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A model for chromatin structure*

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ABSTRACT

A model for chromatin structure is presented. (a) Each of four histone species, H2A (IIb1 or f2a2), H2B (IIb2 or f2b), H3 (III or f3) and H4 (IV or f2a1) can form a parallel dimer. (b) These dimers can form two tetramers, $(H2A)_2(H2b)_2$ and $(H3)_2(H4)_2$. (c) These two tetramers bind a segment of DNA and condense it into a "C" segment. (d) The adjacent segments, termed extended or "E" segments, are bound by histone H1 (I or f1) for the major fraction of chromatin; the other "E" regions can be either bound by non-histone proteins or free of protein binding. (e) The binding of histones causes a structural distortion of the DNA which, depending upon the external conditions, may generate the formation of either an open structure with a heterogeneous and non-uniform supercoil or a compact structure with a string of beads. The model is supported by experimental data on histone-histone interaction, histone-DNA interaction and histone subunit-DNA interaction.

INTRODUCTION

A great progress has been made in extending our knowledge of histone-histone and histone-DNA interaction in chromatin since the initial period during which fundamentals of histone chemistry became clear (1-8). Models of chromatin structure have been developed in several laboratories (9-11). In particular, Van Holde et al (11) proposed a model for particulate structure in chromatin based upon their studies of so called "PS-particles". Although their model represents an advance in our understanding of chromatin structure, it appears to be incompatible with certain important facts, as do the other models proposed.

For purposes of discussion we will start from a brief review of pertinent experimental observations and their implications as obtained from the literature and then present a model of chromatin structure based upon these data. The model will be presented in as great detail as possible,

but not in excess of what the related experiments can support, so that the discussion may serve the purpose of stimulating further experiments designed to enhance our understanding of a more correct and detailed picture of chromatin structure.

This communication will be divided into five parts: (I) histone-histone interaction, (II) histone-DNA interaction, (III) histone subunit-DNA interaction, (IV) chromatin structure: a proposed model and (V) discussion.

Histone-Histone Interaction

Distribution of basic amino acid residues along the histone molecule is uneven in histone H1 (I, f1) (4), H2A (IIb1 or f2a2) (6,7), H2B (IIb2 or f2b) (5), H3 (III or f3) (8) and H4 (IV or f2a1) (2,3). In general, these molecules can be divided into two halves, the more basic and the less basic, or the more basic and the more hydrophobic halves.

Histone-histone interaction has been studied using sedimentation (12), nuclear magnetic resonance (NMR) (13-16), circular dichroism (CD) and fluorescence anisotropy (17-25), gel electrophoresis (26) and electron-microscopy (27). Of the five histones, histone H4 has been studied most extensively (12,13, 15-19, 27,28). When placed in salt this histone rapidly forms an α-helix, dimerizes, then slowly forms a β-sheet within the dimer (17). Based upon this sequence, kinetics and the amino acid sequence of this histone, it was suggested that a parallel dimer is formed rapidly, through hydrophobic contact in the C-terminal regions, followed by a slow but specific hydrogen bonding within the dimer to form some β-sheet structure (28). This suggestion is in agreement with NMR data both of whole histone H4 (13,15) and of histone H4 fragments (16).

Using the kinetic and thermodynamic equations derived for histone H4 (17), Isenberg and colleagues studied interaction among other histones

(20-25) and reported a cross-complexing pattern: strong interaction with H2A-H2B, H2B-H4, and H3-H4, and only weak interaction with H2A-H3. The formation of a histone H3-H4tetramer has been demonstrated (26,29a,29b).

Our recent studies on histone H3 (Yu and Li, manuscript in preparation) show that the formation of a disulfide bond, or bonds, in the C-terminal region of this molecule has no significant effect on both the CD and melting properties of histone H3-DNA complexes. This implies that histone H3 may form a natural parallel dimer, with or without disulfide bonds, possibly through hydrophobic interaction and hydrogen bonding within the dimer. This could also provide a basis for the formation of the histone H3-histone H4 tetramer, wherein two parallel dimers of histone H3 and H4 interact through hydrophobic contact.

