Asymmetric Distribution of Histone on DNA: A Model for Nucleohistone Primary Structure†

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ABSTRACT: The digestion of calf thymus nucleohistone by pancreatic DNase has been reinvestigated. The major macromolecular product is a positively charged complex containing single-stranded DNA and histones associated by a weaker interaction than that seen in native material. We argue that this does not mirror an aspect of the intact nucleoprotein, but rather is generated during the course of the nucleolytic digestion. The protection of a single strand by histones against further digestion may reflect an aspect of the native structure and a model for nucleohistone organization is proposed which encompasses this idea.

Considerable interest was generated recently when Clark and Felsenfeld (1971) proposed a model for native nucleohistone which envisaged rather substantial segments of nucleohistone consisting of DNA relatively free of histone. The basis for this model lies in the following observations: (1) about 50% of the DNA in nucleohistone can be digested by DNase, whereas the remaining DNA is resistant and (2) the nucleohistone can bind an amount of poly(lysine) equal to half that of the histone already present. Clark and Felsenfeld concluded that 50% of the DNA-phosphate groups were available for these reactions and derived their model on this basis.

Itzhaki has also proposed a model for nucleohistone structure which also involves "free" phosphate groups (*i.e.*, phosphate groups which are not directly neutralized by histone positive charges) based on toluidine blue binding studies (Itzhaki, 1970; 1971a). She argues that the free phosphate groups are sterically covered with histone molecules in such a way that they are unavailable to large molecules, though smaller molecules such as toluidine blue can bind and interact with them. Itzhaki (1971b) also noted that nucleohistone is only partially degraded by DNase; however, she interpreted this observation in terms of a release of histone during the initial part of the digestion followed by the formation of insoluble histone-nucleohistone aggregates, thus protecting against further nucleolytic attack. However, Clark and Felsenfeld (1971) also studied cleavage by DNase of nucleohistone which had been pretreated with formaldehyde, which efficiently and covalently binds histone to DNA. Under these conditions these authors still observed a 50% nucleolytic disruption of DNA and thus appear to counter the criticism of Itzhaki.

Thus two distinct models for nucleohistone primary structure have been proposed. Both envisage the presence of free phosphate groups, though the models differ dramatically in that Clark and Felsenfeld have argued that the phosphate groups exist as discrete stretches of DNA within the nucleohistone molecule, whereas Itzhaki considers that the DNA phosphodiester backbone is essentially totally covered with histone but that the free phosphate groups are somewhat sterically hindered by the uncharged regions of the histone molecules. Interestingly the evidence for the existence of "free" phosphate groups is in no instance completely direct.

We have addressed ourselves to two points in an attempt to resolve these issues: (1) can the presence or absence of free phosphate groups be inferred from titration-electrophoresis studies and (2) do the insoluble fragments which appear during DNase digestion of nucleohistone and which contain all the DNase-resistant DNA actually represent a part of nucleohistone preexisting before nucleolytic attack? We will argue that there are in fact few, if any, free phosphate groups in native nucleohistone, that the insoluble fragments are an artifact of DNase digestion (albeit with properties very illuminating in terms of the original nucleohistone structure), and that a reasonable model for the interaction of histone and DNA can be developed which encompasses these observations together with those of Clark and Felsenfeld (1971) and of Itzhaki (1971a).

Materials and Methods

Preparation of Nucleohistone. Nucleohistone was prepared by the method of Panyim *et al.* (1971). Calf thymus tissue was blended in grinding medium (0.25 M sucrose-0.01 M Tris-HCl (pH 8.3)-0.003 M $MgCl_2$-0.05 M sodium bisulfite) and the resulting suspension centrifuged at 750*g* for 10 min. The pellet was resuspended in washing medium (containing all the ingredients of grinding medium with the addition of 0.5% Triton X-100), blended gently and centrifuged at 750*g* for 10 min. The pellet was treated a second time with washing medium and centrifuged. The nuclei were then washed twice with a solution of 0.0125M NaEDTA-0.01 M Tris-HCl (pH 8.3)-0.05 M sodium bisulfite and centrifuged at 12,000*g* for 10 min. Rupture of the purified nuclei was achieved by osmotic shock following their suspension in cold glass-distilled water. The resulting viscous chromatin gel was sheared at high speed in a VirTis homogenizer and the nucleohistone obtained as the supernatant following centrifugation at 23,500*g* for 20 min. Nucleohistone concentration was obtained directly by absorbance at 260 nm (1 mg/ml of DNA has an A_{260} = 19.7). Calf thymus DNA was obtained from Worthington Biochem Corp.

