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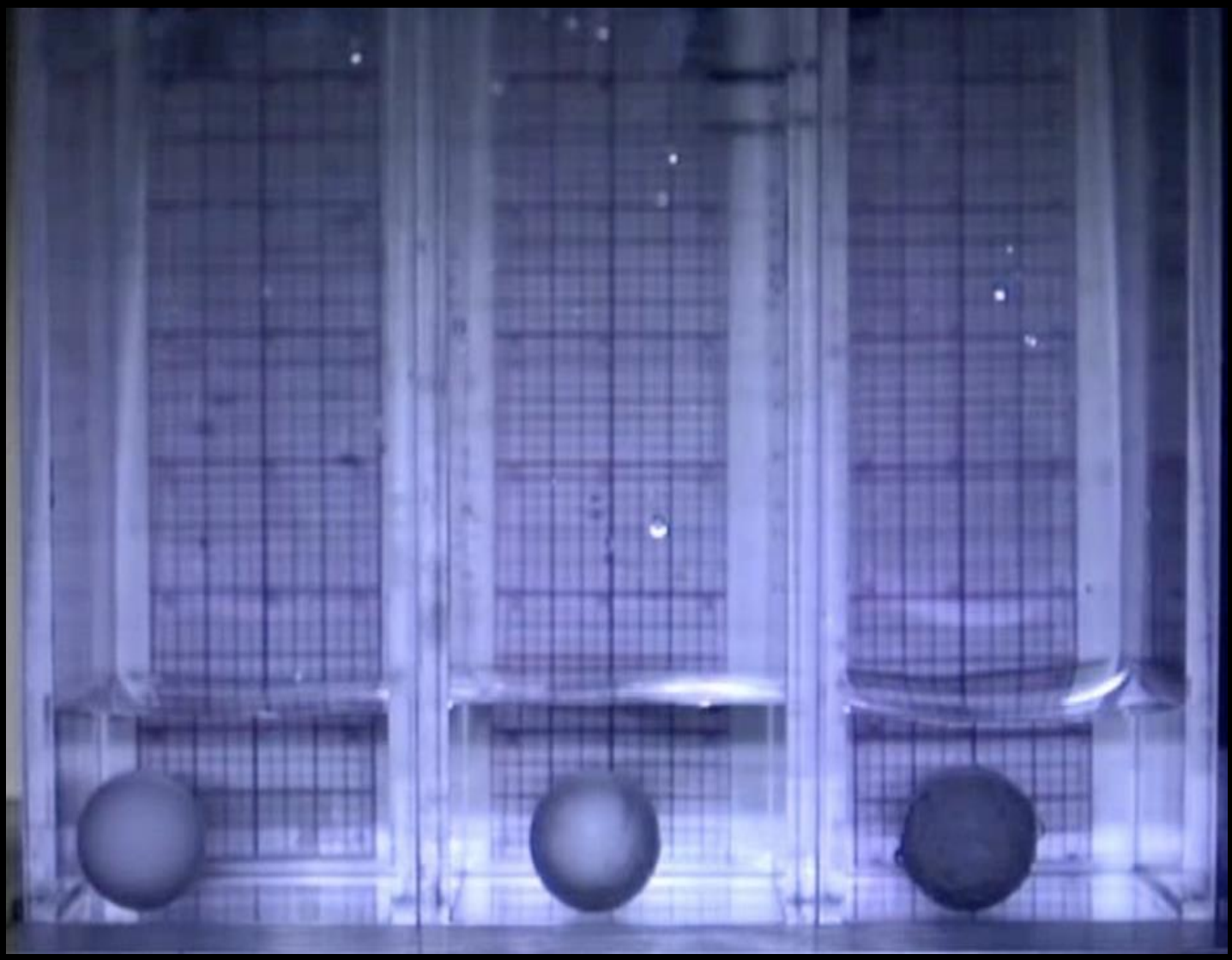

Expulsion Effect of Superhydrophic Materials

Cross-linking of DNA Segments by Histone H1 Explains H1 Associated Phenomena of Chromatin Folding

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Molecular mechanism for the cooperative and non-cooperative binding of histone H1 to DNA, its rearrangement and exchange between chromatin fibers and its role in the folding of interphase chromatin are proposed in this communication. The mechanism of H1 binding to DNA described here is simple and is based on two established facts; (i) histone H1 can crosslink two DNA segments through salt bridge formation between its positively charged lysine and arginine residues and the negatively charged phosphodiester bonds of the DNA segments, (ii) cations reduce the negative charges on DNA segments and thus decrease the force of repulsion between them.