Histone-DNA Interaction

The major role of ionic forces in the bonding between histones and DNA was suggested about a decade ago (30,31). It is this type of bond which has been considered to be the main force in stabilizing chromatin against thermal denaturation (32). Histone binding to DNA in chromatin results in two distinct melting temperatures, one higher than the other (32-35). It was suggested that this difference in stabilization was due to binding by the more basic and the less basic halves of histones to DNA (32). In other words, both halves of a histone molecule bind directly to two adjacent segments of DNA (32,35) rather than two opposite sides or grooves of the same DNA segment; this does not exclude the possibility that parts of the less basic halves can serve as sites for histone-histone interaction.

The above model (32) implies a universal property shared by all histones, such that any histone molecule can be separated into a more basic and a less basic half. In fact, this has proved to be true for

every histone which has been sequenced (2-8). Although the complete sequence of histone H5 (V or f2c) has not yet been published, our recent studies on histone H5-DNA complexes show that this histone induces the same two characteristic melting bands (36), again suggesting unequal distribution of basic residues along this molecule.

Trypsin digestion of chromatin, whether carried out in urea (33) or in its absence (37), leads to a reduction of only the highest melting band. It was suggested that this might represent a preferential digestion of the more basic rather than the less basic regions of histones (35). This suggestion was recently verified using electrophoresis and peptide mapping (38).

CD spectra of chromatin (39-45) show that the presence of histones in chromatin causes a reduction in the positive CD of DNA near 275nm and a big negative CD near 220nm. The latter implies a substantially ordered structure of bound histones. Such structure has been attributed mainly to the binding of histones H2A, H2B, H3 and H4, but not histone H1 (37,45, 46). The CD of trypsin-treated chromatin suggests further that the less basic regions of these histones have more secondary structures than do the more basic regions (37).

Recently we have studied the protection of a protein against trypsin digestion by DNA binding using polylysine, polyarginine and copolypeptides of lysine and alanine of varied α-helical contents (Li, Rothman, and Pinkston, manuscript in preparation). We found that polyarginine is well protected, whereas polylysine is not. Both results of these studies and of experimental manipulations with CPK models of polylysine-DNA and polyarginine-DNA complexes suggest that the bulkier polyarginine favors in the major groove, whereas polylysine favors the minor groove. In the major groove the peptide bond adjacent to the basic residue is protected from approach and subsequent hydrolysis of trypsin. The greater vulnerability

of the more basic regions of chromatin histones to trypsin digestion (33, 35,37,38) could imply that these more basic regions bind mainly inside or on the surface of the minor groove of one segment of DNA, while the less basic regions bind mainly in the major groove of another segment. Binding of histones in the major groove of DNA has been suggested before (47,48).

Previously it was shown that when polylysine binds to chromatin (35), it can bind directly to base pairs already covered by the less basic regions of histones but not to those covered by the more basic regions. This could be explained if polylysine winds along the minor groove as suggested by CPK model and other earlier studies (49-50), because direct polylysine binding to DNA along the minor groove would not be hindered by the less basic regions of histones (in major groove), but would be by the more basic regions (in the minor groove).

Histone Subunit-DNA Interaction

Histone H1 does not seem to be important in maintaining the X-ray diffraction pattern of chromatin (51-53) and CD spectra (37,44-46).

Very recently, using tetranitromethane as a cross-linking reagent, it was shown that, in chromatin, histones H2B and H4 lie next to each other. The presence of H2A seems to be crucial for this cross linkage to occur whereas neither histone H1 nor H3 is required (54).

Using melting properties of chromatin as a criterion, a reconstituted complex between DNA and a mixture of histone H2A and H2B is closer to the native chromatin than a complex made with H2B alone (32,55). This suggests that histone H2A and H2B together may form a more natural subunit for interaction with DNA. A mixture of histone H3 and H4 also yields a better complex with DNA than does either of these two histones complexed separately. Furthermore, employing conditions favorable to the formation of a histone H3-histone H4 tetramer (29b) promotes a complex with DNA which

is even better than the reconstituted complex (Yu and Li, manuscript in preparation). The evidence suggests that complex formation with DNA involves two natural subunits, one composed of histone H2A and H2B and another of histone H3 and H4.

The formation of subunits of two molecules each of all the histones except histone H1 has been proposed for chromatin (53).

