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Hypothesis

Order and disorder in 30 nm chromatin fibers

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The tendency of DNA to form fibers upon condensation with counterions is reviewed. It is shown that chromatin fibers may acquire a relatively constant diameter of about 30 nm simply as an optimal size achieved upon neutralization of DNA, without requiring a repetitive internal structure. Thus the size of chromatin fibers would not be determined by any specific spatial interaction between DNA and histones. The driving force for the formation of fibers in chromatin would be similar to that found in proteins when they acquire a compact globular shape.

Chromatin; DNA; Histone, Protamine

1. INTRODUCTION

During the last few years Dill and coworkers [1] have shown that the globular shape of proteins could result from a collapse of the polypeptide chain due to general thermodynamic forces rather than to specific interactions between amino acid residues. More recently Bloomfield [2] has presented a theory to explain the finite diameter of the complexes formed by DNA with some counterions. In fact both theories could be applied to more complicated systems, such as chromatin. In this paper we explore the consequences of such an approach.

Several models, reviewed by Koch [3], have been suggested in order to explain the structure of the so-called 30 nm chromatin fiber, which in fact has a diameter which fluctuates locally and whose average value depends on the protein composition and on the length of internucleosomal DNA [4,5]. In spite of considerable intellectual and experimental efforts, it is difficult to produce a model in which the irregular diameter of the chromatin fiber is reconciled with definite interactions which allow the nucleosomes to associate in a more or less repetitive fashion. Most models imply interactions to stabilize the fibers which should result in a regular structure. On the other hand, the chromatin fibers show a variable aspect when observed under the electron microscope [6,7]. Furthermore the length of spacer DNA depends on the source of chromatin. Changes in the length of spacer DNA result in a great variability in the relative orientation of nucleosomes [8], so that it does not appear possible to produce a model with a regular organization of nucleosomes inside the 30 nm chromatin fiber. In summary, we are in the presence of an apparent contradiction: an irregular array of nucleosomes on DNA results into a chromatin fiber with a rather regular diameter of about 30 nm.

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2. THE FORMATION OF FIBERS FROM DNA

It has been observed that complexes of DNA with different counterions such as spermidine, trivalent cations, histone H1, other basic proteins, etc., do form fibers or rods with sizes in the 10–50 nm range (references given in Table I). Sometimes toroidal particles are observed which may be considered equivalent to a circular fiber. Fibers with a similar size are also observed in late spermatids–spermatozoa which have a wide variety of protein compositions, many examples of which can be found in the book edited by Baccetti [9]. In Table I we summarize some of the diameters observed for DNA fibers by different authors. From all these observations we conclude that DNA tends to precipitate in fibers with a defined size which varies in a limited range (13–48 nm for the examples given in Table I) depending on the precipitant used: cations, polyamines, protamines, histones, etc. The nature of the counterions appears to have only a marginal influence on the diameter of the fibers.

3. THEORETICAL APPROACHES

From the observations summarized above it appears that the formation of fibers with a defined size is mainly due to a thermodynamic optimization of the DNA–

Table 1

Size of condensed DNA fibers

Counterion/Precipitant/Complex	Approximate diameter* (nm)	Reference
Ethanol	13	[18]
Hexammine cobalt(III)	27	[19]
Spermidine	40–48	[19,20]
Histone H1	13–21	[21]
Nucleosome cores	13	[22]
Dinucleosomes	39	[22]
Chromatin fibers	20–45	[4,5]
Mollusc spermatid fibers	27–47	**
Cricket sperm	30	[14]
Sea urchin sperm	30	[23]

* In most cases the diameter observed depends on the ionic conditions, the method of observation and the composition of DNA and counterions. The original references should be consulted for details.
**Casas, Ausio and Subirana, in preparation.

precipitant interactions which favours an optimal size for the precipitate. In fact, formation of precipitates with a defined size is very common in polymer systems: a spectacular case is the process of phase separation in block copolymers, which results in very regular cylindrical aggregates or fibers in certain concentration ranges as has been recently reviewed [10].