Introduction

Histones H1 and H5 are the most basic histones of molecular weight around 21,500 daltons[1] and play key role in chromatin folding and gene expression.[2] Since chromatin folding is associated with gene activation and inactivation, their role in certain diseases, including cancer, cannot be ruled out and therefore, the mechanism of chromatin folding and the dynamics associated with histone H1 has drawn attention of many scientists.[3] These histones have three domain structures, i.e. a central globular domain flanked by a short $–NH_2$ terminal and a long –COOH terminal tails. The $–NH_2$ and–COOH terminal tails are highly basic and extended while the globular domain is poor in basic amino acid residues but contains all of the aromatic amino acids of the molecule.[4-7] The following phenomena associated with H1 and H5 are known by now: (1) Salt-dependent cooperative binding of H1 and H5 to DNA. (2) Salt-dependent folding of 10 nm poly-nucleosomal chain into 30 nm thick fiber. (3) Salt-dependent rearrangement of H1 and H5 from short oligonucleosomes to longer oligonucleosomes. (4) Salt-dependent exchange of H1 and H5 between chromatin fragments. (5) Salt-dependent aggregation of chromatin fiber. There are two modes of binding for H1 to DNA, non-cooperative and cooperative. Non-cooperative binding takes place at salt concentration below 20 mM and is characterized by uniform distribution of H1 on DNA fragments. H1 binds to all DNA fragments finally saturating and aggregating them together. Co-operative binding initiates on increase of NaCl concentration above 20 mM and is associated continuation of H1 binding to the same DNA molecules leaving other free till their saturation and aggregation is complete. Thus, in non-cooperative binding there is a sudden aggregation of all the DNA fragments just at the point where the ratio of H1/DNA reaches to the saturation level. In cooperative binding, with increase in amount of H1 there is a regular increase in the amount of DNA aggregated till all the DNA fragments are aggregated at the saturation point.[8] Longer DNA fragments are preferred over shorter ones for cooperative binding. H1 shows preference for AT rich DNA during cooperative binding. Supercoiled DNA binds H1 more efficiently as compared to linear and fully relaxed DNA. The globular domain of H1 is responsible for this differential binding affinity.[9] The carboxy terminal extended region of H1

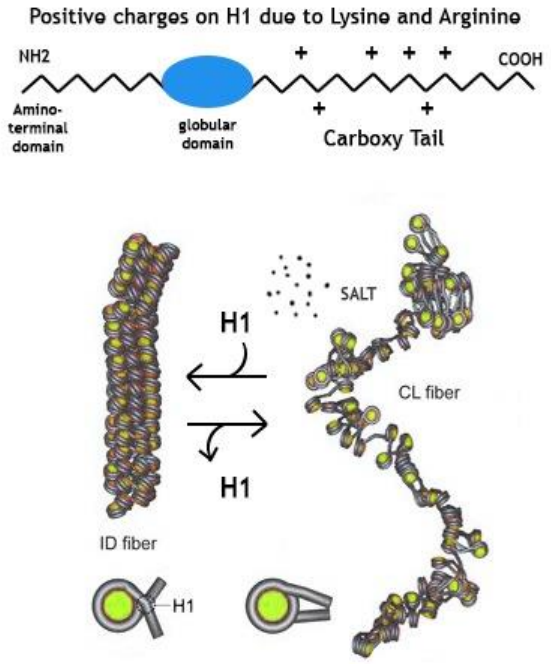

Fig. 1. Schematic diagram of histone 1 (*Top*). Unfolding of chromatin higher order structure is associated with H1 removal and in presence of low salt concentration while the opposite takes place at the time of chromatin folding and gene inactivation. In eukaryotes, in the basic unit of chromatin, the nucleosome, the DNA is wrapped around the histone core (H2A, H2B, H3 and H4) and histone H1 seals the entering and exit points of the DNA strands like a clamp stabilizing the chromatin (Bottom). *Adapted from ref. 29*.