In "PS" particles of chromatin, each particle contains about 120 base pairs and 8 molecules of histones except histone H1. During the preparation of "PS" particles, histone H1 disappears gradually when subjected to a longer nuclease digestion (11). Interestingly enough, we suggested independently (37) that histone H1 might not protect DNA against nuclease digestion as well as do other histones, a suggestion based primarily upon CD results of chromatin and an attempt to reconcile the different estimations of histone-free regions in chromatin, using either thermal denaturation (32, 35,36) or nuclease digestion (57).

Chromatin Structure: A Proposed Model

Two main conflicting models of chromatin structures have been proposed, namely, a uniform supercoil based upon X-ray diffraction (51) and particulate structure based upon electronmicroscopy, sedimentation and neutron diffraction (11,58,59). In an effort to bring these two extremes together (37), we recently suggested a heterogeneous and non-uniform supercoil. To be more specific, the following scheme is proposed (Fig. 1).

1. It is suggested that each histone species of H2A, H2B, H3 and H4 forms a parallel dimer. One histone H3 dimer and one histone H4 dimer form a tetramer through hydrophobic interaction in the less basic regions of the molecules. A similar tetramer is also suggested to form between a H2A dimer and a H2B dimer. Perhaps the $(H3)_2$ $(H4)_2$ tetramer forms a stronger subunit while the $(H2A)_2$ $(H2B)_2$ tetramer a weaker one, because histone

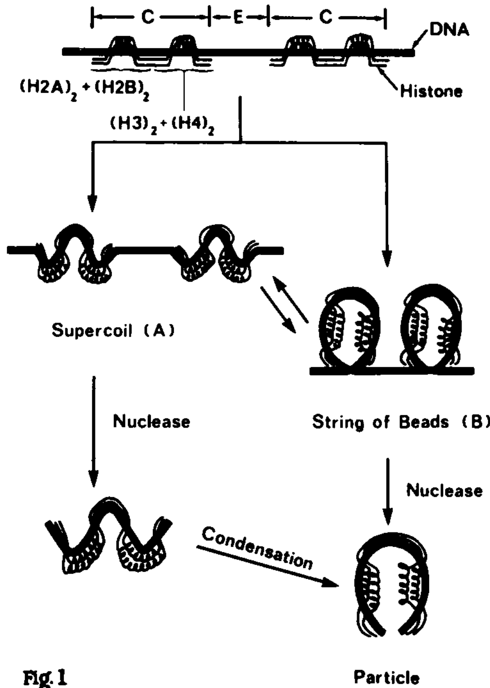

Figure 1.
A model for chromatin structure. The more basic region (——) and the less basic or the more hydrophobic region of a histone molecule (~~~). The parallel dimer of each histone species is represented by ═~~~, while the tetramer formed from the two dimers by ═~~~═. The drawing simply represents the regions of histones and does not represent any secondary or tertiary structure of histones. The histones on both sides of the DNA represents the binding of the more basic and the less basic regions of histones in the opposite grooves of DNA. The shapes of coiled or particle regions in (A) or (B) are schematic. Foldings of DNA in three-dimensional space are likely and not presented here due to the lack of information.

H2B alone can form a complex with DNA which is qualitatively similar to that of either H2A + H2B or chromatin (32,55).

2. Two tetramers, $(H2A)_2(H2B)_2$ and $(H4)_2(H3)_2$, bind next to each other on the DNA and provide a bigger cluster with 8 histone molecules as suggested earlier (11,53). Although the octamer formed from two basic tetramers is a fundamental subunit, minor variations within this subunit, such as a loss of one molecule or one species of histone, could possibly occur in partially dehistonized chromatin or native chromatin from some organisms. These variations may cause a loss of some, but not all, of the physical properties which the chromatin derives from the octamer subunits.

3. Based upon the histone-DNA interactions reviewed above, it is suggested that the more basic regions of histones bind DNA primarily inside or on the surface of the minor groove, while the less basic regions

bind the major groove.

4. Judged by CD results (37), DNA segments bound by the octamers have more histone secondary structure and more distortion in the structure of DNA than the others. Those segments bound by the octamers will be termed the condensed or C segments.

5. Other segments, to be termed extended or E segments can be divided into two groups. For the majority of chromatin (about 80%), the E segments are suggested to be covered by histone H1, one H1 molecule per E segment, or about 30-40 base pairs (35). For the rest of chromatin (about 20%), the E segments can be either free of proteins, or covered (partially or fully) by non-histone proteins. In this case, the length of each E segment can be very heterogeneous.

