The Binding of Deoxyribonucleic Acid and Histone in Native Nucleohistone*

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SUMMARY

The dissociation of histones from DNA by salt has been reinvestigated. Previous methods were examined and found to be unsatisfactory for two reasons. (a) An endogenous proteolytic enzyme is highly active during the separation procedures and the resultant histones are severely degraded. (b) Dissociated histones aggregate at elevated salt concentrations and histones can subsequently cosediment with DNA, thus decreasing the apparent level of dissociation. We describe conditions in solutions of sodium phosphate-urea at pH 5.5 which not only alleviate the above problems, but also permit a highly selective extraction of either histone F1 or of histones F1, F2b, and F2a2 from the other histone fractions. Evidence is presented arguing that histones interact with DNA through two kinds of binding. On the one hand there is a substantial involvement of electrostatic ionic bonds and on the other there is a variable contribution from an interaction which has many of the features of the hydrophobic bond. This latter interaction is characterized by (a) a decrease in strength in solutions of urea, (b) a decrease in strength in hydrophobic solvents such as in the presence of 1-propanol or propylene, and (c) an increase in binding at higher temperatures. The contribution of this additional interaction is most marked for the arginine-rich histones F3 and F2a1 and least so for the lysine-rich histone F1. We have also observed that F3 and F2a1 bind denatured DNA so strongly that they cannot be dissociated at all in high salt or at low pH unless high urea concentrations are also present.

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The nature of the forces involved in the binding of histone to DNA have not been extensively investigated. Most of the work that has been done is concentrated on the involvement of electrostatic interactions which undoubtedly play an important role (1–9). These studies have involved an analysis of the dissociation of histones from calf thymus nucleohistone as a function of either increasing sodium chloride (1–5) or acid concentration (6–9). In general the order of dissociation follows increasing arginine to lysine ratios, i.e. the lysine-rich histones (F1) are removed first, followed by the moderately lysine-rich histones (F2a2 and F2b), and finally the arginine-rich histones (F3 and F2a1) are removed at the highest ionic strengths or the lowest pH. These observations are somewhat surprising since the lysine-rich histone has a higher ratio of basic to acidic residues than arginine-rich histones (4.6 compared to 1.6) and thus on electrostatic considerations one might have expected the lysine-rich histone to bind DNA more tightly than the arginine-rich histones.

Nuclear magnetic resonance studies of isolated arginine-rich histones suggest that if the ionic strength is increased apolar amino acids become involved in a helical segments of histone secondary structure (10, 11). Bradbury et al. (10) have suggested that in addition to electrostatic interactions, the arginine-rich histones might bind DNA through hydrophobic interactions between apolar residues in the α helical segments of histones and the inner regions of the DNA double helix. Certainly one would expect that upon treatment in sodium chloride solutions more concentrated than 0.6 M, essentially all of the electrostatic interactions would be abolished, and yet the bulk of the histones remain associated with the DNA. In the absence of electrostatic interactions one might predict that another form of interaction is playing an important role in the binding of the histones to DNA.

Most workers in this field have previously exploited differential extraction in sodium chloride solutions of different concentration with the procedure initially devised by Ohlenbusch et al. (4). However, using polyacrylamide gel electrophoresis it soon became clear that this approach not only is highly unsuitable in the histone fractions which are dissociated, but also that the histones are severely degraded during the dissociation procedure, thus rendering the results exceedingly difficult to interpret. We have searched for solvent conditions which would not only selectively remove specific histone fractions, but which would also avoid problems of proteolysis of histone during the course of the extraction experiments.

We will show that a highly selective differential extraction of histone from DNA is obtained in solutions of sodium phosphate in urea. The histones so obtained are intact even after the prolonged centrifugation involved in their isolation. By adjusting urea concentrations, solvent polarity, and temperature, it is possible to modify differentially the strength of the binding of various histones to DNA in such a way as to suggest that there is a substantial contribution of a force which has
many of the features characteristic of hydrophobic interactions. This additional interaction is most evident in the arginine-rich histones and least so for the lysine-rich histones.

