Panyim, S., and Chalkley, R. (1969b), *Biochemistry 8,* 3972.
Panyim, S., and Chalkley, R. (1971), *J. Biol. Chem. 246,* 7557.
Panyim, S., Jensen, R., and Chalkley, R. (1968), *Biochim. Biophys. Acta 160,* 252.
Rall, S. C., and Cole, R. D. (1971), *J. Biol. Chem. 246,* 7175.
Ruderman, J. V., Baglioni, C., and Gross, P. R. (1974), *Nature (London), 247,* 36.
Ruderman, J. V., and Gross, P. R. (1974), *Dev. Biol. 36,* 286.
Sahasrabuddhe, C. G., and Van Holde, K. E. (1974), *J. Biol. Chem. 249,* 152.
Seale, R. L., and Aronson, A. I. (1973), *J. Mol. Biol. 75,* 647.
Sherod, D., Johnson, G., and Chalkley, R. (1970), *Biochemistry 9,* 4611.
Sherod, D., Johnson, G., and Chalkley, R. (1974), *J. Biol. Chem. 249,* 3923.
Smerdon, M. J., and Isenberg, I. (1973), *Biochem. Biophys. Res. Commun. 55,* 1029.
Smerdon, M. J., and Isenberg, I. (1974), *Biochemistry 13,* 4046.
Smerdon, M. J., and Isenberg, I. (1976), *Biochemistry 15* (following paper in this issue).
Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30,* 1190.
Spiker, S. (1976), *Nature (London) 259,* 418.
Stout, J. T., and Phillips, R. L. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3043.
Teller, D. C., Kincade, J. M., and Cole, R. D. (1965), *Biochem. Biophys. Res. Commun. 20,* 739.
Thomas, J. O., and Kornberg, R. D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2626.
Truong, T., Bersohn, R., Brumer, P., Luk, C. K., and Tao, T. (1967), *J. Biol. Chem. 242,* 2979.
Van Holde, K. E., and Isenberg, I. (1975), *Acc. Chem. Res. 8,* 327.
Vladimirov, Yu. A., Dobretsov, T. A., Borshchevskaya, T. A. (1970), *Mol. Biol. 4,* 9.
Wetlaufer, D. B. (1962), *Adv. Protein Chem. 17,* 303.
Wickett, R. R., Li, H. J., and Isenberg, I. (1972), *Biochemistry 11,* 2952.
Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H. (1958), *Chem. Rev. 58,* 715.
Woodcock, C. L. F. (1973), *J. Cell Biol. 59,* 368a.
Yphantis, D. A. (1964), *Biochemistry 3,* 297.

# Interactions between the Subfractions of Calf Thymus H1 and Nonhistone Chromosomal Proteins HMG1 and HMG2†

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ABSTRACT: The nonhistone chromosomal proteins, HMG1 and HMG2, interact with the various subfractions of calf thymus H1 with a high degree of specificity. Subfractions 1b and 2 interact very strongly with HMG1 to form heterodimers. In contrast, subfractions 3a and 3b interact much more weakly. HMG2 interacts with 3a and 3b but shows no detectable complexing with 1a and 2.

The nonhistone chromosomal (NHC)[1] proteins HMG1 and HMG2 (Goodwin and Johns, 1973) constitute a relatively large fraction of the NHC pool. They are present in about $10^5$–$10^6$ copies/nucleus (Johns et al., 1975, Walker et al., 1975), but, so far, no functional role has been ascribed to them. Their amino acid compositions are unusual: about 25% of the residues are basic and 30% acidic (Goodwin and Johns, 1973). HMG1 is an acidic protein while HMG2 is basic (Baker et al., 1976), differing from each other probably in the degree of amidation of glutamic and aspartic acids. The proteins are folded over a wide pH range, approximately pH 4–10. In this range, about 40–50% of the residues are in an $\alpha$-helical conformation (Baker et al., 1976). The NHC fraction called P1 by Smith and Stocken (1973) appears likely to be HMG proteins.

Smith and Stocken (1973) stated that P1 interacted with histones H1. Shooter et al. (1974) studied the interaction of HMG1 with unfractionated H1 and concluded that the interaction did not result in specific complex formation.

In this paper, we report studies of the complexing of HMG1 and HMG2 with the subfractions of H1. We find that HMG1 and HMG2 interact with the various subfractions of H1 in a remarkably specific fashion. Some subfractions interact very strongly, some not at all, and some interact with intermediate binding strength.