In the case of DNA complexes it is found that precipitates of both toroidal and fibrous (rod) shapes are formed, as reviewed by Bloomfield [2]. This author also discusses previous theories and suggests a new approach in order to understand the optimal size observed in the condensation of DNA in the form of toroidal particles. He concludes that an optimal size is achieved as a balance of attractive nearest-neighbour interactions (hydration, bending, etc.) and a repulsive electrostatic energy term. Other authors have tried to understand cation-induced DNA condensation [11] and have also enumerated the various energetic contributions to DNA collapse, although they have not suggested any precise mechanism to define an upper limit for the size of the particles formed.

The theory of Bloomfield [2] is concerned with the collapse of DNA into particles of a limited size, as it occurs with viral DNA. When fragments of larger size are used, as well as in the sperm systems discussed above, only cylindrical fibers are found. It is interesting to note that the diameter of such fibers is similar to the diameter of the cylindrical aggregates that bend around to form the toroidal particles. The same forces implied by Bloomfield may determine an optimal diameter for high molecular weight DNA when collapsed in the form of fibers.

It does not appear possible to me to derive an exact theory to determine the optimal diameter of chromatin fibers, but on the basis of the approach suggested by Bloomfield and the counterion condensation theory [12] it is possible to understand that an optimal diameter will appear when DNA is condensed in the form of fibers. In a simplified model we can assume that DNA molecules are oriented parallel to the axis of the DNA–protein fiber complex, with a gain of free energy ε per unit length of DNA molecule upon formation of the complex. The parameter ε includes all the nearest-neighbour interactions which favour the collapse of DNA molecules. If there are n DNA molecules in a cross-section of the fiber, the total gain ΔG_p of free energy per unit length upon formation of the fiber will be:

$$\Delta G_p = n\varepsilon \quad (1)$$

This expression should be corrected for surface effects, but these are rather small since for a fiber with a diameter in the range 30–50 nm, n should be equal to 100–400 molecules. Marquet and Houssier [11] and Bloomfield [2] have estimated the value of ε taking into account the various nearest-neighbour interactions involved.

The attractive forces which favour the collapse of DNA will be accompanied by repulsive electrostatic forces, since DNA, even in a precipitate, has a remaining partial charge q per unit length in agreement with Manning's theory [12]. The charge q should be distributed into two components: q_1, which will migrate to the surface of the fiber, and q_2, which will remain inside the fibers. Unfortunately it is difficult to determine what part of the charge will migrate to the surface; in other words, it is not easy to estimate q_1 and q_2 even if q is known. Thus the repulsive electrostatic force will be:

$$\Delta G_E = k_1 (nq_1/\sqrt{n})^2 + k_2 (nq_2)^2 \quad (2)$$

where k_1 and k_2 are geometric constants, which include the appropiate electrostatic parameters [13]. The first term refers to the free energy at the surface and it is therefore divided by the perimeter of the fiber which is proportional to $\sqrt{n}$.

Equilibrium will be reached when $\Delta G_p = \Delta G_E$, so that from Eqns. 1 and 2 it turns out that:

$$n = \frac{\varepsilon - k_1 q_1^2}{k_2 q_2^2} \quad (3)$$

This equation has only been derived for the purpose of showing that an equilibrium value n will be reached which will strongly depend on the electrostatic interactions. Unfortunately the parameters in Eqn. 3 are difficult to calculate exactly, as discussed by Bloomfield [2] and by Marquet and Houssier [11]. Nevertheless Eqn. 3 shows that when there are few charges left inside the fibers, i.e. q_2 is small, the number of fibers n will increase so that a macroscopic precipitate will appear instead of a fiber with a defined diameter. Fibers will appear when q_2 has a reasonable value, so that it contributes significantly to the free energy ΔG_E given in Eqn. 2.

The theoretical approach we have presented does not

give any direct information on the exact shape of DNA within the fibers formed. It is likely that in those cases in which the fibers are very regular, as in cricket spermatozoa [14], the organization of DNA will be rather regular, whereas in cases where complex counterions are involved, as in chromatin, both the organization of DNA and the diameter of the fibers may become more irregular as it is in fact observed.