6. For the majority of chromatin (about 80%) the binding of histones to DNA in chromatin would result in two plausible structures, a heterogeneous and non-uniform supercoil (A) or a string of beads (B). In the supercoiled structure, the two tetramers in the C segments are not physically bound to each other, but are kept apart, possibly through electrostatic repulsion or through the unfavorable energy required to bend DNA into a more compact structure. On the other hand, a structure similar to a string of beads could also exist, if hydrophobic interaction between the less basic regions of the two tetramers were stronger than the electrostatic repulsion and other unfavorable forces. Both types of structure might exist simultaneously within the same chromatin molecule, or might be in equilibrium under the same solution condition. It is the external factors of ionic strength, type of ions, pH etc, which determine the direction of equilibrium.

7. Nuclease digestion removes the E segments from the chromatin and causes condensation of the C segments into particles.

The suggested structure of chromatin shown in Fig. 1 represents the

most detailed picture we can present which is supported either directly or indirectly by experimental data as to be discussed below. So far there is no convincing evidence to support further speculation of a more detailed structure of histone-DNA complex in the condensed or particulate regions. We prefer to leave this question open for the future research designs.

DISCUSSION

1. The earlier review of histone-histone interaction indicates the existence of dimer in histone H4 (13,15,16,17,28) and H3. Both experimental data and the amino acid sequences of these two histones favor parallel dimers over other arrangements such as anti-parallel dimers. Since the clustering of basic residues in the N-terminal half and the hydrophobic residues in the C-terminal half of a histone molecule exists not only in histone H3 and H4 but also in H2A and H2B, the suggestion of formation of parallel dimers for the latter two histones (Fig. 1) is theoretically feasible.

2. Although the particles in Fig. 1 show the formation of a core of the less basic regions of histones, as proposed earlier by Van Holde et al (11), our model suggests that only about 50% of DNA in the particles is directly associated with this "hydrophobic core". The other 50% is directly bound by the more basic regions of histones. According to the model in Fig. 1 the following hydrophobic interactions among histones are possible: (i) between the dimers of each species of histone, (ii) between $(H2A)_2$ and $(H2B)_2$ dimers or between $(H3)_2$ and $(H4)_2$ dimers, and (iii) between $(H2A)_2(H2B)_2$ and $(H3)_2(H4)_2$ tetramers, which have been shown to exist both in free state (25) and in chromatin (54). The particulate model of Van Holde et al (11) suggests interaction between the two adjacent histone molecules and excludes other interactions.

3. The critical evidence for the particulate model of Van Holde et

al (11) is that, based upon hydrodynamic studies, there are 8 histone molecules complexed with 120 base pairs in the "PS" particles; the diameter of the particle is about 80-100 Å. In order for a particle of this diameter to form, the 120 base pairs (about 400 Å in length) must be condensed or coiled in some way. A complex of 8 histone molecules bound to 120 base pairs is consistent with the model in Fig. 1. According to previous melting results, there are about 3.5 amino acid residues per nucleotide or 7 residues per base pair in histone-bound regions in chromatin (32,35,36). Therefore a segment of 120 base pairs would accommodate 840 amino acid residues, which is equivalent to 8 histone molecules when H1 is excluded. The only modification to be made on our earlier paper (35) is that, since each histone is suggested to form a dimer in this report, the length of DNA covered by the two halves of a histone would be about 30 to 36 base pairs rather than 15 to 18 base pairs when only histone monomers were considered (35). It is emphasized that calculations from thermal denaturation experiments include the melting areas of both those base pairs bound by the more basic and those bound by the less basic regions of each histone molecule (32,35,56). Such calculations are one of the bases for the model of Fig. 1.

So far our discussion of C or E segments has been focused on DNA directly covered by the octamer or histone H1. In this case, melting results (35) suggest that the octamer covers about 120 to 140 base pairs (C segment) and histone H1 about 30 to 40 base pairs (E segment). These two numbers could be the minima for these two segments because of the possible existence of gaps of a few base pairs not directly bound by histones between the two tetramers within C segments or between C and E segments. For instance, in native chromatin, about 20% of DNA base pairs melt at temperatures lower than those bound by histones but higher than that of pure DNA and have been attributed to small gaps of free DNA between

two histone-bound segments or regions bound by non-histone proteins (32,35). Including these gaps a C segment could contain about 130 to 150 base pairs and an E segment about 35 to 45 base pairs when covered by histone H1. These values are in agreement with those obtained by nuclease digestion reported by Corden et al (60).