Histone H1 is heterogeneous both with respect to amino acid sequence (Rall and Cole, 1971; Dixon, 1975; Dixon et al., 1975; Arutyunyan et al., 1975) and the degree of its phosphorylation (Langan et al., 1971). There is evidence that the various subfractions may have distinct roles in embryogenesis (Seale and Aronson, 1973; Ruderman and Gross, 1974; Ruderman et al., 1974) and undoubtedly other distinctions will be found in future studies. However, at present, we have no knowledge re-

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[1] Abbreviations used are: NHC, nonhistone chromosomal; CD, circular dichroism.

lating any functional property to either the structural properties of the subfractions or their locations within chromatin.

The following differences between the various subfractions are now known: (1) the amino acid sequences are not the same (Rall and Cole, 1971; Dixon, 1975; Dixon et al., 1975; Arutyunyan et al, 1975); these serve, in fact, as the operational definition of the subfractions; (2) the affinity to ion-exchange resins vary (Kincade and Cole, 1966; Bustin and Cole, 1968, 1969; Kincade, 1969; Gurley et al., 1975); (3) electrophoretic mobilities are different (Bustin and Cole, 1968, 1969; Kincade, 1969; Panyim et al., 1971; Fambrough and Bonner, 1969; Stout and Phillips, 1973; Spiker, 1976); (4) the antigenic determinants vary (Bustin and Stollar, 1972); (5) in vitro phosphorylation sites are different (Langan et al., 1971). To these differences we now add a striking specificity of binding to HMG1 and HMG2. In the discussion, we shall raise the possibility that at least some of the H1-HMG complexes may exist as such in chromatin.

## Materials and Methods

The four major subfractions of calf thymus H1 were prepared in the presence of phenylmethylsulfonyl fluoride as described in the preceding paper of this issue (Smerdon and Isenberg, 1976). Nonhistone chromosomal proteins HMG1 and HMG2 were kindly provided by Drs. G. H. Goodwin and E. W. Johns.

Protein concentrations were determined spectrophotometrically on a Cary 14 spectrophotometer. A molar extinction coefficient of 1345 $cm^{-1}$ $M^{-1}$ at 275 nm was used for each H1 subfraction (Smerdon and Isenberg, 1976), and $2.1 \times 10^4$ $cm^{-1}$ $M^{-1}$ and $2.0 \times 10^4$ $cm^{-1}$ $M^{-1}$ at 280 nm for HMG1 and HMG2, respectively (Baker et al., 1976).

Stock solutions ($4\text{-}6 \times 10^{-5}$ M) of reduced HMG1 and HMG2 were prepared as described in Baker et al. (1976) and stored either at 4 °C or at −20 °C in 0.01 N HCl-1 mM dithiothreitol. Aliquots of protein stock solutions were electrophoresed during, and at the end of, the time period in which they were used, and neither oxidation nor degradation was observed. In addition, no observable change in the tryptophan spectral properties occurred during the storage period, which, in any case, was never longer than 2 weeks for any given stock solution. Stock solutions ($\sim 10^{-4}$ M) of the H1 subfractions in $H_2O$ were made the same day used.

Samples were prepared by adding the required volumes of stock solutions to a test tube, and then adding the appropriate amount of $H_2O$ to obtain one-third the final volume needed. 0.03 M phosphate was then added to give the final volume, which contained 0.02 M phosphate at the desired pH.

Fluorescence anisotropy was measured on a computer interfaced polarization spectrometer (Ayres et al., 1974). All measurements were at 20 °C. Samples were excited at 295 nm and emission was measured at 340 nm. Under these conditions, essentially only tryptophan fluorescence is observed. The anisotropies expected for noninteracting mixtures were calculated using the Weber addition law (Weber, 1953; Dale and Eisinger, 1975).

All sedimentation experiments were performed at 20 °C, using a 30-mm path length cell, on a Beckman Model E ultracentrifuge equipped with interference, schlieren, and scanner optics. Equilibrium experiments were performed according to the high-speed method of Yphantis (1964) using interference optics. Velocity experiments on HMG1 and HMG1 complexes were carried out using scanner optics at 280 nm. Velocity experiments on H1 were carried out using schlieren optics. A value of 0.728 was used for the partial specific volume ($\bar{v}$) of both HMG1 and HMG2 (Shooter et al., 1974; Goodwin et al., 1975); the $\bar{v}$ values used for the H1 subfractions are those given in Smerdon and Isenberg (1976). A $\bar{v}$ of 0.746 was used for the complexes, and was calculated from the weight average of the partial specific volumes of the two monomers. Molecular weights of 26 500, 26 000, and 24 000 were used for HMG1, HMG2, and the H1 subfractions, respectively (Shooter et al., 1974; Goodwin et al., 1975; Smerdon and Isenberg, 1976). The data were analyzed with a computer program written by Dr. Robert Dyson.

