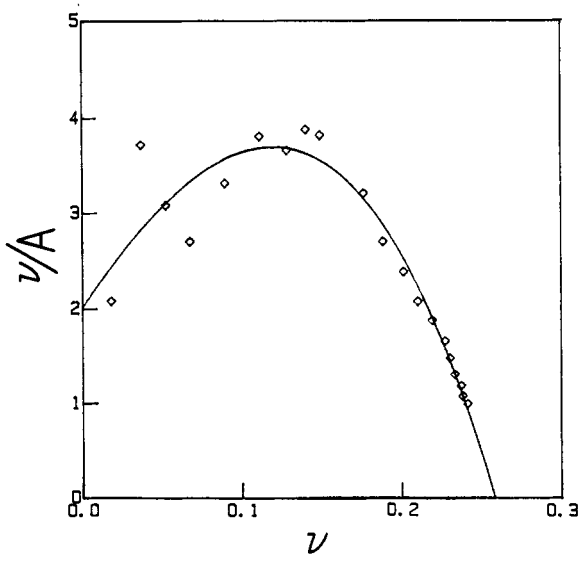

FIGURE 4: Scatchard plot of binding of HMG1 to H1 at pH 6.0. The plot assumed $n = 4$. Concentration of free HMG1 was calculated from the data in Figure 3 by multiplying the distance between the experimental curve and a theoretical curve at infinite HMG1 concentration and $n = 4$ (H1/HMG1 = 0.25 when $\theta = 1.0$) by the total concentration of HMG1.

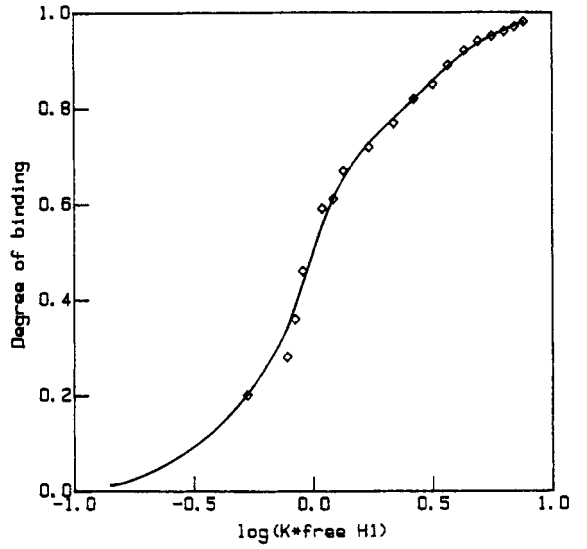

FIGURE 5: Binding of HMG1 to H1 at pH 6.0: degree of saturation as a function of log(K[free H1]).

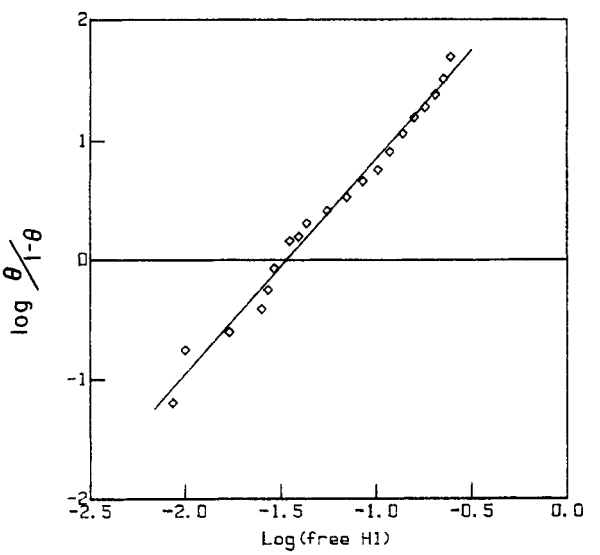

FIGURE 6: Binding of HMG1 to H1 at pH 6.0: plot of log[$\theta/(1-\theta)$] as a function of log[free H1].

