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A MODEL FOR THE NUCLEOSOME CORE PARTICLE SUBUNIT

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Summary

A model derived from electron microscopy and neutron scattering is presented for the nucleosome core particle. The 144 base pairs of DNA in the core particle are distributed in two types of coils. About 35 base pairs spiral up toward the dense core of apolar histones with a 6.5 nm radius and 12 nm pitch. Upon contact with the histone core the coiling changes handedness and the radius and pitch are reduced to 3.0 nm for 1 1/4 turns, having 70 base pairs. At the bottom of the core, the handedness changes and the DNA spirals up again with a 6.5 nm radius. 75 % of the histones fill the cavity of the central coil while the remainder project out and partially neutralize the external coils. Such a core particle is wedge-shaped, with dimensions of 5.5 x 10 x 12 nm. This model is derived from and is a refinement of an earlier model for 200 base pair nucleosomes (1) and appears to provide as good an explanation for the biophysical and biochemical data as other current models.

Chromatin is made up of subunits called nucleosomes (2) which can be isolated after brief nuclease digestion (3,4,5,6). If the nucleosomes, which for most tissues have about 200 base pairs, or chromatin, are extensively digested by nuclease, particles with about 140 base pairs are produced (4,6). These resistant "core particles" (4) contain no histone H1 but have two copies of each of the other four histones (4,7). Nucleosomes and core particles have been studied by electron microscopy (8,9,10,11,12), by neutron scattering (13,14,15) and a large variety of other techniques.

By primarily using the neutron scattering data from core particles in D_2O, Pardon et al. (16,17) were able to 1) determine the height of the core particle $\sim$ 5.5 nm, and 2) propose a detailed model featuring two DNA rings separated by 3.2 nm which surrounded a cylindrical protein core 6.4 nm in diameter. Subsequently, Finch et al. (18) published low resolution ($\sim$ 2.5 nm) diffraction data on core particle crystals from which they could obtain the approximate dimensions $\sim$ 5.7 x 11 x 11 nm - and determine that the structure was wedge shaped. They also proposed one model which could fit their data : a regular DNA

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super coil of 1 3/4 turns with a pitch of 2.8 nm and a center radius of 4.5 nm. (The height of this model is 6.9 nm when the 2 nm width of the DNA is included.)

Another model having a height of 6 nm was presented in 1975 for nucleosomes (see figure 4 of ref. (1), or ref. (19)). However, it must be admitted that, although such a height was a reasonable value, it was not the only possibility for such a double coil structure. On the other hand, the known value for the height of the core particle (17,18) greatly limits the parameters and types of models which can be consistent with all the data. Some indications suggest that the model which will be described here may be a unique solution to all the data, but these arguments will be given elsewhere (20) along wich a detailed development of the model and a direct comparison to all the neutron, X-ray and electron microscopy results. In the present article, it will only be shown that there is at least one double coil type model which is in good agreement with the neutron scattering in D_2O and compares well to other data.

RESULTS AND DISCUSSION

Derivation of the Model. The model results from previous observations of freeze fracture replicas of hydrated chromatin (1, 19). More recent freeze fracture micrographs are of improved quality (Lepault and Bram in preparation) but exhibit the same basic structure. The nucleosomes in these micrographs display a dense core about 7 nm in diameter, and an external, 14-15 nm diameter coil contiguous with and looping over the core. Taking these images (1, 19) at face value, it appears that the outer coil indirectly joins neighboring cores; that is, the bottom of one dense core is linked to the top of the neighboring nucleosome dense core. Measurements of the external coil lengths allowed an estimation of the amount of DNA in the central core to be 70-80 base pairs. Most of the histone was postulated to reside in the dense core. This type of structure was found to be compatible with the low resolution neutron and X-ray scattering on nucleosomes and chromatin (1, 14).

To obtain a core particle of 144 base pairs from such a 200 base pair configuration it was logical to propose (19) that those DNA segments farthest from the histone core would be removed by micrococcal nuclease digestion. This would leave about 35 base pairs on each side of the dense 70 base pair core inside the double coil nucleosome model. Dimensions of the core obtained from the electron micrographs suggested that the DNA in the core is coiled with a center radius of $\sim$ 3 nm.

The height of the core from the freeze fracture micrographs or other data (17,18) and its 70 base pair DNA content then greatly limit the types of models which can be consistent with the images.

Starting with these generalized dimensions, space filling models were constructed and their theoretical neutron scattering patterns were compared to the data (15,17) at all DNA and protein contrasts. After approximately 100 calculations and refinements the model sketched in figure 1 was obtained. Although it is not claimed here that this type of model is the only one possible, it appears that for such types of double coil models the parameters described must be correct to ± 10 %.