Circular dichroism (CD) was measured on a Jasco Model J-10 CD recorder at 20 °C. Data is reported as $\Delta\epsilon$ in units of $cm^{-1}l.$ (mole of protein)$^{-1}$.

Electrophoresis of the NHC proteins was carried out using the methods described in Smerdon and Isenberg (1976). Both HMG1 and HMG2 showed minor bands in overloaded gels. We estimate that about 1-2% of the protein is contamination.

## Results

*Interactions of H1 with HMG1.* To measure the interactions between the non-H1 or inner histones,[2] our laboratory has, in the past, made use of the method of continuous variations (Job, 1928; Vosburgh and Cooper, 1941), where the concentrations of the individual histones were varied but the sum of the concentrations was constant (D'Anna and Isenberg, 1973, 1974a,b; Isenberg, 1976). In those studies, the fluorescence anisotropy, and intensity, of the tyrosine residues of the individual histones served as sensitive parameters characterizing the different molecular species present in a mixture of two inner histones. However, a continuous variation method is not convenient in studying the interactions of H1 with either HMG1 or HMG2. These latter proteins contain tryptophan (Baker et al., 1976) and tyrosine to tryptophan energy transfer is very favorable (Eisinger et al., 1969; Longworth, 1971; Weinryb and Steiner, 1971; Berlman, 1973). Therefore, it is better to use an excitation wavelength outside of the tyrosine absorbance, where only the tryptophans are excited. A continuous variation procedure is, therefore, not possible, since at high concentrations of H1 relative to HMG, the signal to noise will become poor. Instead, we have used the molar-ratio method of Yoe and Jones (1944), where the concentration of H1 is varied keeping the concentration of HMG1 and HMG2 constant. We also note that, in our studies, to avoid aggregation, we have chosen pH conditions that are far from the isoionic points of HMG1 and HMG2 (Baker et al., 1976).

Figure 1 shows the molar-ratio data for unfractionated H1 and HMG1 in 0.02 M phosphate, pH 7.6. As can be seen, there is a pronounced deviation in the data from the values expected for no interaction. The data follow two (approximately) straight lines with a nonintegral break point at about 1.3. (The Appendix shows that two straight lines are good approximations to theoretical expectations.) The nonintegral break point, 1.3, suggested to us that some of the subfractions were interacting differently from others. Therefore, we examined the binding of the four major H1 subfractions to HMG1.

As shown in Figure 2, subfractions 1b and 2 interact strongly with HMG1 and the stoichiometry of the complex is 1:1. In contrast, fractions 3a and 3b interact more weakly. For 3a and 3b, the titration data does not have a break point and does not become linear even at ratios of 5:1.

[2] In this paper, histones H2a, H2b, H3, and H4 will be called the *inner histones* (Isenberg, 1976), since they are inside of the nucleosomes.

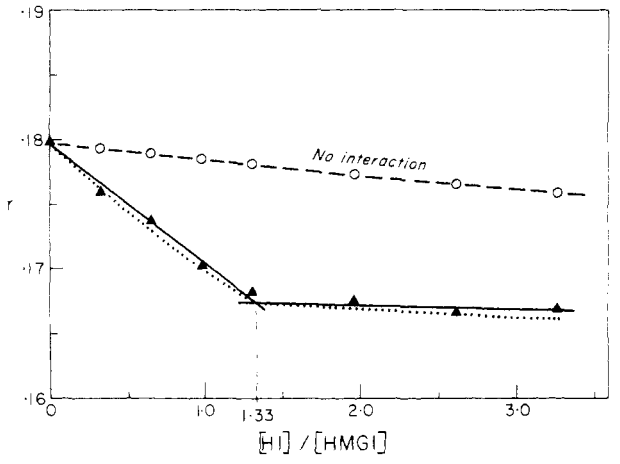


FIGURE 1: Molar-ratio curve for unfractionated H1 and $5.8 \times 10^{-6}$ M HMG1 in 0.02 M phosphate, pH 7.6. $r$ = Anisotropy. Figure shows linear fit to the data (—) and curves given by eq A6 and A10 (···). Note the good approximation of linear fits.