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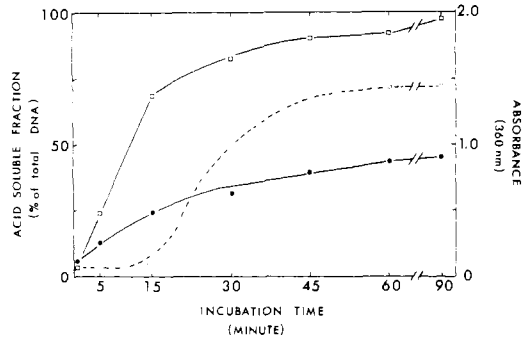

FIGURE 1: The release of acid-soluble nucleotides from deoxyribonuclease-treated DNA (□) and nucleohistone (●). The nucleoprotein ($A_{260} \approx 5.0$) was treated with 5 μg of pancreatic DNase in a buffer containing 0.005 M phosphate (pH 6.8) and 2.5×10^{-5} M $CaCl_2$. Incubation was at 3 min. Samples were removed at the times indicated and treated with 4 N H_2SO_4 to a final concentration of 0.4 N. After centrifugation at 10000 rpm/10 min the optical density of the supernatant material was recorded. Increase in A_{360} before centrifugation is also shown (○). All observations were in triplicate.

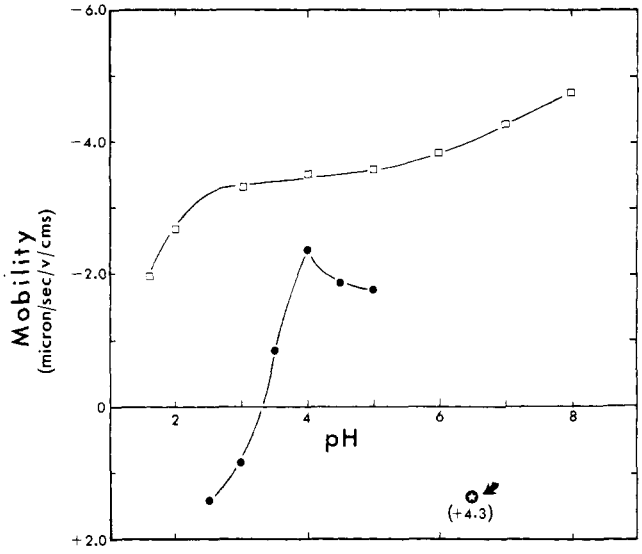

FIGURE 2: Electrophoretic mobilities of nucleohistone (●) and DNA (□) as a function of pH. The data for the electrophoretic mobility of DNA were obtained from Bull (1971). The electrophoretic velocity of the insoluble fragments resulting from nuclease digestion of nucleohistone was determined at a single pH (note arrow). The charge on the molecular aggregates is indicated on the ordinate.

Digestion of Nucleohistone with DNase. Nucleohistone and DNA were digested with deoxyribonuclease I (Sigma Chemical Co.) using those conditions described by Clark and Felsenfeld (1971) at a nucleohistone concentration of approximately 0.25 mg of DNA/ml. The incubation mixture contained 5 mM sodium phosphate buffer (pH 6.7), 2.5×10^{-5} M $CaCl_2$, 5 μg/ml of nuclease I, and either DNA or nucleohistone. The digestion was carried out at 37°. At various times samples were removed from the incubation mixture and adjusted to 0.4 N H_2SO_4 and centrifuged at 23,500*g* for 10 min. The supernatants were analyzed for nucleotide content spectrophotometrically at 260 nm. This assay is based on the insolubility of nucleohistone and polymer DNA in 0.4 N H_2SO_4.

In the course of the incubation a visible precipitate was formed and subsequently collected by centrifugation for further analysis (*vide infra*). The production of these insoluble fragments in the course of the digestion was monitored by continuous absorbance readings at 360 nm.