4. STRUCTURE OF THE 30 NM CHROMATIN FIBERS

In the case of chromatin, this approach implies that the size of the chromatin fibers is not only influenced by specific spatial interactions among nucleosomes, spacer DNA and histone H1, but it is mainly determined as an optimal size achieved upon neutralization of DNA. This interpretation eliminates the problem of placing in a regular fashion spacer DNA segments of variable length, which is a topologically unsolvable problem [8,15]. In the fiber, spacer DNA will run back and forth between contiguous nucleosomes, but without any overall regularity. It will bend easily, since upon neutralization DNA becomes very flexible, as shown by Manning [16,17]. On the other hand, it is likely that there will be local patterns of interaction (solenoids, zigzags, accordeons, bundles of spacer DNA, etc.) which repeat themselves in different regions of the chromatin fiber, depending on the local spacer length and chromatin composition (DNA sequence and proteins present, in particular H1 histone).

5. CONCLUSION

From all these considerations it can be concluded that the 30 nm chromatin fiber appears as a result of charge neutralization of DNA. Its size is mostly defined by an overall energy minimization and not by any specific histone–DNA interactions.

The situation in chromatin fibers may be compared to that of globular proteins, where inside an approximately spherical shape a wealth of secondary structure elements are hidden. Chromatin fibers will vary locally in the organization of their nucleosome cores and spacer DNA, but will probably use a limited number of structural interactions. Such variations in local structure might have an important influence on the biological function of different regions of chromatin.

There is a strong parallelism between this suggestion and the approach of Dill and coworkers [1] for the interpretation of protein structure: the driving force is the formation of a compact shape (globule in proteins, fiber in chromatin), whereas the internal structural elements are of minor energetic importance, although of great biological significance.

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REFERENCES

[1] Chan, H.S. and Dill, K.A. (1990) Proc. Natl. Acad. Sci. USA 87, 6388–6392.
[2] Bloomfield, V.A. (1991) Biopolymers 31, 1471–1481.
[3] Koch, M.H.J. (1989) in: Protein–Nucleic Acid Interactions (Saenger, W. and Heinemann, U. eds.) pp. 163–294, McMillan, London.
[4] Alegre, C. and Subirana J.A. (1989) Chromosoma 98, 77–80.
[5] Williams, S.P. and Langmore, J.P. (1991) Biophys. J. 59, 606–618.
[6] Rattner, J.B. and Hamkalo, B.A. (1978) Chromosoma 69, 363–379.
[7] Olins, A.L. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 325–329.
[8] Subirana, J.A., Muñoz-Guerra, S., Radermacher, M. and Frank, J. (1983) J. Biomol. Struct. Dyn. 1, 705–714.
[9] Baccetti, B. (ed.) (1970) Comparative Spermatology, Academic Press, New York.
[10] Ajdari, A. and Leibler, L. (1991) La Recherche 22, 732–739.
[11] Marquet, R. and Houssier, C. (1991) J. Biomol. Struct. Dyn. 9, 159–167.
[12] Manning, G.S. (1978) Q. Rev. Biophys. 11, 179–246.
[13] Tanford, C. (1961) Physical Chemistry of Macromolecules, pp. 473–475, Wiley, New York.
[14] Suzuki, M. and Wakabayashi, T. (1988) J. Mol. Biol. 204, 653–661.
[15] Trifonov, E.N. (1990) in: Theoretical Biochemistry and Molecular Biophysics (Beveridge, D.L. and Lavery, R. eds.) pp. 377–388, Adenine Press, Guilderland, NY, USA.
[16] Manning, G.S. (1985) Cell Biophys. 7, 57–89.
[17] Manning, G.S. (1989) J. Biomol. Struct. Dyn. 7, 41–61.
[18] Eickbush, T.H. and Moudrianakis, E.N. (1978) Cell 13, 295–306.
[19] Plum, G.E., Arscott, P.G. and Bloomfield, V.A. (1990) Biopolymers 30, 631–643.
[20] Marx, K.A., Ruben, G.C. (1983) Nucleic Acids Res. 11, 1839–1854.
[21] Clark, D.J. and Thomas, J.O. (1988) Eur. J. Biochem. 178, 225–233.
[22] Pérez-Grau, L., Azorín, F. and Subirana, J.A. (1982) Chromosoma 87, 437–445.
[23] Longo, F.J. and Anderson, E. (1969) J. Ultrastruct Res. 27, 486–509.