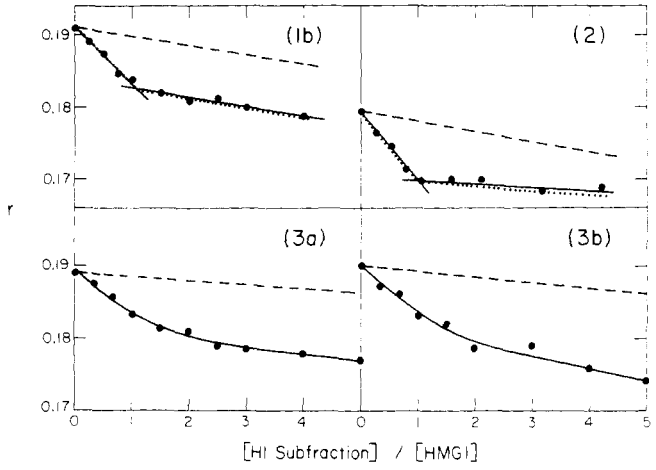


FIGURE 2: Molar-ratio curves for each of the four major H1 subfractions with $4.0 \times 10^{-6}$ M HMG1 in 0.02 M phosphate, pH 7.6. Solid and dotted lines for subfractions 1b and 2 are defined the same as those for Figure 1. The dashed lines represent curves expected for no interaction.

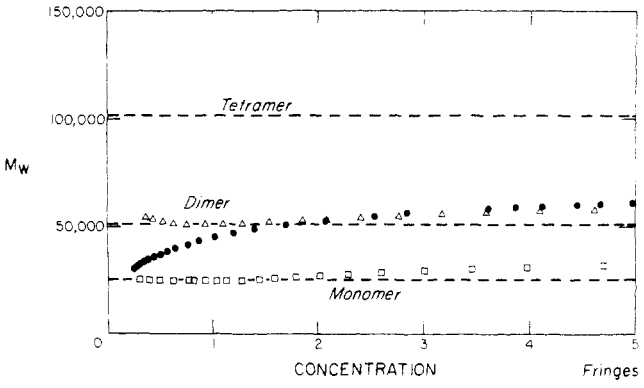


FIGURE 3: Weight-average molecular weight as a function of protein concentration for 1:1 mixtures of HMG1 and subfractions 1b (●), 2 (△), and 3b (□), in 0.02 M phosphate, pH 7.6. Initial concentrations of HMG1 and each of the subfractions was $4.0 \times 10^{-6}$ M. A rotor speed of 30 000 rpm was used for 1b and 2 and 34 000 rpm for 3b.

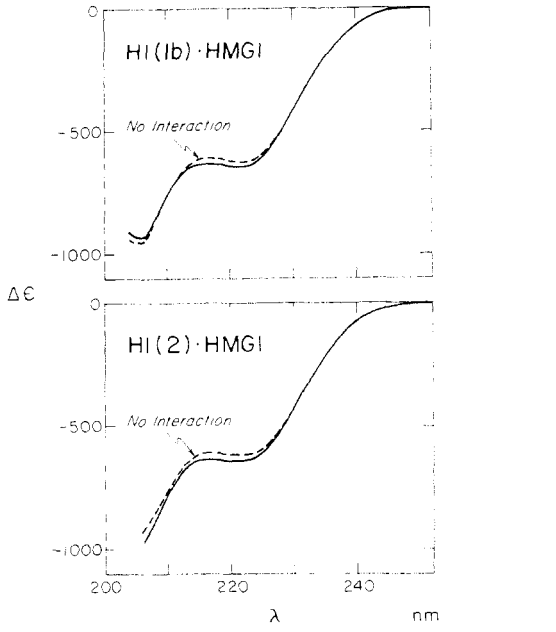


FIGURE 4: Circular dichroic spectra of 1:1 mixtures of HMG1 with subfractions 1b or 2 in 0.02 M phosphate, pH 7.6. In each case, the concentration of each component was $4.0 \times 10^{-6}$ M. A cell of 2-mm path length was used.

By integration of the elution profile of H1 from Bio-Rex 70 columns (Smerdon and Isenberg, 1976), we have estimated the amounts of protein in each peak to be: 38% in peak 1, 27% in peak 2, 33% in peak 3, and 2% in peak X. (See Figure 1 of Smerdon and Isenberg (1976) for an explanation of this nomenclature.) Peak 3 contains subfractions 3a and 3b. If these two subfractions did not interact at all with HMG1, and all of the others interacted very strongly, one would predict the break point of a titration using unfractionated H1 to be $(0.38 + 0.27 + 0.02)^{-1} = 1.49$. The measured value is 1.3. The deviation from 1.49 may be due, in part, to the experimental error in determining the fraction of H1 in each elution peak, but, in addition, it may be due, in part, to the complexing of a small amount of HMG1 by the weaker interaction of subfractions 3a and 3b.