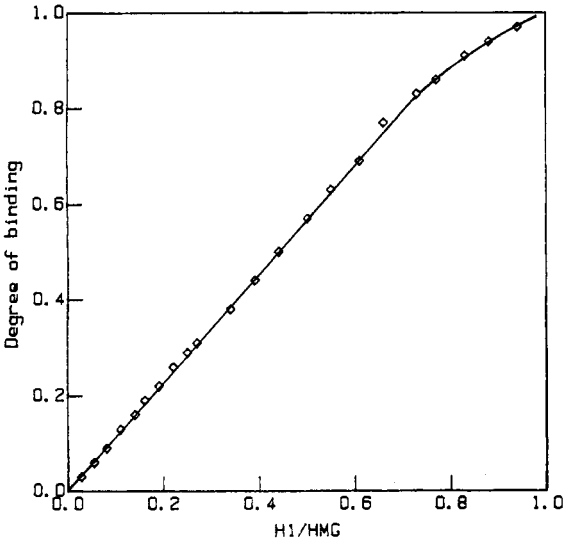

FIGURE 7: Binding of HMG1 to H1 at pH 7.5 and 23 °C. Degree of binding to HMG1 is plotted as a function of the H1/HMG1 molar ratio. Degree of binding was calculated by assuming that the maximum attained quenching represented saturation of HMG1 and that binding was a linear function of fluorescence quenching. The concentration of HMG1 was 1.37 μM.

of the degree of cooperativity. A slope of 1 indicates no cooperativity; a slope greater than 1 indicates positive cooperativity; a slope of less than 1 indicates negative cooperativity. If the slope is equal to n (or $1/n$ if $n < 1$), then the binding can be approximated by an all-or-none model. In the case of the H1–HMG1 interaction, the slope was about 2.6, not steep enough to be approximated by a two-state model, since $n = 4$. The dissociation constant determined by this method was $K_D = 3.5 \times 10^{-8}$ M.

When HMG1 at a concentration of 1.37 μM was titrated with H1 histone at pH 7.5 instead of pH 6.0, a dramatic difference in binding behavior was seen (Figure 7). The interaction at pH 7.5 was best modeled by assuming a stoichiometric number of $n = 1$ and a tighter binding constant. Attempts to analyze these data using a stoichiometry of $n = 2$ or 4 resulted in Scatchard plots that did not agree with the chosen assumption. If $n = 1$, then the binding detected must be tighter than that at pH 6.0. The low quantum yield of reduced HMG1 at the higher pH made it impossible to study the interaction at lower HMG1 concentrations; therefore, we are limited to the conclusion that $K_D < 10^{-9}$ M.

Oxidation of HMG1 affected its ability to bind to H1 histone. At pH 6.0 the oxidized HMG1 had lost the cooperative nature of its binding, and at pH 7.5 no interaction of HMG1 and H1 could be detected. The interaction of H1 with oxidized HMG1 (1.03 μM) at pH 6.0 was observed and interpreted as described for reduced HMG1 above. Assuming a stoichiometric number of $n = 4$, a Scatchard plot was made of the binding data (Figure 8). The plot indicated substantial negative cooperativity. If the binding was assumed to be the combination of two straight lines, two dissociation constants could be calculated from the Scatchard plot: $K_D = 1.2 \times 10^{-8}$ and 1.4×10^{-7} M. The interaction was further analyzed by plotting $\log(\theta/1-\theta)$ as a function of log[free H1] (Figure 9). This plot revealed that the data were a combination of two interactions since there were two straight lines that could be fitted to the data. The first line gave a dissociation constant