is involved in the aggregation of DNA.[10] The complexes formed at lower salt have 6 nm diameter interspersed with naked DNA, while complexes formed on cooperative binding have uniform thickness of 11-15 nm diameter. The H1 molecules on cooperative binding are in close proximity in contrast to the distantly located H1s on non-cooperative binding.[11] Presence of linker histones in 10 nm chromatin fiber is absolutely required for its folding to 30 nm fiber on raising the cation concentration. Approximately one molecule of H1 is present per core nucleosome.[12,13] H1 molecules lie in close proximity to each other as evident from an easy formation of poly H1 on treatment of chromatin with cross-linking reagents.[14] The formation of homopolymers of H1 is observed in both 10 nm as well as 30 nm fibers.[15] The linker histones are arranged in head to tail fashion in the chromatin as concluded on the basis of cross-linking studies.[16] On the folding of polynucleosomal chain to 30 nm fiber cross-linking also occur between CH1-CH1, NGH1-NGH1 and NGH1-CH1 of neighboring molecules.[17] Contact between globular domains of neighboring H1 molecules was proposed by Thoma et al.[18] and later supported by studies of Losa et al.[4] Thus, it seems possible that the contacts between additional sites of neighboring H1 molecules are established on the folding of poly-nucleosomal chain to 10 nm fiber. It has already been mentioned that linker histones have three domain structures. The globular domain of linker histones seals the two turns of nucleosomal DNA.[18,4,5] The $–NH_2$ terminal tail is required for correct positioning at the globular domain of linker histones with respect to nucleosomal structure.[19] The –COOH terminal tail of linker histone alone is capable of folding poly-nucleosomal chain to 30 nm chromatin fiber.[20] The location of $–NH_2$and –COOH terminal tails of linker histones in chromatin is still not clear. Weak binding of H1 with core nucleosome in the chromatin has been demonstrated by cross-linking studies.[21] Very efficient binding of H1 to core nucleosome has recently been reported.[6] The core nucleosomes having H1 binding region long enough to accommodate two molecules at –CH1 shows salt-dependent aggregation on binding of H1 or –CH1.

Although various aspects of the above phenomena have been worked out, the actual molecular mechanism, underlying these processes are not known yet. In this communication, possible mechanisms explaining the above processes are being presented for the consideration of the entire H1.These mechanisms are based on the two facts; (i) H1 and H5 can hold or interact with two DNA segments lying parallel to each other by cross-linking through their lysine and arginine side chains, (ii) cations as well as H1 and H5 help in bringing the two DNA segments close to each other by neutralizing the negative charges in the DNA segments.

Phenomena associated with H1 binding and their explanation in light of our proposed mechanism:

1. Binding of H1 to DNA

Renz and Day[8] proposed two alternative mechanisms for explaining the cooperative binding of H1 to DNA and its preference for AT rich DNA fragments. (1) The binding of H1 to DNA above 20 mM NaCl might be inducing either local change in the secondary structure of DNA or the folding of the whole DNA helix. The changed secondary structure of folded structure of DNA might be preferred for subsequent binding of H1 eventually leading to accumulation of H1 in the same DNA molecule and (2) in case of AT rich DNA, the uniform distribution of AT might provide increased flexibility in DNA molecule making it preferable for binding of H1. The asymmetrical distribution of A, T due to AT rich sequences will provide the high affinity binding centers. The H1 binding will start at these sites and will continue on these molecules. Glotov and his coworkers[10] proposed the following mechanism for the salt-dependent cooperative binding of H1 to DNA. At salt concentrations below 20 mM, H1 is bound to the DNA molecule but there is no interaction between the DNAs. Increase in salt concentration above 20 mM might induce formation of some specific secondary and tertiary structures in H1 and DNA required for cross-linking of DNA strands. This will lead to the cross-linking of DNA strands by H1. Direct specific contacts between DNA and structured region of H1 might be involved in this process. The ‘bridge’ structure can be formed by either the opposite terminal parts of H1 binding to the two DNA strands or by carboxy terminal fragment alone. They suggested that H1-H1 interactions should not be important for cooperative binding. Clark and Thomas[11] have advised against any change in cooperative binding. They have argued that (1) no change in the secondary and tertiary structure of H1 or DNA has been observed in vitro in this range of salt concentration (20-120 mM NaCl). (2) The globular region of H1 is folded even at low salt concentration on binding to DNA. (3) Cooperative binding of H1 to DNA is not associated with change of enthalpy, rather it is entropically driven.[22]They have, however, assigned the role of H1-H1 interaction in cooperative binding. This interaction may be hydrophobic in nature or may be direct or indirect. Indirect interaction may be brought about by the binding of DNA that is caused by the interaction with H1.