Molecular weight determinations of 1:1 mixtures of each of the subfractions and HMG1 were made using high-speed sedimentation equilibrium (Yphantis, 1964). The ln $C$ vs. $\Delta r^2$ plots for 1:1 mixtures of subfraction 2 and HMG1 showed excellent linearity and gave a molecular weight of 53 800. The calculated molecular weight for a heterodimer of subfraction 2 and HMG1 is 50 500. Thus, our data shows that subfraction 2 complexes with HMG1 to form a heterodimer.

Figure 3 shows the values of $M_w$ vs. concentrations of protein for the 1:1 mixtures of subfractions 1b, 2, and 3b with HMG1. In agreement with the anisotropy data, these data show subfractions 1b and 2 interact strongly with HMG1 to form heterodimers, while subfraction 3b interacts more weakly, and shows only a small amount of higher molecular weight species present. Similar results (not shown) have verified the weak interaction in 1:1 mixtures of 3a and HMG1.

From Figure 3 we estimate that the association constant, for the H1(2)-HMG1 complex, is greater than $2 \times 10^6$ $M^{-1}$.

Figure 4 shows CD spectra for the two strong complexes. As can be seen, these data show that there is no appreciable change in CD upon complex formation. This is in marked contrast to what is found in complexes of the inner histones (D'Anna and Isenberg, 1973, 1974a, b). When the inner histones form strong complexes, the $\alpha$-helical content rises.

Table I shows the results of sedimentation velocity measurements on subfraction 2, HMG1, and the complex of these

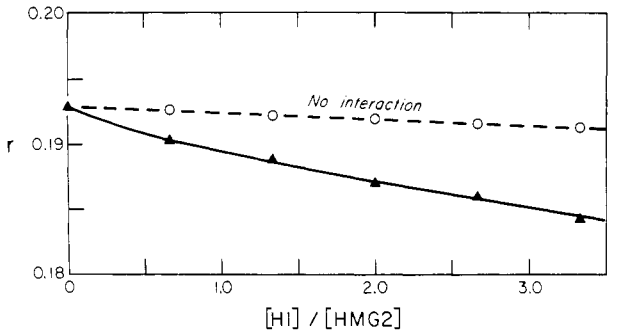


FIGURE 5: Molar-ratio curve for unfractionated H1 and $5.8 \times 10^{-6}$ M HMG2 in 0.02 M phosphate, pH 6.8.

TABLE I: Sedimentation Velocity Results.[a]

| Sample | $s_{20,w}$ | $f/f_0$ |
|---|---|---|
| H1(2) | 1.47 S | 1.72 |
| HMG1 | 2.35 S | 1.37 |
| H1(2) + HMG1 | 3.24 S | 1.41 |

[a] Protein concentrations were: $5.0 \times 10^{-5}$ M for H1(2), $4.0 \times 10^{-6}$ M for HMG1, and $4.0 \times 10^{-6}$ M for each component of the H1(2)-HMG1 complex. A rotor speed of 52 000 rpm was used for H1(2) and 48 000 rpm for HMG1 and the complex H1(2)-HMG1. Each protein solution contained 0.02 M phosphate, pH 7.6.

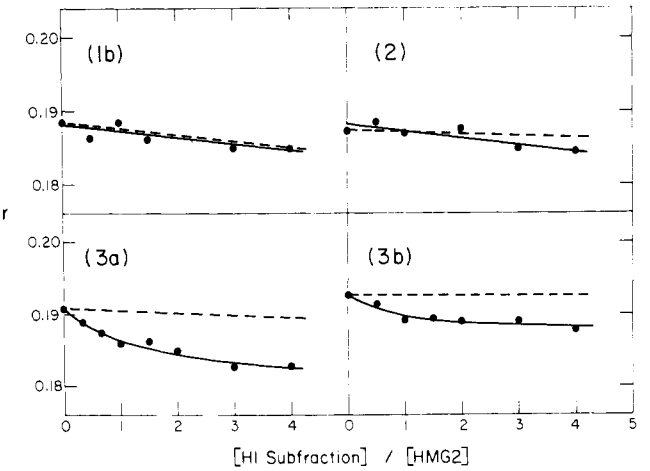


FIGURE 6: Molar-ratio curves for each of the four major subfractions with $4.0 \times 10^{-6}$ M HMG2 in 0.02 M phosphate, pH 6.6. For each subfraction, the dashed line represents the curve expected for no interaction.