Electrophoresis of Intact Nucleohistone. Calf thymus nucleohistone aggregates were formed by gradual titration of nucleohistone with 0.1 N HCl and their electrophoretic velocity determined at various pH's.

Electrophoresis was performed in a microelectrophoresis assembly in a solution of 0.05 M NaCl, over a range of pH. This method measures directly the mobility of particles as observed with a light microscope. A description of the apparatus and the method by which electrophoretic velocity is calculated is described in detail elsewhere (Bull, 1971).

Electrophoresis of Histones. Histone isolation and electrophoresis analysis were performed as described previously (Panyim and Chalkley, 1969).

Results

Enzymatic Digestion of Nucleohistone and DNA. Deoxyribonuclease digestion of both calf thymus nucleohistone and DNA was performed under conditions previously described by Clark and Felsenfeld (1971), except that we have used only pancreatic DNase I, whereas the previous workers used both pancreatic and *staphylococcus* DNase. The methods and data for this digestion are presented in detail in Figure 1. Essentially complete digestion of DNA and 46% digestion of purified nucleohistone is found after a typical 1.5-hr incubation in the presence of DNase. The similarity in the kinetics of nuclease digestion for both DNA and nucleohistone indicates a similar availability of phosphodiester bonds for enzymatic cleavage in the two sources and suggests that the chromosomal protein complement of nucleohistone does not protect at least 46% of the DNA-phosphate backbone. Our value of 46% nucleohistone digestion compares well with that reported by Clark and Felsenfeld (1971) for calf thymus chromatin as well as for that of chromatin from a variety of other sources. During the enzymatic digestion of nucleohistone a precipitate forms. These insoluble fragments presumably consist of a protected DNA–histone complex and are shown in Figure 1 as a progressive increase in turbidity beginning approximately 15 min after the start of the DNase treatment. An analysis of the nucleic acid and protein composition of these fragments will be discussed in detail later.

Titration Analysis. If indeed 46–50% of the DNA in nucleohistone is "free" of chromosomal protein it follows that a sizable contribution to the overall negative charge of nucleohistone at physiological pH would come from DNA-phosphate groups. This conclusion is predicated by the assumption that in native nucleohistone the large bulk of histone positive charges are involved in salt linkages and do not contribute to the overall free charge on the molecule. Most reports indicate that although they apparently exist, the number of free histone positive charges is less than 10% of the total (Walker, 1965; Simpson, 1972).

It follows that if the histone positive charges are involved in salt linkage then that 50% not involved in binding to the "free" phosphates must be bound to carboxyl groups, the only other source of negative charge on the nucleohistone molecule. We have assayed for free carboxyl groups by titration-electrophoresis over the range pH 4.5–3.0 (the p*K* region of free glutamic and aspartic carboxylic acid groups). A decrease in electrophoretic mobility toward the positive pole over this pH range is a reflection of the titration of free carboxyl groups. A reduction in mobility to zero in this pH range would indicate that essentially all the free carboxyl groups had been titrated and that few if any histone positive charges are interacting with carboxyl groups.

On the other hand, little change in electrophoretic mobility of the whole nucleohistone in the range pH 5–3.5, followed by a gradual loss of negative charge at lower pH, would indicate that the bulk of the carboxyl groups were not free. This observation would be consistent with the existence of carboxyl salt linkages and with regions of free phosphate groups.

Previous studies have indicated that in this pH range we would effect only minimal titration of bases in native DNA with its attendant production of positive charge (see Figure 2). Thus, with this approach we should be able to demonstrate whether there are regions of free DNA phosphate in native nucleohistone regardless of their overall length. The results from this experiment are shown in Figure 2, and demonstrate that nucleohistone (but not DNA) becomes electrically neutral at pH 3.3 indicating that the free negative charge contribution has been titrated.

Since phosphate is not titrated over this pH range we conclude that we are monitoring the titration of carboxyl groups. Further, since there are approximately equal amounts of positive charges on histone and negative charge on DNA phosphate groups it is likely that essentially all of the histone carboxyl groups have been titrated when the nucleohistone has no overall charge at pH 3.3.