MATERIALS AND METHODS

Preparation of Calf Thymus Nucleohistone—Calf thymus chromatin was prepared by methods previously described (12). Chromatin (A260 ~ 20) was sheared in a VirTis homogenizer at 50 volts for 1 min. The resulting nucleohistone was clarified by centrifugation (12,000 x g for 10 min at 2°C). The soluble nucleohistone supernatant was collected and stored at 2°C at low ionic strength.

Extraction of Nucleohistone with Extraction Solvents at pH 5.5—Histones were extracted from nucleohistone by either repeated dialysis of the nucleohistone against a large excess of the appropriate extraction solvent or by direct dilution of the nucleohistone with a more concentrated solvent. The nucleohistone was overlaid onto two discontinuous sucrose layers. The bottom layer consisted of 2 ml of 15% sucrose and the top layer of 1 ml of 7.5% sucrose. Both sucrose layers were at the same salt concentration as the extraction solvent. The 7.5 and 15% sucrose layers were incorporated into the centrifugation procedure in an attempt to hinder aggregated histones from sedimenting to the bottom of the tube and becoming enmeshed in the residual nucleohistone pellet, however this procedure was only partially successful.

The salt-treated nucleohistone was centrifuged (100,000 x g for 21 hours at 2°C) in a Beckman L2-65 B preparative centrifuge. After centrifugation the sodium chloride layer was removed from the centrifuge tube to the level of the 15% sucrose-sodium chloride. The tube was then inverted and the 15% sucrose-sodium chloride layer removed leaving the residual nucleohistone pellet at the bottom of the centrifuge tube. Both the sodium chloride supernatant and the 15% sucrose-sodium chloride layer were dialyzed against a large excess of 0.9 N acetic acid for 12 hours (4°C) and concentrated. The solutions were made to 15% sucrose for electrophoresis.

The residual nucleohistone was suspended by homogenization into sodium chloride-0.01 M Tris-HCl, pH 7.5, at the same sodium chloride concentration from which it had been sedimented. The residual nucleohistone was extracted with sulfuric acid (0.4 N H2SO4 final concentration). The sulfuric acid extracts were combined and dialyzed against an excess of 0.9 N acetic acid, concentrated, and made to 15% sucrose for electrophoresis.

Extraction of Histones from Heat-denatured Nucleohistone—Calf thymus nucleohistone was denatured by heating nucleohistone (A260 ~ 20) for 30 min at 100°C. After cooling, the heat-denatured nucleohistone was extracted into an equal volume of sodium chloride-0.01 M Tris-HCl, pH 5.5, or sodium phosphate-urea, pH 5.5, of twice the required molarity. An analysis of dissociation was performed as described above. A melting profile of the heat-denatured nucleohistone indicated that nucleohistone was completely denatured by the heat treatment.

Sedimentation of Free Histone in Dissociating Solvents—Pure calf thymus histone was dissolved in water and dialyzed against, or diluted with appropriate concentrations of extraction solvent, and then sedimented under conditions identical with those used in the extraction of nucleohistone. Analysis for histone in the supernatant or pellet followed methods described above.

Electrophoresis of Histones—Polyacrylamide gel electrophoresis followed the procedure previously reported (13).

Quantitation of Histones after Electrophoresis—Histone bands were quantitated by scanning the polyacrylamide gels on a Gilford 2000 gel scanner and subsequently analyzing the curves on a Dupont curve resolver.