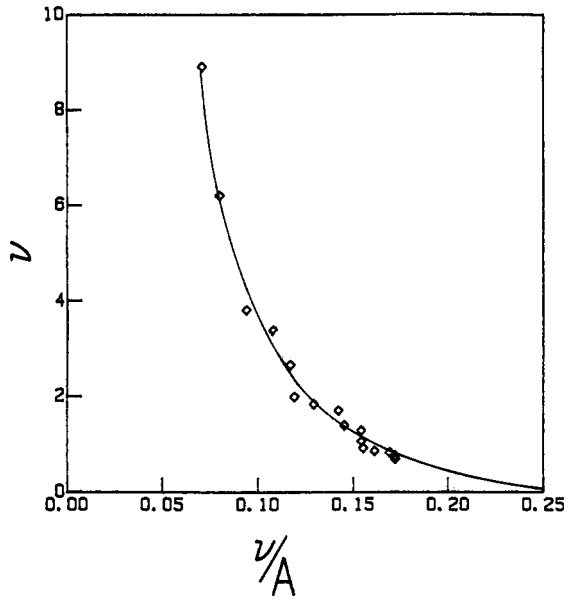

FIGURE 8: Scatchard plot of binding of oxidized HMG1 to H1 at pH 6.0. The Scatchard plot assumed $n = 4$. Concentration of free H1 was calculated as described for reduced HMG1 (Figure 3) by multiplying the distance between the experimental binding curve and a theoretical curve at infinite HMG1 concentration by the total concentration of HMG1, 1.03 μM.

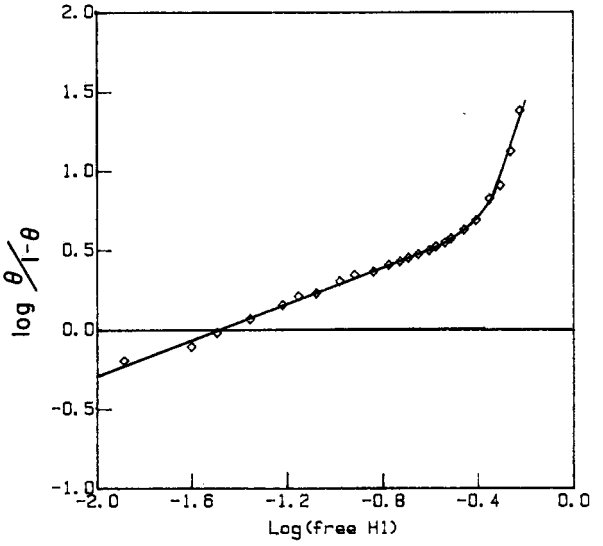

FIGURE 9: Binding of oxidized HMG1 to H1 at pH 6.0: plot of $\log[\theta/(1-\theta)]$ as a function of log[free H1]. The degree of binding and the free H1 concentration were calculated as described in Figures 3 and 8. The HMG1 concentration was 1.03 μM.

of $K_D = 3.2 \times 10^{-8}$ M; the second, if extrapolated to the abscissa, gave $K_D = 2.2 \times 10^{-7}$ M. The first line has a slope less than 1, indicating negative cooperativity. The second has a slope of 4, indicating that the sites filled with this binding constant approximate an all-or-none binding mechanism. One interpretation would be that two or three HMG1 molecules bind to an H1 molecule with negative cooperativity, and then when a critical degree of saturation is reached, another one or two molecules bind with high positive cooperativity that approximates an all-or-none reaction. A conceivable mechanism for this might be that an energetically favorable tetramer of oxidized HMG1 formed when a fourth monomer of HMG1 was added to an H1 molecule which had already bound three monomers of oxidized HMG1 in an energetically unfavored structure.