We conclude that most of the carboxyl groups on nucleohistone are free to interact with the solvent and that as a result the histone positive charge must interact extensively with the negatively charged DNA phosphate groups. Since it has been estimated that >90% of the histone positive charges are involved in salt linkages it seems improbable that more than 10% of the phosphate groups are free. The precision of the experiment does not permit us to exclude the possibility of some free phosphate groups of the order of magnitude estimated above.

Thermal denaturation profiles provide a second line of evidence which argues against the idea of extensive contiguous regions of free DNA. It is well known that histone confers a high degree of stability (and hence resistance to denaturation) to nucleohistone, presumably through the neutralization of the negative charges on DNA which have a destabilizing effect. Hence we would expect that those regions of nucleohistone in which there were no histones would necessarily denature at a lower temperature and have melting properties similar to DNA. We have compared the melting profiles of nucleohistone and DNA at identical ionic strengths in an attempt to identify these regions. Figure 3 shows the results from these experiments. We have shown that there is only a 3% (shaded area) overlap in the melting curves of DNA and nucleohistone. This overlap can be abolished if we preincubate the nucleohistone at 60° for 1.5 hr before thermal denaturation, whereupon the broad melting curve characteristic of nucleohistone is abolished and is replaced with a curve analogous to that seen for DNA except that the thermal transition takes place at a temperature 30° higher. Previous studies have shown that there is no proteolysis of histone at 60° due to inactivation of the protease in chromatin (Bartley and Chalkley, 1970). We do not know the reason for this remarkable behavior, however, it is strongly dependent upon the time of preincubation and certainly is quite uncharacteristic of DNA. It would appear that the early portion of the nucleohistone denaturation is sensitive to temperature and not renaturable though in general nucleohistone shows extensive renaturation if the melting temperature does not exceed 85°.

These results again argue that extensive regions of "free" DNA do not exist in nucleohistone prepared in our hands.

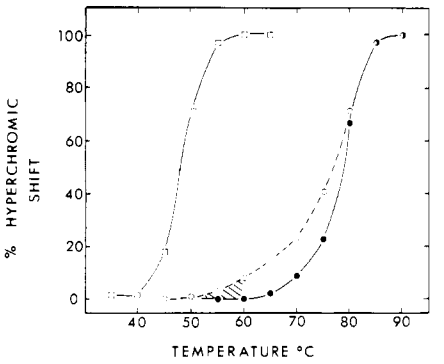

FIGURE 3: Denaturation profiles of native calf thymus DNA (□), nucleohistone (○), and nucleohistone preincubated at 60° for 1.5 hr (●). Thermal denaturation was performed at 1 mM Tris-HCl (pH 7.0) and monitored at 250 nm during continuous temperature changes in 1-cm closed, thermostatically controlled quartz cells, using a Gilford Model 2000 spectrophotometer. One of the nucleohistone samples was first preincubataed at 60° for 1.5 hr, allowed to return to room temperature, and then denaturated as described above. The shaded area depicts that portion of the denaturation curve of nucleohistone which overlaps with the melting curve of DNA.

Electrophoresis. In an attempt to understand the apparently contradictory results of the DNase digestion, the titration, and the thermal denaturation data, we have analyzed the properties of the insoluble fragments produced during DNase digestion of nucleohistone. We reasoned that *all* the DNA–phosphate groups in nucleohistone might be linked to histone, but that the specific organization of the macromolecules might render some parts of the DNA molecule available for nucleolytic attack, *e.g.,* by histone lying to one side of the phosphate backbone thus effectively titrating its negative charge, but permitting nuclease attack on the contralateral side. If this were the case, that histone which had overlain a region of recently digested DNA might by virtue of its highly positive nature either share phosphate groups of residual nucleohistone strands, or, on the other hand, it might become associated with the negatively charged (carboxyl) regions of immediately adjacent histone–DNA complexes, leading ultimately to an insoluble, cross-linked aggregate. One might expect that such an interaction between the histone and nucleoprotein complex involving carboxyl groups in salt linkages might be disrupted at a pH which does not extract histone from native nucleohistone (we have used pH 3.2) (Murray, 1966). On the other hand, if the insoluble particles simply reflected an unmodified part of the original nucleohistone structure released by DNase cleavage of adjacent regions then no histone should be extracted at such a pH. The results from an experiment in which we extracted the insoluble fragments at pH 3.2 are shown in Figure 4. Gel b represents the histones extracted at pH 3.2. Histone fractions F2b and F2a2, in equivalent yield, and to a lesser extent, F3 and F2a1, and some intact and partially degraded F1, were extracted at pH 3.2 and represent approximately 35% of the protein content of the insoluble fragments. The fact that histone fractions F3 and F2a1 were only partially extracted is probably due to their ability to bind more tightly to nucleoprotein (Bartley and Chalkley, 1972; Ohba, 1966). The residue obtained after treating at pH 3.2 was extracted with 0.4 N H_2SO_4 and the histone pattern is shown in Figure 4, gel a. The entire complement of histones is represented in this extraction. That some typical F1 proteolysis (Bartley and Chalkley, 1970) occurs during the 37° incubation is shown in gel d. This sample had