Fig. 1. Proteolysis during extraction of nucleohistone with sodium chloride at pH 7.5. Nucleohistone was dialyzed against sodium chloride and then centrifuged as described under "Materials and Methods." Histones were dissociated at the following concentrations of NaCl: (a) 0.6, (c) 0.8, (e) 1.0, (g) 1.2, and (i) 1.4 M. The histones extracted with 0.4 N sulfuric acid from the pellet of residual nucleohistone at the following concentrations of NaCl are: (b) 0.6, (d) 0.8, (f) 1.0, (h) 1.2, and (j) 1.4 M. A control of calf thymus histones is included (k).
Binding of Phosphate Ion to Histones by Equilibrium Dialysis—One-milliliter aliquots of histone solutions of known concentration (previously dialyzed against 0.01 M Tris-HCl, pH 5.5) were placed into a dialysis tube. The tubes were each placed into 10 ml of the appropriate sodium phosphate solution containing $^{32}$P$_1$ of known specific activity. The test tube was sealed with Parafilm and gently shaken at room temperature for 30 hours. The time for the system to come to equilibrium was established by previous control experiments. The dialysis tubes were then removed and wiped clean. The tube was punctured and the histone solution collected. An aliquot (0.1 ml) was placed into 10 ml of Bray’s solution and counted in a liquid scintillation counter (Nuclear-Chicago Unilux III). The counting efficiency for $[^{32}$P$]phosphate was 93%.

RESULTS

Sodium Chloride Extraction of Nucleohistone at pH 7.5—Fig. 1 shows the polyacrylamide gel pattern of histones dissociated from nucleohistone as a function of NaCl concentration with the method of Ohlenbusch et al. (4). The histones remaining associated with the residual nucleohistone were extracted with 0.4 N sulfuric acid and electrophoretically analysed to give the gel patterns also shown in Fig. 1.

The histones dissociated at each concentration of sodium chloride are extensively degraded. The histones of the residual nucleohistone have undergone less proteolysis than have the dissociated histones. This is most readily apparent when F1, the histone fraction which has the greatest susceptibility to proteolytic degradation (14), has been fully dissociated (Fig. 1e), clearing the degraded bands of F1 from the pattern of the histones extracted from the residual nucleohistone (Fig 1f).

Since the proteolysis of histones is so extensive during the dissociation of the histones from nucleohistone by the method of Ohlenbusch et al. (4), quantitation of the histones extracted at each sodium chloride concentration was not attempted. Furthermore aggregation and sedimentation of free histones at these ionic strengths and at this pH is a serious problem (see below).

Sodium Chloride Extraction of Nucleohistone at pH 5.5—The dissociation of histones from nucleohistone was therefore studied in sodium chloride solutions at pH 5.5, a pH at which the protease has no detectable activity in 0.01 M Tris-HCl. Nucleohistone was extracted either by dialysis against a sodium chloride solution or by dilution with an equal volume of a sodium chloride solution of concentration twice that required. Fig. 2 shows graphically the amount of each of the five histone fractions extracted with either method. Histone F1 is almost entirely dissociated in 0.5 M NaCl. Histones F2b and F2a2 are dissociated coincidentally beginning in 0.8 M NaCl and are almost completely dissociated in 1.4 M NaCl. Histones F3 and F2a1 are also dissociated coincidentally beginning in 1.4 M NaCl and they are completely dissociated in 2.0 M NaCl. Thus the salt ranges in which histones are extracted are in several ways different from those reported by Ohlenbusch et al. (4) for the more degraded histone samples.

The extent of histone dissociation by the direct addition of sodium chloride-0.02 M Tris-HCl, pH 5.5, to nucleohistone is slightly different from that obtained in the dialysis procedure. That the dilution method dissociates slightly more histone than does the dialysis method at a given sodium chloride concentration is most clearly seen in the dissociation of F2b and F2a2 (the comparison is shown in the dialysis portion of Fig. 2).

Over-all there are few differences between sodium chloride extraction at pH 5.5 and 7.5 which cannot be ascribed to the difference in the extent of proteolysis in the two systems.