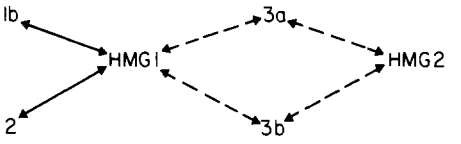


FIGURE 7: Schematic representation of the cross-complexing pattern for the four major subfractions of calf thymus H1 with nonhistone chromosomal proteins HMG1 and HMG2. Solid lines represent strong interactions and dashed lines represent weaker interactions. The pattern shows only the manner in which the proteins interact with each other and is not meant to suggest any particular complexing arrangement in chromatin.

two. The frictional ratio ($f/f_0$ = 1.37) for HMG1 is not very different from what is observed for typical globular proteins (Sober, 1968), while the $f/f_0$ value for H1(2), 1.72, shows that H1(2) is very asymmetrical. Upon complexing, $f/f_0$ drops to 1.41. This could mean that H1(2) changes its structure upon complexing, but it is also possible that H1 does not change its structure, but that the more globular HMG1 interacts along the side of the H1 molecule, thus giving the complex a more spherical shape.

*Interactions of H1 with HMG2.* HMG2 interacts with H1 in a fashion markedly different from HMG1. There is no sharp break point using unfractionated H1 (contrast Figure 1 with Figure 5). Furthermore, subfractions 1b and 2, measured individually, show no observable interaction with HMG2 whatsoever and, while subfractions 3a and 3b do interact, no break point is observed (Figure 6). Sedimentation data on 1:1 mixtures of HMG2 with either 3a or 3b show curves similar to those found with mixtures of HMG1 and 3a or 3b (Figure 3).

## Discussion

We have shown that the NHC proteins HMG1 and HMG2 interact with the H1 subfractions with a high degree of specificity. We believe that this is the first time that any differences in the binding properties of the H1 subfractions have been demonstrated, aside from the finding of Bustin and Stollar (1972) that the subfractions have distinct antigenic determinants. It is noteworthy that very large binding differences are seen for the various subfractions, even though no striking differences are observed in the physical properties of the subfractions themselves (Smerdon and Isenberg, 1976). The high degree of binding specificity that we have found rules out, of course, nonspecific charge–charge interactions as the cause of complexing. Ultimately, the reason for the specificity must be found in the different structural properties of the H1 subfractions. A schematic representation of the cross-complexing pattern is shown in Figure 7.

Our results, incidentally, explain the anomalous finding of Shooter et al. (1974). These authors obtained sedimentation data showing that there was an interaction between HMG1 and unfractionated H1, but concluded that no specific type of complex was produced. Our work shows that this conclusion was due to the use of unfractionated H1; with specific subfractions definite complexes are observed.

We now turn to the question of whether the H1-HMG complexes exist as such in chromatin. There is no evidence to settle this point at present, but we raise the possibility that H1-HMG complexes may already have been detected by cross-linking experiments. Figure 1 of the paper by Thomas and Kornberg (1975) shows a sodium dodecyl sulfate gel pattern of proteins, cross-linked in chromatin by dimethyl suberimidate. Among the bands was a weak one corresponding to a molecular weight of about 50 000–60 000. Thomas and Kornberg marked this band "$(F1)_2$?" and stated that it might be a cross-linked dimer of H1. When H1 was removed, this band was no longer present, so it appeared that the band did contain H1.

Thomas and Kornberg found a similar band when proteins were first extracted from chromatin, and then cross-linked in solution. We note, however, that there is now convincing evidence that H1 does not dimerize (Teller et al., 1965; Haydon and Peacocke, 1968; Edwards and Shooter, 1969; Kornberg and Thomas, 1974; Smerdon and Isenberg, 1976), whereas we have now shown that certain subfractions of H1 and HMG1 do form complexes. The band observed by Thomas and Kornberg was a minor one compared to the amount seen at the H1 monomer position; we note that the ratio of the HMG proteins to H1 is about 0.03:1 to 0.06:1 (Johns et al., 1975;

Walker et al., 1975; Smerdon and Isenberg, 1976). All of these considerations make it more reasonable that the sodium dodecyl sulfate gel band observed by Thomas and Kornberg is an H1–HMG heterodimer rather than an H1 dimer. However, at the present time, there is no direct evidence supporting this speculation, and its validity must await future work.