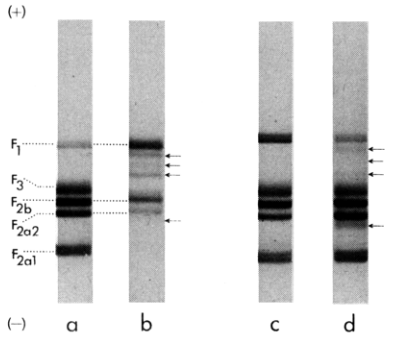

FIGURE 4: Polyacrylamide gel electrophoresis of histone extracted from calf thymus nucleohistone and from the insoluble fragments. Gel b shows the electrophoretic pattern of histone extracted at pH 3.2 from the insoluble fragments which were produced in the course of DNase digestion. Arrows indicate typical lysine-rich histone degradation bands. Gel a shows those histones which were extracted with H_2SO_4 (0.4 N) from the residue remaining after treating the insoluble fragments with HCl at pH 3.2. The histones shown in gel d were extracted from nucleohistone which had been incubated at 37° for 1.5 hr but had not been digested with DNase. Arrows indicate the presence of minor, lysine-rich histone degradation bands. Gel c represents normal calf thymus histone. Electrophoresis was performed as described previously (Panyim and Chalkley, 1969). Histone fractions follow the nomenclature of Johns (Johns and Phillips, 1965).

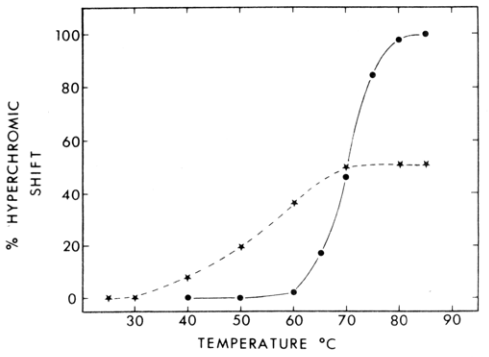

FIGURE 5: Denaturation profiles of nucleohistone (●) and the insoluble fragments (★) in 1% sodium dodecyl sulfate. Thermal denaturation was performed as previously described above (figure legend 3). The insoluble fragments were obtained after DNase treatment of nucleohistone (1.5 hr) and prepared by washing twice with 1 mM NaEDTA (pH 7.0) and twice with 5 mM phosphate buffer (pH 6.7) to remove DNase. Both nucleohistone and the insoluble fragments were adjusted to 1% dodecyl sulfate prior to the thermal denaturation.

been incubated, but not treated with DNase. A gel of normal calf thymus histone is provided for comparison (gel c).

We interpret these results as an indication that the histone which is extractable at pH 3.2 had previously been associated with DNA which was available for digestion. The formation of insoluble particles upon digestion of formaldehyde-treated nucleohistone (Clark and Felsenfeld, 1971) would argue that the histone associated with such DNA must be in close proximity to its final site of binding in the insoluble fragment. It would seem then, that perhaps a region of DNA which is linked to histone need not be totally resistant to nucleolytic attack, and that once digestion begins those histones which are released, promptly bind other regions of nucleoproteins thus providing a resistence to further DNase treatment. One would certainly expect these fragments to be highly positively charged due to the presence of excess histone. We measured the electrophoretic mobility of these fragments, as described in Figure 2, and report that indeed the fragments are highly positively charged whereas native nucleoprotein at the same pH is negatively charged.

Whatever the interpretation of the mode of their formation, it is clear that the insoluble fragments are an artifact of DNase treatment and consequently do not represent an organization which exists in native nucleoproteins.