Sedimentation of Free Histones in Sodium Chloride, pH 5.5—Aggregation of dissociated histones represents a major interpretive problem in the analysis of the extraction of nucleohistone with sodium chloride-0.01 M Tris-HCl, pH 5.5. Fig. 3a shows that the arginine-rich histones (F3 and F2a1) are most likely, and the lysine-rich histone (F1) least likely to sediment during centrifugation typical of this study. In the range 0.8 to 1.2 M NaCl we estimate that greater than 50% of F3 and F2a1 aggregate and sediment from solution together with approximately 30% of F2b. The extent of sedimentation of free histones is less at the higher concentrations of sodium chloride. This is presumably a reflection of the increased density of the more concentrated sodium chloride solutions since it is well known that the aggregation of the arginine-rich histones is not decreased at higher ionic strengths (15, 16). Thus the data obtained by direct salt extraction become impossible to interpret, and one must question the value of utilizing the residual nucleohistone obtained in this way for studies of nucleohistone structure.
Fig. 3. a. Sedimentation of free histone in sodium chloride-0.1 M Tris-HCl, pH 5.5. The sedimentation of free histones is shown in 0.6, 0.8, 1.0, 1.2, and 1.6 M NaCl. The left-hand gel of each pair of gels shows the histones which remained in the supernatant and the right-hand gel of each pair shows these which sedimented.

b. The sedimentation of free histone in sodium phosphate-urea, pH 5.5. The left-hand gel of each pair of gels shows the histones which remained in the supernatant and the right-hand gel the histones which sedimented.
5.6—We elected to study salt dissociation in the presence of urea to try to remove both the problems of aggregation and proteolysis in a single system. Initially we employed sodium chloride-urea solutions at pH 5.5. In this environment aggregation is much reduced, but proteolysis of F3 remained a problem. However since proteolysis is most effectively inhibited in sodium phosphate, pH 5.5, nucleohistone or free histone was dialyzed against sodium phosphate-urea solutions of the required concentration at pH 5.5 and the centrifugation and analysis performed as described above.

The problem of aggregation and subsequent sedimentation is reduced in sodium phosphate-urea solutions. The results of centrifugation of free histones in a series of salt-urea concentrations are seen in Fig. 3b. Sedimentation of free histones is absent in all but one or two concentrations of sodium phosphate-urea, pH 5.5, occurring only seriously in 1.0 M phosphate-1.0 M urea. In the absence of urea, sodium phosphate solutions cause extensive histone aggregation and only the lysine-rich histone (F1) is unaffected.

Urea Enhances Dissociation of Histones—The effect of increasing urea concentrations (at a constant concentration of sodium phosphate) on the dissociation of histones from nucleohistone is seen in graphic form in Fig. 4. Photographs of gels on which this figure is based are not presented but many of them are in fact shown as part of Fig. 6 (see below).

The five histone fractions can be divided into three groups on the basis of their dissociation behavior in sodium phosphate-urea. The lysine-rich histone (F1) is relatively unaffected by increasing urea concentrations. Histones F2b and F2a2 show a dramatic shift in the ease with which they are dissociated from DNA at higher urea concentrations. Thus in sodium chloride alone or in sodium phosphate-1.0 M urea, relatively little of these fractions is dissociated from DNA at an ionic strength of 0.4, yet in 3.0 M urea-sodium phosphate solutions they are fully dissociated at the same ionic strength, an environment in which F1 is not totally dissociated. The arginine-rich histones (F3 and F2a1) also show a much increased ease of dissociation as the urea concentration is increased. The observation that up to 90% of these fractions can be released in 0.6 M sodium phosphate-6.0 M urea provides an impressive comparison with the requirement that in 2.0 M urea a concentration of 1.0 M sodium phosphate is insufficient to dissociate any of these fractions.

Since we have shown that in the range of salt concentration we have used, histone aggregation is abolished at urea concentrations greater than 1.0 M, we conclude that urea effects histone dissociation by a mechanism other than a differential effect upon aggregation and subsequent sedimentation.

The data of Fig. 4 also serve to document that a highly selective removal of histone is now possible. Thus F1 can be totally removed in 0.5 M sodium phosphate-1.0 M urea; and F1, F2b, and F2a2 can be selectively and completely removed in 0.8 M sodium phosphate-2.0 M urea.