Acknowledgments

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## Appendix

Consider the complex formation

$$mA + nB \rightleftarrows A_mB_n$$

with an equilibrium constant

$$K = \frac{[A_mB_n]}{[A]^m[B]^n} \tag{A1}$$

Let $A_0$ and $B_0$ be the total amount of A and B. Let $X = B_0/A_0$. Let $r_A$, $r_B$, and $r_C$ be the respective anisotropies of A, B, and $A_mB_n$, and $I_A$, $I_B$, $I_C$ be the respective intensities.

We consider a titration in which $A_0$ is held constant and $B_0$ is varied. By the Weber addition law (Weber, 1953; Dale and Eisinger, 1975),

$$r = \frac{I_Ar_A + I_Br_B + I_Cr_C}{I_A + I_B + I_C} \tag{A2}$$

We also have, for dilute solutions,

$$I_A = k'_A[A]$$

$$I_B = k'_B[B]$$

$$I_C = k'_C[A_mB_n]$$

where $k'_A$, $k'_B$, and $k'_C$ are constants.

We first consider the titration region $0 \leq X \leq n/m$. For very strong binding, $I_Br_B \ll r_AI_A + r_CI_C$, $I_B \ll I_A + I_C$, and $B_0 \approx n[A_mB_n]$. Therefore,

$$I_C \approx \frac{k'_C}{n}B_0$$

$$= k_CX \tag{A3}$$

$$I_A = k'_A A_0 \frac{m}{n}\left(\frac{n}{m} - \frac{B_0}{A_0}\right)$$

$$= k_A\left(\frac{n}{m} - X\right) \tag{A4}$$

and

$$r = \frac{I_Ar_A + I_Cr_C}{I_A + I_C} \tag{A5}$$

From these,

$$r = \frac{\frac{k_A}{k_C}r_A\left(\frac{n}{mX} - 1\right) + r_C}{\frac{k_A}{k_C}\left(\frac{n}{mX} - 1\right) + 1} \tag{A6}$$

which may also be written

$$\frac{1}{r_A - r} = \frac{\xi_1}{X} + \phi_1 \tag{A7}$$

where

$$\xi_1 = \frac{nk_A}{mk_C}\left(\frac{1}{r_A - r_C}\right) \tag{A8}$$

and

$$\phi_1 = \frac{1 - k_A/k_C}{r_A - r_C} \tag{A9}$$

Knowing $n/m$, data fitted by eq A7 will permit a determination of $\xi_1$ and hence $k_A/k_C$.

In a similar manner, for the titration region, $X > n/m$, we obtain the functional forms

$$r = \frac{\frac{k_B}{k_C}r_B(X - n/m) + \frac{n}{m}r_C}{\frac{k_B}{k_C}\left(X - \frac{n}{m}\right) + \frac{n}{m}} \tag{A10}$$

and

$$\frac{1}{r - r_B} = \xi_2\left(X - \frac{n}{m}\right) + \phi_2 \tag{A11}$$

where

$$\xi_2 = \frac{mk_B}{nk_C(r_C - r_B)} \tag{A12}$$

and

$$\phi_2 = \frac{1}{r_C - r_B} \tag{A13}$$

Figures 1 and 2 show that, for strong binding, eq A6 and A10 can be approximated well by straight lines.

## References

Arutyunyan, A. A., Shlyapnikov, S. V., and Severin, E. S. (1975), *Bioorg. Khim. 1,* 1188.

Ayres, W. A., Small, E. W., and Isenberg, I. (1974), *Anal. Biochem. 58,* 361.

Baker, C. C., Isenberg, I., Goodwin, G. H., and Johns, E. W. (1976), *Biochemistry 15,* 629.

Berlman, I. B. (1973), in Energy Transfer Parameters of Aromatic Compounds, New York, N.Y., Academic Press, p 63-65.

Bustin, M., and Cole, R. D. (1968), *J. Biol. Chem. 243,* 4500.

Bustin, M., and Cole, R. D. (1969), *J. Biol. Chem. 244,* 5286.

Bustin, M., and Stollar, B. D. (1972), *J. Biol. Chem. 247,* 5716.

Dale, R., and Eisinger, J. (1975), in Biochemical Fluorescence: Concepts, Vol. 1, Chen, R. F., and Edelhoch, H., Ed., Marcel Dekker, New York, N.Y., p 256.