The Insoluble Fragments Contain Single-Stranded DNA. It has been shown by Clark and Felsenfeld (1971) that the insoluble fragments contain DNA with a median molecular weight of between 105,000 and 110,000 and a base composition of 58% A + T. We sought to determine the melting characteristics of this material to see if the excess histone provided additional stabilization. Initial attempts to melt the fragments in an EDTA buffer were unsuccessful and we interpreted this to the presence of excess histone. However, when the thermal denaturation of these fragments and native nucleohistone was performed following treatment with 1% sodium dodecyl sulfate (which totally inhibits DNase activity, data not shown) the results in Figure 5 were obtained. The thermal denaturation profile of undigested nucleoprotein was typical of sodium dodecyl sulfate treated material (*i.e.*, DNA-like since 1% sodium dodecyl sulfate fully dissociates nucleohistone); however, the curve obtained for the insoluble fragments was not indicative of intact, double-stranded DNA, but more nearly resembles that of single-stranded DNA, though containing some elements of double strandedness, and we conclude that a substantial amount of the DNA in the insoluble fragments is single stranded.

In order to test this notion further we have exploited the ability of hydroxylapatite to distinguish between single-stranded and double-stranded DNA. A sample of nucleohistone was degraded with DNase as completely as possible and the insoluble fragments isolated by centrifugation. DNA was extracted with sodium dodecyl sulfate applied to an hydroxylapatite column and eluted with a stepwise gradient of potassium phosphate. The results of such analysis are shown in Figure 6. Approximately 50% of the material is eluted between 0.15 and 0.20 M phosphate. Control experiments confirmed that this is the region in which high molecular single-stranded material elutes, as expected from the observations of Bernardi (1969). The elution profile shows only 7% of the material eluting at a phosphate concentration >0.20 M indicating that very little double-stranded DNA is present. About half of the material applied to the column was eluted in 0.10 M phosphate. Subsequent analysis indicated that about 50% of the 0.10 M eluent was precipitable, presumably reflecting its nature as small oligonucleotides. In contrast, the material eluting between 0.15 and 0.20 M phosphate is precipitable in 0.4 N H_2SO_4 confirming that it is at least of moderately high molecular weight.

Discussion

On the basis of the nucleohistone titration experiments and the thermal denaturation profiles we conclude that there is no significant contribution to the effective overall negative charge of the nucleohistone complex arising from phosphate groups of DNA. Further, we would argue that the insoluble fragments produced after DNase digestion of nucleohistone (containing the DNA resistant to nucleolytic attack) do not represent portions of the original nucleohistone simply excised as a result of DNase treatment. This is based on two lines of argument: (1) the fragments contain single-stranded DNA, and certainly 50% of the DNA in nu-

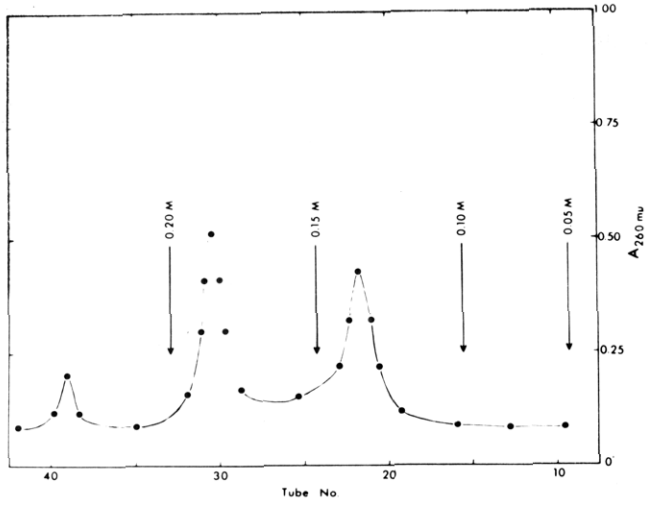

FIGURE 6: Hydroxylapatite chromatography of products from DNase digestion of nucleohistone. Nucleohistone was digested with DNase for 60 min and the insoluble fragments containing 65% of the DNA were then made 1% in sodium dodecyl sulfate and applied to a 5-cm column of hydroxylapatite in 0.001 M potassium phosphate (pH 7.0). It was washed in with 2 × 0.5 ml of 1% sodium dodecyl sulfate before beginning the stepwise elution. Control experiments using both native and heat-denatured DNA showed that when applied in sodium dodecyl sulfate they still behaved as described by Bernardi (1969) in that native DNA elutes at concentrations >0.20 M and denatured DNA elutes between 0.15 and 0.20 M phosphate.