We propose following mechanism for the two modes of binding of H1 to DNA. At low salt concentration (below 20 mM), H1 added below saturation levels binds to the monomeric DNA through electrostatic interactions between positively charged lysine and arginine side chains of H1 and array of negative charges on DNA. The majority of the salt

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bridges should be formed with the –COOH terminal extended tail of H1 as it is the most basic part of the H1. Only the part of the lysine and arginine side chains should be engaged in salt bridge formation and remaining should be free. The DNA-H1 complexes remain in monomeric form as the two DNA-H1 complexes repulse each other and come closer due to remaining negative charges on the DNA–H1 complex. As the H1 is increased to saturation value the negative charges on the DNA are substantially neutralized by the positively charged lysines and arginines of H1. This leads to the bringing together of DNA-H1 complexes and formation of cross-linkage between free lysines and arginines of one DNA-H1 complex with the negative charges on the other DNA-H1 complex, eventually leading to aggregate formation. Increase in the salt concentration in the H1 DNA mixture below saturation stage neutralizes more and more negative charges on the monomeric DNA-H1 complexes. This allows some of the monomeric DNA-H1 complexes to come closer and form dimeric DNA-H1 complex and be held together by the cross-linkage between free lysines and arginines of one DNA-H1 complex with free negative charges on the other DNA-H1 complex and vice versa. The other H1 molecules prefer to bind to this preformed dimeric DNA-H1 complex over monomeric DNA as in the dimeric DNA-H1 complex the H1will be sandwiched between the two DNA segments and thus will be more tightly bound. This would lead to the accumulation of H1 in the dimeric DNA-H1 complex till it gets saturated. Filling up of the dimeric DNA-H1 complex with more H1 molecules will help in further neutralization of negative charges of its DNA. This will eventually allow self-association of several dimeric DNA-H1 complexes, leading to aggregation of DNA. Thus, the role of cations in this process is to neutralize the negative charges on DNA-H1 complex so that the repulsion is less. Preference of H1 for binding to the larger size DNA fragments over shorter fragments during cooperative binding can also be explained by the above model. The larger size dimeric DNA-H1 complexes will be more stable in comparison to the shorter dimeric DNA-H1 complex as the number of cross-linkages per molecule of large DNA-H1 complex will be greater than that of the shorter dimer. Thus, the H1 will prefer to bind more stable DNA-H1 complexes over the less stable ones.

2. H1-induced folding of chromatin fibers

We propose the following mechanism in order to explain the above phenomena. At low salt concentration (below 40 nm), -MH_2 terminal short tail of H1 will bind to one end of nucleosomal DNA while –COOH terminal tail will bind to linker DNA adjoining the other end of nucleosomal DNA. However, part of the –COOH terminal tail will be extended to the DNA of neighboring nucleosomal core. This will lead to the occupation of less than half of the H1 binding region in the nucleosomal core. Thus, each nucleosomal core will have less than half of their H1 binding region occupied by –COOH terminal tail of H1. This will partly resemble to the core nucleosome mixed with one –CH 1 per core nucleosome in vitro. We have shown in our in vitro studies[6] that the core nucleosomes in this case will show increasing tendency of self-association with increase in salt concentration, reaching to a plateau at 100 mMNaCl. Applying same observation in the above case poly-nucleosome chain, the nucleosomal cores will remain apart from each other at very low salt concentration due to the strong repulsive force operating between poly-anionic nucleosomal cores. On increase of salt concentration, the cations will neutralize the negative chanrges on vrepulsion. The neighboring nucleosomal cores at moderate salt concentration will thus be brought together and will be held up by the salt bridges formed between the free lysines and arginines of –CH1 bound to one nucleosomal core and DNA segment of the other nucleosomal cores. Thus, each nucleosomal core will be cross-linked by –CH1 of other nucleosomal core in either side. This will lead to the concentration of the poly-nucleosomal chain. The poly-nucleosomal chain will be folded in helical manner due to fixed inward bending of linker DNA by –CH1. Thus during the condensation of chromatin, neutralization of negative charges on core DNA and crosslinking of nucleosomal cores are required. One molecule of –CH1 tail may not sufficiently neutralize the negative charge of nucleosomal core, therefore, cations also required for this purpose. Interaction of a cross-linker like H1, spermine, spermidine, protamine Mg++, Ca++ to the nucleosomal core should be required for condensation of chromatin because it has been observed that monovalent cations cannot induce self-interaction between core nucleosomes even at high concentrations. Other observations like binding of two molecules of H1 per nucleosome in the reconstituted chromatin can also be explained by the above model. Since each nucleosomal core in the chromatin is only partly occupied by –CH1, this can bind one more –CH1 segment of the second molecules of H1. Globular domain of the second molecule of H1 will thus remain free. This has already been reported by Losa and his coworkers.[4] The nucleosomal cores in this reconstituted chromatin will interact with neighboring nucleosomal cores even at lower salt concentrations, as observed in in vitro studies with core nucleosomes mixed with two –CH1 per core particle.[6] The compactness of these reconstituted chromatin fragments at low salt concentrations has also been reported.[23]