Study on Possible Hydrophobic Interaction between DNA and Histones—The results described above support the idea that the binding of DNA and histones involves interactions other than simply electrostatic bonds. We investigated the possibility that apolar solvents might enhance the dissociation of histones if used with sodium chloride. Extraction of nucleohistone with sodium chloride-1-propanol at pH 5.5 does dissociate some histone fractions. However, an interpretation of the extent of the dissociation is difficult because of the aggregation (and subsequent sedimentation during centrifugation) of some histone fractions. However, an interpretation of the extent of the dissociation is difficult because of the aggregation (and subsequent sedimentation during centrifugation) of some histone fractions.

The addition of urea to sodium chloride-1-propanol does not solve the problem of aggregation and sedimentation of free histones. However extraction of nucleohistone with two specific sodium chloride-1-propanol-urea concentrations was found to dissociate more histones than do corresponding concentrations of sodium chloride-urea or sodium chloride-1-propanol. These
specific data are compared in Fig. 5a. Increasing either the 1-propanol or urea concentration to 3.0 M decreases the amount of histones dissociated. This is probably a reflection of aggregation and subsequent sedimentation of free histones in these solvents.

A similar solvent system which does not facilitate the aggregation of histone is propylurea which has been shown to be capable of disrupting hydrophobic bonds in sperm whale myoglobin, horse heart cytochrome c, and α-chymotrypsinogen (17). Propylurea is more effective in the dissociation of histones than an equivalent concentration of urea as seen in Fig. 5b which shows a comparison of the histones extracted from nucleohistone in sodium phosphate-propylurea, pH 5.5, with those extracted with phosphate-urea, pH 5.5. The extent of dissociation of F2b and F2a2 in 0.4 M phosphate-1.25 M propylurea is intermediate between that in 0.4 M phosphate-1.25 M urea and 0.4 M phosphate-3.0 M urea. Increasing the concentration of sodium phosphate enhances the dissociation of F2b and F2a2 in 1.25 M propylurea (higher concentrations of propylurea cannot be used due to the limited solubility of propylurea in sodium phosphate solutions), 0.5 M phosphate-1.25 M propylurea dissociates more F2b and F2a2 than does 0.6 M phosphate-1.25 M urea and approaches that dissociated with 0.6 M phosphate-3.0 M urea. Histones F3 and F2a1 were not dissociated at the concentrations of sodium phosphate-propylurea employed. The failure to dissociate F3 and F2a1 in this system was not a result of aggregation and sedimentation in sodium phosphate-propylurea as a control sedimentation of free histone indicated that all histones are soluble in both 0.4 M phosphate-1.25 M propylurea and 0.5 M phosphate-1.25 M propylurea.

Effect of Temperature on Dissociation of Histones from Nucleohistones—The strength of hydrophobic interactions has been reported to increase with increasing temperature, at least up to 60° (18). Accordingly, we studied the extraction of nucleohistone at temperatures higher than 2° (the temperature of dissociation used in all procedures previously described). Sodium phosphate-urea, pH 5.5, was selected as the solvent of choice because of the absence of proteolysis during the dissociation at 2°. This solvent system was also attractive because of the selective manner in which the histone fractions are removed from DNA at 2°. Nucleohistone was diluted with appropriate concentrations of sodium phosphate-urea at 20° in a fashion strictly analogous to the dissociation performed at 2°.

Two main points emerge from dissociation studies at different temperatures. (a) There is a marked shift in the affinity of histones for DNA in native nucleohistone as a function of temperature. (b) The affinity of the lysine-rich histone (F1) is less at the higher temperature whereas the affinity of the other fractions (most clearly seen for F2b and F2a2) for DNA is dramatically increased at 20°. This is documented over a range of urea and phosphate concentrations both visually (Fig. 6) and graphically (Fig. 7). Apparently the forces binding the lysine-rich histone differ from those binding the other fractions to DNA. This observation is in agreement with the earlier observations where we have seen the binding of the F1 histone to DNA is very little modified as a function of increasing urea concentration, whereas all the other fractions have shown a dramatic decrease in binding strength as the urea concentration increased. It seems likely therefore that the lysine-rich histone binds DNA primarily through electrostatic bonds and the other histones bind through a contribution of electrostatic bonds and an additional interaction which is sensitive to both urea and increased solvent hydrophobicity and is stabilized at elevated temperature, a description consistent with that of a hydrophobic bond.