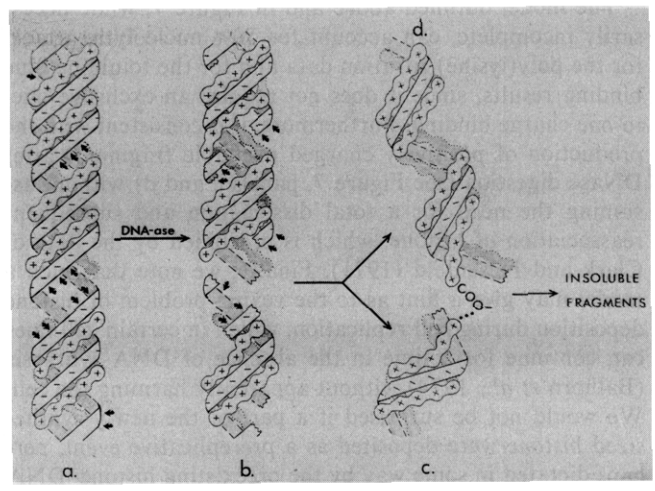

FIGURE 7: A diagrammatic model for the asymmetric distribution of histone on DNA. In this simplified model (panel a) the DNA phosphate groups are shown as negative charges on the backbone of DNA (− − −) whereas the histone molecules (+ + +) are depicted as undulating ribbons bearing positive charges. On the average we expect that one histone molecule would extend along three turns of the DNA helix. It is probable that histone regions devoid of either arginine or lysine residues would either interact hydrophobically with the DNA bases or extend out from the main axis of the complex (not shown). It should be noted (panel a) that those regions of DNA which are available for toluidine blue and poly(lysine) binding and DNase digestion (arrows) always involve the major groove of the DNA molecule. Panel b shows the completed (⊐ ⊏) and progressive (arrows) digestion of nucleohistone with DNase resulting in fragments (c and d) which are (1) extensively single stranded, (2) have a double complement of histone, and (3) through extensive interaction with one another (probably through amino ··· carboxyl electrostatic bands) form insoluble fragments.

cleohistone as we prepare it is not denatured (nucleohistone has a hyperchromicity of approximately 40%), and (2) the insoluble fragments contain histone in a form which is extractable at pH 3.2, a pH at which histones are not dissociated from intact, native nucleohistone.

It was necessary therefore that a new model for nucleohistone primary structure be developed which is capable of encompassing all the pieces of evidence concerning DNA–histone interactions. In attempting to devise such a model we had to reconcile the following points. (1) There is a rather precise equality between the number of charges on DNA phosphate groups and histone positive groups. (2) Histones bind DNA strongly through electrostatic bonds to DNA–phosphate groups, but there is also an important contribution through hydrophobic interactions (Bartley and Chalkley, 1972). (3) There are few free negative charges residing on phosphate groups on intact nucleohistone (this paper). (4) Histones only cover about one-third of the surface of DNA.[1] (5) DNase digests 50% of the DNA of nucleohistone, whereas the other 50% is resistant (Clark and Felsenfeld, 1971). (6) A similar result is obtained even when histones are covalently coupled to DNA to formaldehyde treatment. (7) The DNA which is resistant to DNase is found in highly positively charged aggregates (this paper). (8) The DNA resistant to DNase digestion is primarily single stranded (this paper). (9) About 35% of the histone in the insoluble product from DNase digestion is extractable by titrating carboxyl groups to pH 3.2, a pH which does not normally release histones from nucleohistone (this paper). (10) A substantial number (~50%) of phosphate groups can be induced to react with poly(lysine) (Clark and Felsenfeld, 1971) or toluidine blue (Itzhaki, 1970; 1971a,b).

[1] Calculation based on following points: (1) mass ratio DNA/histone in nucleohistone is 1:1; (2) an "average" histone consists of 120 amino acids; and (3) this "average" histone has 30% α helix when complexed with DNA (Tuan and Bonner, 1969).