A summary of characteristics of the double coil core particle model :

1. The core particle has two types of coils with opposite sense.
2. The inner coil contains 70 base pairs in 1 1/4 turns of 3.0 nm pitch and radius.
3. There are two external helical or semi-helical segments of 37 base pairs each with a radius of 6.5 nm and a pitch of $\sim$ 12 nm.
4. Nucleosomes and core particles in chromatin are indirectly linked bottom to top of neighbor by the external coils or segments.
5. The centers of the two external coils are related by a dyad axis passing through the center of the inner coil.
6. 3/4 of the histone fills the gap of inner coil while the remainder projects out on polar "fingers" and partially neutralizes the external coils or segments of DNA. This accords with the literature on the distribution of polar and apolar histone sequences (21,22,23).
7. It is proposed that the local polar histone-DNA interactions are the same over the 144 base pairs, and that the interactions tend to occur on the interior surface of the DNA.
8. Special and tight histone-DNA interactions are required at the entry and exit regions of the histone core, where the DNA coiling abruptly changes sense and radius. The suggestion of two strategic kinks per nucleosome by Gourovitch et al. in 1974 (24) might provide an explanation.
9. Histones are arranged with the same dyad symmetry as the DNA and, as pointed out by Weintraub et al. (25) and Pardon et al. (16), the simplest interpretation is that the fingers of a given histone tetramer bind to DNA on one side of the dyad, and those of the second bind to the other side.

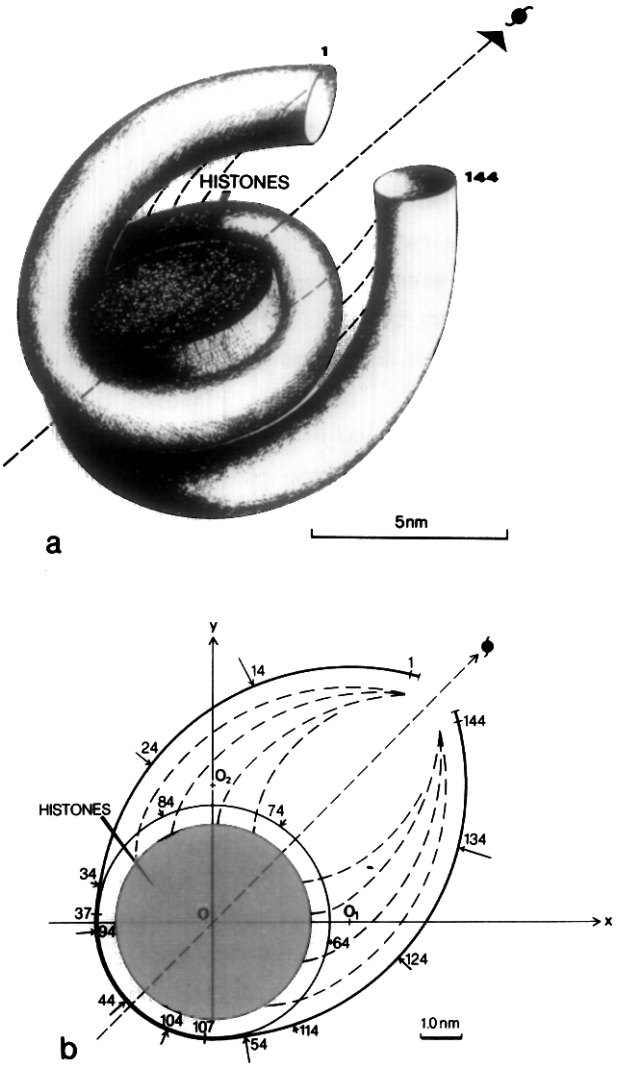

Fig. 1 - Diagrams of the double coil core particle described in the text. The polar histone core is shaded and dashed lines depict the apolar fingers.

a) A sketch of the model viewed down 45° along the dyad axis. The outer coil at base pair 1 projects downward while the DNA at 144 is curving upward. Note the ∿ 2.5 nm hole along the dyad between the outer coils.

b) The XY projection of the model. The DNA (solid line) makes slightly more than one quater turn centered about O_1 and then changes its radius, sense and coiling center (O) at base pair 37. After 1 1/4 turns, the radius and sense of coiling change once again centered on O_2. The DNAse I cutting sites from the 5' end are represented by arrows whose length is proportional to the cutting probability described by Noll (31).

10. The dimensions of the apolar histone core are about 5.0 x 5.0 x 5.0 nm
11. Such a core particle is wedge shaped with external dimensions of about 5.5 x 10 x 12 nm.
12. Nucleosome topology is complex even if changes in DNA secondary structure are neglected, but the core particle model as described has about .8 turns.

The structure of the remaining 50-60 base pairs in the linker between core particles is presently unknown and may either be coiled or project straight out from the core particle ends. The super structure of chromatin has been suggested to have $\sim$ 1/6 of a super helical turn per nucleosome (26). Therefore by adding this .2 to .8, one may conceivably arrive at the value of about 1 turn per nucleosome obtained from the entire SV 40 minichromosome (27). Crick (28) has shown that nucleosome structures of this kind with crossed ends and twistless bends (where the sense of coiling changes) can fit the data on the SV 40 minichromosome topology. However, it is worth mentioning that bends and cross-overs can introduce a considerable contribution to the total linking number.