Dissociation of Histone from Heat-denatured Nucleohistone—Nucleohistone was denatured by heating (100° for 30 min) and then cooled in ice. The heat-denatured nucleohistone was extracted either in elevated concentrations of sodium chloride at pH 5.5 or sodium phosphate-urea, pH 5.5.

A comparison of extraction results for native nucleohistone and denatured nucleohistone is shown in Fig. 8. Several points emerge. (a) Histone F1 behaves the same in both systems. (b) The other histones are more easily dissociated from native nucleohistone, i.e. the binding of the histones to denatured DNA is greater than to native DNA. (c) Histones F2a1 and F3 cannot be isolated from the denatured system as they are present neither in the supernatant, nor can they be extracted with sulfuric acid (0.4 M) from the pellet. Since a portion of histones F2b and F2a2 are still bound to the denatured nucleohistone complex in 1.6 M sodium chloride, a concentration in
some of the histones would still bind to DNA. If this were so, those histones which remain bound to DNA even in the presence of 0.4 N sulfuric acid might be released by the addition of urea. That this was indeed the case was shown by extracting the acid-insoluble pellet with 8 M urea-0.4 N H$_2$SO$_4$ and thus facilitating the extraction of F3 and F2a1.

**Binding of Histones and Phosphate Anion**—The sequence in which histones are dissociated from nucleohistone by either increasing the ionic solvent concentration (1-5) or by lowering the pH (6-9) can be correlated with the molar lysine to arginine ratio of each histone fraction. The greater the molar ratio of lysine to arginine, the lesser is the concentration of ionic solvent or of acid necessary to extract that particular histone fraction, i.e. the ease of dissociation and the molar ratio of lysine to arginine is Fl ($\text{Lys:Arg} = 13.0$) > F2b + F2a2 ($\text{Lys:Arg} = 1.4$) > F3 + F2a1 ($\text{Lys:Arg} = 0.8$) (19).

The results of model-building experiments indicated that there could be an interaction between DNA and histone such as hydrogen bonding between the guanidino group of arginine and the phosphate groups of DNA and that this might account for the order of histone dissociation. Since an analysis of hydrogen bonding is difficult in a complex system such as nucleohistone we wondered if the binding of phosphate anions to histones might reflect the sequence in which the histone fractions are dissociated from nucleohistone and thus provide support for a strong arginine-phosphate interaction. Since purified histone fractions are readily available, we have studied the as-
association of phosphate anions with different histone fractions with the technique of equilibrium dialysis. A rather unexpected result was obtained as shown in Fig. 9. Histone F1 binds much more phosphate per mole than any other histone. The binding curve for F1 does not show signs of reaching saturation although in the higher phosphate concentrations more phosphate is bound than there are positive charges on the histone molecule. There is no evidence that the ability to bind the phosphate anion is correlated with arginine content of a given histone molecule.

It is unlikely that this unexpected result might lie in phosphate becoming irreversibly bound to lysine-rich histones since precipitating F1 (which had been dialyzed against [32P]phosphate) with 20% trichloroacetic acid removed more than 99% of the radioactivity. Furthermore dialysis against sodium phosphate and subsequent precipitation in 20% trichloroacetic acid has no effect upon the electrophoretic mobility of F1.

**DISCUSSION**

The dissociation of histones from DNA has been investigated with several extraction systems and the results indicate that there is a substantial contribution to the binding between DNA and histone of a nonelectrostatic nature. Two problems complicate the interpretation of the dissociation of histone from nucleohistone, these are (a) aggregation and subsequent sedimentation of histones during centrifugation of the extracted nucleohistone and (b) proteolytic degradation of histones during the extraction procedure.

Both aggregation and proteolysis are particularly severe during extraction of nucleohistone with sodium chloride in the method described by Ohlenbusch et al. (4) and the extent of histone dissociation in sodium chloride cannot be accurately assessed. Furthermore degradation of the undissociated histones makes hydrodynamic studies of the residual nucleohistone suspect.