The main feature of the model which is presented in Figure 7 lies in the asymmetric distribution of histone about the two strands of DNA, lying in both the major and minor grooves, thus sterically protecting one strand against nucleolytic attack and titration with poly(lysine), while leaving the other strand more available. The model as drawn conceives of relatively little histone actually overlaying the phosphate backbone itself, thus providing a favorable environment for the development of histone–DNA hydrophobic interactions within the grooves. If the histones were to crisscross the phosphate backbone extensively, the DNase digestion would be unlikely to yield single-stranded DNA in the insoluble fragments of molecular weight as high as is observed (Clark and Felsenfeld, 1971). We do not expect that one continuous strand is available, but rather that the asymmetry shifts from one strand to the other. Certainly, after denaturation of nucleohistone we find no evidence that one strand has a preponderance of histone. We have indicated that each histone molecule binds primarily to one strand though obviously we cannot exclude a measure of binding to both strands particularly within the small groove. However, it may be expected that a minimum of cross-strand linkage would be favorable, since it would reduce the energy expenditure required for strand separation during replication. Nor do we demand that all histone molecules lie within the grooves, for indeed there is some experimental evidence that the lysine-rich histone may cross-link nucleohistone molecules (though still presumably binding extensively to phosphate groups as it cannot be dissociated above a pH of 2.6).

The model outlined above and in Figure 7, while necessarily incomplete, can account for 50% nucleolytic attack, for the poly(lysine) titration data and for the toluidine blue binding results, since it does not require an exclusive one-to-one charge binding. Furthermore, it is consistent with the production of positively charged insoluble fragments after DNase digestion (see Figure 7, panels c and d) without assuming the need for a total dissociation and subsequent reassociation of histone, which is precluded by the data of Clark and Felsenfeld (1971). Finally, we note that such a model may give a hint as to the vexing problem of histone deposition during cell replication, which in certain cell lines can continue for a time in the absence of DNA synthesis (Balhorn *et al.*, 1974) without apparently harming the cell. We would not be surprised if a part of the newly synthesized histone were deposited as a prereplicative event, perhaps dictated in some way by the preexisting histone–DNA organization.

In summary, we have proposed a model for nucleohistone structure which encompasses the results of Clark and Felsenfeld (1971), of Itzhaki (1971a), and those described in this paper. We envisage that histones interact asymmetrically with both strands of DNA along the entire length of the molecule, so that seemingly about half of the DNA of calf thymus chromatin is unprotected by proteins and is available for hydrolysis by nucleases or for combination with poly(lysine) but that in fact very few of the DNA-phosphate groups actually exist with free negative charges.

We emphasize that this concept of nucleohistone structure applies to the primary structure. The manner in which interactions among primary structural elements generate secondary structure, such as that recently described by Olins and Olins (1974), remains an area of future research.

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A Predictable Modification of Enzyme Specificity. Selective Alteration of DNA Bases by Metal Ions to Promote Cleavage Specificity by Deoxyribonuclease†

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ABSTRACT: The specificity of an enzyme that acts on a macromolecule can be altered in a predictable manner. Copper(II), which preferentially binds to guanine sites on DNA, extensively protects guanine sites from action by bovine pancreatic deoxyribonuclease I. Mercury(II), which preferentially binds thymine sites on DNA, extensively protects thymine sites from enzyme action.

Enzymes that act on macromolecules differ from those that act on low molecular weight substrates in that the specificity of the former bears little relation to the dimensions of the substrate. Such enzymes must in fact be able to act on substrates of many sizes. Thus bovine pancreatic deoxyribonuclease I (EC 3.1.4.5) can act on DNA molecules of molecular weights in the millions as well as on tetradeoxynucleotides (Ralph *et al.*, 1962). Obviously this enzyme does not exhibit specificity for the dimensions of the polynucleotide. It is nevertheless sensitive to subtle localized chemical differences; thus the presence of a 2′-OH group on the ribose ring renders the substrate inactive (McCarty, 1946).

It therefore appears that this enzyme recognizes the chemistry and geometry of individual nucleotides even though it is not sensitive to the structure of the polynucleotide. The question arises whether it is possible chemically to

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