D'Anna, J. A., and Isenberg, I. (1973), *Biochemistry 12,* 1035.

D'Anna, J. A., and Isenberg, I. (1974a), *Biochemistry 13,* 2098.

D'Anna, J. A., and Isenberg, I. (1974b), *Biochemistry 13,* 4992.

Dixon, G. H. (1975), Cited in Elgin, S. C. R., and Weintraub, H. (1975), *Annu. Rev. Biochem. 44,* 731.

Dixon, G. H., Candido, E. P. M., Honda, B. M., Louie, A. J., Macleod, A. R., Sung, M. T. (1975), *Struct. Funct. Chromatin, Symp., 1974,* 238.

Edwards, P. A., and Shooter, K. V. (1969), *Biochem. J. 114,* 227.

Eisinger, J., Feuer, B., and Lamola, A. A. (1969), *Biochemistry 8,* 3908.
Fambrough, D. M., and Bonner, J. (1969), *Biochim. Biophys. Acta 175,* 113.
Goodwin, G. H., and Johns, E. W. (1973), *Eur. J. Biochem. 40,* 215.
Goodwin, G. H., Shooter, K. V., and Johns, E. W. (1975), *Eur. J. Biochem. 54,* 427.
Gurley, L. R., Walters, R. A., and Tobey, R. A. (1975), *J. Biol. Chem. 250,* 3936.
Haydon, A. J., and Peacocke, A. R. (1968), *Biochem. J. 110,* 243.
Isenberg, I. (1976), in Search and Discovery, a volume dedicated to Albert Szent-Gyorgyi, B. Kaminer, Ed., New York, N.Y., Academic Press.
Job, P. (1928), *Ann. Chim. (Paris) 9,* 113.
Johns, E. W., Goodwin, G. H., Walker, J. M., and Sanders, C. (1975), *Struct. Funct. Chromatin, Symp., 1974* 95–112.
Kincade, J. M. (1969), *J. Biol. Chem. 244,* 3375.
Kincade, J. M., and Cole, R. D. (1966), *J. Biol. Chem. 241,* 5790.
Kornberg, R. D., and Thomas, J. O. (1974), *Science 184,* 865.
Langan, T. A., Rall, S. C., and Cole, R. D. (1971), *J. Biol. Chem. 246,* 1942.
Longworth, J. W. (1971), *Excited States Proteins Nucleic Acids* 420–422.
Panyim, S., Bilek, D., and Chalkley, R. (1971), *J. Biol. Chem. 246,* 4204.
Rall, S. C., and Cole, R. D. (1971), *J. Biol. Chem. 246,* 7175.
Ruderman, J. V., Baglioni, C., and Gross, P. R. (1974), *Nature (London) 247,* 36.
Ruderman, J. V., and Gross, P. R. (1974), *Dev. Biol. 36,* 286.
Seale, R. L., and Aronson, A. I. (1973), *J. Mol. Biol. 75,* 647.
Shooter, K. V., Goodwin, G. H., and Johns, E. W. (1974), *Eur. J. Biochem. 47,* 263.
Smerdon, M. J., and Isenberg, I. (1976), *Biochemistry 15,* (preceding paper in this issue).
Smith, J. A., and Stocken, L. A. (1973), *Biochem. J. 131,* 859.
Sober, H. A., Ed. (1968), in The Handbook of Biochemistry (Selected Data for Molecular Biology), Cleveland, Ohio, Chemical Rubber Publishing Co., p C10–C35.
Spiker, S. (1976), *Nature (London) 259,* 418.
Stout, J. T., and Phillips, R. L. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 3043.
Teller, D. C., Kincade, J. M., and Cole, R. D., (1965), *Biochem. Biophys. Res. Commun. 20,* 739.
Thomas, J. O., and Kornberg, R. D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2626.
Vosburgh, W. C., and Cooper, G. R. (1941), *J. Am. Chem. Soc. 63,* 427.
Walker, J. M., Goodwin, G. H., and Johns, E. W. (1975), submitted for publication).
Weber, G. (1953), *Adv. Protein Chem. 8,* 415.
Weinryb, I., and Steiner, R. F. (1971), *Excited States Proteins Nucleic Acids,* 308–311.
Yoe, J. H., and Jones, A. L. (1944), *Ind. Eng. Chem., Anal. Ed. 16,* 111.
Yphantis, D. A. (1964), *Biochemistry 3,* 297.