A comparison to biophysical data. The model presented here has been found to be consistent with all the radii of gyration and neutron scattering profiles published for core particles (20). As seen in fig. 2 the small and medium angle scattering profiles in D_2O and that calculated for the model agree rather well. Furthermore the relative intensity and spacings of the 3.7 and 2.1 nm wide angle maxima fit the data to within the experimental error. Preliminary calculations suggest that the model is compatible with the X-ray crystallography results of Finch et al. (18). It can be seen that the dimensions 5.5 x 10 x 12 nm of the core particle model agree with those from X-ray crystallography (18) and electron microscopy (8,11,19).

One observes from figure 1 that a $\sim$ 2.5 nm diameter region of low density or a hole exists along the dyad axis between the inner and outer coils. Such a hole is repeatedly mentioned in electron microscopy studies of unstained (11) and stained (10,12) chromatin. Moreover, loops with radii of 6 to 7 nm are observed in both freeze fracture replicas (1) and STEM images of unstained or lightly stained chromatin (11).

Comparison to nuclease digestion patterns. The 144 base pairs in the model should be protected from micrococcal nuclease digestion by the polar histone fingers emanating from the core; whereas the remaining 50-60 base pairs in a nucleosome project up and away from this core and are offered less

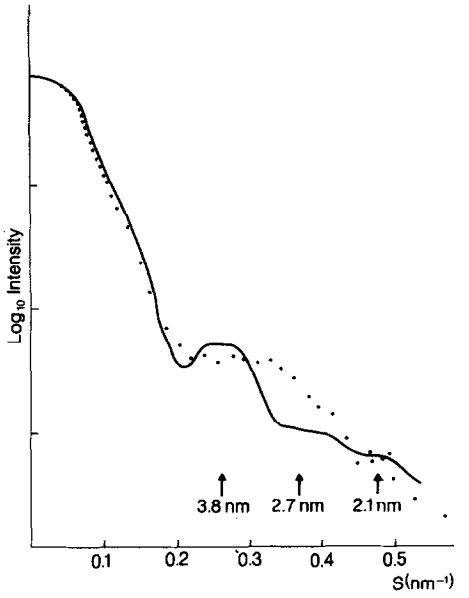

Fig. 2 - Core particle neutron scattering in D_2O from Suau et al. (15) ..., and that calculated for the double coil core particle model (——) described in the text. S is the reciprocal of the equivalent Bragg spacing. Theoretical curves were obtained using 728 appropriately positioned .68 nm cubic volume elements. (The DNA was represented by 289 cubes.) The scattering was then calculated in the normal manner with the Debye equation taking note of the relative scattering of DNA and protein in D_2O. Details will be given elsewhere (20). The theoretical curve agrees with the data in the radius of gyration, general shape and wide angle peak region. An improved fit at larger S could be obtained by slight modification of the model coordinates, however, measurements of neutron scattering intensities at spacings smaller than 4 nm are imprecise due to errors from the incoherent scattering background. Still, the model curve and data display a similar 3.8 nm peak, 2.6 nm inflection and 2.1 nm shoulder.

protection. It is reasonable to assume that tight DNA coiling provides protection for the central core histones against trypsin digestion, while the outer coil fingers should be removed. This could explain the observation that brief trypsin digestion of core particles followed by micrococcal nuclease attack results in a compact 70 base pair particle (4,29).

The DNAse I single stranded digestion patterns of core particles (30,31) contain a wealth of structural information. The occurrence of 10 base pair repeats can be explained by the double coil core particle model with the suggestion of Noll (6) that the DNA is protected from nuclease attack only on the interior side for all 144 base pairs. Finch et al. (17) used the low frequency cuts to suggest the presence of a dyad axis in the core particle but their model does not directly explain the cutting frequency.

The model presented here does suggest a direct rational for the probability of all 14 cuts. Referring to figure (1b) where the arrow lengths reflect the relative probabilities as given by Noll (fig. 14, ref. (31)), the outer segment should be cut with decreasing probability as it spirals toward the histone core. At 34 base pairs from the 5' end the DNA must interact very strongly with protein and bend on itself to change its curvature. Therefore a very low cutting frequency would be predicted. By the 44th base pair the curvature has changed and the DNA is cut with intermediate frequency. The 54th base pair is even more exposed as it is far from the DNA arms and is cut with slightly higher frequency. Then, at base pairs 64, 74 and 84 the DNA is in the interior of the particle. The reader will notice from either projection in figure 1 that these sites are hardly accessible at all to attack by a 3 nm diameter protein. Before base pair 94 the DNA returns to the exterior where it is cut with intermediate probability and the remainder of the diagram repeats.

Conclusion. The model described provides an alternative interpretation to the biophysical and biochemical data for core particles. Its uniqueness lies in its two types of DNA coiling. Hopefully, it will suggest experiments which may help to better understand how the DNA is folded by histones, and vice-versa.

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