The model shown in Fig. 1 suggests that for the majority of chromatin (about 80%), either in a supercoiled structure (A) or in a string of beads (B), there is a repeating unit of about 30 to 40 base pairs (100 to 135 Å) in E segments. Since the ordered structures of histone H1 in E segments are much less than those of other histones in C segments (37), this repeating unit could represent a regular distribution of histone density along chromatin (110 Å) as revealed by neutron diffraction (59).

Although the regular distribution of histone H1 along a chromatin molecule became clearer recently (11,37,53), scattered distribution of histone H1 in chromatin has been reported before based upon electron microscopic studies (61), thermal denaturation (62) and renaturation (63) of histone H1-deprived chromatin.

4. Our model suggests that, if one of the histones is removed, the supercoil may be less coiled and the particles less compacted, but it specifically does <u>not</u> suggest the presence of all four histones in an exact stoichiometric ratio as a prior condition for the formation of a supercoil or a string of beads. This is not implied in either the particulate model (11) or in the chromosomal subunit of 8 histones (53); in fact, "PS" particles similar to those found in calf thymus chromatin have been found in nuclease-treated yeast chromatin in which histone H3 does not exist (64). This model differs from others, in that, according to Fig. 1, a chromatin can be either a heterogeneous, non-uniform supercoil (A), a string of beads (B), or even a mixture of these two, depending upon ionic strength, pH, and other environmental factors surrounding the chromatin.

For instance, although there are roughly equal basic amino acid residues of histones and phosphates of DNA in histone-bound regions, some of the phosphates are not directly bound by these basic residues (32). Electrostatic repulsion among phosphates could still be an important factor in determining the structures of chromatin. However, since the majority of phosphates are already bound by the basic residues of histones, the residual phosphates could possibly be neutralized by low concentration of cations in solution, 0.01 to 0.1M of Na^+ for example.

5. Fig. 1 suggests that "PS" particles could result from the condensation or coiling of nuclease-resistant segments (C regions) after the removal of the E regions which are presumably more hydrophilic. This is not unreasonable since it is well known that removal of hydrophilic groups from a molecule tends to enhance aggregation. In fact, prolonged digestion of chromatin by nuclease yields insoluble products (57).

6. Our model suggests that, the basic and the hydrophobic regions of histones cover separate regions of DNA; this is in agreement with melting results of thermal denaturation experiments (32-35). The particulate model (11) alternatively implies binding of the more basic regions of histones to the outside of segments of DNA already bound by a hydrophobic core from the inside.

7. From the particulate model (11) it was predicted that the protein core and the chromatin structure would be destroyed in the presence of hydrophobic bond-breaking reagents such as urea. Our model, on the contrary, suggests that, although the gross structure of chromatin might be modified, the fundamental histone-DNA complexes would not of necessity be destroyed. Experimental results verified that characteristic melting patterns of both native and partially dehistonized chromatin are preserved in urea (32,43). The effects of urea perturbation on the CD (43,65,66) and melting properties of chromatin as well as their reversibility (43) are in

agreement with our model; they would be difficult to explain under the limitations of the particulate model (11) as originally proposed.

8. For the major fraction of chromatin in which the five major histones exist, the model suggests a regular distribution of histones with one histone H1 molecule per octamer of $(H2A)_2(H2B)_2$+$(H3)_2(H4)_2$. This raises the question of how Nature is able to dictate that histones shall bind the DNA in such regular sequence. The following sequence of events seems to offer a plausible answer: (a) in nuclei, a subunit of $(H1)/(H2A)_2(H2B)_2/(H3)_2(H4)_2$ might be formed before complexing with DNA; (b) these subunits might then bind DNA cooperatively, using energy gained from histone-histone interaction or from binding between histones and DNA; (c) repetition of these subunits of histones on DNA could be interrupted by a signal at the end of an inactive gene, perhaps by a non-histone protein, a RNA chain or some other molecules. This suggestion provides a mechanism for the regular distribution of histone subunits or particles in chromatin. In reverse, it implies a cooperative removal of histone subunits as a means for the cell to "turn on" a gene which initially had been inactivated by histone binding.

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