Sodium phosphate-urea was found to be an excellent system in which to dissociate histones from nucleohistone. In this environment proteolytic degradation was not observed and aggregation and sedimentation of dissociated histones is practically nonexistent. It is also a system in which conditions can be defined for a highly selective dissociation of histones, so that nucleohistone lacking only F1 and nucleohistone totally lacking F1, F2b, and F2a2 can be prepared without removing other histone fractions.

We have shown that the binding of histones to DNA is dependent upon additional forces over and above a direct electrostatic interaction. This is supported by the following observations: (a) the enhanced dissociation of histones with urea and propylene, (b) the enhanced dissociation of histones in apolar solvents, (c) the decrease in the amount of histones dissociated at higher temperature, (d) the ability of the moderately lysine and arginine-rich histones to bind to heat-denatured DNA even when all electrostatic bonds have been neutralized in acid or high salt concentrations.

Urea (with either sodium chloride or sodium phosphate) was found to enhance the dissociation of four of the five histone fractions from nucleohistone, whereas the lysine-rich histone (F1) was little effected. The increased histone dissociation in urea does not arise simply by modifying the extent of aggregation and precipitation, thus keeping dissociated histones soluble.
Free histone (in control experiments) is soluble in the sodium phosphate-extracting system at all concentrations of urea, with only a single exception. This exception was not used in drawing the above conclusion. It is to be expected that all DNA-histone electrostatic bonds will be somewhat weakened due to the slightly increased dielectric constant of urea solutions. However this should apply equally to all histone fractions and is evidently only a minor contributor to the greatly increased ease of dissociation of the nonlysine-rich histones since it has only a small effect on the dissociation of the lysine-rich histone.

The strength of hydrophobic interactions increases as the temperature is raised in the range 0-60°C (18). The decrease in the amount of histone dissociated at a higher temperature under otherwise identical conditions suggests that urea is disrupting a hydrophobic interaction between four of the five histone fractions and DNA; again the lysine-rich histone (F1) is an exception in that more of it is dissociated in higher temperature extractions of nucleohistone. The presence of hydrophobic interactions between histones and DNA have been postulated by several investigators (10, 11, 20), but without any direct evidence for their existence. Smart and Bonner (21) have recently reported that sodium deoxycholate can facilitate the removal of the more arginine-rich histones in agreement with this idea.

We have found no evidence to suggest that hydrogen bonds between the guanidino group of arginine and the phosphate groups of DNA contribute significantly toward the strength of the DNA-histone binding. The results of equilibrium dialysis with the five histone fractions and sodium phosphate show that moderately lysine- and arginine-rich histones bind less phosphate per mole of protein than the lysine-rich histone. However, this result is consistent with the notion that electrostatic interactions account for a greater portion of the binding of lysine-rich histone to DNA than it does for the other histone fractions. Histones can be classified into three groups in terms of increasing amounts of nonelectrostatic interaction with DNA. These are F2a1 = F3 > F2b = F2a2 > F1. We have already described an identical grouping in terms of resistance to evolutionary sequence changes (12). It is only necessary to assume that there is considerable specificity in the nonelectrostatic interactions to explain the varying resistance to evolutionary change seen in the three histone groups. However if this assumption is acceptable, a problem arises since this bonding has many of the characteristics of a hydrophobic interaction but must be of such a high specificity as to so completely restrict evolutionary sequence changes (12, 22) that it might require a more specific binding than is commonly thought to be a characteristic of this type of bond. Perhaps the interaction we have described consists of hydrophobic interactions of unusual specificity, although the data we have obtained could not rule out a minor, but important, contribution of hydrogen bonds to the over-all interaction.

It is interesting that the lysine-rich histone (F1) which has shown many changes in primary sequence during evolution does not appear to have hydrophobic interactions as a significant contribution to its binding to DNA. It has been previously suggested that this histone may function to cross-link different nucleohistone molecules (23, 24) and as such might be required to interact much less intimately with the DNA molecule.

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