3. Rearrangement of H1 from shorter to longer oligonucleosomes

It has been reported by Renz and his coworkers that H1 prefers to bind to longer oligonucleosomes over shorter oligonucleo-

somes.[24] This leads to the rearrangement of H1 from shorter to longer oligonucleosomes. Let us consider this phenomena in light of our model. In the mononucleosomes, H1 will bind along the whole length of linker DNA as well as on the nucleosomal core DNA. In the dinucleosome –CH1 segment of one H1 will be sandwiched between the nucleosomal cores while the -CH1 part of other H1 molecule will remain bound to only one nucleosomal core DNA. H1 molecule which is sandwiched between the two nucleosomal cores will be bound tightly as compared to the other one.

Thus, in nucleosome 100% of the H1 molecules will be bound loosely while in dinucleosome 50% of the H1 will be bound loosely and 50% tightly. Similarly, trinucleosome, tetranucleosome, pentanucleosome and hexanucleosomes will have 66%, 75%, 80%, and 100% H1 respectively in tightly bound form. Thus, it is clear that affinity of H1 binding to oligonucleosomes increases with increase in its chain length. Another factor, which should be considered, is the stability of the condensed state of oligonucleosomes. The condensation in longer oligonucleosomes will be more stable as compared to shorter ones as the number of interaction per fragment responsible for the maintenance of condensed state in longer fragments will be more than the shorter ones. Since, the difference in the binding affinities of H1 molecules arise due to condensed state of oligonucleosomes which in turn requires presence of moderate concentration of cations, the rearrangement of H1 automatically becomes dependent on salt concentration.

4. Exchange of H1 between chromatin fragments and aggregation of chromatin fibers

A fast exchange of H1 between chromatin segments at physiological ionic strength has been reported.[25, 26] Recently Jin and Cole[27] observed the dependence of H1 exchange on the aggregation of chromatin fibers. Rapid migration of H1 from one region to another region in chromatin has been reported by Huang and Cole. [28] Increase in salt above physiological concentration (145 mM NaCl) induces aggregation and precipitation of chromatin fibers. The solubility behavior of chromatin fragments is independent of its size.[7] The divalent cations $Mg^{++,}$ Ca^{++} and multivalent cations spermine and spermidine can also induce the aggregation and precipitation in similar manner.[7,27] This phenomenon can be explained through our proposed mechanism. At lower salt concentration, chromatin fragments will repulse each either due to large negative charges on them. The increase in salt concentration upto physiological one (i.e. 145 mM NaCl) will decrease the repulsive force between the fragments. This will lead to the collision and bringing together of the DNA fragments from the chromatin fragments. On collision of the chromatin fragments with each other, salt bridges will be formed between free lysine, arginine of H1 bound to one chromatin fragment and the negative charges on the DNA of other fragment. After collision, when the chromatin fragments will get apart from each other, they may take away each other's H1 with them. This will lead to exchange of H1 between chromatin fragments. Further increase of salt concentration will weaken the repulsive force between chromatin fragments to such an extent that after collision of fragments these will not be repelled but will remain associated. This will eventually lead to aggregation of the chromatin fibers in which the fibers will be held together through cross-linking by H1.

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