DISCUSSION

HMG1 and -2 both contain four sulfhydryl groups, two of which are easily oxidized under common laboratory conditions (Kohlstaedt et al., 1986). Evidently, the disulfide formation is intramolecular, since SDS–gel electrophoresis in the absence of reducing agents did not reveal dimers or higher oligomers. It is unlikely that the oxidized forms of HMG1 and -2 have any physiological significance. Nevertheless, the rapid oxidation of HMG1 and -2 is of considerable importance to those studying the structure and function of these proteins *in vitro*, especially since oxidation altered the interaction of HMG1 with H1. A possible explanation for the unusually high sensitivity of HMG1 and -2 to oxidation is that the pK of at least one sulfhydryl group is distorted by an unusual environment. There is a cysteine (between residues 45 and 55) that is in the highly folded (Einck & Bustin, 1985), highly basic domain (residues 12–75) that also includes two histidines (residues 26 and 30) and a tryptophan (residue 56). It seems plausible that the sulfhydryl group of that cysteine has an unusually low pK, so that the sulfhydryl group as well as two histidines would be deprotonated near pH 7.0. It would not be surprising then if this whole domain was substantially disrupted in titrating HMG1 from pH 6.5 to 7.2, exposing the tryptophan to increased quenching by solvent, as observed. Moreover, such a picture would provide an easy explanation of our observation that disulfide formation seems to lock HMG1 into a conformation that is much less folded than the conformation in the reduced state (Kohlstaedt et al., 1986). The folding or unfolding of this domain could determine which mode of interaction would occur between HMG1 and H1 producing, respectively, either 4:1 complexes or 1:1 complexes. Our results do not address directly which domains of HMG1 and H1 interact. The oxidation of a sulfhydryl group in domain 12–17 could affect the interaction of HMG1 and H1 either directly or indirectly.

The interaction of HMG1 and H1 histone has been reported previously (Shooter et al., 1974; Smerdon & Isenberg, 1976; Yu & Spring, 1977; Cary et al., 1979), but the specificity of the interaction has been questioned (Cary et al., 1979). There was the possibility that HMG1 and H1 bound each other merely by nonspecific, electrostatic forces between the large number of positively charged lysine residues in H1 and the 40-residue, polycarboxylic amino acid region of HMG1 (Walker et al., 1978). The fact that oxidized and reduced HMG1 interacted differently with H1 makes the latter view untenable. If the interaction was purely electrostatic and nonspecific in nature, the simple creation of a disulfide bond would not have been sufficient to destroy the interaction at pH 7.5. The positive cooperativity displayed in the interaction at pH 6.0 which was destroyed by oxidation is also hard to reconcile with a nonspecific interaction.

Data presented previously on the ternary interaction between HMG1, H1, and DNA also support the view that the interaction between histone H1 and HMG1 is specific (Kohlstaedt et al., 1986). First, if the HMG1–H1 binding was nonspecific, it would be expected that HMG1 would tend to negate the effect of H1 on the circular dichroic spectrum of DNA; instead, HMG1 enhanced the effect of H1. Second, if the H1–HMG1 binding was nonspecific and electrostatic in nature, denaturation of the HMG1 would not be expected to eliminate the effect of HMG1, as was observed. We conclude, therefore, that the HMG1–H1 interaction is specific.

Four HMG1 molecules interact with each H1 molecule at pH 6.0, but the stoichiometry is 1:1 at pH 7.5. Other authors have apparently seen only a 1:1 complex between HMG1 and

histone H1 (Shooter et al., 1974; Smerdon & Isenberg, 1976; Cary et al., 1979). All these reports, however, have employed conditions of pH above pH 7.0. Smerdon and Isenberg performed their experiments at pH 7.6; our results indicate that HMG1 forms only a 1:1 complex with H1 at that pH. Shooter et al. and Cary et al. performed their experiments at pH 7.0. Although the three reports cited here all employed reducing agents, enough oxidation of HMG1 may have occurred to further hinder the detection of 4:1 complexes.

This is the first report of binding constants describing the interaction of HMG1 with H1. The binding constant of native, reduced HMG1 to H1 at pH 6.0 in 150 mM NaCl ($K = 3.1 \times 10^{-7}$ M^{-1}) is of a reasonable magnitude for an interaction between these two very abundant proteins. Since the HMG and H1 used in the present work were not fractionated into their individual sequence variants, the binding constants measured here are averages. Particular combinations of HMG1 variants and H1 variants may vary in their binding properties.

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